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ADVANCES IN GENETICS

VOLUME VI

ADVANCES IN GENETICS

VOLUME VI

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Map Construction in *Neurospora Crassa*

RAYMOND W. BARRATT, DOROTHY NEWMEYER, DAVID D. PERKINS, AND LAURA GARNJOBST

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I. INTRODUCTION

Neurospora was originally selected for studying biochemical genetics because it is an organism in which both formal genetic analysis and the investigation of nutritional mutants appeared possible (Beadle and Tatum, 1941). Its usefulness in both respects has been amply confirmed, but the genetic analysis has lagged considerably behind the biochemical studies. Published segregation data have been inadequate for constructing detailed linkage maps. This has handicapped the investigation of problems that require an accurate knowledge of linkage relations.

The situation has been changed to some extent by two recent publications which present extensive linkage data (Barratt and Garnjobst, 1949, and especially Houlahan *et al.*, 1949). In addition, the present

authors have had access to numerous unpublished data, both from Stanford University dissertations and from individual workers. Therefore an attempt has been made to compile here all available tetrad segregation data relevant to the estimation of linkage. Maps have been constructed on this basis, their reliability has been ascertained, and a variety of problems related to mapping have been considered. Several aspects of the work may be of interest not only for Neurospora but also for other organisms, and especially those in which tetrads can be analyzed.

Three major problems were encountered which are concerned with estimating linkage from tetrad segregations. First, the Neurospora tetrad data are from two different types of intervals (depending on whether the centromere is used as a marker), and it was necessary to combine both types into uniform maps. Second, many map intervals in Neurospora are so long that it appeared important to find a method of correcting for undetected multiple crossovers. Third, most of the data consist of small samples (partly because technical difficulties have prevented the analysis of large numbers of asci), and criteria of reliability were therefore needed for application to linkage estimations. The small sample sizes have also made it necessary to evaluate carefully criteria for establishing linkage and allelism, and to make use of all available methods for determining gene order.

Maps constructed from the compiled data indicate that many uncertainties exist regarding linkage relations. It is hoped that presentation of maps at this time will prove valuable for visualizing experiments necessary to improve their reliability, but even more for planning basic investigations in gene action and cytogenetics.

No attempt has been made to deal comprehensively with aspects of the formal genetics of Neurospora which are not directly concerned with map construction or which have been adequately treated elsewhere. For example, special aspects of interference have been discussed as fully as present data warrant by Whitehouse (1942), Lindegren and Lindegren (1942), and Papazian (1952). For background material on genetic analysis in Neurospora and similar forms, the reader is referred to the publications of Whitehouse (1942), Lindegren (1942), Beadle (1945), Ryan (1950), Ludwig (1937), Knapp (1937), Mather (1938), Mather and Beale (1942), Catcheside (1951), and Papazian (1952).

II. Problems in Estimating Linkage

A number of problems will be discussed below that were encountered in the process of estimating map distances. Estimation of linkage values is, of course, meaningful only for genes known to be linked; the criteria used for establishing linkage are presented in Section IV.

1. *Uniform Treatment of Intervals*

a. *Types of Segregation Data.* Segregants from Neurospora crosses may be collected either as random single spores or as tetrads, which in turn may be obtained either in order or unordered. The great majority of existing segregation data are recorded from ordered tetrads, which consist of spores that have been dissected according to their linear order in the ascus. It would be possible to weigh and combine the few data based on random isolates with the tetrad linkage data (Papazian, 1952; see also Mather and Beale, 1942), but this would make it difficult to solve a much more pressing problem, namely the integration of centromere and linkage data (see Section II, 1, c below). Map distances computed in this paper have therefore been based solely on tetrads, although random isolates have been used for such other purposes as determining allelism and establishing linkage.

b. *Types of Intervals.* Analysis of ordered tetrads makes it possible to map centromeres (Lindegren, 1933), since the occurrence of second-division segregation is revealed by the arrangement of ascospores. The frequency of second-division segregations (postreductions) provides an approximate measure of the recombination frequency between a gene and a centromere (the so-called centromere distance), because homologous centromeres are known to segregate at the first meiotic division (prereduction).

Thus two types of intervals may be encountered when an ordered tetrad is analyzed. One type is bounded by a gene and a centromere, and is measured by one-half the percentage of second-division segregation (Lindegren, 1933); the second is defined by recombination between two genes. Map distances as ordinarily computed for these two types are not equivalent, because 4-strand double crossovers are detectable as recombinants in gene-gene intervals, but not in gene-centromere intervals. If data from both types of interval are to be plotted on the same map, it is necessary to put them on an equal basis.

Figure 1 illustrates the problem for a zygote from a cross of AB × ab. Three segregation types are possible: parental ditype (AB + AB + ab + ab), tetratype (AB + Ab + aB + ab), and nonparental ditype (Ab + Ab + aB + aB). These will be abbreviated PD, T, and NPD throughout the text. Four-strand double exchanges are detectable as nonparental ditype segregations when both ends of the interval are marked by genes. But in the absence of a marker at the centromere, like gene A in the figure, it would be impossible to distinguish a tetrad with a 4-strand double exchange from a tetrad having no exchange between the centromere and locus B, because both the PD and NPD tetrads give

first-division segregations for the B alleles. Thus, first-division segregations for a gene-centromere interval are equivalent to the sum of PD plus NPD segregations for a gene-gene interval, whereas second-division segregations are equivalent to tetratypes. This equivalence also holds for the results of higher multiple exchanges.

c. *Integration of Data from Gene-Centromere and Gene-Gene Intervals.* Uniform treatment of the two types of data could be achieved by ignoring the difference between the two ditype classes in the case of gene-gene

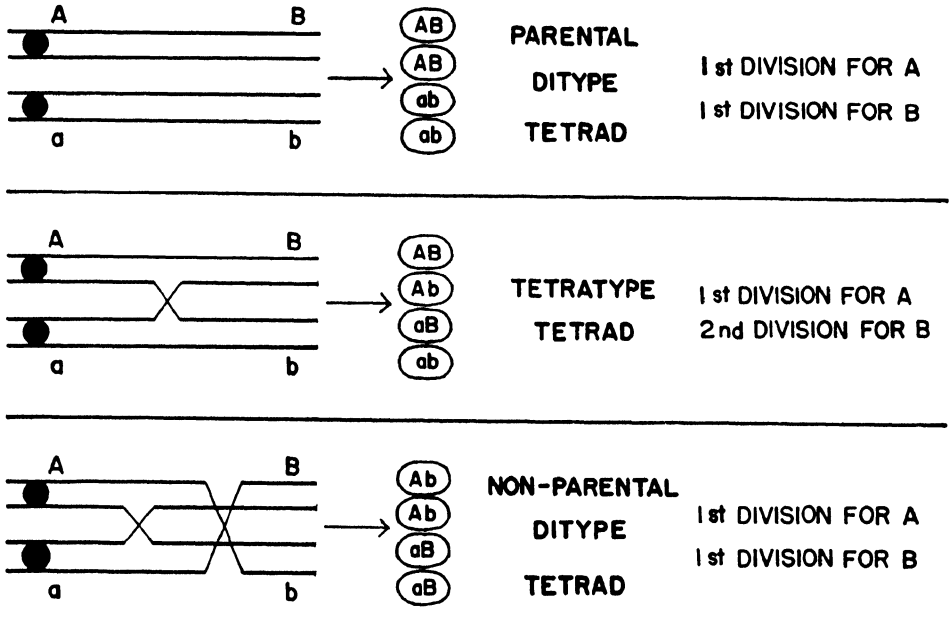


FIG. 1. Diagram showing the relation of parental ditype, tetratype, and nonparental ditype segregations to first- and second-division segregations. (Centromeres are shown as black circles.)

intervals, and using as a measure of linkage either the frequency of tetratype or of second-division segregations, depending on the type of interval. If this be done, those few multiple crossovers that can be detected in gene-gene intervals, but which appear as noncrossovers in gene-centromere intervals, are scored consistently as noncrossovers in both cases.

The proposed procedure unfortunately prevents incorporation of data from random isolates into the same maps, except for short intervals. However, not only do gene-centromere intervals constitute the majority of all information available for mapping, but also there are so few intercrosses that at present it is impossible to map most loci without using gene-centromere intervals. There is therefore little choice except to exclude the few data on random isolates, as well as the information on the few detectable multiple crossovers, and integrate the two types of tetrad data in the manner proposed.

Maps constructed directly from data so integrated are completely uncorrected for multiple crossovers, *i.e.*, they assume complete interference. A correction for multiple crossovers that is applicable to both gene-centromere and gene-gene intervals will now be described.

2. *Correction for Undetected Multiple Crossovers by Means of Mapping Functions*

a. *Introduction.* Few Neurospora crosses involve intervals short enough to preclude a significant amount of multiple crossing over, so that it has not been possible to build up accurate maps from data based on a series of short intervals. It has therefore seemed essential, as a temporary expedient, to seek a correction for the errors due to undetected multiple exchanges within the long intervals which contribute the bulk of existing data. This might be accomplished by a suitable mapping function. (Other possible methods of estimating the frequency of undetected crossovers (Whitehouse, 1942; Perkins, 1949) either provide incomplete corrections or are impractical for present data because of large sampling errors.) Although a mapping function might be only of theoretical interest in Drosophila or maize, where data suitable for mapping are abundant, it becomes a practical necessity for Neurospora, where data are fragmentary, and have mostly been collected incidentally to studying gene action.

Mapping function signifies a mathematical expression relating the observable results of crossing over to the actual map distance. In the case of single strands the observable results are recombination frequencies. In the case of tetrads, second-division or tetratype segregation frequencies are the only observable results common to all intervals; expressions relating these to map distance will be referred to as *tetrad mapping functions.*

Existing single-strand functions are inadequate for the Neurospora data. A simple model has therefore been proposed from which corresponding functions for both single strands and tetrads can be derived for any specified interference intensity. From among these possibilities a specific function has been chosen which agrees satisfactorily with various single-strand and tetrad data. Neurospora maps corrected by this function will later be presented for comparison with the uncorrected maps.

b. *Inadequacy of Existing Mapping Functions.* The true extent of interference in any Neurospora linkage group cannot be known until it is so well mapped that a correction is unnecessary. The best that can be done at present is to use as a correction a function that is based on the amount of interference found in some better known organism, and that agrees with the available Neurospora data.

A number of single-strand mapping functions have been proposed at various times for relating recombination frequencies to map distance. Several of these are shown in Fig. 2, where curve *A* describes the theoretical relation that would obtain with complete interference, and curve *B*, with zero interference (Haldane, 1919, 1931). Curve *C* is stated to agree with data for the mouse (Carter and Falconer, 1951). Curve *D*, proposed

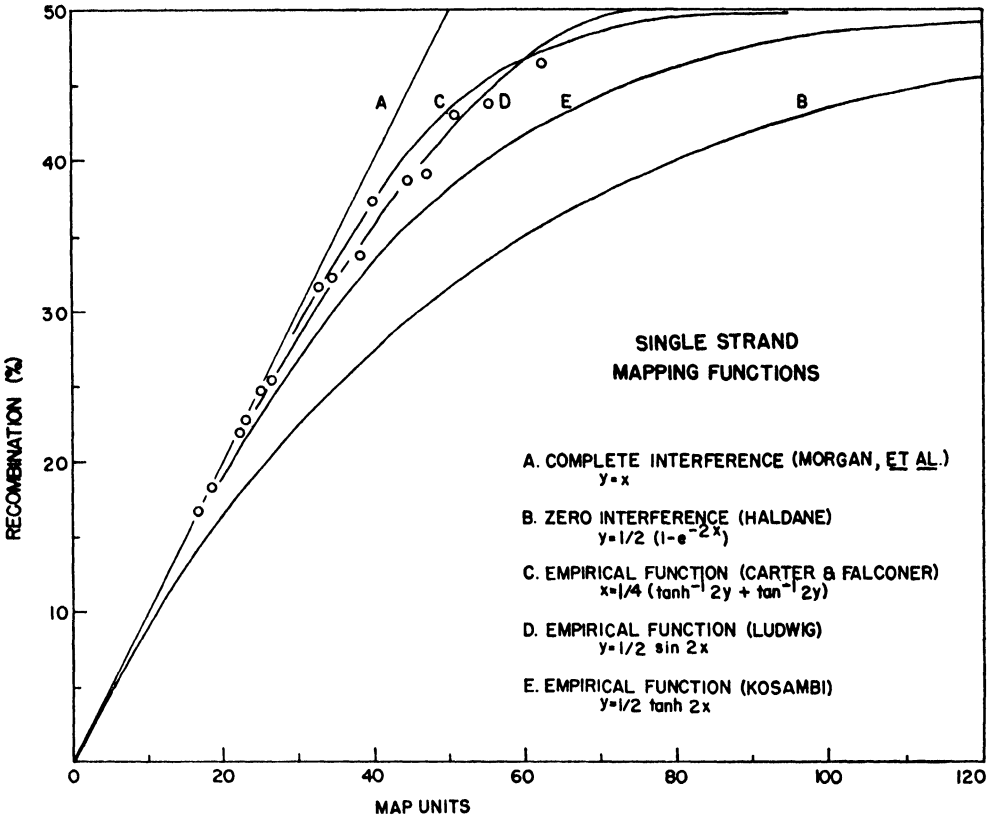


FIG. 2. Single-strand mapping functions in previous use. *A* and *B*: theoretical limiting curves. *C*, *D*, and *E*: empirical functions. Open circles: data for *Drosophila melanogaster* X-chromosome (Anderson and Rhoades, 1931). The source of each equation is indicated in the figure and in the text.

by Ludwig (1934) on the basis of his "gene curves," fits extensive data for the X-chromosome and individual autosome arms in Drosophila. Curve *E* was proposed on the basis of mathematical simplicity (Kosambi, 1944) and is consistent with rather inadequate data in several plant species. For discussions of these and other mapping functions see Owen (1950), Glass (1951), Carter and Falconer (1951), and Carter and Robertson (1952).

In order to be generally useful for ordered tetrads, a function should be capable of relating map distance to second-division (or tetratype)

segregation frequency rather than merely to recombination frequency, which cannot be determined accurately from centromere-distance data. Consequently, a single-strand function, even though it described recombination correctly, would be useful for present needs only if a corresponding tetrad function could be derived from it. This is impossible for the empirical curves shown in Fig. 2, since they do not predict the distribution of exchanges among bivalents. A few single-strand functions do exist that are based on theoretical models (*e.g.*, Owen, 1950; Carter and Robertson, 1952; Rademacher, 1932). These involve special assumptions of uncertain general validity, are mathematically complicated, and have been fully developed only for application to single strands.

c. *Mapping Functions for Tetrads.* The only tetrad mapping functions which have been proposed in the past describe the two theoretical limiting cases of complete and zero interference (curves *A* and *B*, Fig. 5). The complete interference relation, $y_t = 2x$, has been used for mapping ever since 1933, when Neurospora centromere distances were first computed by Lindegren. (The term y_t signifies the frequency of second-division or of tetratype segregations; x signifies map units times 10^{-2}.) The zero interference tetrad function $y_t = \frac{2}{3}(1 - e^{-3x})$ was first given in explicit form by Rizet and Engelman (1949) and by Papazian (1951).

Although there is evidence that interference across the centromere may be negative in Neurospora (Lindegren and Lindegren, 1942; Whitehouse, 1942), the few available data indicate that interference is positive but incomplete within chromosome arms. What is needed for use within arms is a tetrad function intermediate between the existing complete and zero interference curves, analogous to the single-strand functions devised by Ludwig or Carter and Falconer. Such functions have been derived by making simple assumptions regarding the effect of interference on the distribution of exchanges among bivalents in a model chromosome segment.

d. *Development of New Functions.* Assumptions underlying a family of functions will be stated, and the hypothetical model on which they are based will be described before equations are derived.

(i) *Description of hypothetical model.* We have taken as a working model a chromosome segment marked genetically at both ends. Assume that interference between exchanges ('chiasma' interference) within this segment can take on any intensity from zero to complete interference, and that chromatid interference is absent.

When there is no interference, exchanges will be distributed at random among bivalents, following a Poisson distribution whose mean depends on the map length of the segment. We have assumed that when positive interference is introduced into the model the probability of nonexchange

tetrads remains unchanged, but that interference progressively decreases the proportion of multiple-exchange tetrads, relative to the frequency of singles. It is proposed to approximate the new proportions of tetrads that will have r exchanges ($r > 0$) by multiplying the original (zero interference) probability of each successive Poisson term by a factor k^{r-1}. The term k is a coefficient proportional to coincidence, and can

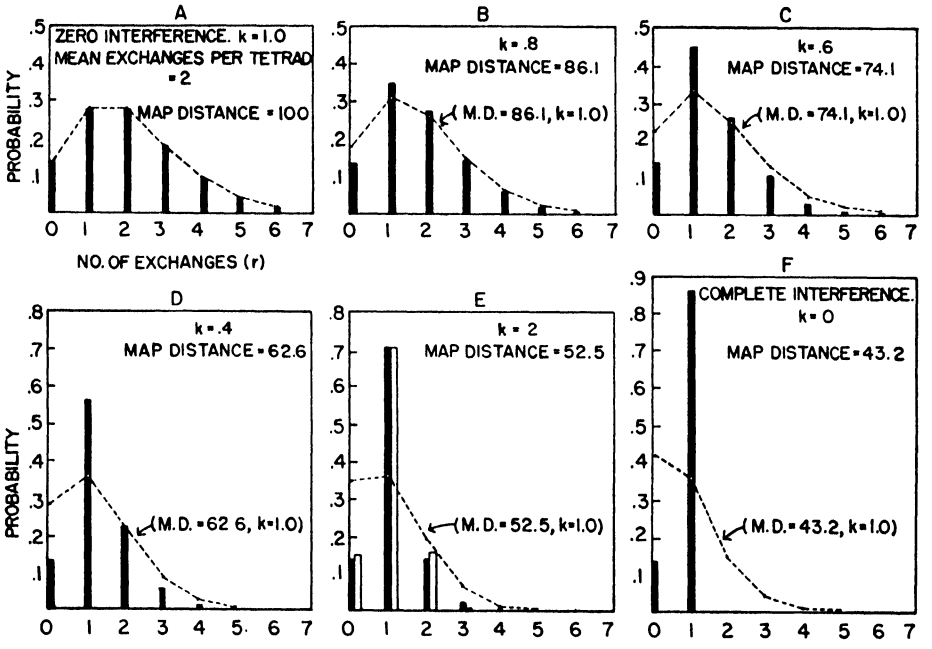


FIG. 3. Effect of changing interference on the frequency distribution of exchanges in a model chromosome segment described in the text. Ordinates: probability. Abscissae: number of exchanges. M.D.: map distance. k: coefficient proportional to coincidence, described in the text. Solid histograms: probability of exchanges in the model. Open histograms (frame E): distribution of exchange frequencies in the $sc - g$ interval, *Drosophila melanogaster*, computed from data compiled by Anderson and Rhoades (1931). Dashed lines indicate the frequencies that would obtain with zero interference in segments having the same map lengths.

assume any value between zero (complete interference) and one (no interference).

The effect of interference on the distribution of exchanges, according to these assumptions, is shown by the histograms in Fig. 3, where the results of introducing different levels of interference into a model chromosome segment are shown in successive frames. As interference increases in this model segment from zero to complete (frames A to F), the probability of exchanges of small rank increases at the expense of exchanges of larger rank, but nonexchange tetrads retain their original probability. Therefore for each level of interference a new map length is realized by the model. For comparison the distribution of exchanges that would

be found in a segment having the same map length, but zero interference, is indicated in each frame by dashed lines.

(ii) *Derivation of equations based on the model. Zero interference.* Consider first the situation when zero interference obtains. The probability of bivalents with 0, 1, 2, . . . r exchanges in the model chromosome segment is given by the Poisson distribution that has a mean equal to the mean exchange frequency per bivalent.

$$p(r) = \frac{(2x)^r}{r!} e^{-2x} \tag{1}$$

where $2x$ = mean exchange frequency per bivalent in the segment;
x = map length of segment (in map units $\times 10^{-2}$) = mean crossover frequency per strand;
r = number of exchanges in individual tetrads (or tetrad "rank": Weinstein, 1936);
$p(r)$ = probability of occurrence of a tetrad of rank r;
e = base of natural logarithms.

Multiplying each term by its rank, and summing, will give back the mean exchange frequency per bivalent; dividing by two will give the map length. Thus, map length is given by the equation

$$x = \frac{1}{2} \sum_{r=1}^{r=\infty} r \cdot \frac{(2x)^r}{r!} e^{-2x}. \tag{2}$$

The proportion of recombinant single strands expected from tetrads of any rank whatever except zero is uniformly ½ (Emerson and Rhoades, 1933). Therefore single-strand recombination frequency, y_s, can be obtained by multiplying all terms except the $p(0)$ term of equation 1 by ½

$$y_s = \sum_{r=1}^{r=\infty} \frac{(2x)^r}{r!} e^{-2x} \cdot \frac{1}{2}. \tag{3}$$

The frequency of tetratype (or second-division segregation) tetrads, y_t, expected at zero interference can be obtained by multiplying each successive term in equation 1 by the proportion of tetrads of each successive rank that are expected to be tetratypes. This proportion is $\frac{2}{3}[1 - (-\frac{1}{2})^r]$ (Mather, 1935) and the equation is

$$y_t = \sum_{r=1}^{r=\infty} \frac{(2x)^r}{r!} e^{-2x} \cdot \frac{2}{3} \left[1 - \left(-\frac{1}{2} \right)^r \right]. \tag{4}$$

Equation 3 is equivalent to $y_s = \frac{1}{2}[1 - p(0)]$ and thus to Haldane's expression $y_s = \frac{1}{2}(1 - e^{-2x})$, plotted as curve B in Fig. 2. Similarly equation 4 is equivalent to the simpler tetrad equation $y_t = \frac{2}{3}(1 - e^{-3x})$ plotted as curve B in Fig. 5. However, the uncontracted equations 3 and 4 will be more useful for predicting the effect of interference, which is expected to change the relative frequencies of different specific ranks.

When interference is absent ($k = 1$) the sum of terms of rank > 0 is given by the summation of Poisson terms

$$S_{k=1} = \sum_{r=1}^{r=\infty} \frac{(2x)^r}{r!} e^{-2x}. \tag{5}$$

Positive interference. Consider now the effect of interference on the probabilities of exchanges of different rank within the same model segment. When interference is positive ($k < 1$), the factor k^{r-1} is introduced and the sum of terms of rank > 0 is correspondingly decreased.

$$S_k = \sum_{r=1}^{r=\infty} \frac{(2x)^r}{r!} e^{-2x} \cdot k^{r-1}. \tag{6}$$

On our hypothesis, the probability of nonexchange tetrads, $p(0)$, is not changed by interference. Therefore, the sum of probabilities for ranks greater than zero must continue to equal $1 - p(0)$, even when interference obtains. This will be the case if each term is multiplied not only by k^{r-1} but also by the factor $S_{k=1}/S_k$, which will reconvert the term to a probability. In the presence of interference, the probability of tetrads of any rank greater than zero can then be expressed

$$p(r) = \frac{(2x)^r}{r!} e^{-2x} k^{r-1} \cdot \frac{S_{k=1}}{S_k}. \tag{7}$$

Interference decreases the probability of higher multiple exchanges and must therefore decrease the map length from x (the value at zero interference) to a new value, x'. Boost (1939) has used the term "*a priori* map length" to describe the map length, x, that would obtain at zero interference in a model similar to ours. Since $p(0) = e^{-2x}$, the *a priori* distance, x, equals $-\frac{1}{2} \log_e p(0)$. *A priori* map length depends only on the probability of nonexchange tetrads, $p(0)$, and is independent of interference. Its value can be obtained from observed recombination frequencies by the equation $x = -\frac{1}{2} \log_e (1 - 2y_s)$.

In contrast, the new map length x', as well as the new tetratype frequency, y_t', does vary with interference. Their values can be obtained by

substituting the new $p(r)$ values from equation 7 for the Poisson terms in the original x and y_t equations (equations 2 and 4).

$$x' = \frac{1}{2}\sum_{r=1}^{r=\infty} r \cdot \frac{(2x)^r}{r!} e^{-2x} k^{r-1} \frac{S_{k-1}}{S_k}; \tag{8}$$

$$y_t' = \frac{2}{3}\sum_{r=1}^{r=\infty} r \cdot \frac{(2x)^r}{r!} e^{-2x} \left[1 - \left(-\frac{1}{2}\right)^r\right] k^{r-1} \frac{S_{k-1}}{S_k}. \tag{9}$$

The single-strand recombination frequency, y_s, is not changed when new $p(r)$ terms from equation 7 are substituted in equation 3; y_s depends only

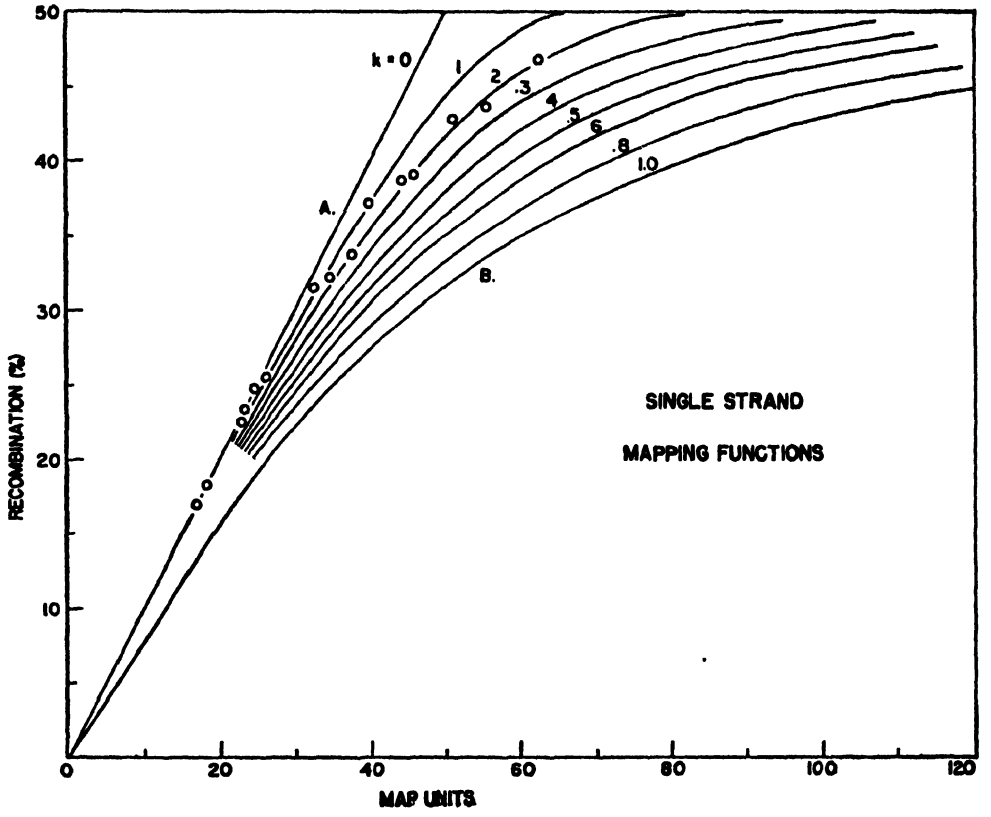


FIG. 4. Single-strand mapping functions based on the model described in the text. Curves are shown for seven graded interference intensities between the theoretical limiting curves *A* and *B*. Open circles: data for *Drosophila melanogaster* X-chromosome (Anderson and Rhoades, 1931).

on $p(0)$, and thus resembles *a priori* map length in being independent of interference.

By substituting various values of x in equations 3, 8, and 9, we have obtained the curves shown in Figs. 4 and 5, where corresponding single-strand and tetrad mapping functions based on our model are shown for a

series of interference levels. This not only gives a series of tetrad functions from which to choose, but enables the extensive single-strand data available from other organisms to be used in making the selection.

e. *Choice of a Specific Function for Neurospora.* The shapes of the single-strand mapping function curves agree qualitatively with expecta-

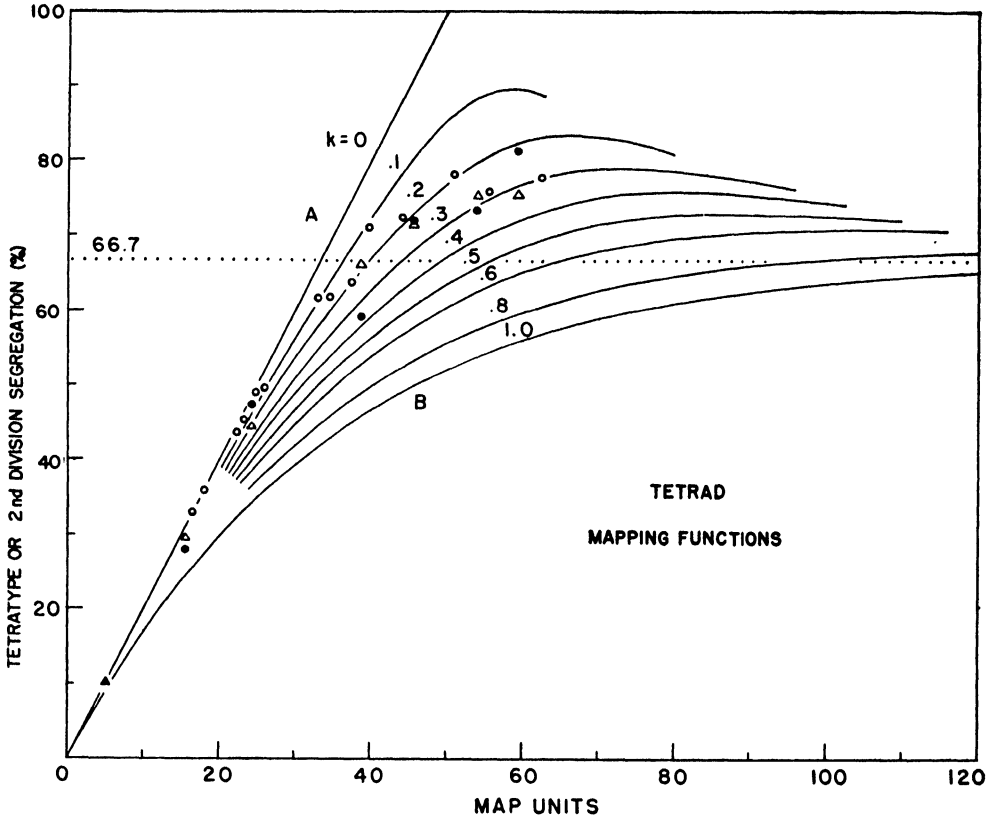


FIG. 5. Tetrad mapping functions based on the model described in the text. Curves are shown for seven graded interference intensities between the theoretical limiting curves *A* and *B*; these are the same intensities used for intermediate curves in Fig. 4. The sources of the two limiting curves are given in the text. Three sets of points are plotted: (a) open circles: postreduction frequencies computed from single-strand data, *D. melanogaster* X-chromosome (Anderson and Rhoades, 1931); (b) postreduction frequencies observed (solid circles) and calculated (triangles) from Drosophila attached-X-chromosome data (Beadle and Emerson, 1935).

tions based on experience, and suggest that the model may be adequate as a first approximation. Use of a particular curve to estimate Neurospora map intervals must be justified, however, by quantitative correspondence with actual data.

(i) *Agreement with Drosophila data.* Probably the best data from any organism that relate map distance to recombination frequency and record all exchanges within a long chromosome segment, are those compiled by

Anderson and Rhoades (1931) for the X-chromosome of *Drosophila melanogaster*. These data can be used in at least four different ways to check the soundness of the model and to determine what particular function shows best agreement.

1. Recombination frequencies can be plotted against map distance, as was done originally by Anderson and Rhoades (Fig. 4). Their data, comprising 26,908 chromosomes tested by Bridges and Olbrycht (1926) and by Anderson (1925), give good agreement with the $k = 0.2$ curve.

2. Postreduction frequencies can be computed from the single-strand data (Sax, 1932; Beadle and Emerson, 1935) and plotted against map distance (Fig. 5). Agreement of computed values with the $k = 0.2$ tetrad function is fair except for the two rightmost points (for $sc - f$ and $ec - f$).

3. Computed postreduction frequencies can be plotted against recombination frequencies (Fig. 6). The data fall near the 0.2 and 0.3 curves.

4. Frequency distributions of exchanges among bivalents can be computed from single-strand data (Sax, 1932; Mather, 1936) and compared with probabilities derived from the model (Fig. 3). Probabilities are tabulated below for bivalents with different numbers of exchanges in the $sc - f$ interval (Mather, 1936) and in the $sc - g$ interval. These are compared with the probabilities expected in models having *a priori* map distances similar to the two intervals and having interference intensities corresponding to $k = 0.2$ and $k = 0.3$.

		Frequency of bivalents of rank						*Map length*	
Interval	*Interference*	0	1	2	3	4	>4	*A priori*	Realized
$sc - g$	observed	0.143	0.701	0.152	0.002	0.001	—	97	50.9
model	$k = 0.2$	0.135	0.703	0.140	0.018	0.003	—	100	52.5
model	$k = 0.3$	0.135	0.663	0.189	0.037	0.005	—	100	57.1
$sc - f$	observed	0.072	0.629	0.287	0.011	0.001	—	132	62.0
model	$k = 0.2$	0.074	0.706	0.184	0.032	0.004	—	130	59.0
model	$k = 0.3$	0.074	0.610	0.237	0.063	0.013	0.003	130	71.5

Agreement with frequencies predicted from the model are good for the $sc - g$ data and $k = 0.2$. These values are plotted as open bars in frame 5 of Fig. 3. The $sc - f$ data agree rather poorly with frequencies expected from $k = 0.3$. (This discrepant interval is represented by the rightmost point in Fig. 5, where it also deviates in the same direction from $k = 0.2$.)

Drosophila recombination data from several sources other than Anderson and Rhoades (Morgan *et al.*, 1935 (X-chromosome); Reck, 1937

(X-chromosome at 13.5°, 25°, 30°C); Bridges and Morgan, 1919, 1923 (autosome arms)) have also been plotted against map distance as in Fig. 4 but have not been shown. The points mostly fall near the 0.2 curve, although agreement may be better with $k = 0.3$ in some cases (notably Morgan *et al.*, 1935).

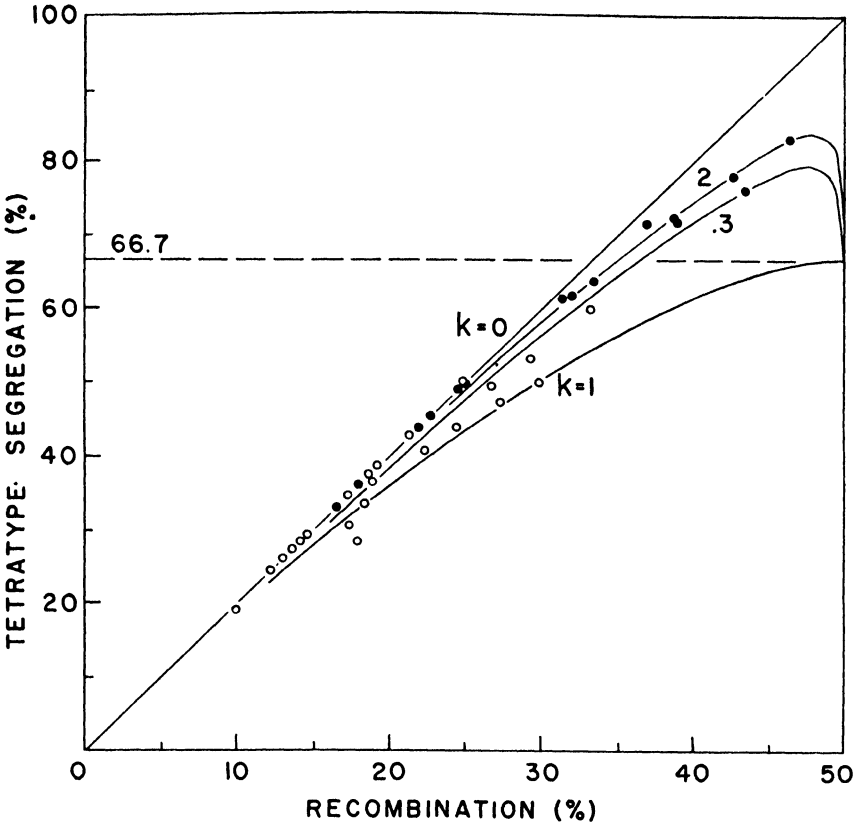


FIG. 6. Curves relating tetratype segregation frequency to single-strand recombination frequency based on the theoretical model described in the text. Two sets of points are plotted: (a) open circles: observed values for *Neurospora crassa* (from Tables 11–16). All intervals of 10 map units or more, within chromosome arms, and for which at least 25 tetrads have been recorded, are used; (b) solid circles: observed recombination and computed postreduction values for *Drosophila melanogaster* X-chromosome (Anderson and Rhoades, 1931). Curves are shown for the limiting cases of zero interference ($k = 1$) and complete interference ($k = 0$) and for interference corresponding in intensity to $k = 0.2$ and to $k = 0.3$ in the model.

A somewhat different type of comparison can be made using homozygosis frequencies from attached-X stocks of Drosophila. The most detailed analysis of this type was made by Beadle and Emerson (1935). Their observed and calculated recessive homozygosis values, multiplied by four so as to correspond with postreduction frequencies, have been plotted in Fig. 6. These could be interpreted to support use of either the $k = 0.2$ or the $k = 0.3$ curves.

(ii) *Agreement with Neurospora data.* Available Neurospora data cannot be used to choose a mapping function by testing recombination or second-division segregation frequencies against map distances, because the map distance axis is unknown; determination of map intervals is in fact the purpose of the investigation. But the two observable values, recombination frequency and tetratype frequency, can be used as coordinates for plotting the data so as to provide information regarding interference and regarding correspondence with the model, without its being necessary to make any assumptions whatever about map distance. This is identical with method 3 applied to the Drosophila data above. All data from crosses comprising 25 or more tetrads have been taken from Tables 11 through 16 for Neurospora genes known to be linked within individual chromosome arms. These are plotted in Fig. 6. As might be expected, they are inadequate for choosing between the curves that come closest to describing the results of crossing over in Drosophila, but do not seem to be inconsistent with either the $k = 0.2$ or $k = 0.3$ functions.

As a result of all these considerations, the $k = 0.2$ mapping function was adopted for converting observed tetratype or second-division segregation frequencies in Neurospora into "corrected" map distances. An enlarged curve, suitable for carrying this out in practice, is reproduced as Fig. 7.

An attempt was made to check the fit of the $k = 0.2$ function with the Neurospora data by testing the agreement of the sum of corrected short intervals with the length of the corrected over-all interval in all possible cases. No conclusions could be reached because of sampling errors.

It is apparent both in Drosophila (see Ludwig, 1934, 1938; Mather, 1938) and in Neurospora (Whitehouse, 1942; Lindegren and Lindegren, 1942) that a correction of this type is justified only for intervals within chromosome arms, because of centromere effects on interference. Consequently, the curve has not been applied to data from intervals spanning the centromere.

3. *Determination of Confidence Limits*

Most of the crosses involve such small numbers of asci (often 20 or fewer) that it seemed essential to have some measure of the magnitude of sampling error. For each observed map interval we have therefore determined the limits within which the true value of the map distance may reasonably be expected to lie, using the 95% level of significance. To facilitate this task, a series of confidence limits curves has been constructed, from which the limits for each datum may be read directly.

Points for constructing the curves were obtained by the rapid graphical method of Mosteller and Tukey (1949), using a photographic en-

largement of their binomial probability paper. Limits were thus obtained for tetratype (or second-division) segregation percentages of 2, 5, 10, . . . 75 at 5-unit intervals, and for values of N (number of asci) of 10, 20, 30, 50, 75, 100, 200, 400, and 600. The resulting points were plotted

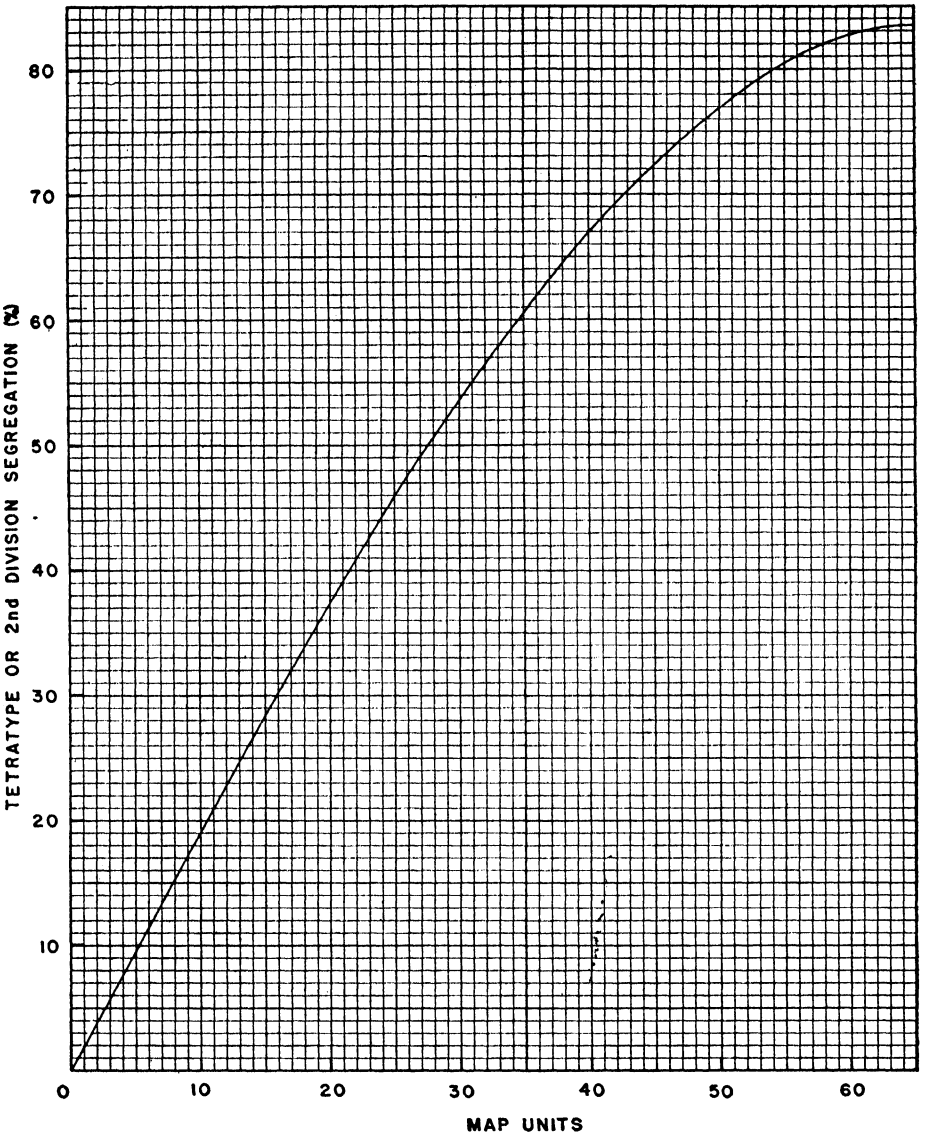


FIG. 7. Tetrad mapping function curve based on the model discussed in the text, with interference corresponding to $k = 0.2$.

to give the curves shown in Fig. 8, in which each sample size is represented by two curves, corresponding to the upper and lower confidence limits. N = infinity is a single curve separating the two sets of limits. A semi-logarithmic plot was used to spread the curves for greater readability at the lower tetratype frequencies. (The curves for $N = 75$ and $N = 400$

have been omitted from Fig. 8 to permit clearer reproduction.) Similar, accurately computed, curves have been published elsewhere (Eisenhart *et al.*, 1947) but were not useful for our purpose because they included too few sample sizes and had too small a scale.

From Fig. 8 the approximate upper and lower limits can be read directly for any observed per cent tetratypes (or second-division segregations) by using the curves for the appropriate value of N and interpolating where necessary. The limits for each interval in the tables of centromere and linkage data (Tables 4 to 16) have been determined in this manner. In order to convert into *uncorrected* map distance, both the observed per cent tetratypes and the corresponding confidence limits were divided by two. To convert into *corrected* map distance, both the observed per cent tetratypes and the confidence limits were corrected by the $k = 0.2$ mapping-function curve (Fig. 7).

Precise confidence limits from the binomial and Poisson distributions may be obtained from a table prepared by W. L. Stevens (Fisher and Yates, 1949, p. 48); the use of this table is described more fully by Stevens (1942). However, reading the limits from our curves is considerably more rapid than computing them from the numbers in Stevens' table and is sufficiently accurate for the present purpose. Representative limits obtained from binomial probability paper fall within one-tenth of a map unit of those from Stevens' table when the range (the distance between the upper and lower limits) is 7 units or less; they never differ from his limits by more than ½ unit except when the ranges are so great (20 to 30 map units) that the error is negligible.

Where the observed tetratype frequency is zero, binomial probability paper is not sufficiently accurate, and the upper limits have therefore been calculated directly from the first term of the binomial expansion by the formula $1 - 0.025^{1/N}$, which is equivalent to the formula of Stevens. (The lower limits in such cases are zero.) Binomial probability paper is also inadequate for certain points (5%, $N = 10$; and 2%, $N = 10$, 20, and 30). Approximate values for these points were obtained by graphical interpolation on an arithmetic plot. These points do not actually occur in the discontinuous binomial distribution and thus cannot be calculated, but were needed to give smooth curves for interpolation. The portions of the curves derived from these points are indicated by dashed lines in the figure.

For sample sizes of more than 600 asci, the limits have been calculated from the standard error. The standard error has not been used in the other cases because, as pointed out by Stevens (1942), it becomes extremely inaccurate when the distribution is not normal—in this case, when the tetratype frequency deviates appreciably from 50% and the sample size is small.

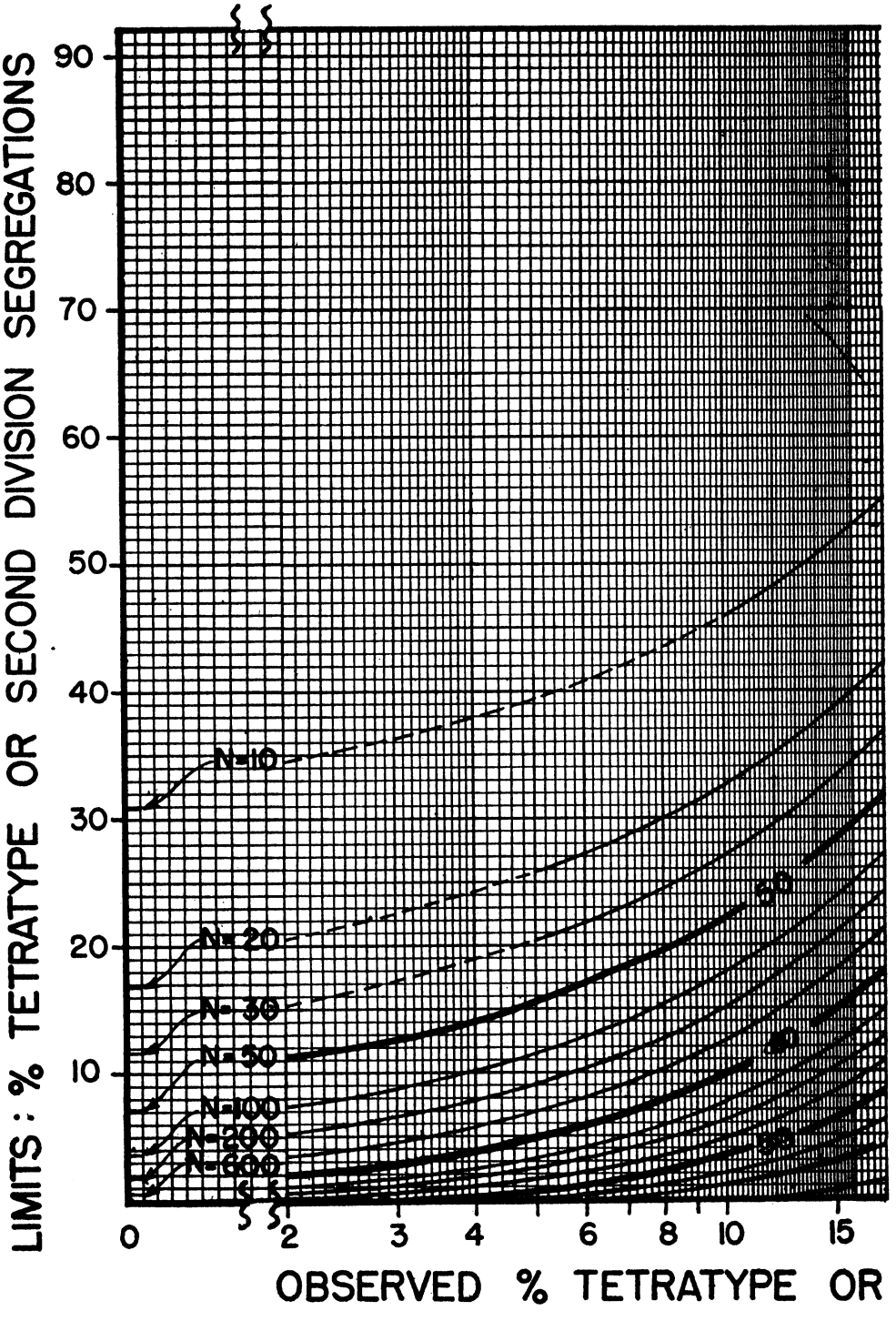
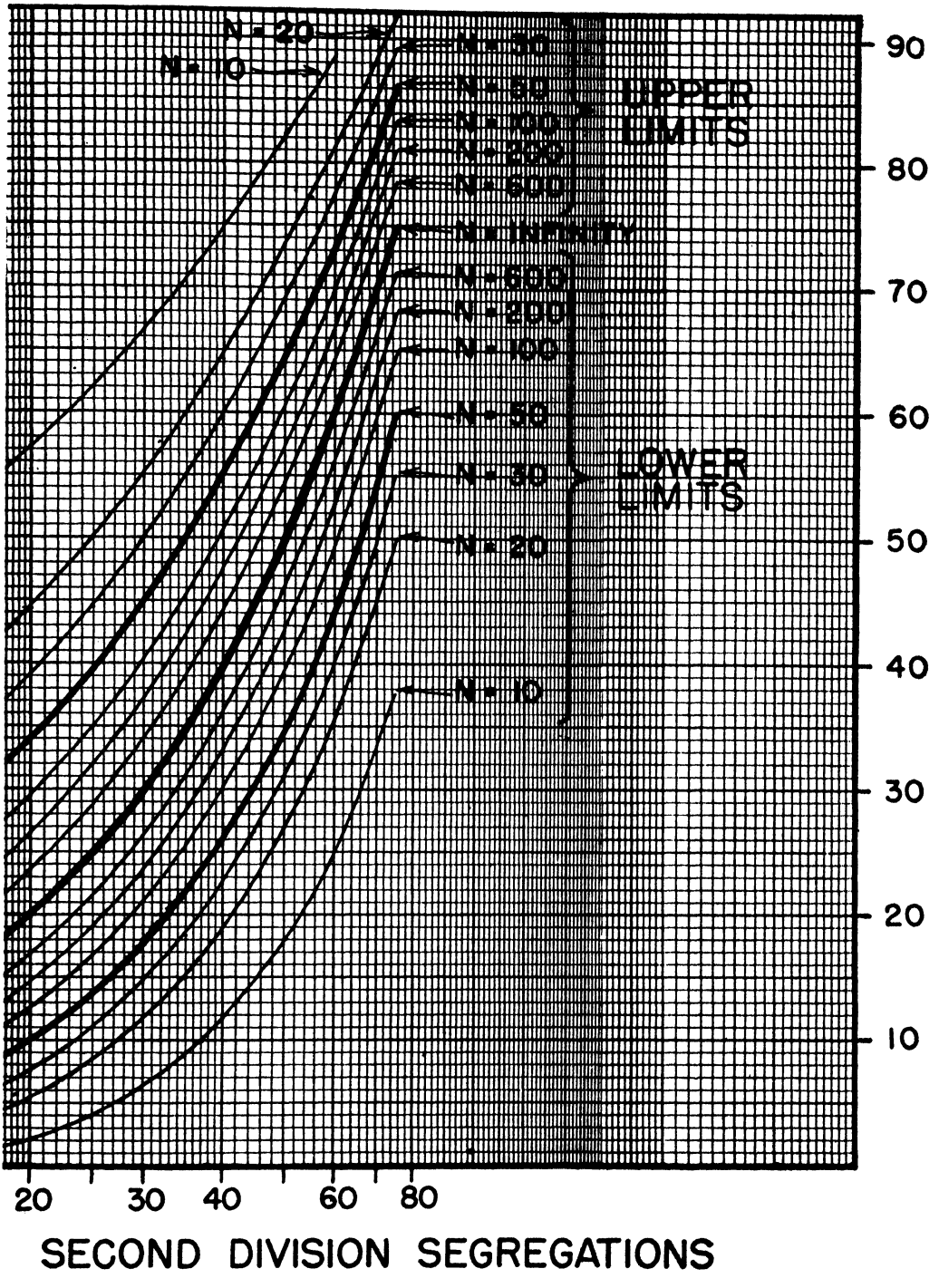


FIG. 8. Confidence limits curves (95 % signifi-



cance level), drawn as described in the text.

For a given small sample size the range is largest at 50% tetratypes, becoming smaller and skewed as the observed value departs from 50% in either direction. Thus on the maps in Section IV the bars representing confidence limits for loci mapped from any given point, *e.g.*, the centromere, are symmetrical only at a distance of 25 map units, being skewed, in opposite directions, for longer or shorter distances.

III. Linkage Groups and Chromosomes

1. *Status of the Linkage Groups*

a. *Designation of Linkage Groups.* Linkage was first demonstrated in Neurospora by Lindegren (1933), who later (1936a) published a map of the sex chromosome containing five genes and centromere. Three years later, a second linkage group was reported containing three genes, peach, tuft, fluffy, and the centromere (Lindegren and Lindegren, 1939). Hungate (1946) and Regnery (1947) described a third group containing serine, scumbo, proline, leucine, thiamine, tryptophan, and centromere. They concluded that this was distinct from the two established groups on the basis of crosses involving markers close to centromere. In 1946, Beadle reviewed the biochemical genetics of Neurospora and published a tentative map showing loci concerned with known biosyntheses. This map contained five undesignated linkage groups, one of which contained the sex locus, and was thus equivalent to Lindegren's first group, whereas another was identical with the linkage group described by Hungate and Regnery. Barratt and Garnjobst (1949), mapping a new mutant, showed that it belonged to Lindegren's second linkage group, and that it segregated independently from the five groups illustrated by Beadle. They designated the six groups thus established by Roman numerals, according to the chronological order of their discovery. An additional group, designated VII, was represented by a single gene discovered by Emerson and Emerson (unpublished).

Later in 1949, Houlahan and co-workers published extensive linkage data on biochemical mutants and designated six groups by letters A through F. The synonymy is summarized below:

Designation of Barratt and Garnjobst	*Designation of* Houlahan, Beadle, and Calhoun	*First described by*
I	A	Lindegren (1936a)
II	—	Lindegren and Lindegren (1939)
III	C	Hungate (1946), Regnery (1947)
IV	D	Beadle (1946)
V	E	Beadle (1946)
VI	B	Beadle (1946)
VII	F	Emerson and Emerson (unpublished)

An alphabetical identification of groups, such as that used by Houlahan and associates, was probably best so long as the independence of all seven groups had not been rigorously demonstrated, because, in the event that two of the lettered groups should turn out to be on two arms of a single chromosome, they could then be redesignated by a single number (Hagerty, 1952). It has now been established by statistical tests that each of the seven groups must indeed be located on a separate chromosome (see Section III, 1, c below). Therefore, the numerical designations of Barratt and Garnjobst have been adopted for use in the present publication.

b. *Designation of Arms.* In order to facilitate descriptions of gene order, one well-mapped locus has been arbitrarily chosen to define the right or left arm in each linkage group, as follows: I—*sex* = left; II—*pe* = right; III—*ser* = right; IV—*pdx-1* = right; V—*iv-1* = right; VI—*rib-1* = right; VII—*nl* = right. In groups VI and VII, respectively, *ad-1* and *sfo* are more precisely located than the markers chosen, but they have not been used because they are too close to centromere to be useful in assigning a new locus to its proper arm.

c. *Criteria and Evidence for the Independence of Linkage Groups.* (i) *General description of methods.* As yet, only three of the seven linkage groups have been identified with cytologically distinguishable chromosomes (see the section on Cytogenetics, III, 2). However, even before the relationships between linkage groups and cytological chromosomes are known, tetrads can provide critical genetic evidence that two genes are located on different chromosomes. This may be accomplished by either of two methods. The first is based on the fact that if two genes are both known to have short centromere distances, they can show a high recombination frequency in a direct cross with each other (or likewise a high frequency of nonparental ditype segregations) *only* if they are different chromosomes.

The second method depends on the fact that a high ratio of NPD to T tetrads can be obtained only if genes are on different chromosomes. The first method will be described here, because it is the most useful where centromere data are abundant, as in Neurospora. The second method, which is applicable to unordered as well as ordered tetrads, is described in the Discussion (Section V, 2).

The first method has been used by several workers in establishing the seven linkage groups, but in most cases the reliability of the evidence for independence has not been statistically evaluated. Thus Lindegren (1936a) concluded that peach is not in group I because it gave 54.5% recombination with gap (in group I) and both loci have small centromere distances. Hungate (1946) concluded that serine (centromere distance

2.1) and Regnery (1947), that leucine (centromere distance 10.0) were on a new chromosome because they gave high NPD frequencies when crossed to markers in Lindegren's groups I and II, where some of the markers were close to centromere. Barratt and Garnjobst (1949) reported that peach is not in linkage groups I, III, IV, V, VI, or VII by crossing with markers having similar small centromere distances and noting that the observed recombination values exceeded those expected from summing the centromere distances. Subsequently it was found that the *sfo* culture, provided by Dr. S. Emerson for testing linkage of peach to group VII, was actually a *pab* mutant in another linkage group. However, using a *bone fide sfo* culture, Emerson and Emerson (unpublished) concluded that *sfo* (centromere distance 0.2) was on a seventh chromosome, because it gave a high frequency of NPD's when crossed to markers close to centromere in the other chromosomes, including group II.

This general method (low recombination or NPD frequencies in crosses between genes close to centromere) may be treated statistically by testing whether the two genes are separated by a map distance significantly greater than the sum of their centromere distances, since this sum is the greatest distance which can separate them if they are on the same chromosome. By applying this criterion to existing data, it can be shown that the seven known linkage groups are actually located in separate chromosomes. The relevant data are given in Table 1. At least one cross is shown for each of the 21 possible combinations of the seven groups. In every case the observed recombination value is significantly greater (5% level) than the maximum expected from the summed centromere distances; and in at least one cross for each combination the deviation is significant at the 1% level, or lower.

(ii) *Details of the method used.* Crosses were chosen that involved genes closest to centromere in order to increase the sensitivity of the test and to increase its reliability by decreasing errors due to undetected multiple exchanges. Centromere distances were computed from all available data (Tables 4–10). The upper confidence limits of centromere distances (uncorrected for multiple exchanges) were summed for each pair of genes being tested, in order to determine the maximum distance that could separate the genes if they were linked. This maximum expected distance was compared with observed recombination frequencies between two genes as follows. The observed numbers of parental and recombinant half-tetrads were divided by two in order to make the sample size equal the number of whole tetrads analyzed. (Segregation in one half-tetrad cannot be assumed to be independent of segregation in the other half (Perkins, 1953).) The probability that the resulting numbers are random deviates from the expected maximum ratio was determined in each case

TABLE 1
Establishment of Independence of Linkage Groups

A.	B.		C.			D.	E.		F.	G.	
Linkage groups involved	*Markers*		*Observed numbers of asci*			*Observed % recombination a with b*	*Centromere distance (upper limit)*†		*Expected maximum % recombination (Col. E; a + b)*	*Probability that D. is random deviate from F.*	*Reference*
	a	b	PD	T	NPD		a	b			
I & II	*gap*	*pe*	35	28	40	52.4	5.5	16.0	21.5	<0.001	84
I & III	*sex*	*thi-4*	11	18	8	46.0	7.0	5.0	12.0	<0.001	68
I & III	*sex*	*ad-4*	10	1	9	47.5	7.0	7.5	14.5	<0.002	68
I & IV	*sex*	*pyr-1*	11	5	7	41.3	7.0	8.2	15.2	<0.01	68
I & IV	*sex*	*pyr(44101)*	5	7	6	52.8	7.0	8.3	15.3	<0.002	68
I & V	*sex*	*lys-1*	16	0	12	42.9	7.0	6.6	13.6	<0.002	37
I & VI	*sex*	*rib-1*	5	1	14	72.5	7.0	6.0	13.0	<0.002	68
I & VII	*sex*	*sfo*	1	1	6	81.3	7.0	0.7	7.7	<0.001	39
II & III	*pe**	*leu-1*	19	18	15	46.2	16.0	12.5	28.5	<0.01	7, 131
II & IV	*pe*	*pdx-1**	10	7	11	51.8	16.0	10.5	26.5	<0.01	7
II & V	*pe*	*lys-1*	6	10	10	57.7	16.0	6.6	22.6	<0.002	7
II & VI	*pe**	*ad-1*	14	33	11	47.4	16.0	1.7	17.7	<0.001	7, 8
II & VII	*pe*	*sfo*	5	5	12	65.9	16.0	0.7	16.7	<0.001	39
III & IV	*sc*	*pyr-1*	4	3	6	57.7	2.5	8.2	10.7	<0.002	72
III & V	*ad-4*	*iv-1*	7	2	11	60.0	7.5	12.7	20.2	<0.002	68
III & VI	*prol-1*	*ad-1*	7	0	4	36.4	7.0	1.7	8.7	<0.05	68
III & VI	*prol-1*	*rib-1*	8	1	9	52.8	7.0	6.0	13.0	<0.002	68
III & VII	*leu-1*	*sfo*	14	3	9	40.4	12.5	0.7	13.2	<0.002	39
IV & V	*pyr(44101)*	*lys-1*	8	0	7	46.6	8.3	6.6	14.9	<0.01	68
IV & VI	*pdx-1**	*rib-1*	4	0	5	55.6	10.5	6.0	16.5	<0.02	68
IV & VII	*pdx-1**	*sfo*	1	4	5	70.0	10.5	0.7	11.2	<0.001	39
V & VI	*iv-1*	*ad-1*	8	3	9	52.5	12.7	1.7	14.4	<0.002	68
V & VI	*iv-1*	*un(66204)*	7	5	7	50.0	12.7	11.5	24.2	<0.05	68
V & VII	*lys-1*	*sfo*	10	0	6	37.5	6.6	0.7	7.3	<0.01	39
VI & VII	*rib-1*	*sfo*	3	0	11	78.6	6.0	0.7	6.7	<0.001	39

* Partly or wholly from an allele other than the standard mutant.
† Taken from Tables 4–10.

by means of the binomial distribution, using the graphical method of Mosteller and Tukey (1949).

(iii) *Validity of the method.* The method used involves three sources of error: (1) undetected multiple crossovers; (2) use of upper confidence limits for centromere distance values; and (3) use of the number of whole tetrads as the sample size. However, the effect of each of these is to decrease the sensitivity of the test rather than to give false indications of independence (with the one exception noted below).

Undetected multiple crossovers affect the observed and the maximum expected recombination values unequally, giving rise to two errors. Error *a*: Four-strand double exchanges are not detectable in the gene-centromere intervals (basis of the expected value, column F, Table 1), but they are detectable in the interval between the two genes (observed value, column D) (see Section II, 1). Error *b*: If the genes are linked and on opposite sides of the centromere, more multiple exchanges of all kinds should occur in the over-all interval between the two genes than occur in the component gene-centromere intervals. For genes close to centromere, error *a* will be small, and in any case should be more than counterbalanced by error *b*, unless the two centromere distances are grossly unequal. The combined result will usually be to bias the test against proving independence of the two genes.

In estimating centromere distances and population sizes, of which the exact values are unknown, we have purposely chosen the approximations that will bias the test against giving false evidence of independence. The use of upper confidence limits for the centromere distances usually leads to overestimating the maximum expected recombination. Use of whole tetrad numbers (treating half-tetrads as though each were completely dependent on its sister half) usually leads to an underestimation of the significance of deviations.

Since, despite these biases, the observed values significantly exceed the expected ratios in all cases, it can be concluded that the seven described linkage groups are actually in different chromosomes.

2. *Cytogenetics*

To date four contributions have appeared correlating cytological and genetical investigations in *Neurospora crassa* (McClintock, 1945; Singleton thesis, 1948; St. Lawrence thesis, 1952; Singleton, 1953). McClintock devised and applied the basic cytological techniques, overcoming the belief that fungus chromosomes present insuperable difficulties. She described the divisions in the ascus, designated the seven chromosomes by Arabic numbers, and showed that the nucleolus organizer is in chromosome 2. Her finding that all the centromeres are decidedly nonterminal

suggests that markers should eventually be found in both arms of all seven linkage groups.

Singleton gave a detailed description of cytological methods and diagrammed the pachytene structure of the seven chromosomes, including apparently heterochromatic regions adjacent to the centromeres.

Both McClintock and Singleton made preliminary observations on several aberration stocks (including 4637T and 45502T), and McClintock described methods of analyzing the patterns of aborted spores produced in crosses involving translocations. More recently, St. Lawrence has made a detailed analysis of a complex translocation stock, R55, as well as of 4637T and 45502T in conjunction with McClintock, with the object of assigning linkage groups to specific chromosomes. The pyrimidine mutant (*pyr-3*) in 45502T shows linkage to both groups IV and VI (Houlahan *et al.*, 1949). 4637T (*al-1*) involves linkage groups I (Houlahan *et al.*, 1949) and II (Hagerty, 1952; St. Lawrence, 1952). R55 involves linkage groups I, II, and IV (St. Lawrence, 1952). The conclusions are that linkage group I is carried on chromosome 6, with sex in the short arm; linkage group II is carried on chromosome 1, with fluffy in the long arm; and linkage group IV is carried on chromosome 2, with *pan* probably in the short arm (St. Lawrence, 1952).

Thus, three linkage groups have been assigned to chromosomes. Groups III, V, VI, and VII have not yet been located.

Several other stocks appear to involve aberrations but are incompletely analyzed. Translocation 44105T (McClintock, 1945) may involve chromosomes 4 and 5 (Singleton, 1948); no linkage relations are known. Arginine strain 36703T shows linkage to groups I and V (Srb, quoted by Singleton, 1948) and contains a translocation not involving chromosome 2 (Singleton, 1948). Leucine strain 5936T, which gives patterns of aborted spores (Regnery, 1947), was found to involve a translocation (Singleton, 1948); it gives no recombination with *leu-3* in linkage group I (Regnery, unpublished).

Adenine 27663Ab appears to be allelic with 70004 (*ad-2*) but gives much less recombination with the centromere and with *ad-4* than does 70004 (Houlahan *et al.*, 1949). Thiamine 17084T gives no recombination with either 56501 (*thi-1*) or with 18558 (*thi-3*), although *thi-1* is in group I (Houlahan *et al.*, 1949) and *thi-3* is in group VII (Reisner, unpublished). Histidine C1710Ab (see *hist-3*) gives patterns of aborted spores (Haas *et al.*, 1952). Nicotinic 4540Ab (*nic-2*) gives abnormal spore patterns and also very different linkage with sex depending upon whether spores are analyzed at random or taken from asci with eight ripe spores (St. Lawrence, 1952).

Arginine strain S1229Ab, an X-ray mutant which also uses ornithine

and citrulline, appears to be an aberration because it produces patterns of aborted spores and because certain crossover types are found only in the six-spored asci (Barratt and Garnjobst, unpublished). Linkages calculated from asci with eight ripe spores give greatly reduced centromere distances for *pe* and *fl* and no recombination between *pe* and S1229Ab, indicating a break in the right arm of linkage group II (Garnjobst, unpublished). No cytological investigations have been made, nor have the allelism relations of this mutant with the other arginine mutants been determined.

There is no evidence that the mutant phenotype in any of these stocks is separable from the aberration, except in the case of T45502 (see Descriptive Index of Mutants).

IV. Data and Mapping

Included in this summary are all extant mutants of *Neurospora crassa* which can be assigned to specific linkage groups on the basis of available data. Mutants are not included in the centromere and linkage tables if there is insufficient evidence to satisfy the criteria for linkage (Section IV, 1, a) or if there is reason to believe they may involve aberrations. They are, however, discussed in the Descriptive Index of Mutants when there is some evidence that they may be allelic with mapped genes. All data from any cross that appeared to involve an aberration stock have been excluded because of the danger of introducing serious errors.

We have attempted to compile all relevant data available in published or thesis form through 1952 (see page 93). Coverage since then is not complete. Additional, unpublished data were contributed by numerous workers, chiefly in 1950 (see Section VIII, Acknowledgments). Linkage data that were published by Barratt and Garnjobst (1949) in terms of parental and recombinant spore-pairs have been redetermined from the original data and are now given as whole asci. Every effort has been made to avoid counting the same data twice by identifying all sources. Random isolates were used to evaluate gene order and allelism and to establish the existence of linkage but were not used for estimating map distances.

1. *General Methods and Conventions*

For the sake of clarity and consistency it has been necessary to establish certain criteria and conventions.

a. *Criteria for Linkage.* Genes were assigned to specific linkage groups on the basis of linkage to at least one marker, as evidenced by a significant excess of parental ditype over nonparental ditype asci (5% level, two-sided) (Perkins, 1953). In a few cases where only random isolates

were available, a significant excess of parental over recombinant spores was used to establish linkage.

b. *Criteria for Allelism.* The data on all alleles at each locus have been pooled in order to decrease sampling error. Therefore, sufficiently rigorous criteria for allelism were chosen so that if two mutants should later prove to be nonallelic they must at least be closely linked. Thus a serious error will not have been introduced into the linkage calculations. In order to be considered alleles, mutant strains were required to satisfy the following four criteria:

1. Phenotypes must be similar.
2. There must be evidence that they lie in the same linkage group (see Section IV, 1, a above).
3. *Either* (a) there must be a direct cross giving no more than 1 % wild types (randoms, whole perithecia, and tetrads were all used for this purpose), *or* (b) if no direct cross, they must have homogeneous centromere distances, and, if crossed to the same marker, homogeneous map distances.
4. If a heterocaryon test was made, it must have been negative.

Allelism was not excluded on the basis of a very low wild type frequency, because in such cases it is very difficult to distinguish recombination from contamination and reverse mutation.

When there appeared to be some doubt from inspection that the data on two mutant strains were homogeneous, a statistical test was made using the graphical method of Mosteller and Tukey (1949). In a few cases two mutants showed no recombination in a direct cross, yet had heterogeneous centromere and/or linkage relations. Such controversial cases are discussed individually in the Descriptive Index of Mutants.

A positive heterocaryon test is considered conclusive evidence against allelism (Beadle and Coonradt, 1944). However, a negative heterocaryon test is not adequate evidence *for* allelism. This has recently been confirmed by the demonstration that certain strains are genetically incapable of forming stable heterocaryons (Garnjobst, 1953).

Mutant strains listed as alleles or possible alleles in previous publications, but for which available data are insufficient to satisfy the criteria for allelism listed here, have been designated "possible" or "probable" alleles in the index of mutants. The data on these strains have been excluded from the linkage and centromere tables.

c. *Determination of Gene Order.* Because of the rarity of multiple-point crosses and the paucity of data on the two-point crosses, it has usually been impossible to determine gene order simply from relative map distances. When the confidence limits do not overlap, the order of two mutants known to be within the same arm can be determined by the

relative centromere distances. But by far the most useful method for determining gene order has been the analysis of spore arrangements in the individual asci. This not only makes it possible to use the centromere as a marker but also permits deduction of the crossovers which must have taken place at each meiosis for any given order of genes and centromere. The method of analysis has been described in detail by Whitehouse (1942). Such an analysis can be made for each possible arrangement of the markers, and the one requiring the simplest crossover patterns chosen as the most likely. This method is referred to in the remainder of the text simply as "ascus segregation" and has been employed for all critical crosses.

When the combined methods are still inadequate for determining order, the questionable loci are indicated appropriately on the maps.

d. *Nomenclature and Symbols.* The principles listed below have governed choice of names and symbols in this paper. Prior usage has been decisive in choosing names and symbols, except where contrary to any of the following rules, or where several alternatives have been used in the past.

1. Biochemical mutant strains are named for the terminal compound in a synthetic series. (For example, all mutants in the ornithine-arginine series are named "arginine.")

2. For the sake of brevity, growth-factor mutants are named for the appropriate compound without adding the suffix "-less."

3. Symbols are constructed to contain two or more letters because one-letter symbols could be interpreted ambiguously in the case of most biochemical mutants (see Table 2). (Exception: *A* and *a* are retained for the sex locus.)

4. Symbols are chosen to achieve a balance between brevity, unambiguity, and pronounceability. In a few controversial cases, unambiguity seemed more important than pronounceability, *e.g.*, *pdx* for pyridoxine (Beadle and Tatum, 1941) rather than *py* or *pyro*, which could easily be confused with pyrimidine, pyrophosphate, etc.

5. Mutants resistant to drugs or toxic metabolites are given the symbol of the drug prefixed by *r-*. Mutants unable to utilize a normal compound (nonutilizers) are given the symbol of the compound prefixed by *n-*. Such mutants are alphabetized under the symbol of the compound.

6. Suppressors are given the symbol *su*, followed by the symbol of the mutant which is suppressed and separated from it by a dash.

7. Mutant strains having the same name and symbol but representing mutations at different loci (mimic loci) are distinguished by adding a locus number which follows the symbol and is separated from it by a dash.

Precedent has been followed wherever numbers have already been used for this purpose (see Table 3).

8. Where assignment of a locus number following the symbol is not warranted (either because of unknown allelism relations, or because of an unknown requirement), the locus is designated by the mutant symbol followed by the isolation number in parentheses, *e.g.*, *me*(*35809*), *un*(*46403*).

2. *Presentation of Data*

The data are presented in the following four parts:

A key relating mutant name to mutant symbol (Table 2).
A key relating isolation number to locus designation (mutant symbol plus locus number) (Table 3).
A Descriptive Index of Mutants arranged alphabetically according to symbol. This also serves as a key relating locus designation to isolation number.
A series of tables presenting the data on centromere distances and on linkage in 2-, 3-, and 4-point crosses (Tables 4–18).

a. *Name-Symbol Key.* Table 2 lists mutant names in alphabetical order and relates them to mutant symbols.

b. *Isolation Number-Locus Key.* Table 3 relates isolation numbers to locus designations and gives bibliographic references to the origin of the locus numbers. For mutants with uncertain allelism relations, the locus designation is prefixed by "see" and is intended only to indicate the locus under which the mutant is discussed in the Descriptive Index of Mutants. All mutants are listed in numerical order regardless of letter prefixes. Capital letters preceding mutant isolation numbers refer to the original source as follows: C, California Institute of Technology; E, Dr. Sterling Emerson; H, Dr. Frank Hungate; S, Stanford University after 1948; Y, Yale University; and JH, Johns Hopkins University. Strain numbers without letter prefixes refer to mutants in the original Beadle and Tatum (1945) numbering system, obtained chiefly at Stanford University prior to 1945. Capital letters following isolation numbers signify aberrations as follows: T, translocation; Ab, suspected aberration of unknown type. Capital R, either as a prefix or suffix, is an exception to the above rules, having been used by several workers for other purposes.

c. *Descriptive Index of Mutants.* This index lists all mapped loci in order of their symbols, and gives (1) phenotype, (2) linkage group and arm, and (3) data on gene order. This information on the locus is followed by: (4) isolation number of the standard mutant in boldface type, with its mode of origin and description of phenotype, and (5) isolation num-

bers of alleles in italics, their modes of origin, descriptions (if different from the standard mutant), and data establishing allelism. In the case of loci with two or more mutant alleles, one allele has been chosen as the standard mutant. Usually this is the one on which most of the genetic

TABLE 2
Key Relating Mutant Name to Mutant Symbol

Name	*Symbol*	*Name*	*Symbol*
acetate	*ac*	nitrate nonutilizer	*n-nit*
adenine	*ad*	ornithine (see arginine)	
albino	*al*	pale	*pa*
arginine	*arg*	pantothenic acid	*pan*
aromatic	*arom*	*p*-aminobenzoic acid	*pab*
asparagine	*asp*	peach	*pe*
canavanine-resistant	*r-can*	proline (also see arginine)	*prol*
choline	*chol*	pyridoxine	*pdx*
citrulline (see arginine)		pyrimidine	*pyr*
colonial	*col*	quinolinic acid (see nicotinic acid)	
crisp	*cr*	riboflavin	*rib*
cysteine	*cys*	scumbo	*sc*
dirty	*dir*	serine	*ser*
fluffy	*fl*	sex	*sex* or *A* and *a*
gap	*gap*	succinic acid	*suc*
heterocaryon-formation	*het*	sulfonamide	*sfo*
histidine	*hist*	suppressor	*su*
homoserine	*hs*	thiamine	*thi*
inositol	*inos*	threonine	*thr*
isoleucine-valine	*iv*	tryptophan	*tryp*
leucine	*leu*	tuft	*tu*
lysine	*lys*	tyrosine	*tyr*
mating type (see sex)		unknown	*un*
methionine	*me*	valine	*val*
natural death	*nd*	visible	*vis*
nicotinic acid	*nic*	yellow	*ylo*
nicotinic-tryptophan	*nt*		

or biochemical data have been obtained. Descriptions contain information or references to information useful in scoring isolates, but irrelevant biochemical data are not included. Reference numbers in parentheses are given except where statements are derived from data documented in the tables. Unmapped loci which have been numbered by previous workers, *e.g.*, *me-1*, *me-2*, *me-4*, are listed briefly in order to avoid future confusion in numbering.

The following abbreviations have been used; m.d. for map distance, c.d. for centromere distance, w.t. for wild type, PD for parental ditype

TABLE 3
Key Relating Isolation Number to Locus Designation

Isolation number	*Locus*	*Reference for locus number*	*Isolation number*	*Locus*	*Reference for locus number*
R1	*su-1-me*	48	Y7655	*arom*	—
R2	*su-1-me*	48	Y8743c	*col-1*	7
R3	*su-1-me*	48	Y8743m	*pe*	—
R4	*su-2-me*	48	8839	*leu-2*	144
C83	*tryp-3*	31	9185	*thi-2*	31
C84	*hist-1*	*	9666	*me-5*	*
C85	*hist-1*	*	10575	*tryp-1*	12
C86	*nt*	—	15069	*lys-4*	71
C91	*hist-1*	*	E15172	*sfo*	—
C94	*hist-2*	*	15300	*al-2*	12
H98	*me-2*	62	16117	*iv-1*	*
C140	*hist-3*	*	16275	see *al-1*	—
C141	*hist-4*	*	Y16331	*tryp-4*	*
H193	*pab-2*	*	16808	see *al-1*	—
H263	*pyr-1*	69	17084T	see *thi-1*	31
H605	*ser*	—	17295	see *al-1*	—
830	*pab-1*	*	18558	*thi-3*	31
S1007	*asp*	—	20705	see *ad-2*	—
1298	*pyr-3*	69	21502	*arg-4*	31
1633	*pab-1*	*	21850	*pab-1*	*
1710T (C1710Ab)	see *hist-3*	—	21863	*prol-1*	112
			27663Ab	see *ad-2*	—
S1908	see *tryp-4*	—	27947	*arg-5*	31
S1952	*tryp-3*	31		(= *orn-1* in 112)	
Y2198	*tryp-4*	*	28610	*ad-6*	31
Y2492	*ac*	—	28815	*lys(28815)*	—
JH2626	*inos*	—	29997	*arg-6*	31
3254	*ad-1*	31		(= *orn-2* in 112)	
3416	*nic-1*	12	Y30166	*nic-1*	12
3717	*vis(3717)*	—	30300	*arg-3*	31
Y3786	see *iv-1, iv-2*	—		(= *cit-1* in 112)	
4540Ab	see *nic-2*	12	Y30539r	*rib-2*	*
4545	*lys-3*	71	Y30539y	*ylo*	—
4637T	see *al-1*	12	31379	see *al-2*	—
JH5202	*inos*	—	Y31455	see *nic-2*	—
Y5296	*col-3*	7	Y31873	*nic-1*	12
Y5331	*col-2*	7	33442	*arg-2*	31
5359	*pab-1*	*		(= *cit-2* in 112)	
5531	*pan*	—	33757	*leu-1*	144
5801	*sc*	—	33933	*lys-1*	71
5936T	see *leu-3*	—	34105	*arg-7*	31
Y6994	*tyr*	—		(= *orn-3* in 112)	

* Signifies locus numbers first assigned in present paper.

TABLE 3. (*Continued*)
Key Relating Isolation Number to Locus Designation

Isolation number	*Locus*	*Reference for locus number*	*Isolation number*	*Locus*	*Reference for locus number*
34486	*chol-1*	65	39401	*nt*	—
34508	*al-1*	12	39404	see *suc*	—
34542	*chol-1*	65	39701	*tryp-2*	12
34547	*n-nit*	—	39706p	*pdx-1*	31
34547-10	*tryp-2*	12	39709	*iv-2*	*
34556	see *pan*	—	39801	*tryp-2*	12
35203	*ad-3*	*	39816	*me-4*	62
	(= *ad-p* in 31, 68)		40008	*tryp-2*	12
35307	*pab-1*	*	43002	*nic-2*	12
35401	*arg-9*	31	44008	*tryp-2*	12
	(= *prol-2* in 112)		44020	*tryp-2*	12
35402	*suc*	—	44101	*pyr(44101);*	—
35405	*pdx-1*	31		see *pyr(44302)*	
35420	*tryp-2*	12	44204p	*pdx-2*	31
35809	*me(35809)*	—	44206t	*ad-4*	31
35810	see *iv-1, iv-2*	—	44207	*arg-8*	31
36001	see *al-1*	12		(= *prol-3* in 112)	
36104	*me-3*	62	44302	*pyr(44302)*	—
36105	*me-3*	62	44409t	*un(44409)*	—
36601	*pyr-4*	112	44411	*ad-1*	31
36607	*tryp-2*	12	44415t	see *ad-4*	—
36703T	*arg-1*	31	44602p	*pdx-1*	31
37101	*lys-2*	71	44707	*tryp-2*	12
37102	*inos*	—	45217	*tryp-2*	12
37301	*pyr-3*	69	45302	*tryp-2*	12
37401	*inos*	—	45303-1	*tryp-2*	12
37602	see *suc*	—	45304	*tryp-2*	12
37803	*pdx-1*	31	45502T	see *pyr-3*	—
37815t	*pyr-3*	69	46003R	*r-can*	—
37903	*chol-1*	65	46006t	see *un(44409)*	—
37907	*tryp-2*	12	46109	*tryp-2*	12
38113	*pab-1*	*	46316	*inos*	—
38502	*pyr-2*	69	46403	*un(46403)*	—
38701	*ad-3*	*	46404	*tryp-2*	12
	(= *ad-p* in 31, 68)		46405	*tryp-2*	12
38706	*me-1*	62	46406	*tryp-2*	12
38709	*ad-3*	*	46802	*inos*	—
	(= *ad-p* in 31, 68)		46807	*iv-2*	*
39106p	*pdx-1*	31	46904p	*pdx-1*	31
39113	see *nic-1*	—	47101	see *iv-1*	—
39115	*tryp-2*	12	47203	*tryp-2*	12
39303	*nic-2*	12	47313	*leu-3*	*
39311	see *suc*	—	47317	*tryp-2*	12

* Signifies locus numbers first assigned in present paper.

TABLE 3. (*Continued*)
Key Relating Isolation Number to Locus Designation

Isolation number	*Locus*	*Reference for locus number*	*Isolation number*	*Locus*	*Reference for locus number*
47807	see *suc*	—	66204t	*un(66204)*	—
47904	*chol-2*	65	66702	see *suc*	—
48009	*tryp-2*	12	67602	*pyr-3*	69
48613	*tryp-2*	12	67701	*pyr(44302)*	—
48614	*tryp-2*	12	70004t	*ad-2*	31
51504	*hs*	—	70007	*col-4*	*
51602t	*rib-1*	*	71104	*ad-5*	31
55701t	*un(55701)*	—	71301	*pab-3*	*
56501	*thi-1*	31	75001	*tryp-2*	12
64001	*inos*	—	75102	*tryp-2*	12
65001	*nt*	—	80801	*tryp-2*	12
65205	*tryp-2*	12	83201t	*inos*	—
65612	*tryp-2*	12	84605	*cys*	—
66108	*leu-3*	*	85902	*thi-4*	31
66110	*tryp-2*	12	89601	*inos*	—

* Signifies locus numbers first assigned in present paper.

ascus, NPD for nonparental ditype ascus. For definition of PD, NPD, and tetratype see Section II, 1, also Fig. 1. T and Ab following isolation numbers signify translocation and aberration, respectively. *p* or *t* following isolation numbers indicate pH- or temperature-sensitive mutants. These symbols are omitted from the centromere and linkage tables and from the maps because they are properties of the allele and not necessarily of the locus. For the significance of capital letters preceding isolation numbers, see Section IV, 2, b. For the meaning of "ascus segregations," abbreviated "ascus segs.," see Section IV, 1, c on Determination of Gene Order. Information on allelism given under the heading "*Alleles*" always refers to a cross, a heterocaryon, or to homogeneity with the standard mutant, unless otherwise stated. For example, under *leu-3*, allele 66108, the statement "no w.t. in 20 asci, and similar linkage with *sex* and *al-2*" means that there were no wild types recovered from 20 asci from a cross of 66108 × 47313 (the standard mutant), and that 66108 and 47313 both give similar map distances from *sex* and similar map distances from *al-2*. Distal and proximal are always relative to the centromere. Information on gene order beginning "ascus segs. indicate" means ascus segregations in a 2-point cross with the marker indicated. For example, under *asp*, ORDER, the statement "Ascus segs. indicate right, distal to *pab-1*" means that ascus segregations in the two-point cross *asp* × *pab-1* indicate that

asp is on the right arm and that the order is centromere—*pab-1*—*asp*. The phrase "right (or left) arm by definition" designates the loci chosen to define the arms of each linkage group (Section III, 1, b).

Descriptive Index of Mutants

A,a sex (mating type). Linkage group I, left arm.
ORDER: Left arm by definition. COMMENT: A = + (plus) in Lindegren's terminology (157).

ac acetate. Linkage group II, right arm.
ORDER: Ascus segs. indicate right, distal to *arom* and proximal to *fl*.
Y2492. N-mustard (156). Requires acetate plus ethanol (160). Usually produces black pigment in agar. *ac* spores often ripen and/or germinate more slowly than spores of most other mutants; ∴ difficult to use as a marker since critical timing is necessary to obtain good germination. Germinate best on sucrose minimal agar plus yeast extract plus ethanol (47).

ad-1 adenine. Linkage group VI, arm unknown.
ORDER: No data.
3254. X-ray (15). Uses adenine or hypoxanthine (125). None of the adenine mutants can use guanine alone (106).

ad-2 adenine. Linkage group III, probably right arm.
ORDER: M.d. and c.d.'s in cross with *ad-4* indicate on right. However, location by m.d. from *ad-4* places it nearer to centromere than lower limit of range from centromere data.
70004t. U.V. (15). Dosage-response curve; uses adenine or hypoxanthine (106).
ALLELE: *27663Ab*. U.V. (15). No w.t. in 33 asci and 715 randoms (106). Mentioned as probable aberration (68) because c.d. and linkage to *ad-4* are heterogeneous with those of 70004t. POSSIBLE ALLELE: *20705*. X-ray (15). Listed as possible allele (68) but no data given.

ad-3 adenine purple. Linkage group I, right arm.
ORDER: Ascus segs. from multiple-point crosses #3 and #15 indicate on right, proximal to *mc(35809)*.
35203. U.V. (15). Dosage-response curve; accumulates purple pigment; uses adenine or hypoxanthine (106).
ALLELES: *38701, 38709*. Both U.V. (15). Only purple progeny when crossed with 35203 or with each other; number of isolates not stated (106).

ad-4 adenine. Linkage group III, right arm.
ORDER: Ascus segs. indicate on right, very close to *prol-1*, with no indication of order.
44206t. U.V. (15). Dosage-response curve; will not use hypoxanthine (101). POSSIBLE ALLELE: *44415t.* U.V. (15). Listed as allele (106); no data presented but fails to respond to hypoxanthine.

ad-5 adenine. Linkage group I, probably left arm.
ORDER: Ascus segs. in cross with *sex* and m.d. from *ad-3* (10.2 units based on 137 random isolates (106)) indicate probably left arm.
71104. U.V. (15). Dosage-response curve; uses adenine or hypoxanthine; requirement is only partial (106).

ad-6 adenine. Not mapped. *28610.* U.V. (15). Uses adenine or hypoxanthine (106).

ad-7 adenine. Not mapped. *44411.* U.V. (15). Uses adenine or hypoxanthine (106).

al-1 albino. Linkage group I, right arm.
ORDER: Cross with *al-2* indicates on right. Seg. in 1 critical ascus in 3-point cross #6 favors *al-1* distal to *mc*(*35809*). *al-1* and *al-2* give no recombination in 32 asci (68), 2 w.t. in 176 random isolates (73), and positive het. (73). ∴ *al-1* and *al-2* nonallelic and approximately 2 units apart; only evidence of order is from seg. in 1 critical ascus from cross of allele *4637T* with *al-2*, which favors *4637T* (and ∴ probably *al-1*) proximal to *al-2* (73).
34508. U.V. (15). Aurescent: white at first, later forming carotenoid-pigmented terminal conidia (73). All data listed under *al-1* in linkage and centromere tables are on 34508.
COMMENTS: Centromere data heterogeneous when all 34508 crosses are included. When greatest deviate (cross with E5297 (68)) is excluded, data are homogeneous; ∴ data from this cross were not used. In different crosses of 34508 × *sex*, both the PD to NPD, and the parental to recombinant ratios are heterogeneous; but tetratype to ditype ratios are not heterogeneous. Since the latter are basis of calculations, and no single cross is responsible for the heterogeneity, all data are included.
ALLELE: *4637T.* X-ray (15). Albino: conidia and hyphae white (73). Negative het. and no w.t. out of 139 randoms (73). Reciprocal translocation (97); see Cytogenetics sec-

tion. POSSIBLE ALLELES: *36001*. U.V. (15); aurescent. *16275*, *16808*, *17295*. All X-ray (15); albino. No direct cross, but all give negative het. tests with 34508 and with each other, and positive het. with *al-2* (73).

al-2 albino. Linkage group I, right arm.
ORDER: Ascus segs. in multiple-point crosses #4 and #15, and ranges of c.d.'s and m.d., indicate right arm distal to *me(35809)*.
15300. X-ray (15). Conidia and hyphae white, with little or no carotenoid pigment; modified slightly by light, media, age of culture, and other genes (7, 73, 145). POSSIBLE ALLELE: *31379*. U.V. (15). No direct cross, but negative het. with 15300 and positive het. with *al-1* (73).

arg-1 arginine. Not mapped. **36703T** U.V. (15). Will not use ornithine or citrulline (151). Reciprocal translocation (146); see Cytogenetics section.

arg-2 arginine. Linkage group IV, probably right arm.
ORDER: Only data are on random isolates; 0.6% w.t. out of 2137 spores when crossed to *pyr-3* (#37815t); 0.4% w.t. out of 3253 spores when crossed to *col-4* (113). Included only in legend of map, because no serial isolations.
33442. U.V. (15). Uses citrulline or arginine (151).

arg-3 arginine. Linkage group I, possibly left arm.
ORDER: One critical ascus in multiple-point cross #16 and c.d. and m.d. from *sex* indicate slightly more likely on left and proximal to *sex*.
30300. U.V. (15). Description (150). Uses citrulline or arginine (151).

arg-4 arginine. Not mapped. **21502.** X-ray (15). Uses ornithine, citrulline, or arginine (151).

arg-5 arginine. Not mapped. Listed in group I (68), but only evidence is cross with translocation 4637T (149). **27947.** X-ray (15). Uses ornithine, citrulline, or arginine (151).

arg-6 arginine. Linkage group I, right arm.
ORDER: Cross with *al-2* indicates on right; seg. in 1 critical ascus favors *arg-6* distal to *al-2*. COMMENT: Not allelic with *arg-5* (149).
29997. U.V. (15). Description (150). Uses ornithine, citrulline, or arginine (151).

arg-7 arginine. Not mapped. **34105.** U.V. (15). Uses ornithine, citrulline, or arginine (151).

arg-8 arginine. Not mapped. **44207.** U.V. (15). Uses proline, ornithine, citrulline, or arginine (150).

arg-9 arginine. Not mapped. **35401.** U.V. (15). Uses proline, ornithine, citrulline, or arginine (150).

arom aromatic. Linkage group II, right arm.
ORDER: Ascus segs. in 3-point cross #10 favor right arm, distal to *pe*; ascus segs. indicate proximal to *ac*.
Y7655. N-mustard (156). Requires all of the following four aromatic compounds for growth; *p*-aminobenzoic acid, tyrosine, tryptophan, and phenylalanine (156). All 4 requirements can be replaced by shikimic acid (155).

asp asparagine. Linkage group V, right arm.
ORDER: Ascus segs. indicate right, distal to *pab-1*.
S1007. X-ray (154). Uses asparagine but not aspartic acid (154).

r-can canavanine resistant. Linkage group I, right arm.
ORDER: No recombination with *al-1*; ∴ cannot determine order, so mapped at same location as *al-1*.
46003R. Canavanine-resistance was found in 46003 threonine stock and shown to be due to a separate nonlinked gene apparently present in parent stock before irradiation (163). Description and dosage-response curve (67, 163).

chol-1 choline. Linkage group IV, right arm.
ORDER: Ascus segs. in 3-point cross #12 and in cross with *pan* indicate on right, distal to *tryp-4* and probably proximal to *pan*.
34486. U.V. (15). Description (64), dosage-response curve (5). Also uses mono- and dimethylaminoethanol (61).
ALLELES: *34542, 37903*. Both U.V. (15). 34542 gives no recombination with 34486 in 18 asci; 37903 gives no recombination with 34542 in 18 asci (68).

chol-2 choline. Not mapped. **47904.** U.V. (15). Also uses dimethylaminoethanol but not monomethylaminoethanol (61).

col-1 colonial. Linkage group IV, right arm.
ORDER: Ascus segs. indicate right, and distal to *chol-1*.
Y8743c. 20-methylcholanthrene (156). Linear growth rate on agar medium less than 1% of w.t. rate; also prevents macroconidiation; frequently reverts to w.t. growth habit (7).

col-2 colonial. Not mapped. **Y5331.** 20-methylcholanthrene (156).

col-3 colonial. Not mapped. **Y5296.** N-mustard (156).

col-4 colonial. Linkage group IV, possibly left arm.
ORDER: One critical ascus in cross with *pdx-1* strongly favors left arm; however, gives only 2.4% recombination (based on 21 w.t. or pseudo-w.t. out of 1717 spores plated (113)) with *pyr-3* (#37301), which is on right.
70007. N-mustard (77). Growth initially colonial, becoming semispreading with age; does not prevent macroconidiation. More stable than *col-1* (48).

cr crisp. Linkage group I, right arm.
ORDER: Ascus segs. in multiple-point crosses #8 and #17 indicate right.
crisp. Apparently spontaneous (84). Very short conidiophores, conidia in tight clusters and more brightly colored than w.t. (85); illustration (84).

cys cysteine. Linkage group VI, right arm.
ORDER: Ascus segs. in cross with *rib-1* indicate same arm and distal to *rib-1*. COMMENT: Nothing published on whether this is allelic with unmapped locus *mc-4*.
84605. X-ray (141). Uses either cysteine or methionine. Also requires tyrosine at 25°C but not at 35°C (66).

dir dirty. Linkage group I, probably right arm.
ORDER: Ascus segs. in 3-point cross #9 indicate slightly more likely on right.
dirty. Apparently spontaneous (84). Conidia few and misshapen; yellowish exudate (85); illustration (84).

fl fluffy. Linkage group II, right arm.
ORDER: Ascus segs. and m.d. and c.d.'s indicate right, distal to *pe*. Ascus segs. indicate distal to *ac*. One critical ascus favors *fl* proximal to *tryp-3*.
fluffy. Spontaneous (83). No macroconidia (83); normally produces few or no microconidia unless culture is wetted down (90); produces abundant microconidia if *pe* is present (7).

gap gap. Linkage group I, left arm.
ORDER: Ascus segs. in 3-point cross #9 indicate left arm.
gap. Apparently spontaneous (84). Conidia produced in upper part of culture tube (85); illustration (84).

het-1 heterocaryon formation. Not mapped. Origin same as *het-2* (46).

het-2 heterocaryon formation. Linkage group II, right arm.
ORDER: Ascus segs. indicate right, distal to *fl*. Found in *inos* (#37401) stock.
heterocaryon formation-2. Not known whether spontaneous or produced by U.V. at same time as *inos* (46). Description (46).

hist-1 histidine. Linkage group V, right arm.
ORDER: Only data are on random isolates; 6.4% recombination with *inos* (calculated from w.t. frequency in 4124 isolates) and 28.4% recombination with *lys-1* (calculated from w.t. frequency in 1691 isolates) (56). Included only in legend of map because no ascus isolations.
C84. U.V. (56, 79). Description (56).
ALLELES: *C85*, *C91*. Both U.V. (56, 79). No w.t. from unstated no. of random isolates, and accumulates a compound with same R_f on chromatograms as does C84 (56).

hist-2 histidine. Linkage group I, arm unknown.
ORDER: Only data are on random isolates; 7.8% recombination with *ad-3* (calculated from w.t. frequency in 1712 isolates) (56). Included only in legend of map because no ascus isolations.
C94. U.V. (56, 79). Description (56).

hist-3 histidine. Linkage group I, arm unknown.
ORDER: Ascus segs. indicate 5.5 map units from *hist-2*. Included only in legend of map because *hist-2* is located only by random isolates.
C140. U.V. (56, 79). Description (56). POSSIBLE ALLELE: *C1710Ab* (called T1710 by (56)). U.V. (56, 79). Description (56). See Cytogenetics section. No w.t. from unstated no. of random isolates and no recombination in 135 asci; also gives negative het. with C140 (56). However, C1710Ab accumulates a compound which appears to be an imidazole derivative while C140 does not (56).

hist-4 histidine. Linkage group unknown.
COMMENT: Listed as a locus in linkage group IV based on 27.8% recombination (calculated from w.t. frequency) with *pyr-3* and 26.2% recombination (calculated from w.t. frequency) with *pyr-2* (56). However, also shows significant linkage based on ratio PD:NPD with *hist-2* (linkage group I) and with C1710Ab (linkage group I) but not with C140 (linkage group I) (from Table 1 in 56). Because of these

inconsistencies, locus was not assigned to a definite linkage group.
C141. U.V. (56, 79). Description (56).

hs homoserine. Linkage group I, right arm.
ORDER: No recombination with *al-1*; ∴ cannot determine order, so mapped at same location as *al-1*.
51504. U.V. (15). Dosage-response curve; also uses methionine plus threonine (164).

inos inositol. Linkage group V, right arm.
ORDER: Ascus segs. in 3 point cross #14 indicate distal to *iv-1*, proximal to *pab-1*.
37401. U.V. (15). Dosage-response curve; grows colonially at low concentrations of inositol (9).
ALLELES: *37102*, *46316*, *46802*, *64001*. All U.V. (15). *89601*, *JH2626*, *JH5202*. All N-mustard (48, 100). Each of the seven alleles gives less than 1% recombination with 37401, based on a minimum of 14,000 ascospores tested. Probably a compound locus (48). JH5202 has high spontaneous reversion rate (48). *83201t*. Origin not published. No w.t. in 29 asci (68).

iv-1 isoleucine-valine. Linkage group V, right arm.
ORDER: Right arm by definition.
16117. X-ray (15). Requires both isoleucine and valine; optimal ratios (20). Also uses corresponding keto acids (165).
POSSIBLE ALLELES: *35810*, *47101*. Both U.V. (15). 35810 gives no w.t. in 20 asci, but could also be allele of *iv-2*, which is only 3 units away, ∴ not mapped. 47101 listed as possible allele (68), but no proof that it is in linkage group V, ∴ not mapped. *Y3786*. See *iv-2*.

iv-2 isoleucine-valine. Linkage group V, right arm.
ORDER: Cross with *iv-1* indicates on right. C.d.'s favor *iv-2* distal to *iv-1*, but ranges overlap.
39709. U.V. (15).
ALLELE: *46807*. U.V. (15). Homogeneous c.d.'s and similar linkage with *iv-1*. POSSIBLE ALLELES: *Y3786*. N-mustard (156). One w.t. out of 17 whole perithecia in cross with *iv-1* (1). *35810*. See *iv-1*.

leu-1 leucine. Linkage group III, right arm.
ORDER: Ascus segs. indicate right, distal to *ser*, and proximal to *thi-2*.
33757. U.V. (15). Description (130).

leu-2 leucine. Not mapped. **8839.** X-ray (15). Description (131).

leu-3 leucine. Linkage group I, left arm.
ORDER: Ascus segs. in 3-point cross #7 indicate left, distal to *sex*.
47313. U.V. (15). Description (131).
ALLELES: *66108*. U.V. (15). No w.t. in 20 asci, and similar linkage with *sex* and *al-2* (132). Adapts more readily than 47313 (131). POSSIBLE ALLELE: *5936T*. X-ray (15). No recombination with 47313 in 25 asci including some 8-spored asci (132). Reciprocal translocation (146). Description (131).

lys-1 lysine. Linkage group V, arm unknown.
ORDER: Gives 31.5% recombination with *inos* (620 random isolates) (53). From m.d. and range of c.d.'s, *lys-1* is probably on left.
33933. U.V. (15). Also uses α-aminoadipic acid (109) and ε-hydroxynorleucine (52); dosage-response curve (109).

lys-2 lysine. Not mapped. **37101.** U.V. (15). Will not use ε-hydroxynorleucine (52).

lys-3 lysine. Linkage group I, right arm.
ORDER: Ascus segs. in multiple-point crosses #2 and #16 indicate distal to *al-2*.
4545. X-ray (15). Also uses ε-hydroxynorleucine (52). Dosage-response curve and inhibition by arginine (35, 36).
POSSIBLE ALLELE: *28815*. See *lys(28815)*.

lys-4 lysine. Linkage group I, arm unknown.
15069. X-ray (15). Will not use ε-hydroxynorleucine (52).

lys(28815) lysine. Linkage group I, right arm.
ORDER: Ascus segs. indicate same arm as *al-2*. C.d.'s suggest proximal to *al-2*, but ranges overlap badly. COMMENT: Listed as possible allele of *lys-3* but no data given (68). Negative het. (37) but c.d.'s are heterogeneous, ∴ tentatively mapped as a separate locus.
28815. U.V. (15). Description (37).

me-1 methionine. Not mapped. **38706** U.V. (15). Uses methionine but not homocysteine (173).

me-2 methionine. Not mapped. **H98.** X-ray (72). Uses methionine and homocysteine but not cystathionine (173).

me-3 methionine. Linkage group V, probably right arm.
ORDER: Ascus segs. indicate probably right, distal to *iv-1*; 23.7 units from *inos* based on 1083 randoms (53).

36104. U.V. (15). Also uses cystathionine and homocysteine (173).

ALLELE: *36105.* U.V. (15). Negative het.; no w.t. in 20 asci; c.d.'s and linkage to *iv-1* homogeneous.

me-4 methionine. Not mapped. **39816.** U.V. (15). Uses methionine, homocysteine, cystathionine, or cysteine (173). See also *cys.*

me-5 methionine. Linkage group IV, right arm.

ORDER: Ascus segs. indicate right, distal to *pyr-3.*

9666. X-ray (15). Also uses cystathionine and homocysteine (173).

COMMENT: Designated as a 5th locus since nonallelic with *me-2* or *me-3* (27), and blocks different biochemical steps from *me-1* and *me-4* (173).

me(35809) methionine. Linkage group I, right arm.

ORDER: Ascus segs. in multiple-point crosses #4, #6, and #15, and ranges of c.d.'s and m.d. from *sex* indicate on right, distal to *ad-3* and proximal to *al-1* and *al-2.*

35809. U.V. (15). Will not use homocysteine (173). COMMENT: Not allelic with *me-2, me-3,* or *me-5* (27); blocks different biochemical step from *me-4* (173); possibly an allele of *me-1* or a new locus.

nd natural death. Linkage group I, right arm.

ORDER: Crosses with *sex* and *al-2* (random and serial isolates) indicate on right, but NPD's are not given (142); ∴ corrected linkage values cannot be calculated. Mapped by c.d.

natural death. U.V. (142). Ceases growth progressively when homocaryotic (142).

nic-1 nicotinic acid. Linkage group I, right arm.

ORDER: Ascus segs. indicate on right, distal to *al-2.* Also m.d. from *lys-3* indicates on right. COMMENT: Called Q locus (17).

3416. X-ray (15). Will not use 3-OH anthranilic acid (21); dosage-response curve (19). Nicotinamide more satisfactory than nicotinic acid for *nic* mutants at most pH's because of permeability.

ALLELES: *Y31873.* N-mustard (156). Negative het. and only 11 w.t. from 350 whole perithecia (22). *Y30166.* N-mustard (156). Negative het., no recombination in serial isolates, and less than 1% recombination from unstated number of whole

perithecia (17). Possibly a compound locus. POSSIBLE ALLELE: *39113*. U.V. (15). Listed as possible allele but no data given (68).

nic-2 nicotinic acid. Linkage group I, arm unknown.
ORDER: No recombination with centromere.
43002. U.V. (15). Accumulates red-brown pigment in medium; dosage-response curve (19).
ALLELE: *39303*. U.V. (15). No direct cross, but similar c.d. and m.d. from *sex. Y31455*. N-mustard (156). No direct cross but similar c.d. and m.d. from *sex. 4540Ab*. X-ray (15). Will not use 3-OH anthranilic acid; accumulates red-brown pigment (19). Negative het. and no w.t. from 20 whole perithecia with Y31455 (22). Probable aberration (138); see Cytogenetics section.

n-nit nitrate nonutilizer. Linkage group I, right arm.
ORDER: Ascus segs. indicate on right and probably distal to *al-1*.
COMMENT: Ditype to tetratype ratios are heterogeneous in 3 crosses with *sex;* c.d. data also heterogeneous (total: 4 crosses). Since in both cases deviations are symmetrical about the mean, all data are included.
34547. U.V. (15).

nt nicotinic-tryptophan. Linkage group VII, right arm.
ORDER: Right arm by definition. COMMENT: 20.3 units from *thi-3* (241 random isolates) (authors, unpublished).
65001. U.V. (15). Grows well on nicotinic acid; extent of growth on tryptophan, phenylalanine, tyrosine, nicotinic precursors, and tryptophan precursors is dependent on modifiers (118); same is true for alleles (60); dosage-response data (60, 118, 119).
ALLELES: *C86*. Apparently U.V. (79, 119). Only 1 w.t. out of 6180 spores (116). *39401*. U.V. (15). Reported allelic with 65001 (18). Negative het. and no w.t. with C86. (17 asci (47); 11 asci and 15000 plated spores (60)).

pa pale. Linkage group I, right arm.
ORDER: Ascus segs. in multiple-point crosses #8 and #17 indicate right.
pale. Spontaneous (83). Conidia clumped and pale in color (83, 85); illustration (84).

pab-1 *p*-aminobenzoic acid. Linkage group V, right arm.
ORDER: Ascus segs. in 3-point cross #14 indicate distal to *inos*.

1633. X-ray (15). Description (157); dosage-response curve (2).

ALLELES: *830*, *5359*, *21850*. All X-ray (15). *35307*, *38113*. Both U.V. (15). All 6 strains give negative hets. in all combinations and no recombination when crossed in all combinations; total number asci involving the respective alleles are 75, 82, 69, 32, and 85 (174).

pab-2 *p*-aminobenzoic acid. Linkage group V, right arm.

ORDER: Cross with *pab-1* indicates on right, either proximal or distal. COMMENT: Evidence for difference from *pab-3* is 2 tetratypes out of 7 asci, although negative het. and no w.t. out of 160 randoms (174). Evidence for difference from *pab-1* is 1 tetratype out of 18 asci, 19.3% recombination out of 352 random isolates (174) and 15.9% recombination out of 314 random isolates (72).

H193. X-ray (72). Description and dosage-response data (174).

pab-3 *p*-aminobenzoic acid. Linkage group V, right arm.

ORDER: Linkage and c.d.'s in cross with *pab-1* indicate on right; c.d.'s indicate distal to *pab-1*, but ranges overlap badly.

71301. U.V. (15). Dosage-response data (174).

pan pantothenic acid. Linkage group IV, right arm.

ORDER: Ascus segs. indicate right, distal to *pdx-1* and *tryp-4*. Ascus segs. in 3-point cross #12 and in cross with *chol-1* indicate probably distal to *chol-1*.

5531. X-ray (15). Requires intact pantothenic acid (166).

POSSIBLE ALLELE: *34556*. U.V. (15). Listed as such but no evidence presented (68, 166).

pdx-1 pyridoxine. Linkage group IV, right arm.

ORDER: Right arm by definition.

35405. U.V. (15).

ALLELES: *37803*, *44602p*. Both U.V. (15). Description (153). *39106p*, *39706p*, and *46904p*. All U.V. (15). The five alleles give no w.t. with 35405 in 37, 37, 20, 20, and 20 asci, respectively (68). C.d.'s heterogeneous but not due to any one allele alone. Even if only those alleles with majority of data (#35405, #37803, #44602p) are retained, the c.d.'s are still heterogeneous although no recombination in 37 asci between those with extreme c.d.'s. ∴ all data included.

pdx-2 pyridoxine. Linkage group IV, right arm.
ORDER: Seg. in 1 critical ascus indicates right arm, distal to *pdx-1*.
44204p. U.V. (15). Description (153). COMMENT: Listed as different from *pdx-1* on basis of 1 tetratype out of 45 asci (68), and preliminary het. evidence (153).

pe peach. Linkage group II, right arm.
ORDER: Right arm by definition.
peach(Lindegren). Spontaneous (84). Peach-colored conidia, less clustered than w.t.; illustration (84).
ALLELE: *Y8743m* (*microconidial*). Designated *pe*^{m} (7). Found in Y8743 colonial stock; shown to be due to separate non-linked gene. Probably present in parent stock prior to methylcholanthrene treatment (7). No recombination in 50 asci with peach (Lindegren); similar c.d.'s and linkage with *fl* (7). Peach conidia occurring closer to agar surface than in w.t.; in some genomes produces greenish-gray microconidia near bottom of slant; in presence of *fl* (which prevents macroconidiation) abundant microconidia are produced. Sometimes difficult to score in absence of *fl*, presumably because of modifiers (7). Illustration (7).

prol-1 proline. Linkage group III, right arm.
ORDER: Ascus segs. indicate distal to *ser*, if on right, while ascus segs. in cross with *tryp-1* indicate definitely on right.
21863. X-ray (15). Will not use ornithine, citrulline, or arginine (150).

pyr-1 pyrimidine. Linkage group IV, right arm.
ORDER: Linkage with *pdx-2* indicates on right.
H263. X-ray (72). Nucleosides (uridine and cytidine) and corresponding nucleotides are 10 to 60 times as active as uracil (93). Apparently true for all pyrimidine mutants investigated (108).

pyr-2 pyrimidine. Linkage group IV, probably right arm.
ORDER: C.d.'s and m.d. in cross with *pyr-3* indicate better agreement if on right, but ranges overlap badly. COMMENT: Evidence for difference from *pyr-1* is 60% tetratypes in 30 asci (68).
38502. U.V. (15). Description (110); dosage-response curve (108).

pyr-3 pyrimidine. Linkage group IV, right arm.
ORDER: Ascus segs. indicate distal to *pdx-1*; also ranges of c.d.'s place *pyr-3* distal to *pdx-1* with no overlap. Ascus segs. in 3-point cross #13 indicate *pyr-3* distal to *rib-2* and proximal to *chol-1*. COMMENT: Evidence for difference from *pyr-1* is 11.6% tetratypes out of 352 asci. Evidence for difference from *pyr-2* is 53% tetratypes out of 100 asci (68).
37301. U.V. (15). Description and dosage-response curve (108).
ALLELES: *37815t.* U.V. (15). No w.t. in 76 asci (68). *67602.* U.V. (15). No w.t. from 39 asci (68); also no w.t. from 104 asci of 67602 × 37815t (68). Requirement of 67602 is only partial at 25°C (108). *1298.* X-ray (15). No direct cross, but gives m.d.'s homogeneous with those of 37301, 37815t, and 67602 in crosses with *pyr-1*. Description (93); dosage-response curve (92); inhibition by adenosine (126). *45502T.* U.V. (15). Reciprocal translocation (97); see Cytogenetics section. One isolate, which was apparently freed of the translocation (based on restoration of normal c.d. and normal asci; not checked cytologically) gave only 1% w.t. or pseudo-w.t. out of 8115 random isolates in cross with 37301 (113).

pyr-4 pyrimidine. Not mapped. **36601.** U.V. (15). Listed as a separate locus but no data given (112).

pyr(44101) pyrimidine. Linkage group IV, probably right arm.
COMMENT: Listed as Possible allele of *pyr-3* (68) and gives no recombination in 26 asci when crossed with *pyr-3*, but m.d.'s in crosses with *pyr-1* are heterogeneous. Could also be allele of either *pyr-1* (no recombination in 16 asci (68)) or *pyr(44302)* (no direct cross, but *pyr(44302)* is only 1.1 units from *pyr-1*). ∴ not mapped.
44101. U.V. (15).

pyr(44302) pyrimidine. Linkage group IV, right arm.
ORDER: Ascus segs. in cross with *pyr-1* indicate on right. COMMENT: Both 44302 and 67701 listed as possible alleles of *pyr-3* (68) but linkage of both with *pyr-1* is heterogeneous with that of *pyr-3*; ∴ tentatively classified as a separate locus but not mapped. Evidence for difference from *pyr-1* is 2.3% tetratypes out of 132 asci. Evidence for difference from *pyr-2* is widely divergent centromere distances. Conceivably an allele of *pyr-4*, since no published data exist on *pyr-4*.

44302. U.V. (15).
ALLELE: *67701*. U.V. (15). No direct cross but c.d. and linkage to *pyr-1* are homogeneous with those of 44302.

rib-1 riboflavin. Linkage group VI, right arm.
ORDER: Right arm by definition.
51602t. U.V. (15). Dosage-response data (107).

rib-2 riboflavin. Linkage group IV, right arm.
ORDER: One critical ascus in 3-point cross #13 and one in cross with *pdx-1* indicate *rib-2* distal to *pdx-1* and proximal to *pyr-3*/but c.d. indicates distal to *pyr-3*.
Y30539r. N-mustard (156). Ascospores germinate best on minimal agar plus riboflavin, and often require up to three weeks to obtain maximum number of germinants.

sc scumbo. Linkage group III, right arm.
ORDER: Ascus segs. indicate right, proximal to *ser*.
5801. X-ray (15). No conidia; irregular, "scummy-like" surface growth (12, 32).

ser serine. Linkage group III, right arm.
ORDER: Right arm by definition.
H605. X-ray (72). Can also use glycine; dosage-response curves (72).

sex See *A*, *a*.

sfo sulfonamide. Linkage group VII, arm unknown.
ORDER: No data.
E15172. Spontaneous (41). Dosage-response curve; reverts frequently via suppressor mutations (41). Best tested on solid minimal growth tubes at 35°C (39).

su-1-me suppressor of methionine. Linkage group I, right arm.
ORDER: Listed as within 1 unit of *al-2* (48). No other data given; ∴ included only on legend of map. COMMENT: Called S-1 locus (48).
R1. Spontaneous (48). Suppresses *me(4894)* and *me-2* (48).
ALLELES: *R2* and *R3*. Both spontaneous (48). Listed as alleles but no data given (48).

su-2-me suppressor of methionine. Not mapped. Called S-2 locus (48).
R4. U.V. (48). Suppresses *me(4894)*.

suc succinic acid. Linkage group I, arm unknown.
ORDER: No recombination with centromere.
35402. U.V. (15). Also uses aspartic, glutamic, fumaric, malic, α-ketoglutaric, acetic, oxalosuccinic, and acetoacetic

acids, glutamine and asparagine; will not use other tricarboxylic acid cycle intermediates (82). POSSIBLE ALLELES: *37602, 39311, 39404, 47807, 66702.* All U.V. (15). Negative hets.; direct crosses not fertile (82). All are sex-linked and have homogeneous low centromere distances, but parental to recombinant ratios in crosses with *sex* are heterogeneous. If the possible allele showing largest recombination with *sex* is omitted, all others fit as a locus on left arm; if the possible allele showing smallest recombination with *sex* is omitted, all others fit as a locus on right arm. ∴ data on possible alleles are not included.

thi-1 thiamine. Linkage group I, arm unknown.
ORDER: Ascus segs. in cross with *sex* give slight preference for right arm.
56501. X-ray (15). Uses thiamine, or pyrimidine plus thiazole moieties. PROBABLE ALLELE: *17084T.* X-ray (15). Dosage-response curve (158). No recombination with either *thi-1* or *thi-3* (68), which are not linked with each other (133). Probable translocation; see Cytogenetics section. Positive het. with *thi-3* and biochemically similar to *thi-1* (158); ∴ probably an allele of *thi-1.*

thi-2 thiamine. Linkage group III, right arm.
ORDER: Ascus segs. indicate right, distal to *prol-1* and *leu-1.*
9185. X-ray (15). Requires intact thiamine; dosage-response curve (158).

thi-3 thiamine. Linkage group VII, arm unknown.
ORDER: No data. COMMENT: 6.0% recombination (calculated from w.t. frequency) with *sfo* (201 random isolates) (133). Best tested on minimal agar slants.
18558. X-ray (15). Uses thiamine or thiazole moiety; dosage-response curve (158).

thi-4 thiamine. Linkage group III, arm unknown.
ORDER: No recombination with centromere. COMMENT: Evidence for difference from *thi-2* is 4 recombination asci out of 17.
85902. Origin not published.

tryp-1 tryptophan. Linkage group III, right arm.
ORDER: Ascus segs. indicate right, proximal to *tyr.*
10575. X-ray (15). Uses indole or tryptophan (159). Accumulates yellow pigment; dosage-response curves (116).

tryp-2 tryptophan. Linkage group VI, arm unknown.
ORDER: No data.
75001. Neutrons (15). Uses anthranilic acid, indole, tryptophan, or kynurenine (16).
ALLELES: *45302*. No w.t. out of over 10,000 viable ascospores plated (116). *39801*. 1 w.t. out of about 8400 spores tested, both by plating and by isolating whole perithecia, in cross with 45302 (116). All remaining alleles tested by crossing with 45302 or a proved allele of 45302, with no w.t.: 1) By plating ascospores—*35420*, *44008*, *45304*, *46405*, *47317*, and *66110*—each with at least 4500 viable spores tested; 2) By whole perithecia and/or perithecial contents—*34547-10*, *33607*, *37907*, *39115*, *39701*, *40008*, *44707*, *45127*, *46109*, *46406*, *47203*, *48009*, *48613*, *48614*, *65205*, *65612*, *75102* and *80801*, each with a minimum of 4500 spores; *46404*, with about 1950 spores; *44020*, with about 600 spores; *45303-1*, with about 450 spores (116). All alleles U.V. except 75102 (neutrons) (15) and 80801 (origin not published).

tryp-3 tryptophan. Linkage group II, right arm.
ORDER: Cross with *fl* indicates right; one critical ascus favors *tryp-3* distal to *fl*.
C83. Called *td-1* (171). U.V. (79, 111). Will not use indole (111). Lacks tryptophan desmolase (171). Not suppressed by suppressor of S1952 (171).
ALLELE: *S1952*. (Called *td-2* (171)). Carcinogen (6). Negative het. (171) and no w.t. out of 6300 spores plated (116). Lacks tryptophan desmolase except in presence of an unmapped suppressor (171).

tryp-4 tryptophan. Linkage group IV, right arm.
ORDER: Ascus segs. in 3-point cross #12 indicate right, and proximal to both *chol-1* and *pan*. (Data from random isolates in crosses with *chol-1* and *pan* agree poorly with tetrad data.)
Y2198. N-mustard (156). Accumulates yellow pigment; uses indole or tryptophan but inhibited by tryptophan; dosage-response curves (116).
ALLELE: *Y16331*. X-ray (156). Negative het. and only 3 w.t. out of 1300 spores (116). POSSIBLE ALLELE: *S1908*. Carcinogen (6). Uses indole or tryptophan. Negative het. and no w.t. out of 35 random isolates in cross with Y2198. Positive het. with *tryp-1* and *tryp-3* (116).

tu tuft. Linkage group II, right arm.
ORDER: C.d.'s, m.d.'s, and ascus segs. in 3-point cross #11 indicate on right, distal to *pe* and proximal to *fl*.
tuft. Origin not published. Lighter in color and slower growing than w.t.; conidia mostly at top of culture in large clusters separated by open spaces (89). Difficult to score in some genomes.

tyr tyrosine. Linkage group III, right arm.
ORDER: Ascus segs. indicate right and distal to *leu-1* and *tryp-1*.
Y6994. N-mustard (156). Description (155).

un(44409) unknown requirement. Linkage group I, right arm.
ORDER: Ascus segs. in cross with *sex* indicate on right.
44409t. U.V. (15). Description (70). POSSIBLE ALLELE: *46006t.* U.V. (15). Cross with 44409t gives no w.t. in 20 asci (68), but c.d.'s are heterogeneous; ∴ data not included.

un(46403) unknown requirement. Linkage group I, probably right arm.
ORDER: One critical ascus in cross with *sex* favors right arm; if on left, is not between *sex* and centromere. COMMENT: No indication from published data that *un(44409)* and *un(46006)* are different from *un(46403)*, but they are listed as different (68), presumably because of unpublished het. or biochemical data. *un(46403)* mapped by c.d. rather than by m.d. from *sex*, because of disproportionately large number of NPD's in cross with *sex* (by methods used, these would be included as noncrossover asci).
46403. U.V. (15).

un(55701) unknown requirement. Linkage group I, left arm.
ORDER: Ascus segs. in 3-point cross #1 and range of m.d. from *sex* indicate on left. COMMENT: Genetically different from *un(44409)* (70).
55701t. X-ray (15). Description (70).

un(66204) unknown requirement. Linkage group VI, arm unknown.
ORDER: No data.
66204t. U.V. (15).

vis(3717) visible. Linkage group I, right arm.
ORDER: Ascus segs. in 3-point cross #1 indicate on right.
3717. X-ray (15). No description published.

ylo yellow. Linkage group VI, arm unknown.
ORDER: No data.
Y30539y. N-mustard (156). Found in same stock as Y30539r. Conidia yellow instead of orange (47).

d. *Tables of Centromere Distance and Linkage Data.* The centromere distance and the two-point cross tables are presented separately for each linkage group (Tables 4–10 and 11–16). In addition a three- and a four-point cross table present the complete ordered ascus segregations on all unpublished multiple-point crosses, and merely list the published ones (Tables 17 and 18). The two-point cross tables list the number of PD, T, and NPD asci (see Section II, 1, b), the recombination percentage, the uncorrected map distance (one-half the per cent of tetratype asci), and the corresponding distance corrected for multiple crossovers by means of the mapping function curve in Fig. 7. The confidence limits (listed as "ranges") are also given for both the uncorrected and corrected map distances. The per cent recombination is the same as the uncorrected map distance wherever nonparental ditype asci were not obtained. For centromere distances no value exists which is comparable to the per cent recombination (see Section II, 1, b); otherwise the centromere tables correspond to the two-point cross tables.

All data on each locus are listed under the standard mutant number, with a footnote reference when another allele was used. The crosses are listed numerically with the lowest number in each cross written first. (For the locus symbols corresponding to mutant isolation numbers see Table 3. The standard mutant at each locus is given in the Descriptive Index of Mutants.)

In order to present complete information on crosses involving two genes and centromere, the complete ordered ascus segregations would have to be listed for each cross, as has been done in the three- and four-point cross tables. This is impractical because of space limitations. The two-point crosses have therefore been tabulated without reference to the centromere, and all the centromere data on each locus have been compiled in a separate table. However, complete ascus segregations from all crosses have been used in determining gene order (see Section IV, 1, c), even though not presented here. The segregations are given in full for the unpublished three- and four-point crosses because they provide much valuable information on gene order and interference. All linkage and centromere data from the three- and four-point crosses, both published and unpublished, have been included in the two-point cross and centromere tables.

In correcting the map distances for multiple crossovers, only the ascending part of the mapping function curve (Fig. 7) was used. For tetratype frequencies greater than 83.5%, the maximum reached by the $k = 0.2$ curve, the corrected distances are indeterminate. In such cases the corrected distances have been listed as greater than 65 units, which is the corrected map distance corresponding to 83.5% tetratypes.

TABLE 4
Centromere Distances, Linkage Group I

Standard mutant	*Locus*	*Number of asci*‡ 1st-div. seg'n (≅ PD + NPD)	2nd-div. seg'n (≅ T)	*Map distance from centromere* Uncorrected Value	Uncorrected Range	Corrected Value	Corrected Range	*Number of crosses*‡‡	*Ref.*
3416	*nic-1*	5	5	25.0	9 –41	28.0	9 –60	1	22*
3717	*vis(3717)*	31	5	6.9	2.5–14.5	7.3	2.5–15.5	—	68
4545	*lys-3*	6	11					1	37
		16	48					—	68
		6	11					1	137
		0	1					1	55
		5	2					2	143
		9	19					1	139
		—	—						
	Total	42	92	34.3	29.5–38.0	41.5	34.0–49.0		
15069	*lys-4*	15	0					1	37
		13	1					—	68
		—	—						
	Total	28	1	1.7	>0.0– 9.0	1.7	>0.0– 9.5		
15300	*al-2*	171	216					20	7§
		(97)	(94)					—	68
		52	69					11	22§
		43	57					**	49§
		41	57					1	149§
		47	50					5	47
		42	31					2	99
		16	34					2	132

			15	24					3	174§
			16	4					1	115a§
			2	12					—	162
			12	16					1	139
			12	12					2	137
			3	3					2	55
			10	13					4	143
		Total	579	692	27.2	25.8–28.6	30.9	29.1–32.7		
28815	*lys(28815)*		16	15	24.2	15.0–34.0	26.8	15.5–40.5	—	68
29997	*arg-6*		52	78	30.0	25.5–34.0	34.8	28.5–41.5	2	149
30300	*arg-3*		23	1					1	149
			27	1					1	139
		Total	50	2	1.9	>0.0– 7.0	1.9	>0.0– 7.0		
34508	*al-1*		35	58					5	27
			17	32					2	36
			91	150					**	162¶
			129	141					—	68
			9	9					1	131
		Total	281	390	29.0	27.2–30.7	33.4	31.0–36.0		
34547	*n-nit*		48	60	27.7	22.5–32.5	31.3	24.5–38.5	—	68
35203	*ad-3*		194	6					—	68*
			59	2					2	99
		Total	253	8	1.5	0.6– 3.1	1.5	0.6– 3.1		

TABLE 4. (*Continued*)

Standard mutant	Locus	Number of asci‡ 1st-div. seg'n (≅ PD + NPD)	2nd-div. seg'n (≅ T)	Map distance from centromere Uncorrected Value	Uncorrected Range	Corrected Value	Corrected Range	Number of crosses‡‡	Ref.
35402	*suc*	18	0	0	0 - 9	0	0 - 9	—	68
35809	*me(35809)*	58	14					2	99
		24	9					2	27
		—	—						
	Total	82	23	11.0	7.0–15.5	11.4	7.0–16.5		
43002	*nic-2*	39	0					—	68*
		14	0					1	22*
		—	—						
	Total	53	0	0.0	0.0– 3.2	0.0	0.0– 3.2		
44409	*un(44409)*	15	5	12.5	4 –25	13.2	4 –27	—	68†
46003R	*r-can*	6	11	32.4	18 –43	38.2	20 –>65	1	162
46403	*un(46403)*	11	4	13.3	3 –25	14.1	4 –31	—	68†
47313	*leu-3*	113	33	11.3	8.0–15.0	11.9	8.0–16.0	**	131, 132*
51504	*hs*	29	38	28.4	22.0–34.5	32.5	23.5–42.5	**	162
55701	*un(55701)*	(195)	(21)	4.9	3.0– 7.5	5.0	3.1– 7.5	—	68
56501	*thi-1*	7	8	26.7	13 –40	30.3	13 –55	—	68
71104	*ad-5*	(50)	(5)	4.5	1.5–10.5	4.6	1.5–11.0	—	68
crisp	*cr*	462	45					—	88
		101	10					—	105
		—	—						
	Total	563	55	4.4	3.5– 5.5	4.6	3.5– 5.7		

dirty	*dir*	75	128	31.5	28.0–35.0	36.8	32.0–42.0	—	88
gap	*gap*	553	54	4.4	3.5– 5.5	4.5	3.5– 5.7	—	88
natural death	*nd*	(17)	(7)	14.6	6.5–26.0	15.5	6.5–28.5	—	142
pale	*pa*	458	158	12.8	11.0–14.5	13.6	11.8–15.4	—	88
sex	*A, a*	233	32					—	42
		964	162					—	88‖
		14	2					1	1§
		48	14					3	7§
		62	9					2	99
		47	7					5	131
		42	8					2	132
		96	15					—	105
		(1601)	(210)					—	68
		51	4					2	149
		24	4					1	139
		23	1					2	137
		18	5					4	143
		5	1					2	55
		12	1					1	27
		10	0					1	22§§
	Total	3250	475	6.4	5.9– 6.9	6.6	6.1– 7.2		

* Some or all of the data are from another allele.
† From Table 1 in ref. 68; discrepancy in Table 6, ref. 68.
‡ Numbers in ( ) calculated from published values for centromere distance and total number of asci.
§ From compilation Table 1A in ref. 7.
‖ Including all data from Table 1A in ref. 7.
¶ Includes all crosses except 34508, 83210, A × Em5297a (68), which is inhomogenous with other data.
** Several crosses.
†† Table 3 in ref. 68.
‡‡ Number given only if from unpublished source.
§§ From compilation Table 1A in ref. 7; other 40 asci in Table 1A, ref. 7, discarded because involve aberration stock 4540 Ab.

TABLE 5
Centromere Distances, Linkage Group II

Standard mutant	Locus	Number of asci‡ 1st-div. seg'n (≅ PD + NPD)	2nd-div. seg'n (≅ T)	Map distance from centromere Uncorrected Value	Uncorrected Range	Corrected Value	Corrected Range	Number of Crosses‡‡	Ref.
C83	*tryp-3*	37	71	32.8	27.5–38.5	39.0	31.0–49.0	—	171*
Y2492	*ac*	154	159	25.4	22.0–28.5	28.5	25.0–32.5	11	47
Y7655	*arom*	82	63	21.7	17.5–26.0	23.7	18.5–29.0	5	47
fluffy	*fl*	42	68					—	83§
		124	154					—	89§
		14	13					—	72§
		89	128					16	7§
		85	89					9	47
		16	26					1	46
	Total	370	478	28.2	26.5–29.9	32.0	30.0–34.6		
het. formation	*het-2*	9	33	39.3	29.5–45.0	52.2	34.0–>65	2	46
peach	*pe*	80	23					—	84§
		215	63					—	89§
		10	5					—	72
		14	5					—	131
		54	34					6	47*
		74	44					8	8*
		279	113					26	7*
	Total	726	287	14.1	12.5–16.0	15.0	13.5–16.5		
tuft	*tu*	188	90					—	89
		26	13					2	47
	Total	214	103	16.2	13.5–19.5	17.3	14.0–21.0		

Footnotes: See footnotes to Table 4.

The correction was not applied to distances spanning the centromere, since this is not justified (see Section II, 2, e). For samples of 20 asci or less, ranges were rounded off to the nearest whole unit; for samples of more than 20 asci, to the nearest half-unit; and for ranges of five units or less, to the nearest one-tenth unit.

3. *Maps*

The material in the Descriptive Index of Mutants and in the centromere and linkage tables have been used to construct the uncorrected and

TABLE 6
Centromere Distances, Linkage Group III

Standard mutant	Locus	Number of asci‡ 1st-div. seg'n (≅ PD + NPD)	2nd-div. seg'n (≅ T)	Map distance from centromere Uncorrected Value	Uncorrected Range	Corrected Value	Corrected Range	Number of Crosses‡‡	Ref.
H605	*ser*	346	15	2.1	1.2– 3.6	2.1	1.2– 3.6	11	72
5801	*sc*	39	1					—	68
		73	1					5	72
		59	1					4	131
		55	1					3	27
	Total	226	4	0.9	0.8– 2.5	0.9	0.8– 2.5		
Y6994	*tyr*	53	69	28.3	23.0–33.0	32.3	26.5–39.0	6	47
9185	*thi-2*	87	48					4	72
		(153)	(74)					—	68
	Total	(240)	(122)	16.8	14.5–19.5	18.0	15.0–21.0		
10575	*tryp-1*	142	87					7	72§
		3	10					1	7§
		41	18					—	68
		11	12					1	47
		11	17					1	49
		5	2					1	22
	Total	213	146	20.3	18.0–23.5	22.2	19.0–25.5		
21863	*prol-1*	79	7					3	72
		(164)	(19)					—	68
	Total	(243)	(26)	4.8	3.0– 7.0	5.0	3.0– 7.2		
33757	*leu-1*	65	8					2	72§
		107	22					9	131
		(55)	(12)					—	68
		22	6					1	7§
		33	23					2	47
	Total	(282)	(71)	10.0	8.0–12.5	10.6	8.3–13.3		
44206	*ad-4*	(354)	(45)	5.6	4.0– 7.5	5.8	4.2– 7.8	—	68
70004	*ad-2*	(110)	(63)	18.2	14.5–22.0	19.5	15.5–24.5	—	68
85902	*thi-4*	37	0	0.0	0.0– 5.0	0.0	0.0– 5.0	—	68

Footnotes: See footnotes to Table 4.

TABLE 7
Centromere Distances, Linkage Group IV

Standard mutant	Locus	Number of asci‡ 1st-div. seg'n (≅ PD + NPD)	Number of asci‡ 2nd-div. seg'n (≅ T)	Map distance from centromere Uncorrected Value	Uncorrected Range	Corrected Value	Corrected Range	Number of Crosses‡‡	Ref.
H263	*pyr-1*	(201)	(26)	5.7	3.7– 8.2	5.9	3.9– 8.5	—	68
Y2198	*tryp-4*	8	16					3	22
		36	33					4	116
	Total	44	49	26.3	21.0–31.5	29.8	22.5–37.0		
5531	*pan*	(122)	(135)					—	68§
		35	40					3	7§
		4	5					1	22
		6	22					1	49
		2	5					1	137
		3	2					1	55
		11	20					1	116
	Total	(183)	(229)	27.8	25.3–30.2	31.6	28.5–35.0		
Y8743c	*col-1*	83	93					—	7
		18	22					2	8
		35	52					16	49
	Total	136	167	27.6	24.5–30.5	31.3	27.5–35.5		
9666	*me-5*	50	57					6	27
		15	5					—	68
	Total	65	62	24.4	19.5–29.0	27.0	21.5–33.0		
Y30539r	*rib-2*	210	121	18.2	15.5–21.0	19.6	16.5–23.0	15	47
34486	*chol-1*	(100)	(88)					—	68*‖
		16	11					—	7§
		13	10					1	47
		12	19					1	116
	Total	(141)	(128)	23.8	20.0–27.5	26.3	22.5–30.5		
35405	*pdx-1*	(499)	(102)					—	68*‖
		25	3					—	7*§
		25	12					4	49*
		29	11					2	47*
	Total	(578)	(128)	9.0	7.5–10.5	9.5	7.8–11.0		
37301	*pyr-3*	(512)	(175)					—	68*
		40	18					3	27
		16	8					1	47
	Total	(568)	(201)	13.1	11.6–14.8	13.9	12.2–15.6		
38502	*pyr-2*	(35)	(59)	31.4	26.0–36.0	36.7	29.0–45.0	—	68
44204	*pdx-2*	(63)	(17)	10.6	6.0–16.0	11.2	6.5–17.0	—	68
44302	*pyr(44302)*	(30)	(4)	5.9	1.5–14.0	6.2	1.5–15.0	—	68*
70007	*col-4*	181	52	11.2	8.5–14.0	11.8	9.0–15.0	29	49

Footnotes: See footnotes to Table 4.

TABLE 8
Centromere Distances, Linkage Group V

Standard mutant	Locus	Number of asci‡ 1st-div. seg'n (≅ PD + NPD)	2nd-div. seg'n (≅ T)	Map distance from centromere Uncorrected Value	Uncorrected Range	Corrected Value	Corrected Range	Number of Crosses‡‡	Ref.
H193	*pab-2*	5	6	27.3	11 –42	31.0	12 –>65	1	174
S1007	*asp*	27	40	29.9	23.5–36.0	34.6	26.0–45.0	5	154
1633	*pab-1*	9	12					1	174
		25	21					1	49
		6	14					1	47
	Total	40	47	27.0	21.5–32.5	30.6	23.5–40.0		
16117	*iv-1*	19	9					—	7§
		53	7					3	27§
		(193)	(42)					—	68
		31	15					1	49
		12	8					1	47
		2	1					1	143
	Total	(310)	(82)	10.5	8.5–12.7	11.0	8.9–13.5		
33933	*lys-1*	61	2					2	37§
		25	3					—	7§
		47	5					—	68
	Total	133	10	3.5	1.7– 6.6	3.5	1.7– 6.9		
36104	*me-3*	94	106					10	27*
		8	12					—	68
	Total	102	118	26.8	23.5–30.0	30.5	25.5–36.0		
37401	*inos*	36	43					6	22§
		49	41					—	7§
		33	47					12	49§
		(159)	(179)					—	68*
		12	7					1	47
	Total	(289)	(317)	26.2	24.2–28.0	29.5	27.0–32.0		
39709	*iv-2*	20	6	11.5	4.5–22.5	12.2	4.5–24.0	—	68*
71301	*pab-3*	6	18	37.5	26.0–45.0	48.0	30.0–>65	1	174

Footnotes: See footnotes to Table 4.

TABLE 9
Centromere Distances, Linkage Group VI

Standard mutant	Locus	Number of asci‡ 1st-div. seg'n (≅ PD + NPD)	2nd-div. seg'n (≅ T)	Map distance from centromere Uncorrected Value	Uncorrected Range	Corrected Value	Corrected Range	Number of Crosses‡‡	Ref.
3254	*ad-1*	9	1					1	72§
		34	3					2	27§
		81	1					—	7§
		20	0					1	131§
		20	1					1	8
		(263)	(3)					—	68‖
		22	0					1	47
		21	0					1	116
	Total	(470)	(9)	0.9	0.5– 1.7	0.9	0.5– 1.7		
Y30539y	*ylo*	130	10	3.5	1.5– 6.5	3.6	1.5– 6.7	6	47
51602	*rib-1*	11	1					1	7§
		107	4					—	68§
		21	5					1	47
	Total	139	10	3.4	1.5– 6.0	3.4	1.5– 6.2		
66204	*un(66204t)*	(74)	(12)	7.0	3.5–11.5	7.3	3.5–12.5	—	68
75001	*tryp-2*	(84)	(29)					—	68*
		16	5					1	116
	Total	(100)	(34)	12.7	9.0–17.0	13.5	9.4–18.0		
84605	*cys*	133	13	4.4	2.5– 7.5	4.6	2.6– 7.7	5	141

Footnotes: See footnotes to Table 4.

corrected linkage maps shown in Figs. 9–15. Sampling errors were usually so great that it seemed inadvisable to attempt to combine all the known bits of data relevant to a given locus. Therefore, the numerical map position and range of each locus are based only on the single most reliably determined interval, *i.e.*, either its centromere distance or its distance from some other well-mapped marker. The same interval has been used for both the corrected and the uncorrected maps. In choosing the interval, preference was usually given to small range, and only secondarily to small map length, since with the usual numbers of asci, sampling errors are more serious than errors from undetected multiple crossovers; in all

TABLE 10
Centromere Distances, Linkage Group VII

Standard mutant	Locus	Number of asci‡ 1st-div. seg'n (≅ PD + NPD)	2nd-div. seg'n (≅ T)	Map distance from centromere Uncorrected Value	Uncorrected Range	Corrected Value	Corrected Range	Number of Crosses‡‡	Ref.
sulfonamide	*sfo*	229	1	0.2	>0.0– 0.9	0.2	>0.0– 0.9	—	39
18558	*thi-3*	20	4					—	158
		37	4					4	133
		—	—						
	Total	57	8	6.2	3.0–11.5	6.4	3.0–12.0		
65001	*nt*	6	4					—	68††
		(63)	(55)					—	68*
		—	—						
	Total	(69)	(59)	23.8	19.5–28.5	26.3	21.0–32.5		

Footnotes: See footnotes to Table 4.

cases the arrangement was made to harmonize with all information on gene order.

a. *Description of Maps:* The map of each group consists of a heavy horizontal line numbered in map units in both directions from the centromere, which serves as the origin. Each locus is represented by a vertical line with a horizontal bar indicating the 95% confidence limits for its position. An open bar indicates that the gene was mapped by centromere distance; a stippled bar, that it was mapped by linkage with some other locus (which is listed in the legend).

A thin vertical line indicates that the position of the locus is uncertain with respect to one or more of the loci represented by thick vertical lines or with respect to the centromere. Actually uncertainty regarding gene order ranges all the way from doubt about the position of the locus with respect to one other locus to complete uncertainty about which arm it lies on. For complete information regarding the position of a locus, reference must be made to the Descriptive Index of Mutants. Thick lines are used to designate only the most useful (usually the longest) series of markers whose sequence is fairly certain. For example, in linkage group II the sequences *pe-tu-fl* and *pe-arom-ac-fl* are established, but not the order of *tu*, *arom*, and *ac*; therefore, *tu* was designated by a thin line. The maps have been so arranged that the confidence limits bars of the best mapped loci are usually nearest to the map unit scale.

TABLE 11

Two-Point Crosses, Linkage Group I

Ditto marks refer only to standard mutant number and not to footnote references.

Cross		Number of asci PD	T	NPD	Per cent recombination	Map distance Uncorrected** Value	Range	Corrected‖ Value	Range	Reference†
3416	× 4545	16	0	0	0.0	0.0	0 –11	0.0	0 –11	22
"	× 15300	6	3	0	16.7	16.7	3 –36	17.8	3 –45	22
"	× 43002*	4	7	2	42.3	26.9	12 –41	—	—	68
3717	× 55701	13	3	0	9.4	9.4	2 –24	—	—	68§[1]
"	× sex	13	3	0	9.4	9.4	2 –24	—	—	68§[1]
4545	× 15069	6	5	0	22.7	22.7	8 –39	—	—	37
"	× 15300	9	6	2						137§[4]
"	× "	0	1	0						55§[4]
"	× "	4	0	0						143§[4]
"	× "	21	7	0						139§[16]
	Total	34	14	2	18.0	14.0	8.0–21.5	14.9	8.5–23.5	
"	× 30300	9	19	0	33.9	33.9	23.5–42.0	—	—	139§[16]
"	× sex	6	10	1						137§[4]
"	× "	0	0	1						55§[4]
"	× "	2	2	0						143§[4]
"	× "	11	17	0						139§[16]
	Total	19	29	2	33.0	29.0	21.5–36.0	—	—	
15069	× sex	15	0	0	0.0	0.0	0 –11	0.0	0 –11	37
15300	× 29997	97	1	0	0.5	0.5	>0.0– 3.0	0.5	>0.0– 3.0	149
"	× 30300	11	17	0	30.3	30.3	21.0–39.5	—	—	139§[16]
"	× 34508	32	0	0	0.0	0.0	>0.0– 5.5	0.0	>0.0– 5.5	68
"	× 35203*	13	24	1						68§[3]
"	× "	35	25	1						99§[15]
	Total	48	49	2	26.8	24.8	19.5–30.0	27.7	21.0–34.5	
"	× 35809	6	5	0						99§[4]
"	× "	37	22	0						99§[15]
	Total	43	27	0	19.3	19.3	13.0–25.0	20.8	15.0–29.5	

"	× 47313*	14	33	3	39.0	33.0	25.5–39.5	—	—	132(2)§[7]
"	× sex	2	4	2						8
"	× "	12	24	2						68§[3]
"	× "	14	11	4						47
"	× "	16	29	5						132(2)§[7]
"	× "	11	11	2						137(2)‡[8]
"	× "	1	4	1						55(2)‡[8]
"	× "	5	17	1						143(4)¶‡[8]
"	× "	4	6	1						99§[4]
"	× "	33	27	1						99§[15]
"	× "	13	14	1						139§[16]
	Total	111	147	20	33.6	26.4	23.5–29.5	—	—	
28815	× 34508	28	3	0	4.8	4.8	1.0–13.5	4.9	1.0–14.0	68(2)‡[8]
"	× sex	8	11	1	32.5	27.5	15.5–38.5	—	—	68§[5]
29997	× "	12	20	0	31.2	31.2	22.0–39.5	—	—	149
30300	× "	23	1	0						149
"	× "	25	3	0						139§[16]
	Total	48	4	0	3.8	3.8	0.5–11.5	3.8	0.5–12.0	
34508	× 34547	9	1	0	5.0	5.0	>0 –23	5.2	>0 –26	68
"	× 35809	5	7	1	34.6	26.9	12 –40	30.5	13 –55	27§[6]
"	× 46003R	17	0	0	0.0	0.0	0 –10	0.0	0 –10	162
"	× 51504	67	0	0	0.0	0.0	0.0– 2.6	0.0	0.0– 2.8	162(2)
"	× sex	9	9	0						131
"	× "	1	12	0						27§[6]
"	× "	32	43	13						68(4)‡[6]
	Total	42	64	13	37.8	26.8	22.0–32.0	—	—	
34547	× sex	42	52	4	30.6	26.5	21.0–31.5	—	—	68(3)
35203	× 35809	52	9	0	7.4	7.4	3.5–13.0	7.6	3.5–14.0	99§[15]
"	× sex	31	7	0						68§[3]
"	× "	52	9	0						99§[15]
	Total	83	16	0	8.1	8.1	4.5–12.5	—	—	

TABLE 11. (*Continued*)

Cross	Number of asci PD	T	NPD	Per cent recombination	Map distance Uncorrected** Value	Uncorrected** Range	Corrected‖ Value	Corrected‖ Range	Reference†
35809 × sex	20	13	0						27(2)‡[6]
" × "	7	3	0						99§[4]
" × "	48	13	0						99§[15]
Total	75	29	0	13.9	13.9	9.5–19.0	—	—	
43002* × sex	38	1	0						68(2)
" * × "	10	0	0						22
Total	48	1	0	1.0	1.0	>0.0– 5.5	1.0	>0.0– 5.5	
44409 × sex	14	5	1	17.5	12.5	4.0–25.0	—	—	68
46403 × "	13	1	1	10.0	3.3	>0 –17	—	—	68
47313* × "	56	11	0	8.2	8.2	4.0–14.0	8.5	4.0–14.5	131, 132(6)‡[7]
51504 × "	10	9	1	27.5	22.5	11.5–34.5	—	—	161
55701 × "	76	0	0	0.0	0.0	0.0– 2.4	0.0	0.0– 2.4	68‡[1]
56501 × "	7	8	0	26.7	26.7	13 –40	—	—	68
71104 × "	17	3	0	7.5	7.5	1.5–19.5	7.8	1.5–21.0	68
crisp × gap	378	40	1	5.0	4.8	3.5– 6.5	—	—	88§[17]
" × pale	401	94	2	9.9	9.5	7.7–11.3	9.9	8.0–12.0	88§[8, 17]
" × sex	403	90	4						88§[8, 17]
" × "	85	25	1						105
Total	488	115	5	10.3	9.5	7.9–11.0	—	—	
dirty × gap	32	75	8	39.6	32.6	27.5–37.0	—	—	84§[9]
" × sex	31	63	11	40.5	30.0	25.0–35.0	—	—	84§[9]
gap × pale	301	115	3	14.5	13.7	11.5–16.0	—	—	88§[17]
" × sex	531	62	4	5.9	5.2	4.0– 6.5	5.3	4.1– 6.7	84, 88‡[9, 17]
pale × "	414	183	9	16.6	15.1	13.0–17.0	—	—	88‡[8, 17]

* Some or all the data are from another allele.
† Numbers in parentheses designate number of crosses, if more than 1.
‡ Data in part from 3- or 4-point cross; number in italics designates which cross (see Tables 17, 18).
§ Data wholly from 3- or 4-point cross; number in italics designates which cross (see Tables 17, 18).
‖ No corrected values are given where the loci are on opposite sides of centromere.
¶ Same data are given in reference 142 but not broken down into ascus types.
** Uncorrected map distance equals one-half the per cent tetratype asci.

TABLE 12

Two-Point Crosses, Linkage Group II

Ditto marks refer only to standard mutant number and not to footnote references

Cross	Number of asci PD	T	NPD	Per cent recombination	Map distance Uncorrected** Value	Range	Corrected‖ Value	Range	Reference†
C83 × fluffy	33	1	0	1.5	1.5	>0.0– 8.0	1.5	>0.0– 8.5	172(4)
Y2492 × Y7655	15	5/	0	12.5	12.5	4.0–25.0	13.2	4.5–28.0	47
" × fluffy	65	11	0	7.2	7.2	3.5–12.5	7.5	3.5–13.0	47
" × peach	37	19	1	18.4	16.6	10.5–24.0	17.8	11.0–26.5	47
Y7655 × fluffy	25	15	0	18.7	18.7	11.5–27.5	20.2	12.0–31.2	47(2)‡[10]
" × peach*	17	3	0	7.5	7.5	1.5–19.5	7.8	1.5–21.0	47§[10]
fluffy × het-2	21	21	0	25.0	25.0	17.0–33.0	28.1	18.0–39.0	46
" × peach	143	131	4						89§[11]
" × " *	61	66	10						7(10)
" × " *	18	14	1						6(3)
" × " *	29	30	5						47(4)‡[10]
Total	251	241	20	27.4	23.5	21.5–25.5	26.0	23.5–28.5	
" × tuft	175	101	2	18.9	18.2	15.0–21.5	19.5	16.0–23.5	89§[11]
peach × "	231	47	0						89‡[11]
" × "	17	2	0						47
Total	248	49	0	8.2	8.2	6.0–10.5	8.5	6.5–11.5	

Footnotes: See footnotes to Table 11.

There are a few genes for which no tetrad data are available but which have been assigned to linkage groups on the basis of random isolates. These are included only in the legends of the maps but are discussed fully in the Descriptive Index of Mutants.

b. *Uncertainties Concerning Gene Location.* A few loci could equally well be indicated on the map in either of two places. These are loci whose order with respect to centromere, or to the locus by which they were mapped, is not indicated from the data. Such loci are arbitrarily mapped at only one position, but their situation is discussed in the Descriptive Index of Mutants. The authors felt that showing a locus in two places would both clutter up the map and in the case of centromere-mapped genes would indicate two arms of a linkage group where only one has been definitely established. (The problem is illustrated by markers in group VI.) For genes showing no recombination with the marker by which

TABLE 13

Two-Point Crosses, Linkage Group III

Ditto marks refer only to standard mutant number and not to footnote references

Cross	Number of asci PD	T	NPD	Per cent recombination	Map distance Uncorrected** Value	Range	Corrected‖ Value	Range	Reference†
H605 × 5801	22	2	0	4.2	4.2	0.5–13.5	4.3	0.5–14.0	72
" × 9185	39	32	2	24.6	21.9	16.0–28.0	24.0	17.0–34.0	72(2)
" × 10575	76	30	0	14.2	14.2	10.0–19.0	15.0	10.0–20.5	72
" × 21863	32	3	0	4.3	4.3	1.0–11.5	4.5	1.0–12.0	72
" × 33757	37	5	0	6.0	6.0	2.0–13.0	6.2	2.0–13.5	72
5801 × 21863	9	1	0	5.0	5.0	>0–23	5.2	>0–26	68
" × 33757	40	4	0	4.5	4.5	1.5–11.0	4.6	1.5–11.5	131(2)
Y6994 × 10575	13	10	0	21.7	21.7	11.0–33.5	23.7	12.0–39.0	47
" × 33757	17	9	0	17.3	17.3	9.0–27.5	18.5	9.0–32.5	47
9185 × 21863	23	8	0	12.9	12.9	6.0–22.5	13.7	6.0–24.5	72
" × 33757	25	6	0	9.7	9.7	3.5–19.0	10.1	3.5–20.5	72
" × 85902	13	3	1	14.7	8.8	2 –22	9.3	2 –24	68
10575 × 21863	16	4	0	10.0	10.0	2.5–22.0	10.5	2.5–24.5	72
21863 × 44206	20	0	0	0.0	0.0	0.0– 8.5	0.0	0.0– 9.0	68
44206 × 70004	40	3	1	5.7	3.4	1.0– 9.5	3.4	1.0–10.0	68(2)

Footnotes: See footnotes to Table 11.

they were mapped, however, the only doubt concerns the direction of the range. Such loci are mapped with two confluent ranges extending in both directions.

Where a gene is mapped by linkage, the range indicated is inadequate to the extent of the range of the marker by which it was mapped. However, no genes have been mapped by linkage with markers whose sequence is uncertain. In the one case where at first it was necessary to make an exception, the sequence has since been determined. In group I, the location of a large number of genes depended on the position of the two closely linked albino loci. It appeared probable that the albinos were in the opposite arm from *sex*, but the scarcity of data from intervening markers, plus the great inequality of the centromere distances of *sex* and of the albinos, had made the conclusion questionable. Mr. Hugh McDevitt in our laboratory undertook to settle the question by means of multiple-point crosses involving intermediate markers; his unpublished results, given in Tables 17 and 18, leave little doubt that the albino loci are indeed on the opposite arm from *sex*.

No attempt has been made to check the agreement of all the intercrosses involving each locus, since the ranges on most crosses are too

TABLE 14

Two-Point Crosses, Linkage Group IV

Ditto marks refer only to standard mutant number and not to footnote references

Cross	Number of asci PD	T	NPD	Per cent recombination	Map distance Uncorrected** Value	Range	Corrected‖ Value	Range	Reference†
H263 × 37301*	311	41	0	5.8	5.8	4.0– 7.8	6.0	4.1– 8.1	68(8)
" × 38502	11	18	1	33.3	30.0	20.0–39.0	34.8	21.5–50.5	68
" × 44204	30	0	0	0.0	0.0	0.0– 6.0	0.0	0.0– 6.0	68
" × 44302*	129	3	0	1.1	1.1	0.2– 3.6	1.1	0.2– 3.7	68(2)
Y2198 × 5531	25	6	0	9.7	9.7	3.5–19.0	10.1	3.5–20.5	116§[12]
" × Y30539r	12	8	0	20.0	20.0	9.5–32.0	21.7	9.5–38.5	47
" × 34486	26	5	0	8.1	8.1	2.5–17.0	8.4	2.5–18.0	116§[12]
5531 × Y8743c	47	0	0	0.0	0.0	0.0– 3.8	0.0	0.0– 3.8	7
" × 34486*	41	1	0						68(2)
" × "	30	1	0						116§[12]
Total	71	2	0	1.4	1.4	0.2– 5.0	1.4	0.2– 5.2	
" × 35405*	28	20	1	22.4	20.4	13.5–28.0	22.2	14.2–31.8	68
" × 37301	11	5	0	15.6	15.6	5 –30	16.6	5 –35	68
Y8743c × 34486	23	4	0	7.4	7.4	2.0–16.5	7.7	2.0–17.5	7
" × 35405*	16	12	0	21.4	21.4	12.5–31.5	23.3	13.0–37.5	7
9666 × 37301	11	7	0	19.4	19.4	8 –33	21.0	9 –39	27
Y30539r × 34486	48	18	0	13.6	13.6	8.5–20.0	14.5	9.0–21.5	47‡[13]
" × 35405*	39	1	0	1.2	1.2	>0.0– 7.0	1.2	>0.0– 7.0	47
" × 37301	23	1	0	2.1	2.1	>0.0–11.0	2.1	>0.0–11.5	47§[13]
34486 × 37301	19	4	0	8.7	8.7	2.0–20.0	9.1	2.0–21.5	47§[13]
35405* × 37301	38	1	0	1.3	1.3	>0.0– 7.0	1.3	>0.0– 7.0	68
" × 44204	44	1	0	1.1	1.1	>0.0– 6.0	1.1	>0.0– 6.0	68
" × 70007	16	4	1	14.3	9.5	2.5–21.5	—	—	49(2)
37301* × 38502	44	53	3	29.5	26.5	21.5–31.5	30.0	23.5–37.5	68(4)

Footnotes: See footnotes to Table 11.

large to merit the time required. An investigator interested in a particular locus can use the tabular data to check the relevant intercrosses. Such a detailed evaluation may frequently provide a more accurate estimate of the most likely position of the locus.

V. Discussion

1. *Mapping Functions*

a. *Evaluation.* The general subject of mapping functions was taken up in this paper because some practical method was needed for estimating

TABLE 15

Two-Point Crosses, Linkage Group V

Ditto marks refer only to standard mutant number and not to footnote references

Cross	Number of asci PD	T	NPD	Per cent recombination	Map distance Uncorrected** Value	Range	Corrected‖ Value	Range	Reference†
H193 × 1633*	63	11	0	7.4	7.4	4.0–12.5	7.7	4.0–13.5	174(3)
" × 71301	5	2	0	14.3	14.3	1 –36	15.2	1 –46	174
S1007 × 1633	13	8	0	19.5	19.5	9.0–32.0	21.0	9.5–37.5	154
" × 16117	9	13	0	29.6	29.6	18.0–40.0	34.2	19.5–53.0	154
" × 37401	23	5	0	8.9	8.9	3.0–18.5	9.4	3.0–20.0	154
1633 × 16117	31	14	1	17.4	15.2	9.0–23.0	16.2	9.0–25.5	49§[14]
" × 37401	43	2	0	2.2	2.2	>0.0– 8.0	2.2	>0.0– 8.5	49§[14]
" * × 71301	111	35	0	12.0	12.0	8.5–16.0	12.7	9.0–17.0	174(7)
16117 × 36104*	18	20	2	30.0	25.0	17 0–33.0	28.0	18.0–40.0	27(2)
" × 37401	33	12	0						49§[14]
" × "	13	7	0						68
Total	46	19	0	14.6	14.5	9.5–21.0	15.5	9.5–23.0	
" × 39709*	56	2	1	3.4	1.7	>0.0– 6.5	1.7	>0.0– 6.5	68(3)

Footnotes: See footnotes to Table 11.

map lengths of long intervals from the observed frequencies of different tetrad types. Had the data consisted of single strands an empirical mapping function might have filled the need, but empirical functions for tetrads were not available, nor were experimental data adequate for drawing a tetrad correction curve or even for establishing its form. A model was therefore devised (Section II, 2, d) from which corresponding mapping functions were derived for both single strands and tetrads. It is possible that some features of this model, and of the method used to derive mapping functions from it, may prove generally useful, aside from its application to the present work.

The mapping function adopted for use is based on hypotheses regarding the distribution of exchanges among 4-stranded bivalents and rests on the assumptions: (1) that chromatid interference can be ignored within chromosome arms; (2) that the probability of zero exchanges occurring in a genetically marked interval is independent of interference; and (3) that positive interference shifts the proportions of bivalents with one or more exchanges in an orderly manner. It is recognized that these assumptions may be subject to modification.

TABLE 16
Two-Point Crosses, Linkage Group VI
Ditto marks refer only to standard mutant number and not to footnote references

Cross	Number of asci PD	T	NPD	Per cent recombination	Map distance Uncorrected** Value	Range	Corrected‖ Value	Range	Reference†
3254 × Y30539y	20	2	0	4.5	4.5	0.5–15.0	4.6	0.5–16.0	47
" × 51602	17	0/	0	0.0	0.0	0 –10	0.0	0 –11	68
" × 66204	14	5	0	13.1	13.1	4 –26	13.9	4 –29	68
" × 75001	15	5	1	16.7	11.9	3.5–24.0	12.5	3.5–26.0	116
51602 × 84605	39	1	0	1.2	1.2	>0.0– 7.0	1.2	>0.0– 7.0	141

Footnotes: See footnotes to Table 11.

The statement has been made that "consistency is the sole criterion (other than mathematical simplicity) in preferring one mapping function to another" (Owen, 1950). Our model has the additional advantages that it involves few arbitrary assumptions, that it is flexible, and that it defines basic features of crossing over in tetrads. Flexibility can be achieved quantitatively by changing the magnitude of the coefficient k, or qualitatively by substituting another progression for the present k^{r-1}. Because the model specifies the distribution of exchanges in tetrads, a variety of testable predictions can be made (Section II, 2, e), and light may be thrown on interference, conceived of as a continuous property of the linear chromosome.

Comment on other mapping functions. The data plotted in Figs. 5 and 6 make one hesitate to accept Spiegelman's (1952) suggestion that maps might better be based on the tetrad mapping function for zero interference (curve B, Fig. 5) than on the customary function for complete interference (curve A).

Kosambi's (1944) empirical function shows far less satisfactory agreement with the data shown in Fig. 2 than do the other empirical functions.

b. *The Upper Limit of Second-Division Segregation Frequencies.* An interesting consequence of the proposed model is the prediction that the proportion of second-division segregations may exceed two-thirds as a normal consequence of interference. When interference is positive, the curve passes through a maximum in excess of two-thirds and then approaches two-thirds asymptotically from above (Fig. 5). Two-thirds has commonly been considered to be the upper limit for tetratype or second-division segregations in the literature of crossing over in organisms like Neurospora; yet the prediction of a higher maximum follows from the

TABLE 17

Crosses Involving Three Loci and Centromere

Data are given only for unpublished crosses. All data are included in the two-point cross tables.

Cross number	*Linkage group*	*Cross* X	Y	Z	*Ascus segregation* No. of asci	From centromere X	Y	Z	Between genes X & Y	X & Z	Y & Z	*Reference*
1	I	3717	− 55701	− sex								68
2	I	4545	× 15300	− a	4	1st	1st	1st	PD	PD	PD	137
					2	2nd	2nd	1st	NPD	T	T	137
					4	2nd	1st	1st	T	T	PD	137
					3	2nd	2nd	1st	PD	T	T	137
					1	2nd	1st	1st	T	T	NPD	137
					1	1st	2nd	1st	T	PD	T	137
					1	2nd	2nd	2nd	PD	PD	PD	137
					1	1st	1st	1st	PD	NPD	NPD	137
					1	2nd	1st	2nd	T	NPD	T	55
		4545	− 15300	× A	2	1st	1st	1st	PD	PD	PD	143
					2	1st	1st	2nd	PD	T	T	143
3	I	15300	− 35203*	− sex								68
4	I	15300	× 35809	− a	4	1st	1st	1st	PD	PD	PD	99
					3	2nd	1st	1st	T	T	PD	99
					1	1st	2nd	1st	T	NPD	T	99
					1	1st	1st	2nd	PD	T	T	99
					1	2nd	2nd	?	PD	T	T	99
					1	1st	2nd	2nd	T	T	?	99
5	I	28815	− 34508	− sex								68
6	I	35809	× 34508	− A	1	1st	1st	1st	PD	PD	PD	27
					1	1st	1st	2nd	PD	T	T	27
					3	2nd	2nd	1st	PD	T	T	27

			7	1st	2nd	1st	T	PD	T	27
			1	2nd	2nd	1st	NPD	T	T	27
7	I	15300 × 47313* – a	23	2nd	1st	1st	T	T	PD	132
			10	1st	1st	1st	PD	PD	PD	132
			4	1st	2nd	1st	T	PD	T	132
			2	2nd	2nd	2nd	PD	PD	PD	132
			2	2nd	2nd	2nd	T	T	PD	132
			2	2nd	2nd	2nd	NPD	NPD	PD	132
			2	2nd	2nd	1st	PD	T	T	132
			1	1st	1st	1st	NPD	NPD	PD	132
			1	2nd	2nd	1st	T	T	T	132
			1	1st	2nd	1st	T	NPD	T	132
			1	2nd	1st	2nd	T	NPD	T	132
			1	2nd	1st	2nd	T	T	T	132
8	I	crisp – pale – sex								88
9	I	dirty – gap – sex	(not all scored for sex)							84
10	II	Y7655 × peach – fluffy	9	1st	1st	1st	PD	PD	PD	47
			3	2nd	2nd	2nd	PD	PD	PD	47
			3	2nd	2nd	2nd	PD	T	T	47
			2	2nd	1st	2nd	T	T	T	47
			1	2nd	2nd	1st	PD	T	T	47
			1	2nd	2nd	2nd	T	PD	T	47
			1	1st	1st	2nd	PD	T	T	47
11	II	peach – tuft – fluffy								89
12	IV	Y2198 – 34486 × 5531	15	2nd	2nd	2nd	PD	PD	PD	116
			10	1st	1st	1st	PD	PD	PD	116
			3	1st	2nd	2nd	T	T	PD	116
			1	2nd	2nd	2nd	T	T	PD	116
			1	1st	1st	2nd	PD	T	T	116
			1	2nd	1st	1st	T	T	PD	116

TABLE 17. (*Continued*)

Cross number	*Linkage group*	*Cross* X	Y	Z	*Ascus segregation* No. of asci	From centromere X	Y	Z	Between genes X & Y	X & Z	Y & Z	*Reference*
13	IV	Y30539r	− 34486	× 37301	13	1st	1st	1st	PD	PD	PD	47
					6	2nd	2nd	2nd	PD	PD	PD	47
					3	1st	2nd	1st	T	PD	T	47
					1	2nd	?	2nd	T	PD	T	47
					1	1st	2nd	2nd	T	T	?	47
14	V	1633	× 16117	− 37401	21	1st	1st	1st	PD	PD	PD	49
					9	2nd	2nd	2nd	PD	PD	PD	49
					8	2nd	1st	2nd	T	PD	T	49
					2	2nd	2nd	2nd	T	PD	T	49
					2	1st	2nd	1st	T	PD	T	49
					1	2nd	2nd	2nd	T	T	PD	49
					1	1st	2nd	2nd	T	T	PD	49
					1	2nd	1st	2nd	PD	PD	PD	49
					1	1st	1st	?	NPD	?	?	49

* Some or all of the data are from another allele.

TABLE 18

Crosses Involving Four Loci and Centromere

Data are given only for the unpublished crosses. All data are included in the two-point cross tables.

Cross				No. of asci	*Ascus segregation*									
					From centromere				Between genes					
W	X	Y	Z		W	X	Y	Z	W & X	W & Y	W & Z	X & Y	X & Z	Y & Z
Cross no. 15*—Group I														
15300 − 35809 × 35203 − A				30	1st	1st	1st	1st	PD	PD	PD	PD	PD	PD
				1	2nd	1st	1st	2nd	T	T	T	PD	T	T
				16	2nd	1st	1st	1st	T	T	T	PD	PD	PD
				2	2nd	2nd	1st	1st	PD	T	T	T	T	PD
				3	1st	1st	1st	2nd	PD	PD	T	PD	T	T
				1	2nd	2nd	2nd	1st	T	T	T	PD	T	T
				1	2nd	2nd	2nd	1st	PD	PD	T	PD	T	T
				1	2nd	2nd	1st	2nd	T	T	T	T	PD	T
				1	2nd	2nd	1st	2nd	T	T	PD	T	T	T
				1	2nd	2nd	1st	2nd	PD	T	PD	T	PD	T
				1	1st	2nd	1st	1st	T	PD	PD	T	T	PD
				1	1st	2nd	1st	1st	T	NPD	NPD	T	T	PD
				2	2nd	2nd	1st	1st	?	T	T	T	T	PD
Cross no. 16†—Group I														
a × 30300 − 15300 − 4545				8	1st	1st	1st	1st	PD	PD	PD	PD	PD	PD
				11	1st	1st	2nd	2nd	PD	T	T	T	T	PD
				1	2nd	2nd	1st	2nd	PD	T	T	T	T	T
				3	1st	1st	1st	2nd	PD	PD	T	PD	T	T
				1	1st	1st	2nd	1st	PD	T	PD	T	PD	T
				2	2nd	1st	2nd	2nd	T	PD	PD	T	T	PD
				1	1st	1st	2nd	2nd	PD	T	T	T	T	T
				1	2nd	1st	2nd	2nd	T	NPD	T	T	T	T
Cross no. 17‡—Group I														
gap − sex − crisp − pale														

* Reference 99.
† Reference 139.
‡ Reference 88.

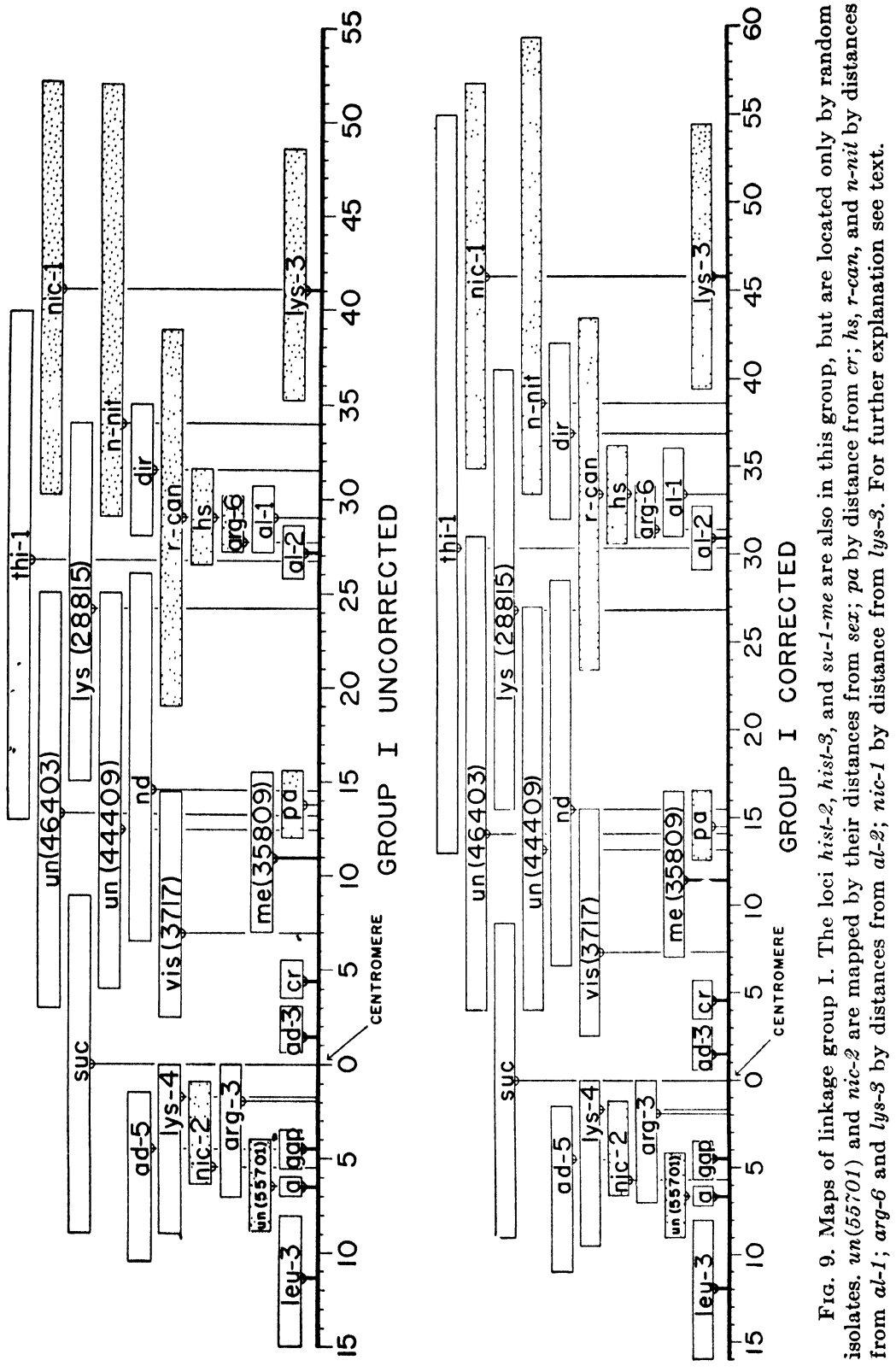


FIG. 9. Maps of linkage group I. The loci *hist-2*, *hist-3*, and *su-1-me* are also in this group, but are located only by random isolates. *un(55701)* and *nic-2* are mapped by their distances from *sex*; *pa* by distance from *cr*; *hs*, *r-can*, and *n-nit* by distances from *al-1*; *arg-6* and *lys-3* by distances from *al-2*; *nic-1* by distance from *lys-3*. For further explanation see text.

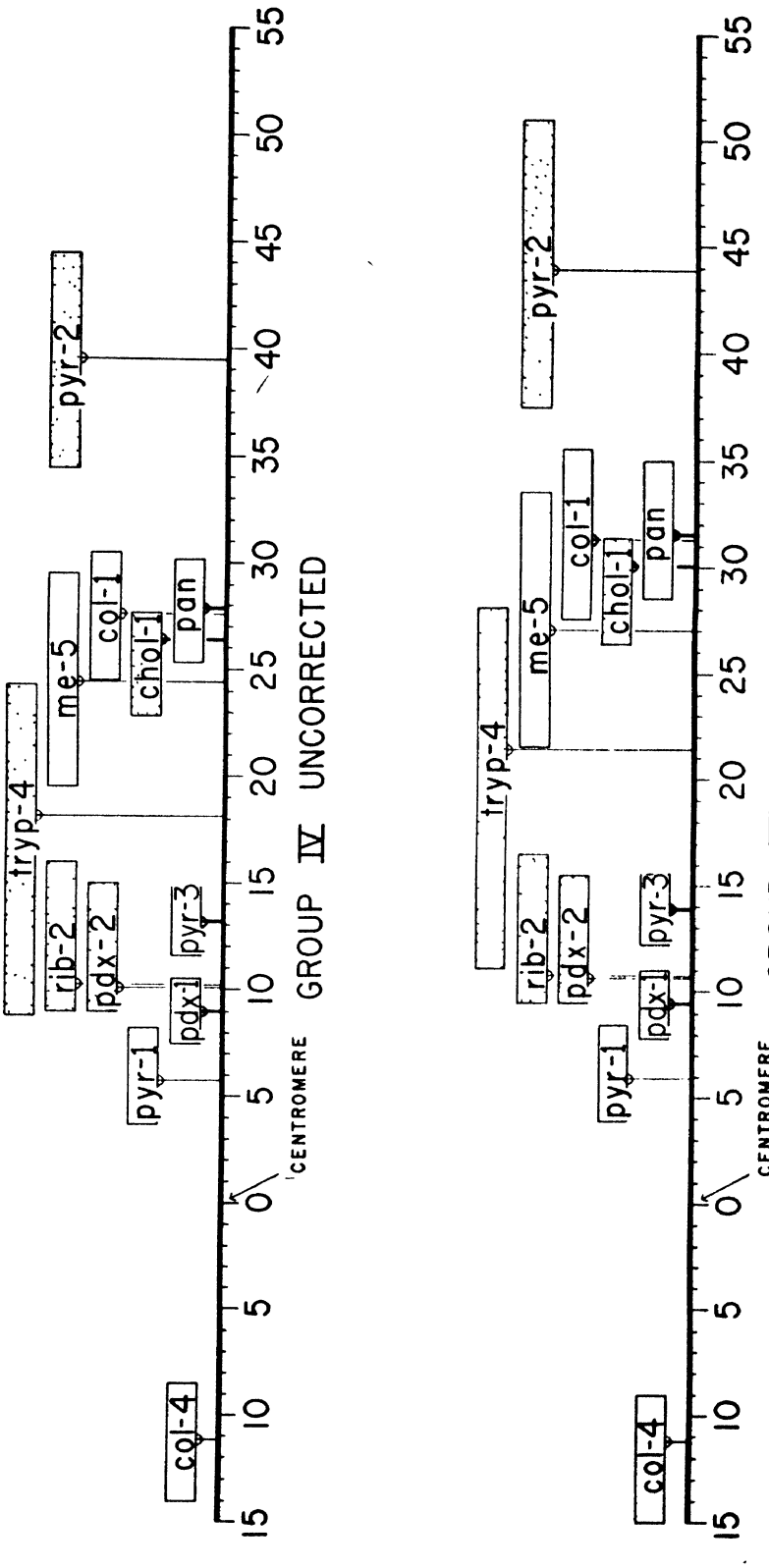


FIG. 10. Maps of linkage group IV. *arg-2* is also in this group, but is located only by random isolates. *rib-2* and *pdx-2* are mapped by their distances from *pdx-1*; *tryp-4* and *chol-1* by their distances from *pan*; *pyr-2* by its distance from *pyr-3*.

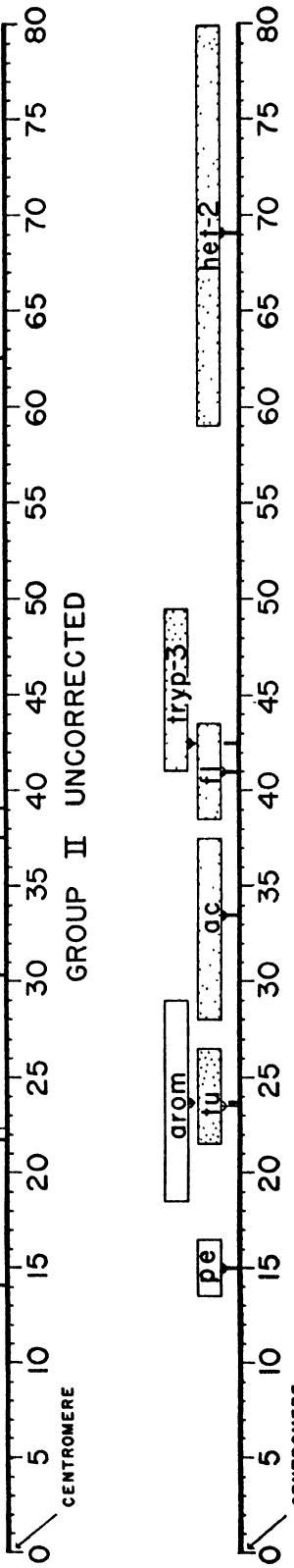


FIG. 11. Maps of linkage group II. *tu* and *fl* are mapped by their distances from *pe*; *tryp-3*, *ac*, and *het-2* by their distances from *fl*.

reasonable assumption that interference will cause single-exchange tetrads to predominate at the expense of higher exchanges in intervals of appropriate length. Occurrence of such a maximum was long ago suggested by homozygosis frequencies and other data in Drosophila (Rhoades, 1931; Beadle and Emerson, 1935; Demerec and Lebedeff, 1934; Ludwig, 1937–

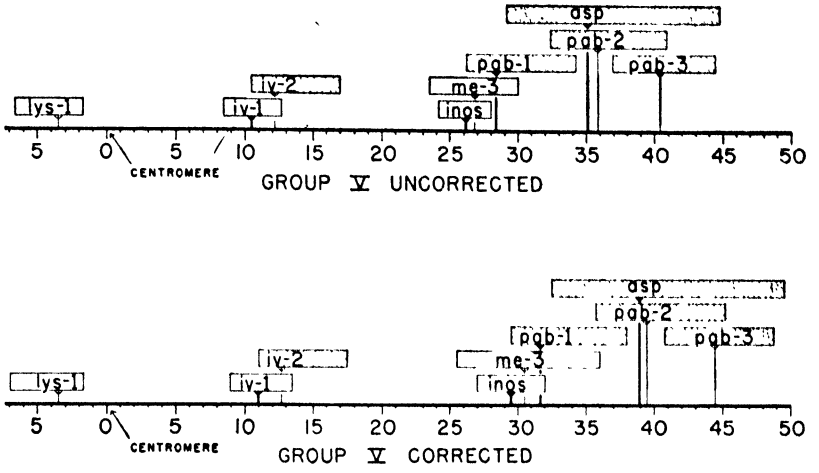


FIG. 12. Maps of linkage group V. *hist-1* is also in this group, but is located only by random isolates. *iv-2* is mapped by its distance from *iv-1*; *pab-1* and *asp* by distances from *inos*; *pab-2* and *pab-3* by distances from *pab-1*.

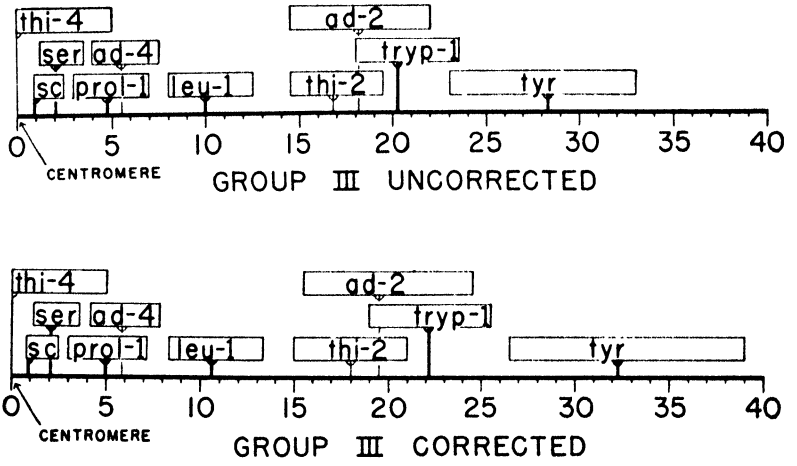


FIG. 13. Maps of linkage group III.

1938; Mather, 1938. See points in Fig. 5). The maximum postreduction attained by X-chromosome loci is around 80%. Tetratype or second-division segregation frequencies significantly greater than two-thirds have been reported for a variety of organisms (see for example Hüttig, 1931, Knapp, 1936; Lindegren, 1949; Rizet and Engelmann, 1949; Leupold, 1950). Neurospora segregations significantly above two-thirds have

not been reported, possibly because the markers that have been employed are not sufficiently far from centromere or from each other.

2. *A Criterion for Independence Not Requiring Ordered Tetrads*

The location of seven independent linkage groups in separate chromosomes was established genetically for Neurospora by comparing recombination frequencies with summed centromere distances (Section III, 1, c).

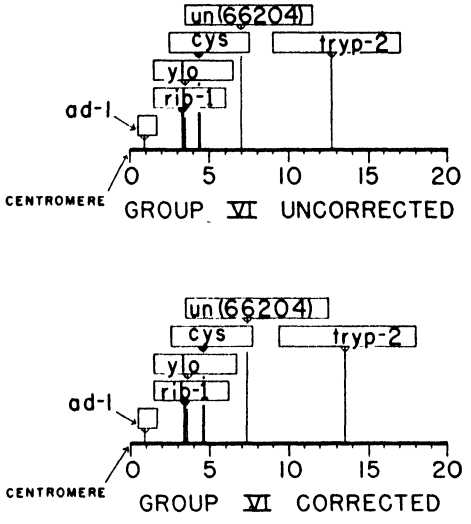


FIG. 14. Maps of linkage group VI.

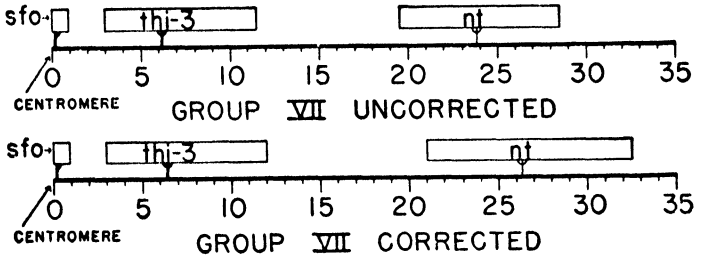


FIG. 15. Maps of linkage group VII.

This method proved effective because much information already existed concerning the second-division segregation frequencies of genes close to the centromere in each linkage group. But another genetic method might have been used for proving independence, which is simpler and which can be applied to unordered tetrads. This method should be especially valuable for organisms where first- and second-division segregations cannot be distinguished, *e.g.*, Saccharomyces or Chlamydomonas.

It was pointed out by Whitehouse (1949) that compatibility loci in certain mushrooms must be independent because many nonparental ditype tetrads occurred compared with few tetratypes, and he first

brought to our attention the possibility of using tetrad ratios as a criterion for establishing nonlinkage (Whitehouse, 1949, and personal communication). It is unlikely that two genes are linked if the NPD:T ratio significantly exceeds 1:4, which is the maximum expected for genes far apart in the same chromosome. The rationale for this criterion is straightforward.

If genes are in separate chromosomes, half of all ditype segregations will be nonparental, and there is no upper limit to the NPD:T ratio. This is not so with linked genes. NPD segregations of genes in the same linkage group can result *only* from $\frac{1}{4}$ of double exchanges + $\frac{1}{8}$ of triples + $\frac{3}{16}$ of quadruples + $\frac{5}{32}$ of quintuples, etc., provided there is no chromatid interference. For very long intervals, $\frac{1}{6}$ of all segregations are NPD'S and $\frac{2}{3}$ are T's.

When many NPD segregations occur, genes must either not be linked or else be far removed in the same linkage group. When tetratype segregations are rare, genes must either be closely linked or else be close to the centromeres of nonhomologous chromosomes. Consequently, whenever genes segregate so as to produce many NPD's and few T's at the same time, location in different chromosomes is indicated.

Chiasma interference could increase the ratio somewhat for linked genes, but only to a maximum of 1 NPD:2T, which would result only if all exchanges in the interval occurred as doubles. Chromatid interference could also increase the ratio attainable with linked genes by producing an excess of 4-strand double crossovers. Until these sources of error are evaluated, a significant excess over 1 NPD:4T would seem to be a practical criterion for assigning genes to separate linkage groups, whereas an excess over 1 NPD:2T would appear to be a very conservative criterion.

An example of the application of this method to Neurospora is furnished by the data from gap × peach. Here 40 NPD and 28 T segregations were observed (Table 1). This is significantly in excess of the maximum numerical ratio of 23:45 expected from the conservative hypothesis, and it could therefore have been concluded that the two genes are in separate chromosomes, without any information being required about centromere distances.

The ability to establish cytological independence by genetic means is a very real advantage conferred by tetrads. This can be appreciated by contrasting the ease with which this has been accomplished in Neurospora to the difficulty of establishing independence in the mouse, where thousands of animals were required to establish genetic independence of three groups, without providing decisive information regarding location of the groups in separate chromosomes (Carter and Falconer, 1953).

Use of pooled data in establishing independence. The number of tetrads available from crosses between two specific genes may be too small to provide significant evidence regarding independence. In such cases, it might nevertheless be possible to reveal nonlinkage by pooling small amounts of data from crosses involving other genes. Consider two groups of genes, where all the members of each group are known to be linked, but independence of the two groups themselves is uncertain. One could combine data from different crosses between any member of the first group and any member of the second group, and use the totaled NPD and T numbers for establishing independence. Similar reasoning would apply to establishing linkage, which would be indicated if PD's were significantly in excess over NPD's in the combined data. Maximum sensitivity could be achieved in both cases without biasing the selection of data, by introducing crosses into the pool in the order of increasing frequencies of T segregations. This is because low T frequencies occur either when genes are closely linked or when genes are close to the centromeres on different chromosomes.

3. *Data*

a. *Status.* The chief weakness of the Neurospora data is failure to show gene order. This results primarily from the limited number of multiple-point crosses and also from the small number of tetrads analyzed. This situation has come about quite naturally. A primary object of the original studies in biochemical genetics was to show that biochemical mutants segregated as single gene differences, and to discover whether mutants concerned in different reactions involved mutations at different loci. From the point of view of biochemical genetics, there was little reason at that time to carry the formal genetic analysis beyond this point. However, the Mendelian inheritance of biochemical mutants is now an established fact, and it has become increasingly evident than an understanding of gene action will require new methods of attack. Many of these methods depend on the existence of adequate genetic maps.

The earlier work has provided invaluable data to use as a starting point. The maps constructed from them in the present paper should enable intelligent planning of critical multiple-point crosses, so that reliability of the maps can be rapidly increased. Also, determination of the proper gene sequence should often make sampling errors less important, because loci would be pinpointed between well-established markers.

Information on unmapped genes, not compiled here, should be useful in placing more loci on the maps. Houlahan *et al.* (1949) present extensive centromere data on such genes, as well as data excluding linkage to certain

groups. Additional data of this sort are scattered throughout unpublished theses.

In the past many of the linkage data have been published in a form either not containing the complete information provided by tetrad segregations or else coded in such a way that their interpretation requires considerable labor. The format used in multiple-point cross Tables 17 and 18 is suggested as one which presents all the pertinent data clearly and concisely.

b. *Technical Advances.* Several improvements in technique may aid in the development of better maps. For crosses involving two biochemical markers efficiency may be increased by dissecting asci on minimal medium, germinating *in situ*, and scoring directly for growth (Haas *et al.*, 1952), providing centromere distances are not required. Plating of ascospores is an efficient method for allelism tests (Haas *et al.*, 1952; Newmeyer, 1954), and often a rapid plating method can also be used for linkage detection (Newmeyer, 1954). (Plating has not been found satisfactory for determining accurate map distances, possibly because of crowding effects such as have been described by Grigg, 1952.) Linkage detection would also be facilitated if satisfactory multiply marked tester stocks could be developed. Efforts in this direction have been hampered by difficulties in scoring when morphological mutants are combined, and by poor germination when several biochemical markers are combined, although Giles and Lederberg (1948) have had some success in this direction.

c. *Relative Usefulness of Random Isolates and Tetrads.* Development of maps may also be aided by the increased use of random isolates. Analysis by dissection of asci in order was admirably suited to the earlier phases of the work, particularly because it is the most efficient way of determining the number of genes that are segregating. Use of ordered tetrads has provided the extensive centromere data which form the backbone of the present maps. The centromere data have greatly speeded establishment of the independence of linkage groups and have been a valuable guide in choosing the most useful markers for testing linkage in particular cases. In the future, however, random isolates may prove to be more efficient for a number of purposes, *e.g.*, establishing linkage and gene order, and determining accurate map distances over short intervals.

In the limited number of cases where the same interval has been examined using both random isolates and ordered tetrads, the agreement has frequently been poor. This may be due to various types of selection which affect random isolates and tetrads differentially. Undetected chromosome aberrations may be an important source of differential selection. (Many of the mutants mapped have not been subjected to

tests by which aberrations could be detected.) Tetrad segregation data from crosses involving aberration stocks may be greatly in error, because only those few crossover types giving rise to asci with eight ripe spores will be selected for dissection (see St. Lawrence, 1952). Random isolates should not be subject to such selection. Poor viability of certain classes of segregants is a second source of selection. This error might be minimized by making crosses both in coupling and in repulsion phases (Fisher and Mather, 1936–1937). However, with tetrad analysis it is frequently possible to correct for poor viability simply by inference from the surviving products in individual asci. A third source of error may be genes affecting the rate of spore ripening; these would be expected to cause more serious selection in ordered tetrads, which must be dissected before the ascus wall breaks down.

Tetrad analysis and single-strand analysis are therefore complementary, and the safest as well as the most efficient development of maps may come from intelligent combination of the two methods.

4. *Maps*

a. *Distribution of Genes.* The maps provide an opportunity to examine the spacial distribution of different loci concerned with related steps in single biosynthetic series. Horowitz's (1950) observation is still valid, that if nonrandom distribution throughout the linkage groups exists, it "does not leap to the eye." However, there are a few cases where loci concerned with synthesis of the same metabolite are linked within a short distance of one another. For example, the three *p*-aminobenzoic acid loci are within 12 units in group V; the two pyridoxine loci are less than 2 units apart in IV; a minimum of three pyrimidine loci are in IV, and all but one of them are within a 10-unit interval; two isoleucine-valine loci are less than 2 units apart in V. The last three examples may include loci close enough to be termed compound loci. Other loci concerned with particular syntheses are spaced far apart (*e.g.*, thiamine, nicotinic acid, riboflavin), and restrict the generality of any hypothesis that visualizes "assembly-line" synthesis of all vitamin-like substances by neighboring genes (Pontecorvo, 1952).

b. *Approaches to Map Construction.* Although it appears that random isolates may in some respects be more useful than ordered tetrads for future work, the map distances given here have been computed exclusively from tetratype and second-division segregation frequencies. This has been done only because there was no practical alternative. We would have preferred to draw conventional maps, based on recombination frequencies, so that both single-strand and tetrad data could readily be incorporated, but this would have involved omitting most of the loci.

(Many genes can be mapped only by centromere distances, which are not comparable to recombination percentages except for short intervals. See Section II,1,b.) Similarly, we would have preferred to map by summing short intervals instead of using long ones corrected by a mapping function; but this would have meant excluding an even larger number of loci. Thus the only alternative to the solution chosen was to draw a map based on recombination frequencies or a map based on short intervals, either of which would include a mere handful of genes.

Ultimately, when maps can be built up from short intervals within which multiple crossing over does not occur, all the alternative methods will lead to identical results. Recombination frequencies will then become equivalent to centromere distances, data both from single strands and tetrads can be put on the same map, and the correction for multiple crossovers will disappear. The advantage of the method adopted in the present paper is that it has produced workable maps conveying the maximum information *now*.

VI. Perspectives

It is understandable that exciting developments in phenogenetics, and particularly in biochemical genetics, should have led to the neglect of crossing-over genetics during the past decade. Yet one is faced with the fact that microbial genetics, the field where biochemical genetics has seen its fullest development, has been forced to come back repeatedly to using recombinational methods of analysis in connection with a variety of problems. This has been true for workers investigating gene action, mutation, and even biosynthetic pathways.

Recently, important changes in ideas about the physical organization of genes and chromosomes, and the nature of allelic differences, have evolved from data based on recombination analysis, effectively correlated with cytological observations, chiefly in Drosophila and in maize (Goldschmidt, 1951; McClintock, 1951; Lewis, 1951). In Neurospora, cytogenetic analysis and recombination genetics, of which mapping is one major facet, have now progressed to the point where a direct attack should be possible on the broad problem of delimiting the functional units of the chromosomes.

Studies on compound loci have contributed much to altering ideas about the constitution of genes. With the use of Neurospora, it should be possible to analyze such loci not only in terms of their divisibility by crossing over but also in terms of their biochemical activities. An essential problem is whether pseudoalleles are merely separable parts of a structure controlling a single reaction, or whether they control separate, possibly sequential, reactions (see Goldschmidt, 1951; Pontecorvo,

1952). So far no locus has been found in Neurospora where the three most important prerequisites for studying this problem are fulfilled, namely, the production of a known enzyme, the existence of several alleles (possible pseudoalleles), and location between closely linked markers. However, crossing over within the *inos* locus has been demonstrated (Giles, 1951), and some evidence exists that the same is true for *nic-1* (Bonner, 1950). There are also several series, both of multiple alleles (Horowitz, 1950) and of indistinguishable recurrences, which might be explored for further cases of compound loci. Three of these allelic series are concerned with enzymes which have already been investigated, namely, *tryp-3* (Yanofsky, 1952), *pan* (Wagner and Haddox, 1951), and "amination deficient" (Fincham, 1951b); the first two of these loci have already been mapped. New, efficient enrichment techniques for selecting mutants (Woodward *et al.*, 1952) should make allelic series increasingly available.

Genetic analysis by means of recombination offers an approach to other problems. First, since Neurospora is the best mapped organism in which tetrad analysis is possible, it may be used to throw light on the mechanics of crossing over and of interference, and on the internal and external factors affecting it. The possibilities for investigating regional variations in crossing over have been demonstrated by the pioneer work of the Lindegrens (1939). Second, because biochemical mutant phenotypes are easily measured quantitatively, Neurospora should be useful for investigating not only allelic differences but also the mechanism of action of modifiers and suppressors. Third, Neurospora provides material for comparative studies bearing on the evolution of genetic systems and the relations between species (Dodge, 1931; Fincham, 1951a).

Many problems remain for the future, when there are available not only better maps but also a cytology worked out in greater detail. Then it should be possible to study such problems as the structural nature of the mutational changes produced by different mutagenic agents, the localization of certain kinds of mutants with reference to specific chromosome structures, and perhaps the effects of changes in heterochromatin on the expression of biochemical mutants. We may look forward to the day when it will also be technically feasible to study position effects on a biochemical level, perhaps even in terms of enzymes.

VII. Summary

1. Linkage and centromere data from *Neurospora crassa* have been compiled from all published material as well as from unpublished sources.

2. Tetrad data from gene-centromere and gene-gene intervals have been placed on a uniform basis for mapping by computing map lengths

from second-division segregation frequencies and tetratype segregation frequencies, respectively.

3. In order to correct for undetected multiple crossovers, a mapping function has been devised from a 4-strand model of crossing over. The function is consistent with extensive single-strand data from Drosophila and with the limited tetrad data from Neurospora. The correction curve for tetrads is presented.

4. To estimate the extent of sampling errors, a series of curves is presented from which the 95% confidence limits can be determined for tetratype or second-division segregation frequencies up to 75%, and for numbers of asci from 10 to 600.

5. The independence of the seven linkage groups (corresponding in number to the seven chromosomes) has been established by a statistical evaluation of the tetrad data. An alternative method for establishing nonlinkage is described, which is applicable either to ordered or to unordered tetrads.

6. Progress in chromosome cytology, and in assigning linkage groups to chromosomes, is reviewed briefly.

7. All mutants at mapped loci are described as to phenotype, origin, order with respect to other loci, and allelic relations.

8. Maps of the seven linkage groups have been constructed from tetrad data. Seventy-five loci are shown. Confidence limits are indicated for the position of each locus. Two sets of maps are presented, the first being completely uncorrected for multiple crossovers, and the second corrected by means of the mapping function. (A few additional genes have been assigned to specific linkage groups on the basis of random isolates. The use of random isolates for mapping is discussed.)

9. The maps indicate that genetic and cytogenetic analysis has progressed to the point where Neurospora can now be used to attack a variety of problems regarding the nature of the gene, the mechanism of gene action, and the mechanics of crossing over.

VIII. Acknowledgments

Data presented here have originated from many sources, as is evident from the references given in the tables and text. Special mention should be made of the contribution of unpublished segregation data by Dr. E. A. Adelberg, Mrs. Miriam Bonner, Dr. N. H. Giles, Dr. S. Gross, Mr. Hugh McDevitt, Dr. D. C. Regnery, Mr. Alex Reisner, Dr. P. St. Lawrence, Dr. T. C. Sheng, Dr. C. Yanofsky, and by students of Dr. F. J. Ryan at Columbia University. In addition, Dr. and Mrs. S. Emerson kindly permitted publication of material which had previously appeared in Microbial Genetics Bulletin. It is hoped that the usefulness of the present compilation will be such as to justify the generous cooperation of all those who contributed.

The authors also wish to thank Dr. E. L. Tatum for his encouragement and sug-

gestions throughout the work. The technical assistance of Miss Sarah Guthrie, Miss Ruth Ogren, and Mr. Charles Gowans is appreciated.

Because of the diversity of the problems encountered, there has necessarily been division of labor and responsibility among the authors. The compilation of data and the development of methods for determining confidence limits and establishing independence with ordered tetrads were undertaken by RWB and DN. Development of mapping functions and of the criterion of independence for unordered tetrads are the work of DDP. The mapping of linkage group II is the work of LG. The remaining sections of the paper are the joint effort of the first three authors.

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Notes Added in Proof

The data of Lindegren and Lindegren, 1942 (reference 91) were inadvertently omitted from the compilation. Their paper reports on 1081 asci from a cross involving *sex*, *gap*, *crisp*, and *pale*. It also gives a revised table of data on a previously published, similar cross, including several non-crossover asci not included either in the original publication [Lindegren and Lindegren, 1937 (J. Hered. 28, 105–113)] or in Whitehouse's (1942) compilation of the Lindegrens' data. Addition of these data to our tables would reduce the confidence limits on *cr*, *gap*, and *pale*, and alter their mean values slightly, but would not change gene order.

Genetic Changes in Human Populations, Especially Those Due to Gene Flow and Genetic Drift

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I. Introduction

In the rapidly developing field of population genetics, the contribution made by studies of human populations is at least as great as that derived from studies of any other species. This significant fact stems largely from the growing awareness on the part of physical anthropologists and medical scientists of the insight into problems in their respective fields now offered them by developments in human genetics. The movement may be said to have started with the pioneer work of L. and H. Hirszfeld (1919) on the frequencies of the ABO blood groups among soldiers of different national origins typed in the Balkans during World War I. In volume alone, the work done since that time on the frequencies of blood groups in different peoples is truly staggering. In addition to several hundred studies of the ABO, MN, and P blood groups made prior to 1939, when Boyd (1939a) summarized and evaluated the accumulated data, an even larger volume of studies has been made since that time—what with the addition to the classical blood group systems of the Rh, Lutheran, Kell, Lewis, Duffy, *Ss*, and secretor systems (see Race and Sanger, 1950). In *Genetics and the Races of Man*, Boyd (1950) has clearly brought out the significance of the blood group frequencies in different populations to human population genetics and to physical anthropology.

These, with a number of other studies of the frequencies of alternative phenotypes, enable the worker to compare different populations in genetic terms, provided he can derive from the phenotypes the frequencies of the underlying genotypes. This conversion depends upon the application of the familiar Hardy-Weinberg formula (Hardy, 1908; Weinberg, 1908), which expresses the frequencies of genotypes at a single locus in a population in genetic equilibrium, that is, in a population large (theoretically infinite) in size, and breeding at random with respect to the trait considered. Such a population reaches genetic equilibrium in a single generation and maintains it thereafter so long as positive forces (see p. 97) do not disturb it. The equilibrium thus attained is expressed by the terms of the binomial expansion $p^2 + 2pq + q^2$, where p and q represent the frequencies of the alleles at a given locus. The three terms are thus the relative frequencies of one homozygous genotype, the heterozygous genotype, and the other homozygous genotype. If the frequency q be that of a recessive allele, it is obviously possible to estimate it by extracting the square root of the frequency of the recessive phenotype in the population. Since p equals $1 - q$, the distinction between the genotypes represented by p^2 and $2pq$ may then be readily made. This fundamental theorem is so basic to all studies of population genetics that it needs to be restated in introducing any review or exposition of the subject, in spite of its general familiarity.

Whenever a locus is characterized by a series of more than two alleles, the calculation of allele frequencies is more involved. Yet it is precisely the more numerous parameters introduced by multiple alleles which render such loci of greatest value in comparing and distinguishing populations. Bernstein (1925) and Wiener *et al.* (1929) early developed methods for obtaining from the ABO blood group phenotypes the respective frequencies of the alleles I^A, I^B, and I^O; and Fisher (see Dobson and Ikin, 1946; Race and Sanger, 1950, p. 21) has recently provided a refinement of these formulas that approximates a maximum likelihood solution (see Stevens, 1938). For more complicated situations, such as the estimation of the frequencies of the numerous alleles in the Rh series, the calculation is more laborious but not any more difficult in principle. The maximum likelihood solution has been worked out by Fisher (1946, 1947) for the Rh series, although most workers still resort to the simpler, if less efficient, formulas given by Wiener and Sonn (1946).

Without accurate determinations of the frequencies of alternative phenotypes whose mode of hereditary transmission has been determined, and without accurate methods of estimating the frequencies of alleles once the frequencies of the phenotypic classes are known, the genetic analysis of the dynamics of populations could not be carried out. Much

of the earlier work is rendered valueless for such an analysis because of crude or careless technique, evident from the internal inconsistencies of the data, as Boyd (1939a) indicated in his tabulation of ABO and MN blood group data. Boyd (1949) has also emphasized, like others, the importance of the second requirement, that of using accurate methods of estimating the allelic frequencies; but of the two requirements, the first is the sine qua non. No analysis can be any better in its conclusions than in its primary data. This truism needs reiteration because the laborious task of describing a population, of accurately determining the phenotypes of a sufficiently large sample of a population to give a statistically reliable picture of it, is a task likely to be shirked by workers eager to push on to more exciting pursuits. In this respect geneticists and blood group workers might well emulate the older physical anthropologists, who were careful to amass mountains of meticulous measurements—even though unfortunately these remain up to now immune to genetic analysis. On the contrary, the blood group workers have often seemed content to determine the blood groups of 100 or 200 individuals, without particular care to make sure that these represented a random sample of the population to which they belonged, and then to publish the result as though definitively characterizing the group. Reviewers of the field and other workers who cite the original studies have an even greater responsibility for this situation; for they not only perpetuate such suppositions but create and extend them even where the original worker was more careful and critical. A striking instance of this is to be seen in the following example. For nearly a decade the only published data on the frequencies of the Rh blood types among United States Negroes have remained those of Wiener *et al.* (1944) and of Levine (1945). The former series comprised 223 individuals and the latter included 135. Both series were from New York City. Yet these quite small series have been widely cited and reported in tables as representing the frequencies of the Rh types in "American Negroes." Is the "American Negro" then so homogeneous that the New York City Negro properly represents the entire Negro population of the United States? What, if any, are the real differences between the two series, which are not statistically different to a significant degree, owing to the large standard errors of the frequencies in the seven classes of Rh types occurring in the samples? It is apparent that a real genetic analysis of the American Negro must wait upon far more extensive series, adequately distributed geographically.

The modern genetical theory of evolutionary changes in populations envisages four major factors that affect the frequencies of alleles: mutation, selection, gene flow, and genetic drift. Mutation includes all kinds of physical change of the hereditary material. Selection pressure, as

Wright (1949) has pointed out, includes "differential mating, differential fecundity, and differential emigration, as well as differential mortality." The term "gene flow" may be defined to subsume all movements of genes from one population into another, whether by migration of solitary individuals or groups, and whether leading to occasional hybridization or mass intermixture. It is not synonymous with "migrations" of populations, inasmuch as these might remain genetically unmixed in a new geographical territory; hence, it seems best to avoid the use of the term migration in a genetic sense, and to adopt the new term "gene flow" used by Birdsell (1950) in his analysis of the Australian tribes. The term "genetic drift" refers to the accidents of sampling (in meiosis and fertilization) whereby the allele frequencies in any generation may differ from those in the antecedent generation purely because of chance, and become "compounded by a stochastic process into wide deviations from equilibrium" (Wright, 1949). It is particularly influential in small populations (Wright, 1931, 1940, 1948); whereas mutation, selection, and gene flow affect allele frequencies more significantly in populations above a certain size, namely, $N = \frac{1}{4u}$, $\frac{1}{4s}$, or $\frac{1}{4m}$, where N is the effective population size, u the mutation pressure, s the coefficient of selection, and m the flow of genes into the population. Whenever u, s, and m are less than $\frac{1}{2N}$, genetic drift sets in. That is to say, gene frequencies oscillate about their equilibria, the oscillations become larger in magnitude as the population size becomes smaller, and the cumulative effect over a number of generations may lead to the elimination of some alleles and the fixation of others, as well as to intermediate frequencies. This can happen (Wright, 1948) even in populations of an effective size up to 250,000 for loci with a mutation rate of the order 10^{-6}, provided that selection and gene flow are also extremely low. The formula for estimating genetic drift in a random-breeding population is that of the sampling variance of the allele frequency q, namely, $\sigma_{\delta q}^2 = \frac{q(1-q)}{2N}$ (see Wright, 1943; Li, 1948, pp. 296–298). Thus in any single generation q will vary around its mean value with a standard deviation of $\sigma_{\delta q} = \sqrt{\frac{q(1-q)}{2N}}$. This is largest when q and $(1-q)$ are each 0.50. In a population in a steady balance between genetic drift and incoming gene flow, this variance becomes (Wright, 1943) $\sigma_q^2 = \frac{q(1-q)}{2N - (2N-1)(1-m)^2}$, which is the same as the previously given sampling variance in the limiting case of no isolation whatsoever ($m = 1.0$).

It follows from these general considerations that the genetic loci do

not all follow the same pattern in evolution, but rather that, as Wright (1948) has said, "the genes in a population may be put into 3 classes with respect to the roles of selection and random sampling. One class of segregating genes in any population may be expected to be almost wholly dominated by selection in one way or another, another class almost wholly by accidents of sampling, while an intermediate class, to which special importance was attributed, will show important joint effects." Glass *et al.* (1952) emphasized this further: "Still, to prove that the fluctuations in the frequencies of the alleles at one locus are due to selection rather than genetic drift has no direct bearing on the question whether the fluctuations at some other locus are attributable to the one or the other. If the critical size of the population is $\frac{1}{2s}$, then a population (say of 10^4 individuals) which is 'enormous' with respect to a gene having a selection value of 0.2 will be 'small' with respect to a gene at some other locus for which $s = 0.0001$." The discussion on this question by Cain, Birdsell, and Dobzhansky (Birdsell, 1950) helps to clarify a good deal of common misconception about these interactions, and to point up the fact that genetic drift and selection are not alternative explanations but, as Sewall Wright has constantly maintained, factors which may often act in conjunction. (For further discussion of these relations, see Wright, 1940, 1948.)

Wright (1949) has made a more elaborate classification of the modes of change in gene frequency according to degree of determinacy. The *immediate* factors he puts into three categories:

1. From systematic pressures (Δq determinate in principle).
   a. Recurrent mutation.
   b. Intrapopulation selection.
   c. Recurrent immigration and crossbreeding (= "gene flow").
2. Fluctuations (δq indeterminate, $\sigma_{\delta q}{}^2$ and $\varphi(q)$* determinate in principle). "Random drift" according to Wright's usage.
   a. In the systematic pressures.
   b. From accidents of sampling (= "genetic drift").
3. From unique events (wholly indeterminate).
   a. Mutation favorable from first.
   b. Unique selective event.
   c. Unique hybridization.
   d. Swamping by mass immigration.
   e. Unique reduction in numbers.

* $\varphi(q)$, "the probability curve describing the distribution of these deviations in single populations in the long run" (Wright).

In the evolution of human populations and races, these factors have undoubtedly interacted in complex ways from place to place and from time to time, and the unique events which were of importance can probably never be identified. The answer as to which major factors have on the whole been most important depends in great measure upon the population structure which existed in times past, that is, upon the sizes of populations, the amount of inbreeding, and the system of mating. Until the last few thousand years everywhere, and in many parts of the world up to much more recent times, man has been a Stone Age hunter. Thus "throughout the greater part of his history the . . . breeding populations of man appear to have been very small. Peoples at the lower hunter stage of cultural development at the present day rarely if ever attain a breeding population size of 1,000" (Montagu, 1950, p. 329). Kluckhohn and Griffith (1950) have estimated the size of Andean communities to be about 500 to 3000; those in the Amazon valley range from 150 to 3000, and those in the "Marginal" area from 50 to 150. Birdsell (1950) has stated that the 574 Australian tribes now vary in population size from 100 to upwards of 1500, with the average size about 500. At the time of the modern discovery of Australia, if the continent contained some 300,000 natives, as estimated, the mean value was 523 persons per tribe. In the absence of specific information about mean family size and the age distribution of tribes, it is impossible to calculate the effective size of these populations, but it seems unlikely to be much larger than 200, and might be well under that. In such populations, mutation pressure and selection pressure would have to be above 10^{-3} to be of much effect in determining the frequencies of alleles. This would seem to exclude mutation pressure as an important factor, except possibly for a very few loci (see below). Probably a good many alleles may exceed competing alleles by selection coefficients of 0.001; but at many other loci there may be two or more alleles which differ in existent environments by less than that. *A priori*, one might therefore expect to find that in prehistoric human populations selection, gene flow, and genetic drift had combined to produce the existing diversities of gene frequency, *with gene flow acting uniformly upon all loci, genetic drift acting with determinate variance upon all loci, and selection acting differentially with respect to each locus.* The major problem, then, of human population genetics is to analyze the interaction of these factors in given situations, so as to demonstrate the validity or faultiness of the postulates, and thus to arrive at a real understanding of the dynamics of human populations in genetic terms. In the present review of studies of the roles of gene flow and genetic drift, it is clearly inadvisable to neglect the existence of the mutation and selection pressures with which gene flow and genetic drift interact.

II. Mutation and Allele Frequencies

As has already been indicated, it is unlikely that in the extremely small breeding populations of man in the hunting stage of culture mutation pressure could often have been responsible for the determination of the frequencies of competing alleles. For it to do so would require two conditions: (1) a very high rate of mutation to a given allele from the total of other alleles at the same locus; and (2) a much lower rate of mutation from the said allele to the total of other alleles at the same locus. The same relative difference at lower absolute rates would be ineffective because the small size of the population would mean that the actual number of mutations per generation would be extremely low. Thus, even if the mutation pressure favoring allele A was 10^{-4}, in a population of effective size 200 to 500, it would take 10 to 25 generations to get one net mutation to A. Yet recently the very high mutation rates necessary to be effective in determining allele frequencies have been reported for certain loci (Neel, 1952; Goodman and Reed, 1952). A consideration of the validity of these estimates and the generality of such rates is therefore in order.

In human populations, direct observations of the mutation rate to a particular allele can be made only for dominant alleles, and such studies have been carried out for only a few abnormal hereditary conditions, such as retinoblastoma (Neel and Falls, 1951; Falls and Neel, 1951). Neel (1952) has pointed out the major sources of error in such estimates. First, it is assumed that all mutation bringing about the condition is at the same locus and to the same allele. Second, it is difficult or impossible to distinguish between somatic and germinal mutation. As a result, the estimate obtained is a maximum estimate, except in so far as it is offset by incomplete ascertainment of cases of the trait in the population. With these qualifications, the rate obtained for retinoblastoma, 2.3×10^{-5}, does not seem to be particularly high, especially when cognizance is taken of the added likelihood that human mutation rates will be determined chiefly for a selected group of highly mutable loci (Haldane, 1948).

The mutation rates for completely recessive and incompletely recessive mutant alleles are based not upon a direct count of observed mutations, but upon the frequency of the mutant trait in the population. Such estimates involve a number of unproved assumptions, foremost among which is the assumption that the elimination of a gene that is affected adversely by selection is counterbalanced (solely) by mutation from the normal allele to the unfavorable allele. Haldane (1949), Neel (1952), and Goodman and Reed (1952) have all pointed out that this is a safe assumption only if the population studied is in equilibrium. Yet this point is

often not sufficiently heeded and deserves further emphasis. For a completely recessive gene, the mutation rate in a random-breeding population at equilibrium $u = sR = sq^2$ (see Li, 1948, p. 260), where R is the frequency of recessive individuals, s is the selective disadvantage of the recessive homozygote, and q is the allele frequency. In a population not in equilibrium, and characterized by any inbreeding whatsoever, the frequency of recessive homozygotes R will really be greater than q^2 and lead to an overestimate of q. Unless the inbreeding is allowed for, the estimated mutation rate will be too *high*. On the other hand, as Neel (1952) has noted, when the inbreeding is included in the equation, $u = s[(1 - F)q^2 + Fq] = sR$ (F being the coefficient of inbreeding) and the population is in equilibrium, then any diminution of inbreeding and departure from equilibrium will lead to a fall in the proportion of homozygous recessives in the population, and the estimated mutation rate will then be too *low*. Thus the assumption that there is no inbreeding when there really is, and the assumption that the population is in equilibrium when it is really not, lead to opposite sorts of error in the estimation of mutation rates. In other words, in dealing with a population which is in equilibrium one may derive the mutation rate to a recessive allele from the frequency of recessive persons, but in dealing with a population that is not in equilibrium one must estimate *the amount of inbreeding and the allele frequency*. The proportion of recessives in the population may be very misleading.

A second common assumption in the estimation of the mutation rates of recessive genes is that reverse mutation can be safely ignored. Actually, whenever this is not the case, $sq^2 = u - \frac{vq}{1 - q}$ (s, the selection coefficient; u, the mutation rate to the recessive allele in question; v, the mutation rate from the recessive allele in question; and q, its frequency). Thus what one estimates is actually the *net* mutation rate between u and v. One therefore obtains a minimum estimate of u if one assumes that $v = 0$. If $v = u$ and $q/(1 - q) = 0.5$, the real value of u would be double that estimated; whereas as $q/(1 - q)$ approaches 1.0 (that is, if the recessive allele is itself as common as the dominant allele), the real value of u would approach infinity in comparison to the value estimated simply by equating it to sq^2. This raises several questions. We know literally nothing about opposed mutation rates in man. Mutation has been considered solely as a one-way process. Even in Drosophila very little is known about the frequency of mutation in opposite directions at single loci, except for X-ray induced mutations. It would appear, however, from the extensive reports of family studies of various blood groups that, at least for the ABO groups and the MN groups, the several alleles are in

no case highly mutable and probably have mutation rates of the same order of magnitude. If this is so, it appears wise not to try to estimate the mutation rate of a recessive allele from the value of sq^2 unless the frequency of that recessive allele is very low in the population. At least, it is proper to insist that such estimates are always *minimum* estimates, if the other assumptions, *e.g.*, that the population is in equilibrium, are valid. Inasmuch as some of the estimated mutation rates to recessive alleles found by this method are already extraordinarily high (*e.g.*, 10^{-2} or 10^{-3} for sickle-cell anemia, Neel, 1950), any further increase in estimated rates might well serve to make us question the assumptions rather than accept the estimates.

The third assumption which commonly affects the estimation of mutation rates is the assumption that s, the measure of selection pressure, is constant. This assumption takes two forms, both of which are greatly in need of experimental study and verification. First, it is assumed that the rigor of selection remains unchanged, even though the population may shift from an equilibrium with high inbreeding and, possibly, much assortative mating to a disturbance of equilibrium and ultimately a Hardy-Weinberg equilibrium in panmixia. It hardly needs to be pointed out that the effective size of population, which changes radically in this process, and the pattern of mating may themselves affect the selection pressure. As Wright (1949) has said, "Selection in a random breeding population tends merely to bind the species to one peak, which is not likely to be the highest one. In a subdivided species, intergroup selection operates, by means of differential growth of local populations and differential migration, on the whole genetic complex which, even more than the single genotype, is the object of selection most directly related to the success of the species as an entity."

The second form of this assumption that selection is constant takes the form that dominance and recessiveness are complete, whereas actually there may be some selective differential against or in favor of the heterozygote. Although it is recognized that if q is low, selection favoring the heterozygote may well overbalance the effect of selection against the homozygote (see Neel, 1952), in practice little has been done to attempt to take this into account, except in the case of selection against the heterozygote in Rh maternal-fetal incompatibility. More will be said regarding this consideration in the next section.

The present discussion of mutation in relation to allele frequencies is not meant to imply that the estimation of mutation rates upon given simplifying assumptions is valueless, but only that they should not be taken too literally. The chief value of making such estimates may well come from an appraisal of the assumptions and from recognition that

before mutation rates can really be measured further effort must be devoted to measurement of the effects of selection upon heterozygotes as well as homozygotes, to determination of the effective sizes of populations and the amount of inbreeding, and to demonstration of the presence or absence of genetic equilibrium within them.

III. Selection: Differential Mortality and Fecundity

Birdsell (1950), in the discussion following his paper on some implications of the genetic concept of race, made the following interesting observation: "Strange as it must seem to geneticists, the concept of natural selection operating on man as a powerful evolutionary force has only very recently been reintroduced by anthropologists. The pain of the idea is such that it is by no means universally accepted. To date, no single instance of the operation of selection has been incontrovertibly demonstrated in human population genetics, although a number of instances have been imputed." In the recent book by Boyd (1950), the question is discussed further, and racial differences in skin, hair, and eye pigmentation are held to be adaptive (pp. 175–180). Dobzhansky (1950) has expressed the contemporary genetic view that "natural selection would be abolished only if every person born anywhere in the world were to have equal chance to attain reproductive age, and to produce equal number of offspring. . . . What does happen in any species, and what did happen in mankind, is that the adaptive values of different genotypes undergo changes in the course of time." In yet another recent book, *Races*, Coon *et al.* (1950) have, in Dobzhansky's words, frankly devoted themselves to "conjectures about the possible adaptive significance of racial traits."

We should, then, be at the dawn of an era during which the effects of selection upon allelic frequencies will be investigated actively. This should involve not only studies of differential mortality but also studies of differential fecundity, the latter having been strangely neglected to date in view of the relative ease with which it can be assessed compared to differential mortality.

Our present knowledge of differential mortality is largely confined to lethal and semilethal conditions, such as fibrosis of the pancreas (Goodman and Reed, 1952), thalassemia and sickle-cell disease (Neel, 1952), and hemophilia (Haldane, 1947). Some of these are of particular interest because of their high incidence, *e.g.*, fibrosis of the pancreas, 0.1% of all births (United States). Others are of special interest because of their occurrence wholly or chiefly in certain races and populations, *e.g.*, sickle-cell disease in Negroes and thalassemia in Mediterranean peoples. Yet in all these cases it is only the differential mortality of the homozygote (or hemizygote) that is known, and that of the heterozygote remains

unmeasured. The problem is not so insoluble as sometimes has been thought. It would, for example, be most instructive to find out the mean length of life of all daughters of hemophilic fathers in a population for which good general mortality statistics are available. What is needed is sufficient medical registration of the population, as in Kemp's University Institute of Human Genetics in Copenhagen (Kemp, 1950). Another attack on the problem is to discover, if possible, some way of detecting the genetic carriers (Neel, 1949). For thalassemia and sickle-cell anemia such methods exist, and it will presumably be only a matter of time until definite information is available about the differential mortality of the heterozygotes for these diseases.

The special case of selection against the heterozygote has received much attention, owing to the interest attaching to the Rh maternal-fetal incompatibility and the different Rh-positive and Rh-negative frequencies in different human peoples. First analyzed by Haldane (1942) and Wiener (1942), it has further been considered by Strandskov (1945), Stern (1949), Glass (1950), Boyd (1950), Dobzhansky (1950), and Goodman and Reed (1952). There are three positions of equilibrium for a pair of alleles when selection is directed entirely against the heterozygote, namely, 1.0, 0.50, and 0. The midpoint is a position of unstable equilibrium, for the slightest chance departure from equality in the allelic frequencies would be enough to start selection against the less abundant allele. Thus 0 and 1.0 are the only positions of stable equilibrium. If the net mutation from the commoner to the rarer allele is greater than the reverse, and if selection is against *all* heterozygotes, then, as Li (1948, p. 262) has shown, at equilibrium $u = sq(1 - 2q)$, approximately; or when q is very small, $u = sq$. If selection is only against heterozygotes born to recessive mothers, as in the case of Rh maternal-fetal incompatibility, then $u = sq^2(1 - q)(q - \frac{1}{2})$. Glass (1950) has presented evidence that the value of 0.05 for s (or k in Haldane's symbols), assumed by Haldane (1942) as an approximation, is reasonably correct. It follows that if one were to assume, as Goodman and Reed (1952) have done, that the West European and North American white populations are in equilibrium with respect to D and d (Rh^+ and Rh^-), then the net mutation rate from D to d must be approximately 0.00051, or 5.1×10^{-4}, as estimated by Goodman and Reed using Haldane's formula. On the other hand, the same reasoning indicates that in the Chinese and Japanese populations, where d (Rh^-) is rarer (0.10), the net mutation rate from D to d is less than half as great, 1.8×10^{-4}; whereas in a population with an intermediate frequency of d, say 0.30, the estimated mutation rate would be higher than in either. One is therefore seemingly forced to choose between believing that the mutation rate to a particular allele varies at first

inversely and then directly as its frequency in different populations falls; or on the other hand supposing that the populations are not in equilibrium, just as theory predicts. A third alternative exists, however. The population may be in equilibrium, but as a result of a more complex balance between opposing forces other than mutation pressure and selection due to differential mortality alone. Selection due to other factors, and in particular to differential fecundity, may be involved; and gene flow and genetic drift ought not to be ignored unless evidence is found to exclude them.

The importance of differential fecundity is so obvious that it is surprising how very little has been done to determine the relative fecundity of alternative genotypes. This might be observed by a no more difficult measure than that of determining respective sizes of families (numbers of children born alive, which of course includes prenatal mortality with fecundity; or numbers of living children at some specified age, which likewise includes childhood mortality up to that age). If the numbers of children reaching maturity and living through the reproductive period are used, the criterion very conveniently measures the combined effect of fecundity and mortality, and becomes a fairly accurate measure of the total selection pressure. For a complete study, the productivity of nine types of matings should be compared: namely, the possible combinations of the three types of male parents (AA, Aa, aa) with the three types of female parents. When the heterozygote cannot be distinguished, this reduces to four types of matings; and by randomizing one sex (usually the male) it can be limited to a comparison of two matings: any male $\times$ A female; and any male $\times$ aa female.

A few studies exist which indicate that in human populations genetically determined biological influences upon fecundity may not infrequently be overbalanced by psychological factors arising from individual motivation or cultural patterns. Populations are characterized by each having a certain pattern of family size, and very few cultures probably manifest a completely "natural," *i.e.*, uncontrolled family size. Even where there have been social and cultural pressures in favor of rearing large families, it was often, as in China until quite recent times, only boys that were wanted, so that when a family reached a certain size any additional female infants that might be born were disposed of. Kluckhohn and Griffith (1950) mention a type of selection practiced by the Tanala of Madagascar which would affect not only skin color, the object of the selection, but would naturally tend also to reduce family size. This practice was the selection for light skin color in the Maromena gens and for extremely black skin color in the Zafiakotry gens by killing all infants unusually dark-skinned in the former case, and all infants unusually pale in

color in the latter. The rationalization of this practice need not concern us here—it is sufficient to point out that such practices would inevitably reduce the total number of children below the maximum. The characteristic size of family in a given society becomes a powerful factor in motivating parents not only to restrict family size whenever they exceed the mode but also to increase family size should they not yet have attained the mode. The loss of infants because of biological factors thus often leads to a compensating effort by the parents to replace the dead child; and the very strength of the sense of loss and temporary frustration may not infrequently lead to overcompensation.

This suggestion that parents who for genetic reasons lose offspring may nonetheless exhibit a fecundity superior to that of parents of the alternative genotype was suggested by Fisher (see Race, 1944) and later by Spencer (1947). Families tend to attain a certain average size for a given population or stratum within a population. Some evidence of this was found by Race (1942) in his study of acholuric jaundice, a semidominant condition which causes significant losses of infants. Twenty-one acholuric men and women had 37 children, "more live children than their normal sibs," in spite of having lost 18 offspring through prenatal death or miscarriage. Their 15 normal siblings had 19 living offspring and had lost none. The excess fecundity among the acholurics was more characteristic of the females than of the males. Such data are not extensive enough by themselves to prove the point, but are indeed suggestive. Silvestroni *et al.* (1950) have collected data in their study of thalassemia (microcythemia) which show that in the district around Ferrara, where the incidence of thalassemia minor is about 10%, there is a higher fecundity in marriages of heterozygote with heterozygote than in either marriages of heterozygote with normal or normal with normal. However, the number of *living* children in the $Mm \times Mm$ marriages is not significantly increased. Only a small number of such matings was recorded, and additional data are needed to clarify the matter.

Glass (1950) presented the results of an extensive study of the fecundity of Rh-negative and Rh-positive women, both white and Negro, in the city of Baltimore. The sample was composed of women typed at the Baltimore Rh Typing Laboratory, every woman included being at least in her second pregnancy. In order to include a larger number of Rh-negative women, the collection period was extended for them somewhat longer than for the Rh-positive women; but it was established that the average number of living children per Rh-negative woman was not greater in the extension of the period than in the main period. The collection may be regarded as two random samples, one of Rh-positive women and a second of Rh-negative women. Among 2,723 Negro women,

the Rh-positive women had significantly more living children per woman than the Rh-negative women, as was to be expected from the mortality due to *erythroblastosis fetalis*. Among the 8241 white women, on the other hand, exactly the reverse was found. As expected, the mean number of living children per pregnancy was lower in the 3373 Rh-negative women. However, in the number of their living children per woman, they were significantly above the 5048 Rh-positive women, the difference being 0.076 children per woman. This difference was clearly due to a corresponding excess in the mean number of pregnancies per woman on the part of the Rh-negative women, especially the sensitized ones. Inasmuch as the Negro women had considerably larger families on the average than the white women, this reversal of relative family size among the Rh-positive and Rh-negative white women was attributed to the possibility, on the part of the Rh-negative white women, of compensating—and in fact of overcompensating—for their losses of infants through hemolytic disease by having additional pregnancies that resulted in a certain proportion of healthy *rr* infants. Goodman and Reed (1952) have pointed out that if any such group is not replacing itself in the general population, as has been generally true for United States urban populations, then the effects of differential fecundity would not be expected to outweigh those of differential selection on other bases. That is quite true. However, it is by no means certain that this group is not replacing itself. The study was not made upon completed families, but upon a cross section of women in the reproductive period, excluding primiparas. The mean number of living children per woman for the white women was 1.454—which is sufficiently high that perhaps the replacement level might be reached by the time all families were completed.

A final example of the high fecundity of genetically laden families was supplied by Reed and Palm (1951) in a study of Huntington's chorea. Two branches of the same family, descended from two brothers, one transmitting the condition and the other not, were compared. Fecundity was strikingly greater in the branch transmitting the trait, although both branches far exceeded the average reproductive rate of the general population. The affected branch numbered 787 persons (716 living); the other branch, 186 persons (167 living). It was further shown that the mean number of children of affected persons in this pedigree, which extends over approximately 120 years, was 6.07 ± 0.9, whereas that of unaffected sibs was 3.33 ± 0.5. The difference, 2.74 ± 1.03, is statistically significant.

Such studies point the way into a little explored field of human heredity. Nothing seems clearer in the present state of our knowledge of human population genetics than that it is extremely difficult to estimate

the selection pressure which acts on a particular gene. It is not so much the homozygotes as the heterozygotes that create the difficulty; and it is not so much the viability effects *per se* as the combination of viability and fertility effects that is important. Psychological and cultural factors that bear on the motivation to reproduction cannot be ignored. Consequently, although at the present time it is interesting to predict future changes in allele frequency from what is known of differential mortality and to calculate mutation rates from the usual assumptions, it ought to be fully realized that these are simplifying assumptions which may be grossly in error. The general effect of these assumptions is perhaps most often to lead to an overestimate of directional change in allele frequencies and an overestimate of mutation rates.

IV. Gene Flow in Space and Time

A tremendous literature has by now accumulated describing the frequencies of the alleles at various genetic loci in particular populations. It is not the purpose of the present account to review these, but certain relations of importance to the further discussion may be stated. When such populations are geographically or socially isolated, the comparisons between adjacent populations, and between more distant populations lying along a single geographical line, permit a most interesting analysis of their genetic relationships. When two remote populations are compared, they may sometimes coincide surprisingly in the frequencies of alleles at one particular locus. Thus the Eskimos and West Australian aborigines are astonishingly alike in their ABO frequencies; and so are the Chinese and Russians (see Boyd, 1950, p. 265). However, when the frequencies of alleles at a large number of loci are compared, differences are virtually certain to show up in some instances if the populations are not closely related, just as monozygotic twins may be distinguished from dizygotic twins if a sufficient number of genetic traits be examined. Thus the Eskimos are seen to be quite different from the Australian aborigines as soon as their respective M and N frequencies are compared; and the Russians and Chinese, although alike in their MN frequencies as well as their ABO frequencies, will almost certainly be distinguishable by means of their Rh frequencies, since the Chinese have virtually no Rh-negative persons in the population, whereas the Russians (still unreported) in all likelihood have a percentage approaching that of the Latvians for *r*, namely 36.6% (Race *et al.*, 1948).

1. *Clines*

When populations along a geographic line show a consistent increase or decrease in some phenotypic or allelic frequency, this is referred to as

a cline. A classic example in human populations is the steady decrease in frequency of blood group B as one moves westward from Mongolia across Russia into Eastern and then Western Europe, starting from a high frequency of allele I^B amounting to about 0.35 and diminishing to a low frequency of nearly zero. Many other clines in the frequency of blood group alleles can be discerned in the maps showing their frequencies (see, for example, Boyd, 1950, Figs. 33a, 33b, 34, 35). An interesting cline in the frequency of L^M runs from a high frequency of 0.68 in Java to 0.49 in Sunda, 0.40 in Timor, and 0.34 on the Sahul Shelf area of northern Australia.

The simplest development of a system of clines would occur in a case where a new mutant allele, possessed of some selective advantage over the alleles pre-existing in the population, is found diffusing outward into other populations from its center of origin. Clines will radiate outward from this center of origin in all directions. A graphic illustration of this phenomenon has been described by Birdsell (1950) in his analysis of the Australian tribes. A genetically determined trait, the possession of tawny hair, reaches frequencies in the population of certain adjoining tribes in central Australia (the Pitjandjara and the Jangkundjara) of almost 100%. The trait is apparently of simple Mendelian inheritance, the homozygote being lower in melanin content than the heterozygote, with both genotypes possessing in addition a red-gold pigment. Thus the homozygote has a light-tawny phenotype and the heterozygote a dark-tawny phenotype. In all directions from the apparent center of origin the frequencies of tawny-haired individuals diminish, although the gradients of the clines differ much in different directions. To the southwest the gradients are even and gradual, to the north there is a slow decline succeeded by a precipitous descent, and to the east there is first a precipitous declivity followed by a more gradual decline. The picture (Fig. 1) is clearly that to be expected of a new allele of positive (or possibly neutral) selective advantage in the Australian environment arising and diffusing outward from its center of origin.*

A similar situation will occur whenever a population lacking a certain allele is infiltrated at one point or on one side by migrants from a population containing the allele. An example of this is also to be found in Birdsell's remarkable study. The Australian aboriginal tribes lack the ABO blood group allele I^B entirely. From Papua, migrants carrying this allele have crossed Torres Strait and introduced it into the Cape York tribes; and from Timor and other islands Malay fishermen of late prehistoric

* Even a mutant allele of negative selective value might diffuse outward from its center of origin in this way, if it were associated with something of positive selective value.

and historic times have introduced it along the Sahul Shelf. The study has not proceeded far enough to describe the details of the clines, but the frequency of I^B appears to drop from about 0.17–0.20 in Papua to 0.08–0.09 on Cape York and to zero a little farther south.

Obviously such clines as the two examples just discussed should tell quite a lot about the migrations and intermixture of populations originally genetically distinct, or about the gene flow between adjacent populations

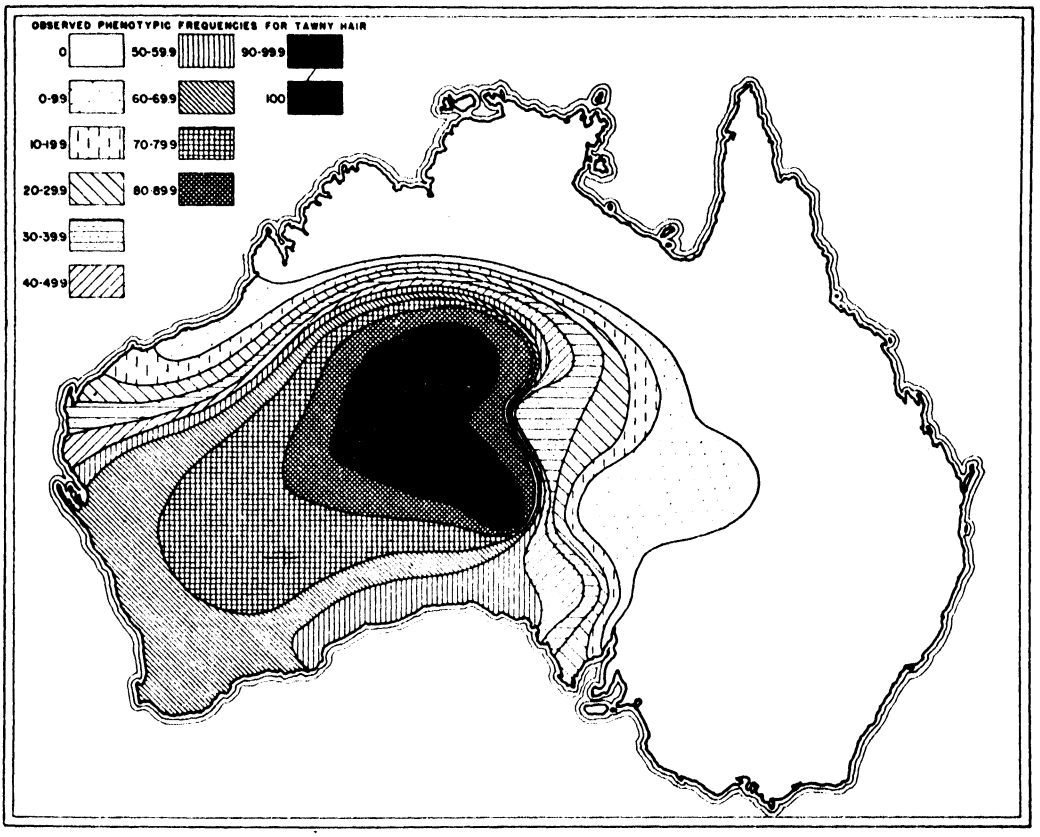


FIG. 1. Isophenic distribution of tawny hair, to illustrate clines radiating from the center of origin of a mutation. (From Birdsell.)

which are relatively stationary. Such conclusions are to be drawn with caution. One cline connecting a series of populations may be a simple product of chance. But if the cline truly reflects gene flow between populations, other clines (at the same or other genetic loci) must also be present between the same populations. If the number of alleles at the initial locus is small, their frequencies cannot vary independently, and any decrease in the frequency of allele A must be accompanied by a reciprocal increase in the frequency of allele a. If there are more than two alleles at the said locus, the situation is more complex; but if analysis were to

show that the frequency of allele A' remains constant in the populations along the line, the reciprocal relations of A and a should still hold. For example, Mourant (1950), in his analysis of the blood group frequencies of the Mediterranean peoples, described a cline in the frequency of R^1 (*CDe*) dropping from nearly 0.70 in Sardinia to 0.50 in Catalonia to 0.38 among the Basques; and inasmuch as the frequencies of two of the other three common Rh alleles, R^2 (*cDE*) and R^o (*cDe*) were approximately equal in all three populations, the frequency of r (*cde*) showed a cline reciprocal to that of R^1, rising from 0.21 in Sardinia to 0.40 in Catalonia to reach 0.54 among the Basques. The existence of this reciprocal cline on the part of another allele at the same locus strengthens the evidence for population intermixture only to the degree that the frequencies of R^1 and r are independent variables.

Much better evidence is obtained if clines are demonstrable at additional loci. Nor should this be difficult, for if there is real intermixture of populations geographically or regular gene flow of any sort between them, then clines should be demonstrable at practically every locus for which allele frequencies are determinable. In fact, it is not necessary to limit work to hereditary traits whose exact mode of transmission is known. If a trait is clearly hereditary, the phenotypic frequencies should also reveal the underlying genotypic clines. These generalizations should apply except where the paths of migration have become entangled and intertwined to a degree sufficient to obscure any main lines of flow; or except where, subsequent to the gene flow, selection has favored particular alleles and disfavored others in one way in certain environments and in an opposite way in others. In the present example, however, the frequencies for the alleles I^A and I^O of the ABO blood groups fail to confirm the existence of a cline, for I^O is lower in frequency in Catalonia than in either Sardinia or the Basque region, whereas I^A is higher in Catalonia than at either end of the line. Consequently it appears to be unwarranted, or at least premature, to draw conclusions about the genetic relations of these three populations.

2. *Isolates*

The development of clines is conditioned by the presence of isolates, that is, of populations across the borders of which gene flow is restricted—or in other words, of populations which are to some degree endogamous. Isolates may be geographical, such as aboriginal tribes, island populations, or rural communities (for example, Hanhart, 1949; Sjögren, 1949; Böök, 1950); or they may be social, as in the case of the castes in India or endogamous religious sects in other countries. In many cases social factors reinforce the geographical barriers. Identification of a genetic

isolate can be based on the divergence of the frequencies of various alleles within it from those in adjacent populations. Thus Birdsell (1950) finds clear evidence that the Australian tribe is essentially a genetic isolate, rendered so not only by its habitation of a distinct territory but also by possession of a common dialect and culture. The frequencies for the blood group alleles of the ABO and MN systems, as well as other genetic traits which have been studied, vary from tribe to tribe in such a way as to imply that the main barriers to gene flow are the tribal boundaries, whereas within each tribe a relatively short time is required to reach a panmictic state. Sanghvi and Khanolkar (1949) have shown that a similar differentiation of allele frequencies from surrounding populations characterizes six endogamous groups in Bombay studied by them. These were three subdivisions of one Brahman subcaste, one subdivision of a second Brahman subcaste, and two other full castes. Data were obtained with respect to the ABO, MN, P, and Rh blood group systems, taste capacity for phenylthiocarbamide, and color vision, and it was found that the subdivisions of the same subcaste do not differ significantly in their phenotypic frequencies. On the other hand, the different subcastes of Brahmans and the two separate castes revealed distinct differences in composition, in every intergroup comparison save one. The subdivisions of the Maharashtra Brahmans were not found to differ from the Marathas caste in these characteristics, although possibly more extensive data on the same and other hereditary traits would reveal differences there too.

In an analysis of this kind, the finding of significantly different phenotypic frequencies cannot be regarded as sufficient to demonstrate the existence of differences in the frequencies of alleles. The possible existence of different degrees of inbreeding in the populations studied must also be reckoned with, for obviously a higher amount of inbreeding will increase the frequency of the recessive phenotypes and conversely decrease those of the dominant phenotypes without of itself introducing any change in allele frequencies. If the amount of inbreeding is not negligible, the customary formulas for deriving allele frequencies from phenotypic frequencies yield false results. It is only for loci, such as the MN blood group locus, where dominance is not a complicating factor, that the allele frequencies can be calculated directly from the phenotypes. It is therefore important to note that if MN phenotypic frequencies in the data of Sanghvi and Khanolkar are converted into the frequencies of L^M and L^N, the statistical significance of the differences reported is greatly reduced. χ^2 is reduced by approximately one-half, and this leaves only a single intergroup MN difference significant at the 1% level, instead of three. It therefore remains uncertain whether the significant phenotypic differences reported in many of these comparisons are attributable to different

degrees of inbreeding or to different frequencies of alleles. Larger samples of the groups will be required to elucidate the matter.

The amount of inbreeding in a population can be estimated in several ways, as Li and Horvitz (1953) have recently indicated. Most familiar is the inbreeding coefficient F, introduced by Wright in 1921 and calculated by the method of path coefficients. In Wright's own words, "the amount of heterozygosis relative to that in a random-breeding population with the same gene frequencies is given by $(1 - F)$. . . . The coefficient F can be shown, moreover, to be the Galtonian correlation between alleles that are brought together at fertilization under the system of mating in question" (Wright, 1950; see also Wright, 1951, for a full consideration of the general structure of populations in terms of panmixia and inbreeding).

Dahlberg (1938, 1947, 1948) has calculated a formula for the size of an isolate based on the frequency of cousin marriages: $N - 1 = 2b(b - 1)/c$ where c is the frequency of cousin marriages, b is the average number of children becoming adult and marrying, and N is the size of the isolate. This formula assumes that cousin marriage is neither favored nor disfavored by social custom, but occurs at random among the available matings (see, for example, Neel, in discussion after Buzzati-Traverso, 1950). In Western Europe and the United States this may rather generally be true, although in certain social groups there is still a tendency to keep family property within the family by encouraging cousin marriages, and conversely, in some states there are laws which prohibit the marriage of first cousins. In other societies, however, this formula is totally inapplicable. Thus in Japan, as Neel *et al.* (1949) have shown, cousin marriages are greatly favored and in Hiroshima, Kure, and Nagasaki amount to 4.30 per cent of all marriages (7.45% for total consanguineous marriages); whereas in India the caste system, as Sanghvi and Khanolkar (1949) point out, rigorously excludes cousin marriage, since it "permits an individual to marry within his own entity [endogamous group] and forbids him to marry within a narrower section inside the entity."

In Western Europe and America the frequency of consanguineous marriages has greatly declined over the past two centuries. Thus, the frequency of first-cousin marriages fell from 0.0071 in Prussia in 1875–1880, and from 0.0087 in Bavaria, to 0.002 in both states in 1921–1926; and data for Vienna show a decline from 0.0077 in 1901–1902 to 0.0068 in 1913–1914 and to 0.0053 in 1929–1930. On the other hand, in France and Denmark today it is still about 0.01. In London, according to records obtained from hospital in-patients, it is 0.004. (All the above data are cited from Table 5 of Neel *et al.*, 1949.) In Baltimore, at the Rh Blood Typing Laboratory, the frequency in over 8000 white private obstetrical

cases was ascertained to be only 0.0005 (Glass, in discussion of paper by Buzzati-Traverso, 1950). Applying Dahlberg's formula to these data, we find that the "isolate," which represents in this case the average size of the population from which an individual's mate is drawn, has increased from about 400 or less to around 25,000. Even this last figure is a very small proportion of the total population of a country or a large city. It follows that the chief factor of isolation tending to prevent panmixia in Western European and American human populations is that of distance, geographic or social.

The part played by geographical distance as a factor in isolation has been analyzed by Wright (1943) in an important paper in which he came to the conclusion that "in the absence of disturbing factors, short range dispersal (N less than 100 in the case of area continuity) leads to considerable differentiation not only among small subdivisions but also of large ones. Values of N greater than 10,000 give results substantially equivalent to panmixia throughout a range of any conceivable size. . . . Recurrent mutation, long range dispersal and selection are factors that restrict greatly the amount of random differentiation of large (but not small) subdivisions of a continuous population." N in this case is the effective population size, or actual number of individuals contributing gametes to the next generation. It is therefore smaller than the size of the isolate taken in ordinary census enumeration, or in Dahlberg's formula. N may be estimated from Wright's formula $N = (4N' - 2)/(\sigma_k^2 + 2)$, in which N' represents the number of parents in the population and σ_k^2 is the variance in the number of gametes contributed to the next generation (Wright, 1938).

3. *Gene Flow in Space*

Bernstein (1931) began the analysis of racial intermixture by means of allele frequencies when he pointed out that if one knew the frequencies of the alleles in two original populations one could predict the frequencies in a hybrid population made up of given proportions of the two original populations; or *vice versa*, if one determined the frequencies in a hybrid population, one could estimate the proportions of the two parent populations which had entered into it. The latter relationship has been widely utilized. For example, Boyd (1939b) has calculated the proportion of white intermixture in American Indians, Wiener (1943) likewise from data of Snyder, and Ottensooser (1944) in Brazilian Indians. Da Silva (1948, 1949) undertook to estimate the proportions of Negro and white intermixture in Maranhao, Brazil, and of Indians and whites in southern Mato Grosso. From estimates of the frequency of allele I^A in mulattoes, he calculated them to be only 32.8 to 52.7% Negro—the difference

depending upon whether Wiener's or Bernstein's formulas for deriving the allele frequencies were used. Stevens (1938) has developed a maximum likelihood method for estimating the ABO allele frequencies, and by applying that method to da Silva's data, Boyd (1949) obtained an estimate of 50.8% of Negro genes in the Brazilian mulattoes. Boyd does not think the analysis in this case is very meaningful, since the frequency of I^B in the same mulatto population is not intermediate between those for the pure Negroes and whites. The data of Glass and Li (1953) for the Rh allele R^o (*cDe*) possess relatively high reliability, and indicate that the North American Negro population now possesses 30.56% of genes derived from white ancestors.

Such studies become spatial when the populations investigated reveal a cline. For example, Boyd (1950) made an analysis of the composition of the Interisland Aleuts—the most mixed of the Aleuts, according to Laughlin (1950)—from data for the MN blood groups in the Angmagssalik Eskimos, the Interisland Aleuts (data from Laughlin), and the Russians. The formula used may be expressed in the form: $(q_x - Q)/(q - Q)$, where Q and q are the frequencies for a particular allele in the parent populations, and q_x is its frequency in the hybrid population. It was found that the Aleuts had 24.45% of Russian mixture, but owing to an error in the Aleut frequency of L^N (which Laughlin, 1950, gives as 0.181 but Boyd took to be 0.171) this estimate should be 28.5% of Russian admixture. Inasmuch as the Aleut group consisted of only 47 persons, the statistical error involved in this estimate is very large; yet some idea of the degree of intermixture is given, provided one accepts the assumption that the Aleuts were originally like the Greenland Eskimos in allele frequencies. This assumption is not completely borne out by the comparison of the ABO frequencies of the Western (purest) Aleuts with those of the Angmagssalik (pure) Eskimos of Greenland.

Using five series of ABO blood group determinations made on the Mato Grosso Indians by E. M. da Silva, Stevens (1952) has estimated the proportions of white intermixture in each series. These populations, and another described as "local whites," can be placed on a graph with coordinates p and q (frequencies of I^A and I^B), in relation to the regression line that joins the points representing the frequencies of I^A and I^B in pure white and pure Indian populations, respectively. By useful developments of the statistical methods for handling such data, Stevens has succeeded in showing which of the populations are significantly different from each other. The "local whites" (0.767), for example, differ significantly from pure whites. The three series of Indians showing most white admixture do not differ significantly from each other and may be pooled, with a mean white admixture of 0.580. The two populations

which are purest Indian reveal white admixture of 0.233 and 0.138, respectively. In spite of the small sizes of the samples in these series, the results indicate the existence of marked differences in degrees of intermixture. Unfortunately, nothing is said about the geographic locations of these groups or the duration of the periods during which they have been exposed to intermixture with whites, so that it is impossible to say anything about the nature of the barriers to gene flow which have operated so much more effectively for certain groups than for others.

The classical study of this type is that by Candela (1942), who made a remarkable analysis on the basis of data collected from many sources and found them to reveal the introduction of the blood group allele I^B into Europe from Mongolia within historic time. Candela first demonstrated the presence of a regular cline in the frequencies of I^B, from the maximum in Buriat and Kalmuck Mongols (over 0.30) diminishing through the part-Mongols, Russians, and Danubian peoples to the western Europeans and finally the refugee peoples of the Caucasus, with less than 0.10 (Fig. 2*A*). Within Western Europe itself decreasing frequencies were also found, ranging from a maximum in Germany to a minimum in Spain and Portugal (Fig. 2*B*). In the Caucasus the highest frequencies of I^B are in the eastern Caucasian populations (up to 0.20), and much lower frequencies characterize the western tribes (all below 0.10), in line with the greater inaccessibility of the western region from the paths of mass invasion and conquest. Candela concluded that not only was the source of most European blood group B identifiable, but also that the time of its introduction could be placed in the millenium between the fifth and fifteenth centuries, when the brachycephalic Mongol hordes, from the Huns to the armies of Tamerlane, made their repeated incursions into western Asia and Europe. This last conclusion is based on the still existing correlation, in the non-European populations of the U.S.S.R., between the frequency of I^B and such physical Mongol traits as presence of the epicanthic eye fold, darker skin, weak growth of beard and body hair, and concavity of the nasal profile. It is quite unlikely, according to Candela, that this correlation would still exist if the introduction of blood group B had been more ancient. On the other hand, since all the part-Mongol elements of the invading armies were brachycephalic, although varying widely in percentage of I^B, there is no such correlation between skull shape and blood group in the peoples of eastern Europe. Although no conclusive proof seems possible, there can be little doubt that in Europe most of the increase in I^B above an original level of perhaps 0.05 to 0.06 owes its origin to this gene flow from the east.

The investigation of gene flow in space became a study of the dynamics of the process with the analysis by Birdsell (1950) of gene flow between

the tribes of Australian aborigines. Having established the basic postulate that the Australian tribe is essentially a genetic isolate, its boundaries being the principal barriers to gene flow in Australia, Birdsell used gene flow through a given number of tribes to develop a novel concept, the

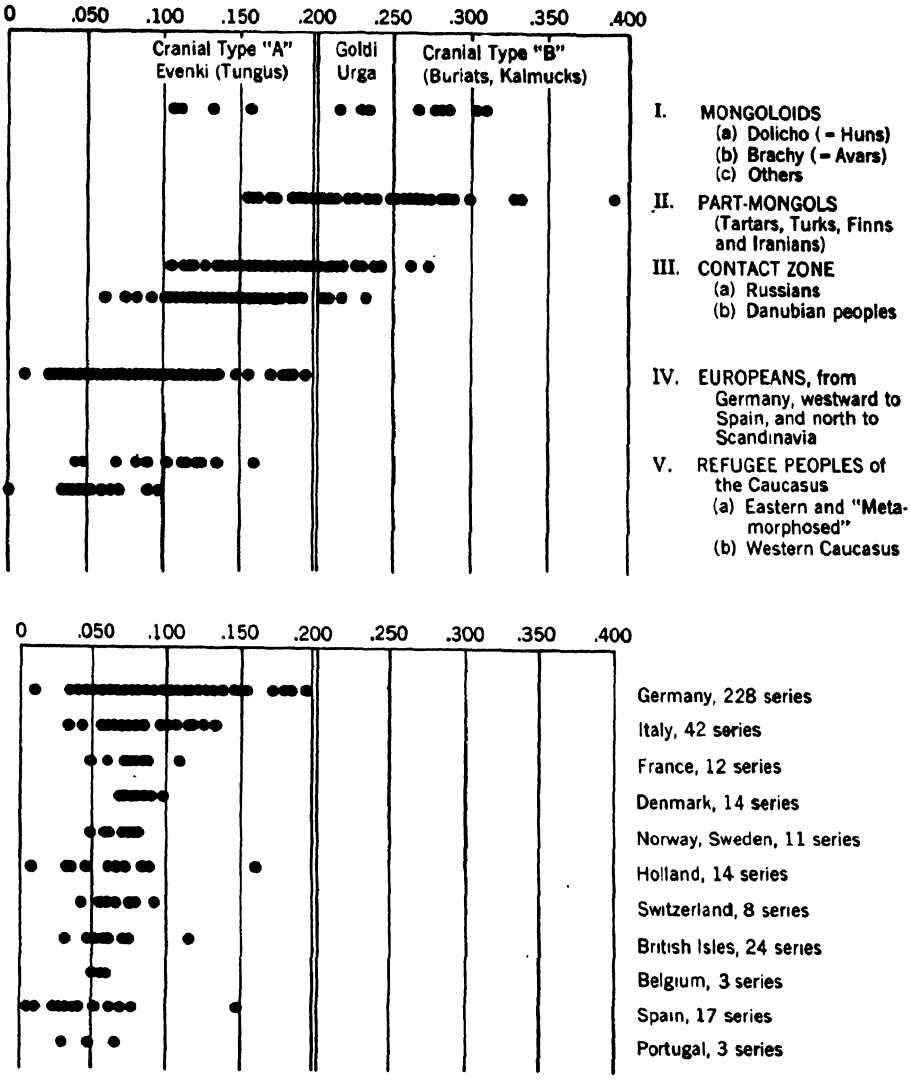


FIG. 2*A*. Allele frequencies of blood group allele I^B in Asiatics and Europeans. (From Boyd, after Candela.) *B*. Blood group allele frequencies of I^B in various European peoples. (From Boyd, after Candela.)

concept of *genetic space*. That is to say, from a genetic point of view the space traversed by a flow of genes can be measured in units of tribes or multiples of tribes. Distance is thus measured in terms of the number of genetic barriers penetrated. By making some simplifying assumptions, such as (1) that gene flow is constant for long periods of time across all

tribal boundaries, and (2) that all tribes are equal in effective size of population, Birdsell then applied his concept to the construction of two models of gene flow for the introduction of a foreign element into an original population composed of Murrayan and Negrito elements. Model A postulated the introduction of the new racial element across Torres Strait from New Guinea into the Cape York region of Queensland, and thereafter diffusion in all directions and at equal rates in terms of genetic

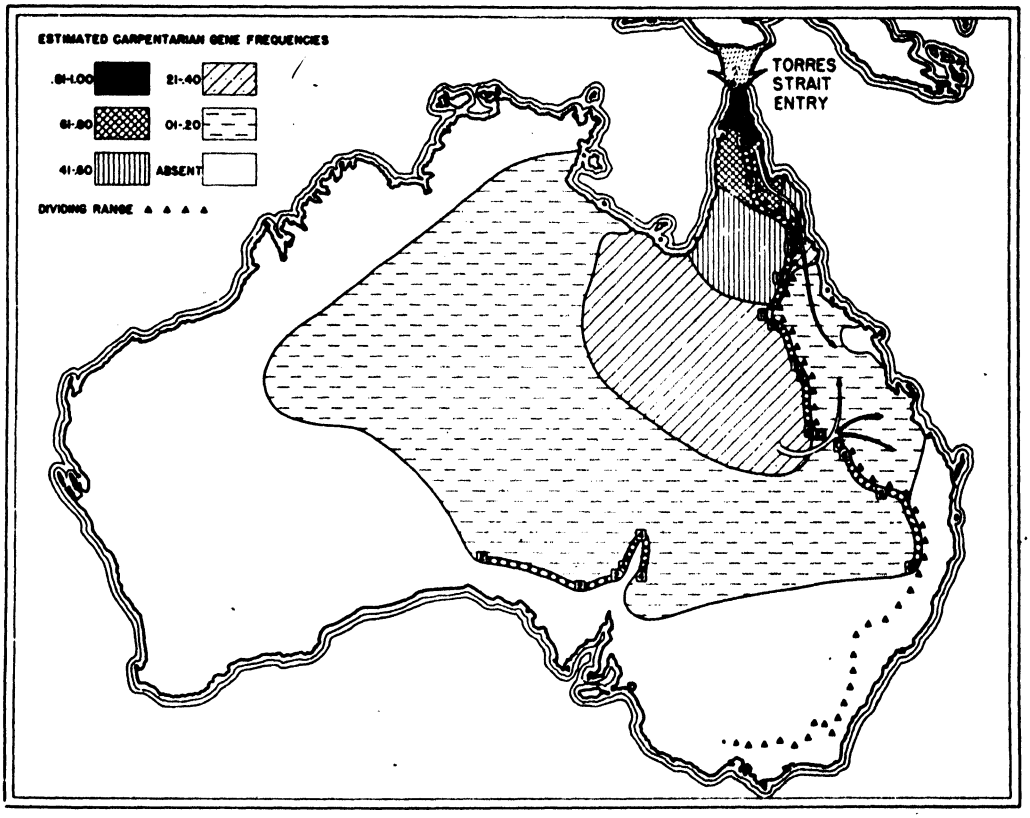


FIG. 3. Gene flow model *A*, based upon the Torres Strait entry into Australia. (From Birdsell.)

space (Fig. 3). Model B (Fig. 4) supposed, on the other hand, that the new element was introduced along the Sahul Shelf from Cape Londonderry on the west to the tip of Cape York on the east, and thereafter diffused from this broad front in accordance with the same postulates as in Model A. Cognizance was also taken of geographical barriers to flow, in the shape of the Great Dividing Range paralleling the east coast of the continent from Cape York southward, and of the waterless Nullarbor Plain parallel to the south coast and of the Flinders Range extending northeastward from Spencer Gulf in South Australia. A central break in the barrier formed by the Great Dividing Range was another important

feature. The geographic barriers, according to their estimated effectiveness, were equated to various numbers of tribal barriers to gene flow. The final result was in each model a series of contour lines representing equal numbers of barriers crossed, *i.e.*, equal units of genetic space traversed. The two models revealed themselves as very different.

The test of such models comes in comparing them with genetic reality, by no means an easy matter. The supposed new element entering

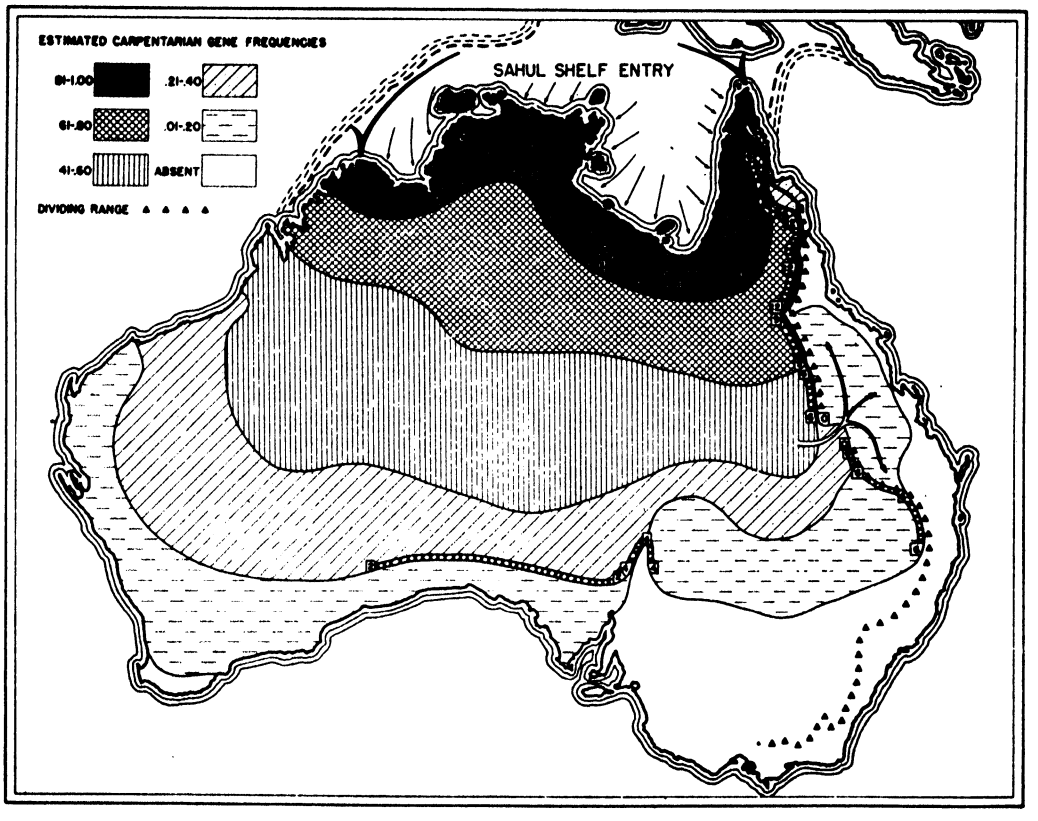


FIG. 4. Gene flow model *B*, based upon the Sahul Shelf entry. (From Birdsell.)

Australia in prehistoric times is called by Birdsell the Carpentarian element. He believes he can distinguish in various populations the amount of intermixture of this element with the Murrayan and Negrito on the basis of physical measurements and marker traits. The Carpentarians were of tall stature and linear in build, their skin color was very dark, their hair form wavy to straight, their body and face hair scanty. The nose was short, broad, and low in relief. In general, they are regarded by Birdsell as most nearly related to the dark-skinned aboriginal people of India and as forming with them a fourth major racial group of mankind, equal in status to the Mongoloid, Negroid, and Caucasoid peoples. On the other hand, the Murrayans, who are found relatively pure in

southeastern Australia (the Murray River basin), are relatively light, short in stature, lateral in body build and inclining to obesity, commonly bald, with excessive facial and body hair, and a nose unique among present-day peoples in being both extremely long and extremely broad. Birdsell describes them as "rough-hewn caucasoid," and regards them as most closely related to the Ainu. The Negritos are characterized sufficiently by their very short stature, dark skin, and woolly hair. Birdsell

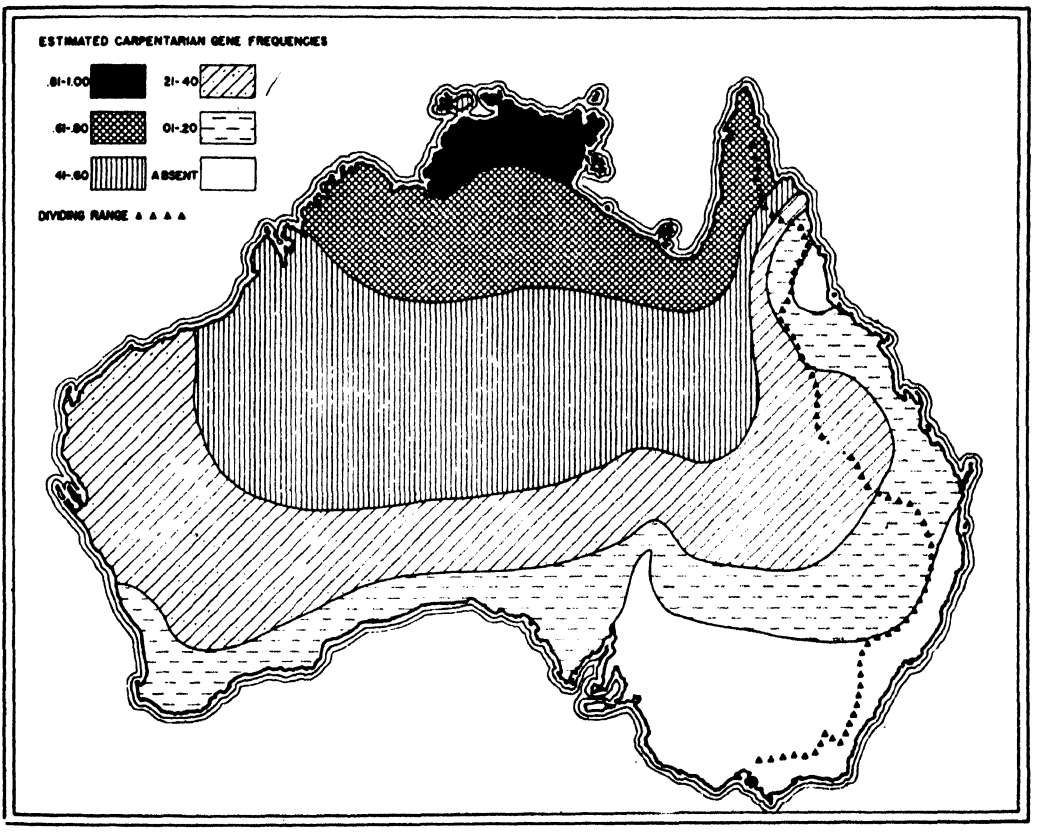


FIG. 5. Distribution pattern of the Carpentarian element in recent aboriginal Australian populations, based upon Birdsell's subjective taxonomic evaluation. (From Birdsell.)

was able, on the basis of a frankly subjective estimate, to draw a map with contours showing the supposed amount of Carpentarian element in the Australian tribes (Fig. 5). This map is completely at variance with the pattern of gene flow postulated in Model A, and invalidates completely the commonly accepted Torres Strait entry of a Carpentarian element. On the other hand, it agrees so strikingly with the gene flow postulated in Model B that one must be amazed. Lest there be doubt, the strongest point in his argument is the fact that this map was "formulated in its essential pattern more than ten years ago, upon the completion

of field work in Australia," and therefore years before the independent construction of the gene flow models.

Anthropologists, however, do not seem agreed about the reality of this Carpentarian element of the Australian population. In a recent study of the Australian aborigine, Abbie (1951) has said: "With regard to the Carpentarians and Murrayans of Birdsell's classification, it may be pointed out that south-eastern Australia can produce skulls as rugged as any, while from the far north-west come examples of the pure 'Murrayan' type. And there are all intermediate grades. The distinction is, in fact, purely statistical; but even were the distinction greater it still would not prove that the Carpentarians and Murrayans are distinct peoples. Within the limited confines of Tasmania there was a similar grouping of more rugged forms to the north-west, less rugged ones to the south-east." Summarizing other evidence, he concluded, "There is no necessity to postulate a trihybrid origin for the Australian aborigine." We must therefore wait for further evidence on the question, although it should be added that Abbie's paper was written without reference to the study of Birdsell published in the Cold Spring Harbor Symposia.

Birdsell has further suggested that the shape of the coastline along which introgression of a genetically foreign element occurs will affect the pattern of gene flow and the concentration of the introduced alleles in the area. Thus, if the coastline is convex, as along the Arnhem Land segment of the Sahul Shelf, the gene flow will tend to come to a focus and the concentration of the introduced alleles will be relatively high. But if the coast is concave, as around the shores of the great Gulf of Carpentaria, the gene flow will tend to diffuse out in radiating directions and the concentration of introduced alleles will become relatively low. It is easy to imagine the same kind of a focusing effect being produced by parallel or converging geographic barriers such as high mountain ranges, and the same kind of radiating diffusion after passage of the gene flow through a pass in a barrier.

If entrance of the Carpentarians into Australia be accepted, the time must be fixed as prior to the submergence of the Sahul Shelf, probably late in the Fourth Glacial Period. Without doubt both tribal migrations and intertribal hybridization were involved in the spread of the Carpentarian element, although the gene flow model is based solely on the latter. However, if the tribal migrations were randomized along the front of gene flow, then the distribution would, as Birdsell has said, not be fundamentally changed. "Migrations, which we know to have occurred, in general merely extended the front of the flow more rapidly."

From the size of the aboriginal population, Birdsell has estimated that the population contained 549,000 genes per locus. This total he calls

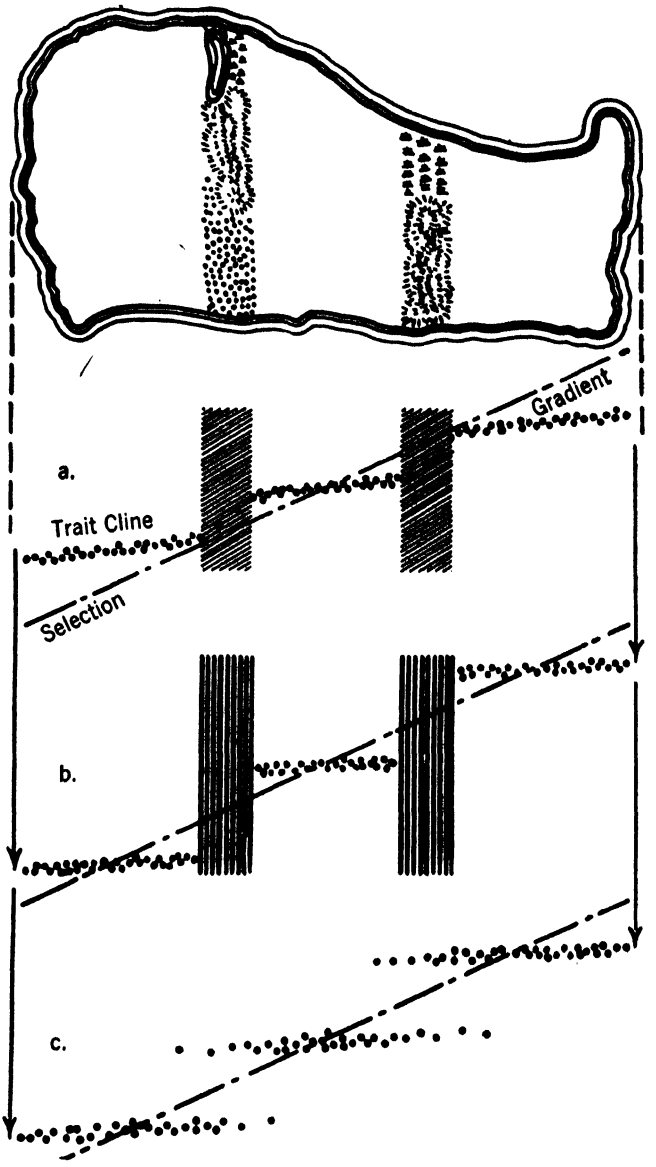


FIG. 6. The role of differential gene flow in variety and species formation: *a*. Zones of relatively reduced gene flow may produce stepped trait distributions, even with selection gradients of constant slope. *b*. Complete isolation results in discontinuous stepped clines with easily recognizable varieties. *c*. If biologic isolating mechanisms develop before the removal of physical barriers, former varieties may inter-penetrate each other's ranges without hybridizing, demonstrating speciation. (From Womble.)

the "genetic mass." The over-all contribution to this genetic mass by the Carpentarian element of the population is estimated at about 40%. From this certain conclusions may be derived. If the population has remained roughly constant in size, which would seem likely from the primitive hunting culture that obtained throughout the time, it might be supposed

that approximately 220,000 genes per locus had been brought in by the Carpentarians. This would mean 110,000 immigrant persons (Birdsell's figures are slightly larger) if none of them succumbed to the stresses of population pressure, and more than that if any were lost, as seems most likely. This tremendous immigration seems quite unlikely to have occurred, and therefore one is forced with Birdsell to the conclusion that gene flow alone is insufficient to explain the situation. Since neither mutation nor genetic drift appear applicable to this situation, the conclusion is almost inescapable that selection of some sort in favor of the Carpentarian element was involved. Following the suggestive reasoning in Coon *et al.* (1950), Birdsell suggests that the extreme linearity of body build and heavy pigmentation of the Carpentarians presented adaptive advantages in the hot, dry climate of northern and central Australia. It may be wondered whether cultural superiority did not have something to do with it too.

In a markedly original theoretical development of the subject, Womble (1951) has clarified many of the relations to be expected of gene flow under different conditions. In a species with a continuous distribution, gene flow of course tends to bring about an equalization throughout the territory of the frequencies of each and every allele. Barriers to gene flow produce stepped clines, and if complete isolation results, recognizable discontinuous varieties arise (Fig. 6). Selection of course may also be involved in the production of either simple or stepped clines. "Both the generalized effect of the intraspecific differentiation of gene flow on all trait distributions and the indirect action of selection gradients on the total genetic balance of the organism establish a common tendency for clines to steepen in the same zones. Therefore, even with gradients inclined in opposite directions, there is a good probability that the high and low absolute values of their slopes will coincide in geographic locus. Consequently, it is reasonable to average them as a mean derivation: $\frac{\left|\frac{dg_1}{ds}\right| + \left|\frac{dg_2}{ds}\right|}{2}$." Here g_1 and g_2 are two traits (or alleles) and dg/ds is the derivative that expresses the slope of the cline (Fig. 7). By weighting (w_i) and combining the several cline slopes available for any population under analysis, Womble derives a function

$$Syf = \frac{\sum_n w_i \left|\frac{dg_i}{ds}\right|}{\sum_n w_i}$$

which he has termed the "systemic function." This function measures the weighted average change with distance of the clines for the individual traits measured, and "approximates the spatial variation of the totality."

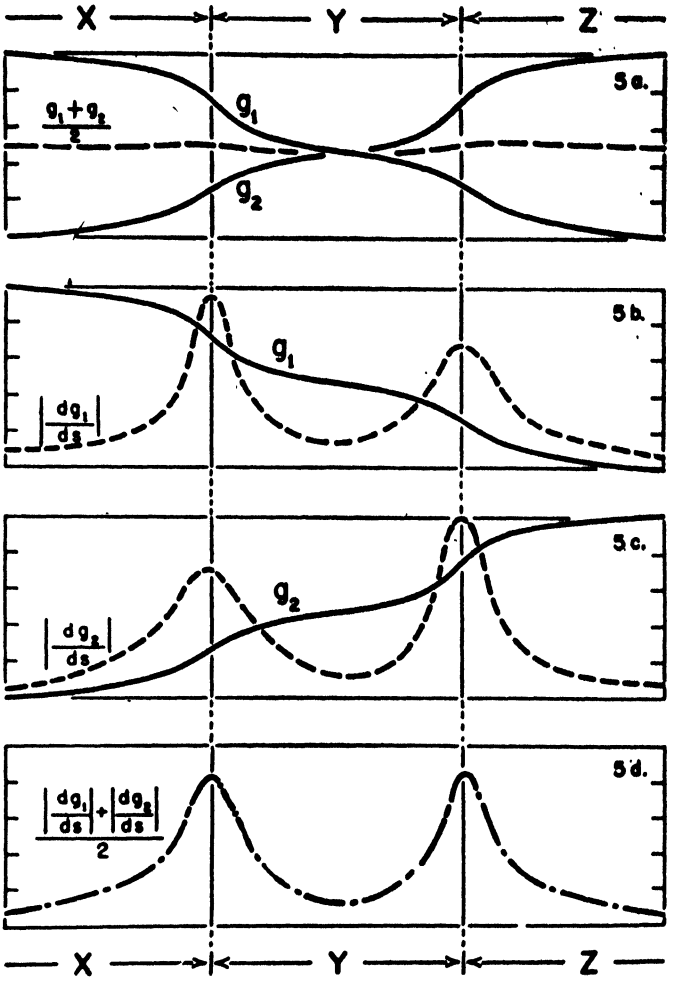


FIG. 7. Profiles demonstrating the synthesis of multiple variable traits into a composite derivative: *5a.* Traits g_1 and g_2 show clines of an opposite slope. Averaging them only confuses the picture of the evident varieties *X*, *Y*, and *Z*. *5b.* The absolute value of the derivative of g_1 is determined from the slope of its cline. *5c.* The derivative for g_2 is similarly developed. *5d.* The two derivatives (and those for other traits as well) are averaged as a composite derivative. Peak values divide the varieties. (From Womble.)

Because Birdsell's concept of genetic space is applicable only to situations with discrete isolates, Womble has preferred to develop a more general concept equally applicable to continuous distributions. It relates genetic distance inversely to *effective gene flow. Systemic distance,* thus derived, is the area of a profile through the systemic topography (which

maps the isogenes or isophenes as contour lines) from one geographic limiting point s_1 to a second limiting point s_2:

$$Syd^{s_1 s_2} \underset{\text{def.}}{=} \int_{s_1}^{s_2} Syf \cdot ds$$

Systemic distances along any line can thus be measured planimetrically from a profile. Ridge lines of the systemic topography bound natural systematic groups and measure by elevation the sharpness of the distinctions between them. Saddles and passes in the ridges point to probable routes of gene flow between groups. "In fact, at any geographic locus, the value of the systemic function *Syf* is the measure of local geographic differentiation of the species. Its reciprocal $1/Syf$ is an index of geographic homogeneity."

It remains to be seen whether systematists and workers in population genetics will undertake to apply and use such a method. It does not seem, however, to involve either overly laborious methods or intricacies difficult to understand. It certainly deserves an adequate trial.

4. *Gene Flow in Time*

Womble (1951) has further suggested that "just as systemic space may be related to physical space by summing average trait differences over short distances, so (when adequate data are available) systemic time may be related to physical time by adding average changes in the heredity over short periods." Any number of varying traits or allele frequencies may be plotted against time, and their slopes may then be determined and averaged as a composite derivative, just as in the case of space.

This is excellent but still far in the future. Whereas we have at the present time many examples of the geographic variation of traits and even of allele frequencies, studies of variation in time are extremely scarce. In human population genetics the study of changes in allele frequencies in time has barely begun. A pioneer study in this direction has been made by Glass and Li (1953), who have utilized allele frequencies at three loci in an analysis of the dynamics of racial intermixture as seen in the North American Negro population. These three loci, those for the ABO and Rh blood groups and for phenylthiocarbamide taste capacity, offer seven usable parameters for the measurement of gene flow. A statistical model was elaborated in which gene flow was considered to occur exclusively from the North American white into the North American Negro population; that is, gene flow in the reverse direction was considered to be negligible in relative amount up to the present time, the basis for this simplifying assumption being that the North American white population has remained on the average about ten times as large as the

North American Negro population, so that the same absolute amount of gene flow would affect the white much less than the Negro population. Evidence for this was found in the present lack of any indication of a rise in the frequency of the R^o (*cDe*) allele at the Rh locus in the North American white population, above the frequency found in the populations of western Europe.

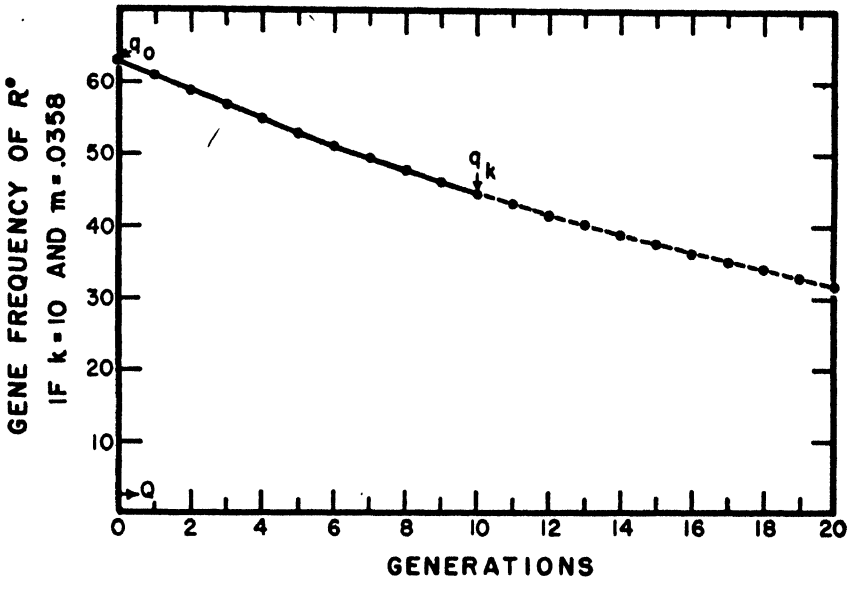


FIG. 8. The change in allele frequency of R^o in the North American Negro population from $q_o = 0.63$ to the present value $q_k = 0.445$ over 10 generations, assuming constancy in the rate of change. The dotted line is the extrapolation of the curve, assuming constancy in the rate of gene flow, for the next 10 generations. The symbol Q is the frequency of the R^o allele in the North American white population today. (From Glass and Li.)

The equation derived to express the rate of gene flow per generation is

$$(1 - m)^k = \frac{q_k - Q}{q_o - Q},$$

where the fraction on the right has the same meaning as in the preceding section, k is the number of generations over which the gene flow has occurred, and m is the gene flow. The curve described by this equation for the frequencies of the R^o allele is reproduced in Fig. 8, according to the estimated lapse of 10 generations of intermixture since slaves were first introduced into the United States. The value of m, estimated from the R^o-frequencies, is 0.0358. This estimate is more reliable than those based on other alleles because the difference in the R^o-frequencies in West African Negroes and North American whites is almost twice as great as the corresponding difference in the case of any other allele in-

cluded in the study. Nevertheless, assurance of the validity of the method and the reliability of the estimate of m is gained from the very similar values of m obtained when the frequencies of the six other available alleles are used. These calculated values of m ranged only from 0.028 to 0.056.

It is obvious that a faulty overestimate of the number of generations of intermixture will lead to an underestimate of m, and conversely for an underestimate. But Glass and Li have pointed out that the change in m with change of k becomes rapidly less as k increases. After k reaches 10 generations, an error of 1 or 2 generations in estimating k will make relatively little difference in the estimate of m in comparison with other sources of error, particularly the experimental error in the determination of the allele frequencies in the several populations under comparison. Therefore it is especially important to note that Glass and Li have indicated that the estimate of k as 10 generations is a *minimum* estimate rather than an average or maximum estimate. This conclusion derives from the fact that the present length of generation in the North American Negro population is 27.5 years and the total period of intermixture is 275 to 300 years; whereas the length of generation before the present century was almost certainly shorter than now.

Figure 8 also shows the extrapolation of the curve for the R^o allele frequency in the North American negro population for another 10 generations, assuming that the gene flow remains constant. This will almost certainly not be the case, but the extrapolation serves to indicate the fact that with constant gene flow the ultimate genetic equilibrium is approached more and more slowly with the passage of time. That is, Δq, the change in gene frequency per generation, becomes less as q_k approaches Q, since $\Delta q = -m(q_k - Q)$. Hence if m in the present case were to remain as at present at a value of 0.0358, and the length of a generation likewise at 27.5 years, the equilibrium frequency of 0.074 R^o would not be reached for 60.7 generations, or 1669 years. Thus, although the North American Negro population, according to the R^o data presented by Glass and Li, is by now genetically constituted of 30.56% genes derived from the white population, it will take about six times as long as the period already elapsed before equilibrium is reached. There is good reason to suppose, however, that the rate of intermixture may accelerate. The authors say: "Complete equilibrium need not be attained before the distinction between the two populations may become indistinguishable to ordinary observation. If, for example, the ordinarily distinguishable limit is taken to be a difference of the gene frequencies of 10 per cent of the original difference, then the limit would be attained in about 39 generations, or between 11 and 12 centuries (1072.5 years). This is only two-thirds of the

time required for complete equilibrium. . . . Secondly, it seems probable that the rate of gene flow will tend to increase as the limit of distinguishability of the two groups by ordinary observation is neared. In the third place, gene flow in the opposite direction should eventually . . . contribute its share to the attainment of the equilibrium."

The analysis assumes for the sake of simplification that the gene flow (m) has remained constant over the period of intermixture. This is highly unlikely, and the estimate obtained is therefore only an average of the amount of gene flow per generation. The method, however, points out a way in which to study the variations in amount of gene flow from generation to generation. Glass and Li say: "At any given time a population will contain individuals sufficiently different in age to cover three generations. It is consequently to be expected that different allele frequencies are to be expected in successive generations. This is a phenomenon which has been given little or no consideration in gene frequency analyses of human populations. If a population is not in equilibrium, however, the allele frequency analysis should separate different age groups, and the analysis of successive generations should yield data of considerable interest." Thus in the analysis of the North American Negro population Δq accumulated over two generations should be approximately 0.03, which might be detected in samples of about 1200 persons each from the oldest and youngest generations, respectively. If the rate of intermixture in earlier times was greater than now, then the expected change per generation would be less than 0.015; and conversely. Of course, much greater samples would be required to determine the significance of differences between the observed values of m and its average value over a period of k generations. It would be more practical to allow Δq to accumulate over more generations, "but at least ascertainment of the gene frequencies which exist in the generations now living will provide a basis for the continuation of the analysis in the future. Unless the rate of gene flow between populations is extremely high, the dynamics of racial intermixture can be studied only over a suitably long period, like other aspects of population change."

Selection may also be expected to bring about differences in the frequencies of alleles or of the respective heterozygous and homozygous genotypes in different age groups making up any single sample of the population, as Nachtsheim (1950) has found may be the case for the heterozygous Pelger anomaly. Hence the analyst would theoretically have to sample the successive generations when the individuals of each were at the same age. On the other hand, if the preponderant direction of gene flow is known, as in the instance of the North American Negro population, an examination of allele frequencies in different age groups

may be used to establish the existence of selection. That is, if the allele frequencies were not to change in the direction predicted from the gene flow but were to remain unaltered or to change in the opposite direction, it might be concluded that selection was significantly opposing the effect of gene flow. Thus, in the case of sickle-cell anemia, data of several authors (see Neel, 1950) indicate that it is less frequent in persons above 50 than below 20 years of age, in the North American Negro population. Because sickle-cell anemia is almost completely limited to Negroid stocks, this age change in frequency is opposite to what would be expected from the prevailing gene flow from the white into the North American Negro population, and also considerably larger. It is also worth noting that if the mutation rate were assumed in such a case to be great enough to maintain the frequency of the gene in the population, it would have to be large enough not merely to compensate for elimination of the gene through selection but also for the effect of gene flow, which is likewise tending to reduce the frequency of the same allele. However, the frequency of sickle cell trait in West Africa (see Fig. 3 of Neel, 1950, and mean of 9 to 12 coastal populations, Senegal to Angola, from the map in Hiernaux, 1952) appears to be about 16.0 per cent (all ages); in the United States (Neel, 1950, Table 3) it is 11.0 per cent (all ages). This is almost exactly the 30 per cent reduction to be expected from the accumulated gene flow, as estimated from the analysis by Glass and Li. It therefore appears that, in spite of the reduced mortality and fertility of homozygotes for the sickling allele, selection has been no more effective in reducing its frequency in North America than in Africa; and presumably selection pressure is balanced either by high mutation pressure, higher fertility on the part of the heterozygotes, or by a combination of both. A further study of the population dynamics of sickle cell disease has just been made by Neel (1953).

V. Genetic Drift

It is hard to see how any factor except genetic drift could possibly account for such divergent gene frequencies as those of the Eskimos of Thule in Northern Greenland. Laughlin (1950) has compared the existing data on the various groups of Eskimos, and it is apparent that the smaller groups differ strikingly from the major Eskimo groups, which "display an impressive similarity." The Polar Eskimos have a very high frequency of the ABO blood group allele I^O and a correspondingly low frequency of the allele I^A. I^B is low in all Eskimos; in Labrador and Baffin Land it has seemingly disappeared altogether.

Birdsell (1950) has considered the role of genetic drift in producing the inter-isolate differences among Australian tibes. An isogenic map for

the distribution of allele I^A among the tribes in the central desert of South Australia illustrates very well the pattern probably produced by genetic drift (Fig. 9). There is a high frequency peak exceeding 0.45 of I^A among the Pitjandjara; to the west is a tribe with a much lower frequency, so that the isogenic contours show a steep declivity between the peak and the western region. To the east, the gradient is more gradual until one comes to the territory of the Aranda, a migrant Carpentarian tribe from the north with a very low frequency of I^A. In respect to the

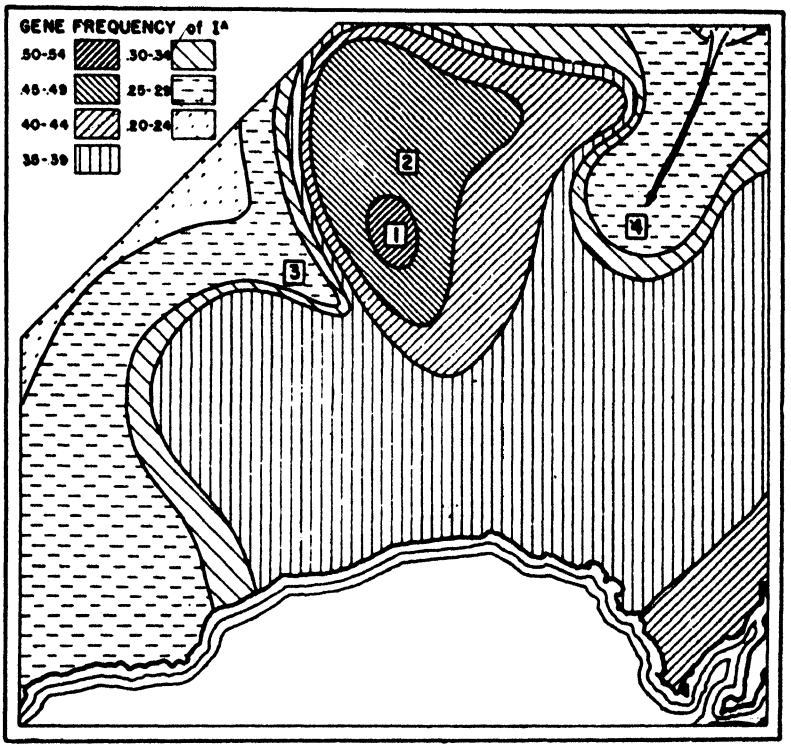


FIG. 9. Isogenic map for the allele I^A in the South Australian tribes. (From Birdsell.)

frequencies of the MN blood group alleles, however, the gradients are in all directions much more gradual. Although the action of selection cannot be excluded in such situations, the combination of small, isolated reproductive units with extreme variation in the frequencies of some but not of all alleles known not to be strongly affected by selection does lead to the suspicion that genetic drift is here responsible for the variations.

The next part of Birdsell's analysis is particularly original and interesting. He has shown that a multifactorial trait, namely, stature—which is not expected to manifest any effect of genetic drift because the effects of drift upon the alleles at the different loci concerned will cancel out—in fact shows a distribution (isophenic) throughout these same tribes

that varies by the most gradual changes (Fig. 10). On the other hand, if such minute differences in facial height as were measured are indeed reliable and statistically significant, then conversely one could interpret the discontinuous distribution of frequencies of a trait into peaks, valleys, and steep gradients as implying an underlying genetic mechanism that is not multifactorial, but more simply inherited. The same thing appears to be true of the distribution of fourth distomolars, which are apparently limited in occurrence to five adjacent tribes.

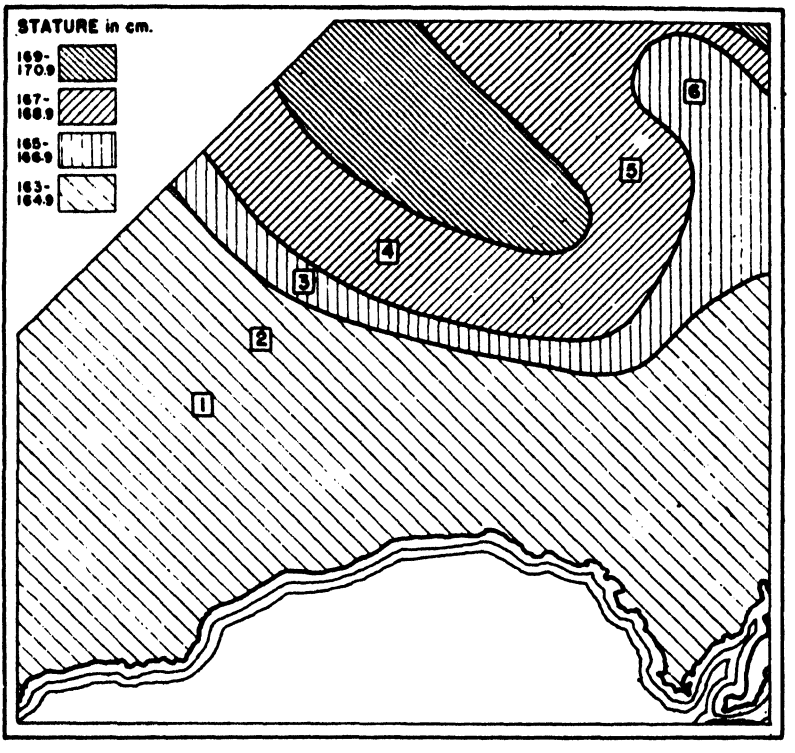


FIG. 10. Isophenic map for stature in the South Australian tribes. (From Birdsell.)

Considering the nature of the desert region inhabited by these tribes, and their frequent suffering and decline in population during droughts, Birdsell says: "The present data seem to show that climatic accidents, such as localized droughts, may produce accelerated changes due to genetic drift. . . . All Australian tribes fall within classification of small effective breeding populations, but those of the dry interior stretches seem subject to climatic disasters which cause considerable cyclical fluctuation in population size, particularly through the elimination of children. Hence there the size of the effective breeding population tends to be nearer the minimum density this country can sustain in times of climactic crisis than the maximum. Drift would be expected to be more

extreme among such desert populations." Unfortunately, inasmuch as the strength of selection upon polymorphic traits in human populations cannot be measured at the present time, the demonstration of the effect of drift is not conclusive in the present case.

A brief study has been made by Lasker (1952) of the probable force of genetic drift and gene flow in a small Mexican mountain town, Paracho. The population is given by the 1940 census as 3304; and the number of parents within it is estimated to be 1180. From this and the variance of family size in the town the effective size of the population is calculated to be 336. The gene flow from the surrounding region into the town was estimated from the number of parents in the town who were born outside Paracho, and amounted to 0.23. The estimate for the amount of genetic drift, assuming allele frequencies of 0.50, is $\sigma_{\Delta q} = 0.019$. It seems probable that in this community tendencies of the allele frequencies to drift would be checked rather effectively by the gene flow into the population from outside; but no studies of actual frequencies were made.

Similar data have been provided for the Ramah Navaho by Kluckhohn and Griffith (1950), and Lasker (1952) has used these for comparison with the Paracho study. He has estimated the effective size of population to be 64.2, giving genetic drift $\sigma_{\Delta q} = 0.044$. Not only is the genetic drift in this case larger but the gene flow into the isolate is smaller, 11.8%. Such a relationship should definitely permit alteration of the allele frequencies by means of genetic drift—a likelihood that cries for genetic investigation.

It occurred to Glass *et al.* (1952) that conclusive evidence of the operation of genetic drift might be obtained from study of "a genetic isolate of known size, age, and origin and which in particular shares an environment indistinguishable from that of the major population with which it is to be compared. The situation ideal for study would be that of a genetic isolate interspersed within a larger population, so intermingled that the individuals of the isolate do not differ from those of the general population in any aspect of life except their assortative breeding restrictions . . . an isolate neither geographic nor economic, since such isolation factors might indeed be correlated with selective factors. Nor is an ethnic isolate very suitable, since in such a case one would presumably have to deal with initial differences in gene frequency as well as with possible differences in environment that might have a selective effect. The very type of isolate desired exists, however, in the communities of certain endogamous religious sects."

Such a community was located in Franklin County, Pennsylvania—a community of Old German Baptist Brethren, or Dunkers. It included 298 persons, or 350, if nonmember adults who are children of members are

included. The effective size of the population was estimated directly to be about 90. This community, like the 54 others of the sect in the United States, is composed of descendants of emigrants from the German Rhineland early in the eighteenth century. Formerly a larger sect, the German Baptist Brethren split into three denominations in 1881. Of these the community studied belongs to the smallest and most orthodox branch. Approximately one-fourth of the children in each generation leave the religious group upon arriving at adulthood, but these losses are made up by the large average family size. Gene flow into the isolate is estimated to be between 0.10 and 0.15. It is hard to estimate exactly, because the degree of general relationship between the outsiders who marry into the community and the "natives" cannot be ascertained. Probably most of these outsiders are also of German descent.

The genetic characters analyzed included the ABO, MN, and Rh blood groups; mid-digital hair; distal hyperextensibility of the thumb; ear lobes; and handedness. In the case of the blood groups, comparisons could be made not only with the frequencies in the North American population but also with those in the Rhineland, or in England (Rh). In the absence of genetic drift, it would of course be expected that the frequencies in the Dunker isolate would fall between those of the United States and of the country of origin. For the Rh frequencies, no significant differences were found. The ABO and MN frequencies, on the other hand, showed very striking departures from expectation. Thus the frequency of blood group A, which was expected to fall between 0.446 and 0.395, had risen to 0.593. The recessive group O, which one might expect to see increasing in frequency in an inbreeding community, had fallen below the value for West Germany instead of rising toward the characteristic American frequency. Allele I^B had practically disappeared altogether. It was represented by 7 individuals of group B and 5 of Group AB, of whom only one person did not definitely trace his I^B allele to the outside within the past generation. The evidence of genetic drift in the case of the MN groups was even more striking, because in this case the West German and United States frequencies are practically identical. Type M had increased from 29% or 30% to 44.5%, type MN had decreased somewhat, and type N had declined from 20% or 21% to 13.5%.

Frequencies of the other traits studied could be compared only with frequencies in the American white population, and the evidence is therefore only confirmatory. The mid-digital hair types and ear lobes were found to differ strikingly and significantly from these in the control population. Distal hyperextensibility of the thumbs also differed strikingly, but because only a part of the isolate was examined for the trait, the statistical significance of the difference remained borderline ($P = 0.05$

— 0.02). The frequency of right- and left-handedness was identical in the religious isolate and the general population.

The study thus included analyses of 7 loci, at 3 of which there are series of multiple alleles that to some extent can vary independently, giving 15 degrees of freedom. Eleven of these manifest deviations from the control population(s) significant at the 5% level. Thus gene differences can develop in a sufficiently small genetic isolate and be maintained in spite of a gene flow into the community of some 10 to 15% per generation.

A further study (Glass, unpublished) has utilized the technique of analysis by age groups to make the interpretation conclusive. When the individuals of the community are grouped by age into the three generations 3–27, 28–55, and 56 or more years of age, no significant differences were found between the three generations in ABO and Rh blood groups, mid-digital hair pattern, or ear lobes. But the analysis of the MN blood groups revealed a striking fact: the allele frequencies in the oldest generation were 0.55 L^M, 0.45 L^N; in the second generation they were 0.66 L^M, 0.34 L^N; and in the youngest generation they were 0.735 L^M, 0.265 L^N. Hence only two generations back the MN alleles had frequencies identical with those in West Germany and the United States; and in two generations, whereas the frequencies of the other genes tested remained sensibly unaltered, the MN alleles had shifted to frequencies more characteristic of American Indians or Eskimos. It is thus demonstrated that the marked differences in frequency of various alleles in the Dunker community arose at different times, and that changes of great magnitude can occur within a period of two or three generations. These facts serve to establish definitely that "genetic drift can in fact determine gene frequencies to a considerable extent in small human isolates."

This being so, it still remains a question whether such differences, having arisen in small isolates, could take any significant part in the origin of the hereditary differences between major ethnic groups and racial stocks. Small genetically differentiated isolates would surely tend, upon coming in contact, to merge and lose their differences in a common gene pool. Explosive increases in population size, such as took place with the advent of agriculture, might allow the particular genetic characteristics of one isolate to become stamped upon a population too large to be swamped when absorbing smaller groups. A suitable model for the study of this side of human population genetics is not immediately apparent.

VI. Conclusions

In Volume 2 of *Advances in Genetics*, Dahlberg (1948) surveyed numerous aspects of the genetics of human populations. Although many of the

same topics have been covered in the present instance, a striking difference may be noticed. This difference, I believe, aptly characterizes the development of this new field of genetics. The earlier review was largely concerned with the mathematical theory of the subject. The present survey demonstrates the application of theory, partly old, partly new, to actual field studies. This healthy movement has grown equally out of the development of theory by Wright, Fisher, Haldane, Dahlberg, Hogben, and others, on the one hand; and out of the laborious determinations of the frequencies of genetic phenotypes and the frequencies of alleles by a host of blood group analysts, physical anthropologists, and geneticists, on the other. It seems for the moment to be focused particularly on the demonstration and analysis of gene flow and genetic drift. Yet the effects of these upon the composition of populations are so inextricably knit with the effects of selection and mutation that it is impossible to discuss them altogether apart. And it may be confidently expected that the development of our understanding of gene flow and genetic drift will make it possible to arrive at a better grasp of the interactions of mutation and selection with them and with each other.

In the study of both gene flow and genetic drift, age group analysis has opened up new methods of attack. The indication is clear that it may be equally promising in clarifying the effects of selection. Far more studies along these lines are needed. It is a scientific tragedy that in the past such a tremendous amount of blood grouping was done throughout the world with so little effort to obtain random—and adequately large—samples of the populations (see the emphasis on this point by Thieme (1950, 1952) in his studies of the geographic variation in Puerto Rico of stature and other physical measurements, ABO and Rh blood types, and phenylthiocarbamide tasters; and by Glass, in the discussion of Thieme, 1950). It is a further, and more needless, tragedy that not only in all the earlier blood group work, but even in the numerous excellent studies now being so widely published, age grouping has not been attempted, though so easy to do. Invaluable data for studying the effects of gene flow, genetic drift, and selection in human populations are thereby lost. It is worth reiteration that the proper genetic study of populations requires *random and adequate sampling in both time and space.* A population is a living thing, and cannot be properly treated as though it were static. Human population genetics is moving into a period when attention will be directed chiefly upon the dynamics of the processes of change.

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Monozygotic Twins in Cattle

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I. Introduction

Since the early 1930's when Kronacher (1932) published his first major paper on his researches on monozygotic cattle twins, there has been an increasing interest in this subject all over the world. World War II caused the cessation of work of the Berlin school, but large-scale operations begun during the last fifteen years at the Animal Breeding Institute, Wiad, Sweden, Ruakura Animal Research Station, New Zealand, and the Animal Breeding and Genetics Research Organisation, Edinburgh, Great Britain, have ensured a practically uninterrupted continuation of the work started by Kronacher. At the present day the interest in this work is widespread, as evidenced by the twin collections established in research

institutions in such various countries as Great Britain, Denmark, Norway, Sweden, New Zealand, the United States (Minnesota, Maryland, Ohio, South Dakota, Wisconsin) (De Wit, 1950), and Australia (White, 1951). The present review is an attempt to collect and collate the available knowledge on the subject in order to facilitate further efforts.

As many of the problems which confront the research worker interested in monozygotic twins, *i.e.* diagnosis, heritability studies, and suitability as experimental material, are inevitably concerned with comparisons with dizygotic twins it is also necessary to consider fairly fully the phenomenon and implications of dizygotic twinning.

In the following the term monozygotic is used for the various synonyms: monochorionic, monovular, one-egg, monozygous, and identical, to indicate twins of a type which are assumed to have developed from one ovum fertilized by one sperm and which thus possess identical germ plasm. The corresponding term dizygotic describes twins which in contrast have developed from two different ova and sperms; and which are thus as genetically unlike as full sibs.

II. Evidence of the Occurrence of Monozygotic Twins in Cattle

Newman *et al.* (1937) enumerated the lines of evidence available to prove the existence of monozygotic twins in man. Of these it is possible to apply the following to cattle: (1) inference from authenticated cases in other species, (2) indications from embryological material, (3) the occurrence of conjoined twins (double monsters or Siamese twins), (4) statistical studies of sex ratios in twins, and (5) an unusually high degree of similarity between the members of some sets of twins.

1. *Inference from Other Species*

The evidence accumulated through half a century of investigation into human twinning leaves very little doubt as to the authentic occurrence of monozygotic twins in man. However, the only species in which the zygote has actually been observed to undergo a division is the nine-banded Armadillo, which regularly produces one-egg quadruplets (Newman and Patterson, 1910). There is some evidence (see below) of monozygotic twinning in other species, but it is neither sufficiently exhaustive nor reliable to indicate that it may be a common phenomenon.

2. *Indications from Embryological Material*

As the result of studies of slaughterhouse material, Lillie (1923) and Keller (1933) came to the conclusion that monozygotic twins did occur in cattle as great rarities. On the assumption that the presence of two

embryos together with only one corpus luteum indicated that the embryos were of monozygotic origin, Lillie (1916) found only one set of monozygotic twins amongst a total of 126 sets. However, Lillie himself called attention to the fact that the butchers who collected the uteri containing twins were likely to overlook early twin pregnancies confined to one horn. As it may be assumed that the majority of monozygotic twins lie in the same horn, it is thus likely that a number of monozygotic twin embryos could have been omitted from the material investigated.

The existence of monochorionic and dichorionic twins in man was long thought good evidence of the occurrence of two different types of twins. However, in practice it was not always easy to distinguish between fused dichorionic membranes (which occur quite frequently) and truly monochorionic ones. As it was further shown by Curtius (1928) and Lassen (1931) that monozygotic twins often have separate chorions and even separate placentae, it is now generally conceded by human biologists that the evidence provided by the chorions is not reliable.

Hamlett and Wislocki (1934) reviewed a number of cases of synchorial embryos in mammals. The degree of fusion of the two originally separate chorions varied widely and included some so completely fused as to render them microscopically indistinguishable from a single one. They stated that synchorial twinning is most common in simplex uteri and that it may be suspected that autonomous development of twin ova is practically impossible in this type of uterus. Despite the fact that the bovine possess a bicornuate uterus, it is known, since the pioneer work of Lillie (1916), that the great majority of all cattle twins are not only synchorial but also establish vascular anastomosis in early fetal life. This latter is a phenomenon peculiar to cattle and allows an interchange of fluids between the two fetuses, thus causing the "freemartin" condition, *i.e.*, underdevelopment of the sexual organs of the female twin born co-twin to a bull.

As will be seen later vascular anastomosis, which, according to Swett *et al.* (1940), occurs in approximately eleven out of twelve cases of all cattle twins, has far-reaching consequences also in like-sexed twins in that it may cause dizygotic twins to resemble each other more than would be expected on the basis of their genetical similarity alone, thereby creating difficulties in distinguishing between the two types of twins, and rendering the genetic interpretation of twin comparisons hazardous.

3. *The Occurrence of Conjoined Twins*

The occurrence of conjoined twins has been taken by many authorities as the best available evidence of the existence of twins monozygotic in origin. Embryos showing various degrees of splitting were described by Johansson and Hansson (1943) in sheep; Streeter (1924) and Hughes

(1930) in pigs; Gluecksohn-Schoenheimer (1949) and Bodemann (1935) in mice; Boyer (1947) in hamster; Patterson and Hartman (1917) in opossum; Assheton (1914) in ferret; Byerly and Olsen (1934), Sturkie (1946), Newman (1940), and Waddington (1941) in hens; and Hildebrand (1938) in turtles.

On the basis of a search of the literature, Johansson and Venge (1951) concluded that double monsters are more frequent in cattle than in any other farm animals. Keller and Niedoba (1937) reported as a result of a nation-wide survey in Austria that double monsters occurred at least once in every 100,000 calvings. Referring to work by Gurlt (1832), Schwalbe (1907), Keller and Niedoba (1937), and Kröning (1924), and also to their own observations on preserved specimens at various veterinary institutions, Johansson and Venge (1951) noted that conjoined cattle twins formed a graded series from specimens showing only slight duplications of an extremity to almost separate individuals. They also stated that present-day authorities seem to be of the unanimous opinion that double monsters, irrespective of degree or type, always arise from a single fertilized ovum. Reported cases of conjoined twins of different sex could be explained on the basis of their having developed from an intersexed embryo. From reasoning along these lines it is but a short step to conclude that if the fission is complete two separate individuals, *i.e.*, a monozygotic set of twins, would arise, representing thus the ultimate stage in the series of double monsters.

4. *Statistical Studies of Sex Ratios*

Statistical studies on human twin populations applying the method originated by Weinberg (1902) provide one of the most convincing proofs of the existence of two kinds of twins in man. The method is based on the assumption that sex is genetically determined and that all unlike-sexed twins are dizygotic. An excess of like-sexed over unlike-sexed twins thus provides evidence of the existence of two types of twins.

Despite the fact that the proportion of monozygotic twins to all twins is much lower in cattle than in man, several studies on cattle twin populations summarized by Johansson and Venge (1951) revealed that the statistical approach may yield evidence of monozygotic twinning also in this species. (Further details of the methods employed are given in the section on frequency of monozygotic twins.)

5. *Noticeable Similarity within Sets of Twins*

Galton (1883) (cited by Newman *et al.*, 1937) took as evidence for monozygotic twinning in man the existence of two distinct classes of twins:

1. Those in which the members were so alike that they could hardly be distinguished from each other.

2. Those which were no more alike than brothers or sisters.

Throughout the times this thesis has been universally adopted and constitutes even at the present time the basis for the identification of monozygotic twins. Kronacher (1932) and Kronacher and Sanders (1936) were the only ones who attempted to prove the occurrence of monozygotic twins in cattle by the same reasoning. The evidence provided of the existence of two distinct classes of twins was however not very convincing. Despite this the diagnosis of monozygotic twins in cattle (as in man) is largely based on the specific similarities shown by members of certain twin sets.

III. Causes of Twinning

1. *Dizygotic Twins*

In normally monoparous species such as man and cattle, double ovulation (which gives rise to dizygotic twins) is influenced by heredity as well as by environmental factors. Evidence of hereditary influence may be based on pedigree analyses and on differences in twinning frequencies shown by different races or breeds situated in similar environments.

In man the evidence of hereditary determination of dizygotic twinning is inconclusive, although Newman (1942) considered that the pedigree analyses by Bonnevie and Sverdrup (1926) indicated strongly that genetic factors were involved. Strandskov and Edelen (1946) found statistically significant differences in the frequencies of dizygotic twins in the "coloured" and "white" populations in the United States which they took to be of mainly genetic origin. Pedigree analyses in cattle undertaken by Korkman (1948) showed significant differences in frequencies of twin births between daughter groups of different sires, thus supporting earlier evidence by Johansson (1932), Hewitt (1934), Löwe (1939), Weber (1944), and others, that there was a hereditary disposition for twinning in cattle. As no dam-daughter correlation could be established, Korkman concluded that recessive factors must be involved. As, in this study, no other means were available of distinguishing between monozygotic and dizygotic twins than the division into like and unlike sexed twins, it was not possible to state with certainty whether inherited factors influenced both types of twinning. However, Korkman inferred that hereditary factors principally affected dizygotic twin births from the fact that the variation between cows was greater in the births of unlike-sexed than of like-sexed twins.

The marked breed differences in over-all twinning rate noted in cattle (see Table 1) also provide fairly convincing evidence of the fact that inherited factors do influence twin births in this species, as many of the

TABLE 1
Frequency of Twin Births
(Mainly after Johansson and Venge, 1951)

	Total No. births investigated	*No. twin births and freq.*	*M.T.F.*	*D.T.F.*	*Monozygotic as % of all twins* Method* (50:50)	Method* (2 × ♀)	*Monozygotic as % of like-sexed twins* Method* (50:50)	*Monozygotic ♀ as % of like-sexed ♀ twins* Method* (50:50)
Simmentaler (Strebel, 1909; Weber, 1944)	12,625	582 4.61	0.14	4.47	3.1	0.7	6.0	1.3
Swedish Friesian (Magnusson, 1919; Johansson, 1932; Bonnier, 1946b)	24,670	820 3.32	0.11	3.21	3.5	5.2	6.8	10.0
Brown Swiss, Red Danish, and Red Polled Norwegian (Engeler, 1933; Frederiksen, 1927; Tuff, 1928)	23,719	648 2.73	0.20	2.53	7.6	12.9	17.1	23.9
Swedish Red and White (Johansson, 1932; Bonnier, 1946b)	53,554	992 1.85	0.20	1.65	5.9	3.2	11.1	6.1
Swedish Polled (Johansson, 1932; Bonnier, 1946b)	3,751	68 1.81	0.10	1.71	15.5	18.3	26.8	36.7
German Lowland Cattle (Löwe, 1939)	12,502	246 1.97	0.28	1.69	11.5	12.0	20.7	21.5
Finnish Ayrshire (Johansson, 1932)	57,082	750 1.31	0.05	1.26	3.7	5.6	7.2	14.6
New Zealand (mostly Jersey) (Ward, 1944, 1946, and 1947)	87,926	901 1.02	0.09	0.93	8.9	12.0	16.6	22.1
South African Friesian (Joubert, 1952)	26,247	350 1.33	0.18	1.15	10.9	13.7	19.6	30.8

* See pages 149, 150 for explanation of method.
M.T.F. = Monozygotic twinning frequency.
D.T.F. = Dizygotic twinning frequency.

populations enumerated are geographically intermingled or exist side by side. The significance of these breed differences must apply mainly to the dizygotic twins for the reason that monozygotic twins in cattle are numerically comparatively negligible.

Of the environmental factors influencing dizygotic twinning the age of the mother seems the most important, as evidenced by the findings of the many authorities on twinning in man referred to by Korkman (1948). Johansson (1932), Hewitt (1934), Weber (1944), and Korkman (1946, 1948) found that the same situation applied to cattle.

Nutritional factors may also be important in view of the reported effect (Wallace, 1952) of an increased twinning rate in ewes which had been subjected to a high level of nutrition three to four weeks prior to mating. It seems likely that the greater ovarian activity resulting in multiple ovulations is mediated through hormones from the pituitary gland. Injection of pregnant mare serum, which has the same action as the follicle stimulating fraction (F.S.H.) of the anterior pituitary, into cows three to four days before oestrous resulted in multiovulations and consequent multiple birth in a great number of cases (Hammond and Bhattacharya, 1944; Hammond, 1949; Hancock, 1947).

2. *Monozygotic Twins*

Because monozygotic twins are very rare in cattle and because experimental work for several obvious reasons is difficult, there is only little direct knowledge of the causes and mechanism of monozygotic twinning in the bovine. The present-day theory of the phenomenon of monozygotic twinning stems from the work on the nine-banded Armadillo (*Dasypus novemcinctus*) which Newman and Patterson (1910) found to produce regularly one-egg quadruplets. In this species the very early embryo, at an inner-cell stage, comes to a complete developmental stop lying free in the uterus for over two months before placentation. When finally placentation occurs, twinning quickly follows. From this Newman (1923) formed the general theory that monozygotic twinning was the result of a period of embryonic quiescence. In 1942 Newman (1942) still adhered to this theory despite strong criticism (Hamlett, 1933) based mainly on the fact that other species failed to produce monozygotic multiple births although showing a delayed placentation similar to that in the Armadillo. That a check in embryonic development has a tendency to cause a split, was shown experimentally by Stockard (1921) in fish; Newman (1921) in starfish; Sturkie and Williams (1945) and Sturkie (1946) in the fowl. From twin material in man, Newman (1931) reasoned that the period of embryonic development, during which twinning of a single embryo can take place, may begin at the stage of cleavage prior to the trophoblast

and inner-cell stage and end very late in gastrulation. Twins separated before gastrulation should develop into duplicate individuals showing no mirror-imaging. During gastrulation the bilateral axis (the axis of symmetry) is however determined and twins separated during this period will therefore show asymmetry reversal, which becomes progressively more pronounced with delay in splitting.

In a recent paper Gluecksohn-Schoenheimer (1949) described a dominant mutation (Ki) in the house mouse which in heterozygous condition produces fission and absences of tail vertebrae resulting in kinkiness and shortening of the tail. When homozygous, Ki acts as an early lethal, all affected embryos dying at about eight or nine days after conception. These embryos showed duplications varying from partial duplicity to complete twinning. It was suggested that the splitting of the embryo may be caused by the abnormal composition of the embryonic fluid, which results in changes in the osmotic pressure, causing rupture of the membranes and tissues—a process wholly unlike the stoppage in embryonic development assumed by earlier authorities to be the cause of monozygotic twinning.

Although the general situation in Armadillo and the Ki mutant in house mouse are undoubtedly examples of inherited types of monozygotic twins, it does not seem likely that in man hereditary factors are involved (Newman, 1942), although recently Strandskov and Edelen (1946) reasoned from the significant differences they found in the monozygotic twinning frequency between the "coloured" and "white" populations in the United States that heredity may possibly have an effect.

Data from Ruakura Animal Research Station (Hancock, 1953a) showed no evidence of an hereditary tendency for monozygotic twins in cattle, as 437 matings of monozygotic twin bulls and 226 matings of single-born bulls to monozygotic twin females resulted in 660 single births and three sets of dizygotic twins. However, in the course of twin collection work, four bulls were noted (Hancock, 1949a) to have sired two sets of monozygotic twins each. As their occurrence could hardly be ascribed to chance or to specific environmental factors, it seemed that the sperms of these bulls were so constituted as to cause the fertilized ova to split. Newman and Gardner (1942) postulated that the mechanism for this may lie in the underactivation of defective sperm.

IV. Frequency of Twinning

Hancock (1949a) argued that to the cattle research worker the frequency of monozygotic twins is of more than theoretical interest in that it has a direct bearing upon the potential number of twins available and upon the ease of collection. In this connection two statistics are of impor-

tance: (1) The ratio of monozygotic twin births to the total number of calvings provides an estimate of the total number of monozygotic twins available within a given population; and (2) the ratio of monozygotic twins to all like-sexed twins is closely related to the ease of collection, since this ratio determines in practice the number of twin sets that have to be inspected for every set of monozygotic twins found. Twin collection work will thus be efficient both in respect to the total number of sets procured and to the effort spent in locating each twin set, if the monozygotic frequency is high and the dizygotic twinning rate low. As further, the number of heifer twins required for experimental work is in general many times greater than the number of bull twins, it follows that knowledge of these statistics as applied to heifer twins especially is most desirable.

The frequency of monozygotic twins can be estimated by applying Weinberg's (1902) "differential" method to sex combination data of twins. This method is based on the theory that the three sex combinations ♂♂, ♂♀, and ♀♀ of the dizygotic twins in a population should appear in the proportions 1:2:1, weighted according to the square of the birth sex ratio of the total population. The difference between the total number of like-sexed twins and like-sexed dizygotic twins computed in the above manner is then considered to consist of monozygotic twins. The sex ratio in twins should be used in the computations if it differs markedly from that in singles.

Strandskov and Edelen (1946) showed that if the sex ratio in twins is near 50:50, little error is introduced if the estimate of monozygotic twins is simply obtained by subtracting the number of unlike-sexed twins from the total number of like-sexed twins. With these methods it is not only possible to calculate the total number of monozygotic twins in a population, but it is also possible to obtain estimates of the number of male and female monozygotic twin sets separately. It must, however, be noted that the method is valid only on the assumption that there are no differences in prenatal mortality amongst the twin sets of various sex combinations or zygocity.

Johansson (1932) made the first estimate of monozygotic twinning frequency in cattle and concluded that in Swedish breeds and Finnish Ayrshires over 6% of all twins should be monozygotic. This corresponds to approximately 11% of the like-sexed twins. Working on his own data, Bonnier (1946b) found marked differences amongst Swedish breeds—10.1%, 4.8%, and 27.9% of like-sexed twins for Swedish Red and White, Swedish Friesian, and Swedish Polled Landrace, respectively; however, as these differences were not statistically significant, he concluded that approximately 8½% was the best estimate of a common true monozygotic

twinning rate. Johansson and Venge (1951) computed and tabulated (see Table 1) the monozygotic twinning frequencies for a number of breeds. It is clear that the breed differences shown are of such magnitude that if they were true the difficulties in collecting monozygotic twins would vary greatly from one population to the other. However, as the differences were nonsignificant, Johansson concluded that in all breeds of dairy cattle it might be expected that approximately 10% of all like-sexed twins are monozygotic. Hancock (1949a) noted that the monozygotic twinning frequencies for the three Swedish breeds computed from Johansson's (1932) data showed breed differences of the same order as calculated by Bonnier (1946b) from independent material. From this he concluded that it was somewhat rash to plan twin collection work on the assumption that monozygotic twinning frequency is the same in all cattle populations. In the course of fairly large-scale collection work, it was noted (Hancock, 1949a) that in the sample of twins inspected it was almost twice as difficult to find a set of monozygotic bull twins as a set of heifer twins. From this it was concluded that a differential prenatal mortality may exist amongst bull twins of different zygocity. It is well known (Johansson, 1932) that in cattle as in man the sex ratio of twins is different from that of singles in that more female than male twins are born, while the opposite holds for singles. This is not important from the point of view of estimating the monozygotic frequency in a population by the Weinberg (1902) method, which, however, is rendered invalid if mono- and dizygotic twins are subject to different degrees of prenatal hazards.

In view of the present uncertainty in regard to the significance of the differences between populations and the possibility of differential prenatal mortality, it seems that the safest estimates of monozygotic twinning rates that can be made in a cattle population are: (1) a maximum frequency of monozygotic twin heifers derived by subtracting half the number of unlike-sexed twin sets from the number of like sexed heifer twins; (2) a minimum frequency of monozygotic bull twins derived in an analogous manner. The above estimates apply naturally only to populations where male twins suffer greater prenatal mortality than female twins. The figures in the last column of Table 1 have been calculated to show the highest possible frequency of monozygotic heifer twins in the various populations. It may be noted that the frequencies vary very widely between breeds (or between the regions in which the breeds are situated), and although they are not statistically significant, it seems that the ease whereby monozygotic twin heifers may be located may be very variable, depending on the population concerned.

It is likely that research with monozygotic cattle twins as material was unduly retarded owing to pessimism about the prospective number

available, evoked by the low monozygotic twin frequency found by the early workers in the field. Hancock (1951a), however, pointed out that, whereas it was granted that in cattle the proportion of monozygotic twins amongst all twins was very low, the total number of monozygotic twins born annually in a given population may be quite high, owing to the fact that almost the whole of the dairy cattle population consists of females breeding regularly each year. The success of the twin collection work by Bonnier (1946a), Hancock (1949a), Donald (1951), and Petersen (1951) and others, shows indeed that monozygotic cattle twins may be obtained in sufficient numbers to make even large-scale experimentation possible.

Newman (1942) suggested that Hamlett's (1935) data on quadruplets in man indicated that, in this type of multiple birth, monozygotic progeny were more frequent than in twins. No such tendency, however, can be noted in this data on triplets. Data collected from the literature pertaining to the frequency of monozygotic sets of calves of higher order than twins, were presented by Johansson (1932) (triplets) and Donald *et al.* (1951) (quadruplets, quintuplets, and sextuplets). There were more like-sexed triplet sets than would be expected from monozygotic twinning frequencies found in other populations. However, as the number of sets involved was very small (37), no definite conclusion can be drawn. The sex distribution in quadruplets and quintuplets did not suggest that monozygotic twinning was important in their production.

V. Diagnosis

The first set of monozygotic cattle twins was described already in 1924 by Lush. Progress, however, was slow, no doubt partly owing to the lack of a simple accurate method of distinguishing between monozygotic and dizygotic like-sexed twins. Kronacher (1932) and Kronacher and Sanders (1936) first attempted to solve this problem and devised a system based on the hypothesis that, if the members of a set of twins were very similar in a large number of morphological and physiological characters, this could only be explained by assuming that the twins were of monozygotic origin. This is the similarity method employed by Siemens (1927) and Verschuer (1928), in their twin studies in man; and thereafter employed with great success in the diagnosis of zygocity in this species. In cattle, the method was, however, not as easily employed, for the reason that, owing to intensive selection over many generations, even unrelated individuals of a cattle breed show great similarity in conformation, hair pigmentation, and other characters strongly determined by genetic factors, which otherwise would be most suitable as points for comparison. Another difficulty lay in the fact that certain conspicuous traits in cattle

are to a marked extent influenced by prenatal environment, thus making it possible for monozygotic twins to exhibit marked differences.

Nadai (1949) and Hancock (1949b) enumerated optimal conditions involved in a practical diagnosis of zygocity of cattle twins. The most important of these seem to be:

1. The number of characters on which comparisons can be made between the members of twin-sets should be as great as possible.
2. The characters should be influenced as little as possible by nongenetic factors, especially by those operating before birth.
3. The characters should be inherited independently of each other.
4. The genetic variability within the population should be as great as possible, in order to increase the chances that even as closely related animals as dizygotic twins are relatively unlike.
5. The characters should be capable of being measured or described as objectively as possible.

The pioneer work on methods of diagnosis of zygocity in cattle twins was done by Kronacher and his school at the University of Berlin (Kronacher, 1932; Sanders, 1935; Kronacher and Sanders, 1936). Kronacher (1932) selected twenty-two different characters out of which two—*i.e.*, head and body shape—involved, respectively, six and eleven different measurements. His followers (Schmidt and Kliesch, 1938; Haak, 1942) mainly tried to simplify this method by dropping many of the characters employed. In the following, the most important characters used in similarity comparisons will be discussed.

1. *The Similarity Index as an Aid in Diagnosis*

Kronacher (1932) attempted to distinguish between mono- and dizygotic sets of twins with the aid of a "similarity index," which he constructed by allotting points on a 1–3 scale according to the relative degree of similarity shown by the members of the sets under classification. The final index was represented by the average of the sum of the ratings for all individual characters. According to this system, 35 sets of like-sexed twins were classified as follows:

A	2 sets certainly monozygotic 3 sets very probably monozygotic
B	9 sets possibly monozygotic
C	17 sets probably not monozygotic 4 sets not monozygotic.

The work of the Berlin school and also that of Newman *et al.* (1937) indicated clearly that twin sets cannot be separated into nonoverlapping classes on the basis of similarity indices. However, if indices are computed

for all sets in a mixed population of twins, the average index for the monozygotic twins will be appreciably greater than for the dizygotic twins, showing that as a class the former are much more similar than the latter. This situation contains a tacit admittance that in the final classification of a set of twins some characters of an intangible nonmeasurable quality are given greater weight than the relative similarity in some of the measurable characters which are contained in the index. As in practice the task of diagnosing twins involves a decision on each set separately, it follows that similarity indices are not very useful as diagnostic aids. Similarity indices, such as those of Kronacher (1932) and Eriksson (1948), which are based on characters which can only be appraised subjectively, are open to the objection that conscious or unconscious prejudgment of the zygocity of the twins may influence the similarity rating given for each character.

Later workers (Eriksson, 1948; Hancock, 1949b; Johansson and Venge, 1951) used similarity indices mainly in order to compare the relative diagnostic values of various characters. The above studies (and those of Bonnier and Skarman, 1938, and of Nadai, 1949) contain most of the systematic investigations on methods of diagnosis of zygocity in cattle twins. Descriptions of the methods used in the recognition of odd cases of monozygotic twins were given by Lush (1924, 1929), Chapman and Casida (1942), and Deakin (1944), and of a set of quadruplets by Donald *et al.* (1951).

2. *Body Dimensions and Shape*

The eighteen linear body dimensions assumed an important role in the similarity indices of Kronacher and his followers (see especially the work by Sanders, 1935, and Haak, 1942, 1943, which Nadai, 1949, reviewed critically). An evaluation of the relative value of the various body dimensions was also attempted by Johansson and Venge (1951) in a critical study, in which they estimated the diagnostic value of five head and ten body linear dimensions, and six ratios of dimensions. The study fell into two parts:

1. The repeated measuring (4 times) on the same animals of fourteen different linear body dimensions in order to estimate the error of measurement of each linear dimension.

2. The measuring of 123 sets of twins and an equal number of randomly paired cows of the same age classes and the same breed as the twins. (The twins were of unknown zygocity but presumably the majority were dizygotic.)

In the final analysis the diagnostic value of a dimension was considered to depend on the variance ratio $\frac{\text{intra cow pair}}{\text{intra twin set}}$, with due regard to the

magnitude of the error involved in the measurement of the trait. On the basis of this study it was concluded that head length, height at withers, width at hips, and length of pelvis had the greatest diagnostic value. Height at sternum was also quite valuable despite the large error of measurement involved. Large error, however, spoiled the use of length of forehead and width at pin bones. The orbital and frontal width of head had only slight value and flank girth had none. It was also concluded that the various ratios of body dimensions were of only slight diagnostic value in addition to that provided by the body dimensions themselves.

Bonnier and Hansson (1946a) and Hancock (1949b), who can claim the greatest practical experience of any workers in the location and diagnosis of monozygotic cattle twins, also considered that body and especially head conformation were very important criteria. Despite this, they did not attempt to take any body measurements during the actual process of diagnosis. Hancock (1949b) justified this by the claim that:

1. Body measurements taken under field conditions on young calves were very inaccurate.

2. Some undoubtedly monozygotic twins showed in any case appreciable differences in body size.

3. Certain important characters (curvature of forehead, height of eye arches, size, shape and angle of muzzle and nostrils, and set and size of ears) were of such nature that they could not be measured.

For these reasons it seemed that eye appraisal by a trained observer of the relative similarity of head and body conformation could provide a more definite decision, concerning the zygocity of a given set of twins, than could be obtained by a series of measurements. Hancock (1949b) thus claimed that over 70% of dizygotic twins would be diagnosed correctly on the basis of differences they showed in shape of head, over 70% on shape of lower jaw, over 50% on general body conformation, and finally 25% on the basis of differences in length of tail.

3. *Muzzle Prints*

The work of Petersen (1922), Habu (1935), and Hering (1931) showed that it was very unlikely that two individuals could be found with identical muzzle prints. According to Hering (1931) only three main types of prints can be distinguished. Later attempts at classification were made by Nadai (1949) and Hirsch *et al.* (1952). Kronacher (1932), Dry (1942), Bonnier (1946c), Bonnier and Hansson (1946a), and Nadai (1949) claimed that the muzzle prints were one of the most useful criteria in the diagnosis of zygocity for the reason that differences within sets of monozygotic twins were very small compared with the variations occurring between unrelated animals. Hancock (1949b), however, pointed out that many

dizygotic sets showed no greater differences than even the most similar monozygotic sets, whereas the detailed differences within some sets of the latter type were rather wide. It was therefore concluded that in a population of mainly Jersey and Jersey crossbred cattle, in which other more suitable aids to diagnosis were available, muzzle prints are of so little additional value that they are hardly worth taking. These findings were largely substantiated in a critical study by Johansson and Venge (1951), in which they compared the relative similarity of the muzzle prints of 123 sets of twins (mostly dizygotic) with the prints of a similar number of randomly paired cattle.

4. *Hair Patterns*

The main traits of hair pattern used in diagnosis of zygocity are the whorls and the escutcheons (*i.e.*, the patterns of lighter and darker areas formed on the portion of skin between vulva and udder by hair growing in opposite direction). The early workers (Kronacher, 1932; Dry, 1942; Bonnier, 1946a) claimed that the relative similarity of whorls was a good criterion of zygocity. With this, Hancock (1949b), after an exhaustive study of whorl patterns in twins, disagreed on the ground that many sets of presumably monozygotic twins showed marked dissimilarities. Thus, it was noted in a sample of 90 sets of monozygotic twins that in 8 cases one calf possessed a forehead whorl which was missing in the co-twin; in 16 sets the twins had different numbers of whorls above the eyes; and in 12 cases one twin had two poll whorls, whereas the other had only one. It was emphasized that in addition to relatively rare major differences such as those described above, monozygotic twins always showed at least some minor differences in regard to type and position of the various whorls. It was therefore stressed that valuable experimental material might be lost if great similarity of whorl pattern were made an important criterion of monozygocity. In a further study, where the relative similarity of the whorl patterns was compared on a strictly objective basis, on three samples of 40 sets of presumably monozygotic twins and equal numbers of sets of dizygotic twins and randomly paired calves, it was observed that the similarity indices (on a 1–3 scale) were 2.43, 1.85, and 1.51, respectively. However, the distribution of the indices showed clearly that much overlapping occurred. Ten per cent of the lowest scoring monozygotic twin sets were, for instance, no more alike than a similar proportion of the most similar calves paired at random. This led Hancock (1949b) to the conclusion that whorls were of real use in the diagnosis only when they showed features which were unusual in the general population, and he therefore described the various types of whorls and investigated their frequency and position in a sample of 400 calves selected at

random. He noted that only approximately 40% of all dizygotic twins could be judged correctly on this criterion alone. Scoring the relative similarity of whorl patterns on a 1–3 scale, Eriksson (1948) found in a sample of 48 monozygotic and 83 dizygotic sets that the former averaged 2.91 and the latter 1.80 points. However, Johansson and Venge (1951) pointed out that, as the scoring was based on a subjective judgment after the zygocity of the sets had been determined by other methods, the results were probably biased. On the basis of their own studies, these authors concurred in general with Hancock's (1949b) findings in regard to the usefulness of whorls.

Hancock (1949b) is the only one who studied the escutcheon patterns as aids in diagnosis, and he found that they were very useful as confirmatory evidence in that the pattern of a monozygotic twin cow matches almost perfectly that of its co-twin, whereas unrelated animals show a very wide variety of easily distinguishable patterns. The escutcheon pattern cannot be used as an early criterion, as this character is not recognizable in calves as young as those generally inspected. However, as the relative shape of the escutcheon pattern does not change even under very variable environmental conditions, this character provides a useful check on previous early diagnosis, even when the members of a set have been exposed to different environments from an early age.

5. *Coat Color*

Owing to the fact that coat colors in cattle are determined by a great number of independent or interacting genes (see reviews by Ibsen, 1933; Shrode and Lush, 1947) and are relatively little influenced by environmental factors, they should be of great importance in the diagnosis of zygocity of twins. However, as coat color is a conspicuous, and fairly easily definable character, it became, ever since the beginning of the "pure breed" concept of cattle breeding, the most important distinguishing mark between many breeds of cattle. Through rigid selection this resulted in a great uniformity as regards coat color in any breed, thus making it unlikely that so close relatives as dizygotic twins will show marked differences. However, in cattle populations in which cross breeding is practiced coat color is probably the most useful aid in the diagnosis of zygocity (Dry, 1942; Hancock, 1949b). The usefulness of any one color or color pattern depends on: (1) how many allelic or interacting genes determines the extent of variability and (2) the degree to which the expression of the genes is influenced by environment (particularly prenatal). In the following a brief outline is given of the usefulness of some of the most common coat colors:

1. White markings: The most common of the nonallelic series of white

markings (of which "belted" color-sided, inguinal white, and Hereford pattern are other examples), is the character "recessive white spotting," which consists of a seemingly continuous series of white markings which varies from almost wholly white to almost totally colored. Briquet and Lush (1947) postulated that at least four alleles were necessary to explain the genetic variations in this character in a herd of Holstein Friesians. They also observed that as heritability of individual differences was high (over 90%) there was not much room left for dominance, epistasis, or environmental effects to be important. All twin authorities, who have considered this criterion, agreed that while monozygotic twins may show easily observed differences in the details of the white spotting pattern, they also show a remarkable likeness in regard to the estimated total proportion of white area. The importance (in relation to other criteria) various authorities on twin diagnosis attached to this character depended obviously on the breed to which the twins they were attempting to diagnose belonged. Thus, Kronacher and Sanders (1936), Bonnier and Hansson (1946a), Deakin (1944), Donald *et al.* (1951), and Johansson and Venge (1951), who all worked with such spotted breeds as Holstein-Friesians, Ayrshires, Shorthorns, were of the opinion that white spotting was a very useful criterion in the diagnosis of the zygocity, whereas Hancock (1949b), searching for twins in a predominately Jersey population, considered this character of relatively little use. Finally, it must be noted that in practice differences in white spotting between members of twin sets offered for inspection, are likely to be small owing to strict preselection by the farmers, who generally can easily appreciate even small differences of this type.

2. Brindle: Brindle describes a color consisting of irregular stripes of black hair on colored background. The gene for brindle can act only in presence of the gene for blackish hair (Cole, 1923). Phenotypically, brindle shows a great variability, according to Hancock (1949b and 1952a). Thus, the lightest type of a brindle animal shows only a few bluish streaks on an otherwise brown muzzle, while at the other extreme an animal may appear almost jet black owing to the density of the black stripes. Hancock (1949b) found that the members of monozygotic twin sets may show sufficiently great differences in the intensity of brindle color to be noticeable by eye estimation, but that the differences within sets are relatively small compared with the range commonly occurring in unrelated animals, or between dizygotic twins. As Brindle color is not well developed at birth, this character is obviously of little use as an aid in early diagnosis.

3. Roan: The roan color effect is produced by the intermingling of white and colored hairs. Hancock (1949b) showed that great variations

in the proportion of white hairs occurred between sets of monozygotic twins, while within-set differences generally were small, and concluded that this character was of appreciable use in the diagnosis of zygocity. However, as no comparisons between relative likeness in monozygotic and dizygotic twins were made, it seems possible that the likeness in degrees of roaning within monozygotic twins might not be of genetic origin but be due to common maternal environment, as was indeed suggested by Chester-Jones (1947). If this is the case, roan color may be of relatively little use in the diagnosis of zygocity.

4. Red: The gene for red coat color is presumed (Ibsen, 1933) to be present in all breeds in the homozygous state, and is thus useful in diagnosis only insofar as its expression is variable owing to modifying genes. Recent work by Koger and Mankin (1952) indicated that differences in intensity of red color in Herefords were determined to the extent of 75% by inheritance. They postulated that three genes with major, and possibly a number with minor, effects were necessary to explain the observed segregation. Several modifiers which changed the red coat color of cattle of the Jersey breed were noted by Ibsen (1933). Hancock (1949b) stated, as a result of his experience gained in search of twins in this breed, that monozygotic twins soon after birth showed no differences in shade of red, but that it was possible to diagnose correctly over 90% of all dizygotic twins offered for inspection on the basis of differences in the shade of red color.

5. Blackish: This color effect is caused by black pigmented hair being concentrated only in certain areas. The gene is almost universal in the Jersey breed. However, owing to modifiers the genetic expression of blackish is very variable: At one extreme a blackish animal may appear totally black, whereas at the other extreme black hair is present only in the switch of the tail. All intermediate types occur (Hancock, 1952a). The blackish pattern is not discernible in calves until they lose their birth coat and is therefore of limited use in diagnosis. As certain environmental factors, such as lactation, hot climate, and differences in developmental rates, tend to cause differences in the intensity of blackish, it was also concluded that this color pattern is not a very reliable criterion of zygocity if the members of a twin set have been subjected to widely different environments.

6. *Skin Pigmentation*

Hancock (1949b) noted that Jersey and Jersey cross cows showed characteristic pigmentation patterns on the bare or slightly hairy areas of the skin. Of these it seemed that relative similarity in color gradients of muzzle and in the pigmentation patterns of the lips and inside the

ears were specially useful adjuncts in the diagnosis. For instance, it was possible to diagnose correctly 36% of dizygotic twins on the basis of the differences they showed in ear pigmentation. In a further study Hancock (1952a) observed that ear pigment patterns were to some degree pleiotropic effects of hair color genes (mainly "blackish" and a new gene "black ears"). This, however, does not detract from the value of this character as an aid in diagnosis, as the above hair colors are only poorly developed at the early age when in practice cattle twins have to be inspected. Other skin pigmentation patterns, such as spotting of palate, tongue, and udder, were not of the same use because they seemed to be influenced to a marked degree by prenatal environmental differences in relation to the genetic range generally shown.

7. *Antigenic Characters*

a. *Red Cell Antigens.* Genetically determined antigenic red cell differences are ideal criteria on zygocity in twins, in that they can be determined strictly objectively and with a great degree of precision. For this reason, much effort was spent by the early workers Kronacher (1932), Lodemann (1933), and Kronacher *et al.* (1934) on the development of serological technique applicable to cattle. However, they had little practical success in their attempt to use the method in the diagnosis of the zygocity of twins, and their followers, Schmidt and Kliesch (1938) and Haak (1943) considered that the blood types belonged to the less important criteria.

The work of the Wisconsin school by Ferguson (1941), Ferguson *et al.* (1942), Stormont *et al.* (1945), Stormont and Cumley (1943), and Stormont (1950) demonstrated that there were at least 38 distinct antigenic components in cattle red cells. Their studies caused considerable interest among workers involved in investigations on methods of twin diagnosis (Bonnier and Hansson, 1946a; Hancock, 1949b), and are still a subject of numerous enquiries by prospective searchers for twins who are not familiar with the difficulties involved. For this reason, a short account of the technical and serological aspects of blood typing in cattle is given.

In contrast to the A. B. O. system in man, a bovine lacking a given red cell factor does not normally possess antibodies against it. Monospecific antisera for blood typing in cattle must thus be produced by a technique of blood transfusion (to elicit antibody formation), and subsequent removal, through absorption, of all antibodies except one. As the final step, red cells of unknown antigenic composition can be typed through serial testing against monospecific sera produced as described.

Up to 1951, it was thought that these antigens were inherited as separate entities (with presence dominant over absence). On this basis,

it would be computed (Irwin, 1947) that the number of possible combinations was so enormously large that the chance of finding two animals (except monozygotic twins) with the same red cell combination was practically nil. However, the later work by Stormont *et al.* (1951) established that many of the factors were not inherited independently of each other but rather in groups of several factors together. As a result of the analysis of a series of test matings, evidence was presented that, of the 38 blood factors, 21 were the members of one system (B), 7 were members of another system (C), and 2 were members of a third system (FV). Thus, the maximum number of independent genes controlling the various antigens could not be greater than 11. The B system consists of at least 80, and the C system of at least 22 allelic groups.

Even with only 11 independent genes, the red cell antigens would have provided a most useful criterion on zygocity, had it not been for the discovery by Owen (1945) that dizygotic cattle twins in the majority of cases possessed identical red cell factors. The explanation given for this unexpected situation was that the vascular anastomosis which occurs in the majority of cattle twin fetuses (Lillie, 1916) allows an admixture of fetal blood. Since the blood factors exchanged in this way would not persist in the host calf after the normal life span of red cells and yet are found there throughout the life of the host, it was concluded that there is an interchange of erythrocyte precursor cells which become established in the hemopoietic tissue of the host twin. Here they produce, throughout the life of the host, red cells of an antigenic pattern determined by the genotype of the donor. As a dizygotic cattle twin of this type derives its cells from two distinct zygote lineages, it is technically a "genetical chimaera" or, in botanical parlance, a "graft hybrid" (Anderson *et al.*, 1951). A practical consequence of this condition is that it should be possible to determine at a very early age whether a heifer calf born co-twin to a bull will be a nonbreeder ("freemartin") or not, in that difference in antigenic properties of the blood in unlike-sexed twins automatically excludes the probability of fetal vascular anastomosis, which is necessary to cause the occurrence of the "freemartin" condition.

At the present time, the potential usefulness of blood typing as an adjunct in the diagnosis of zygocity in cattle twins is based on two findings (Stormont, 1952).

1. Mixture of red cells with different antigenic properties can be detected in some cases by quantitative serological tests.

2. The factor J, which is not a true red cell antigen but only adsorbed to the cells, may cause dizygotic twins with fetal anastomosis to differ in their reactions to J antiserum (Stormont, 1949). It seems further that in some cow populations the gene controlling the J factor is of an optimum

frequency (*i.e.*, about 0.5) from the point of view of greatest possible usefulness in the diagnosis of zygocity. Thus both of these tests can serve to eliminate a number of perhaps otherwise doubtful cases of dizygotic twins.

In conclusion, it may be said that blood typing in cattle is a major laboratory undertaking which requires much labor and a high degree of technical skill. It is therefore not a technique to be attempted except where very large-scale twin collection work is contemplated.

b. *Epithelial Antigens.* As a result of previous experimental evidence on the behavior of skin grafts in mammals, it was thought (Anderson *et al.*, 1951) that skin transplantations between members of sets of twins would provide a very good criterion of the zygotic origin of the twins. Such skin grafts (homografts) are destroyed in genetically diverse animals by a mechanism which belongs to the general category of actively immune reaction. The initial healing is as good as in grafts from one part of the body to another of the same animal (autografts), but the survival time of homografts in unrelated cattle is only approximately ten days. If an animal is given another graft from the same donor, the new reaction is much more rapid. This effect provides a valuable confirmatory test of incompatibility. The results of several exhaustive series of skin transplantations involving unrelated animals, close relatives, dizygotic and monozygotic twins were described by the previous authors and by Billingham *et al.* (1952). The conclusions were as follows:

All cattle twins show either absolute tolerance or a high degree of tolerance to homografts. The mutual exchange of skin grafts is therefore a technique which does not allow differentiation between monozygotic and dizygotic cattle twins.

Of 42 individual cattle of either sex (*i.e.*, members of unlike-sexed sets) that received homografts from their co-twins, 36 (or approximately 86%) proved to be completely tolerant within the limits of observation. The close correspondence of frequency of homograft-tolerant unlike-sexed twins with the frequency of "freemartins" (see p. 143) justified the presumption that the tolerance to homografts exchanged between dizygotic twins is also a consequence of anastomosis of the fetal circulation. Medawar (1946) showed that there was some degree of overlap between the antigens of leucocytes and of skin epithelium. Thus it seems that fetal anastomosis in cattle twins allows not only the exchange of hormones and red cell precursors, but also that of leucocyte precursors.

There was some evidence that, when skin was transplanted from an unrelated test animal to each member of a dizygotic twin set, the twins reacted with slightly different intensity. However, when grafts were transplanted from a dam to each of her dizygotic twins, these reacted in

markedly different ways. Why twins who are tolerant to grafts from each other should be intolerant to grafts from their mother was not quite clear, but this asymmetry of response provides the best basis for the development of a reliable method of identifying dizygotic cattle twins.

8. *Miscellaneous*

a. *Supernumerary Teats.* Kronacher (1932) had already pointed out that concordance in supernumerary teats was a poor criterion of zygocity. However, Bonnier and Hansson (1948) in their method of diagnosis rejected as not monozygotic any twin sets showing difference in this character. After a systematic study; Hancock (1949b) indicated that the relative similarity in the character was no guide to zygocity.

b. *Hair Structure.* Bonnier and Skarman (1938) considered that cross sections of hair bundles provided a good indication of zygocity, but on the basis of further experience (Bonnier, 1946a) this method was abandoned, on the grounds that hair cross-section patterns did not occur in a sufficiently wide range to make the technique worth-while. Johansson and Venge (1951) reinvestigated the usefulness of this character and reached the conclusion that the repeatability of similarity scores was so poor that the method was of little value. Dry (1942) found himself unable to make use of hair cross-section patterns in the diagnosis of cattle twins and preferred to compare the "hair landscapes" or the pictures presented by the fibers of a small tuft spread out one by one placed roughly in order of size. Relative similarity in length, coarseness, and curvature were compared by eye estimate. It was claimed that in monozygotic twins the hair landscapes were always alike, while in dizygotic twins they were nearly always recognizably different.

c. *Eyes.* Color of eyes was mentioned by Bonnier and Hansson (1946a) as an important criterion of zygocity. As, however, no studies have been made of the range of differences met with in a cattle population, it is not easy to evaluate the usefulness of this character. Nadai (1949) studied the blood-vessel patterns in the fundus region of eyes in many sets of twins (mainly dizygotic) but could not reach a definite conclusion of the usefulness of this characteristic.

d. *Physical and Biochemical Properties of the Blood.* Kronacher (1932), Nadai (1949), and White (1951) studied the possibilities of using various properties of the blood, such as hemoglobin content, red cell fragility, red cell count, plasma chlorides, as a basis for the diagnosis of zygocity. In general, the results were either inconclusive or negative.

e. *Mirror Imaging.* The phenomenon of mirror imaging (or asymmetry reversal) has provided important evidence on some aspects of the

etiology of monozygotic twinning in both man and cattle (see p. 148). In the diagnosis of twins in man, Newman *et al.* (1937) considered that mirror imaging in a set of twins provided strong contributory evidence of monozygocity. Many workers on cattle twins (Kronacher and Sanders, 1936; Schmidt and Kliesch, 1938; and Bonnier 1946a), having noted the high frequency of mirror imaging existing in conjoined twins, followed this lead and attached great importance to reversal in color patterns, hair whorls, muzzle prints, and other external characteristics. However, as no systematic study has ever been carried out, and as some mirror imaging may be due to mere chance (Hancock, 1949b), the value of this phenomenon is doubtful at the present state of knowledge.

9. *Conclusion*

It must be admitted that, despite the fact that more than two decades have passed since Kronacher began his studies on cattle twins, no single test or series of tests yet exists through which it would be possible to distinguish between monozygotic and dizygotic cattle twins in an objective manner. However, studies on diagnostic methods have shown that the number of strongly inherited characters on which cattle twin diagnosis can be based is as great as, if not greater than, in man. Newman *et al.* (1937) showed clearly that, in man, a trained observer, after a cursory examination, was able to distinguish between monozygotic and dizygotic twins with a very great degree of certainty. Much the same situation applies to cattle. Superficially it may seem that most cattle belonging to the same breed are so alike that they cannot be distinguished from each other (just as all people of a strange race seem indistinguishably alike) but an observer with highly developed critical faculties can easily note a great number of often subtle characteristics which make each individual different from the others. As this applies even to members of monozygotic twin sets, the real difficulty lies in knowing what kind and degree of differences are compatible with a diagnosis of monozygocity. Full assurance in regard to the problem can come only from further systematic work of the type described in this review.

An important aspect of diagnosis, to which Johansson and Venge (1951) drew attention, is that the relative usefulness of the various characters differs from breed to breed. Hair colors for instance, in the Jersey and Jersey cross cattle, are genetically so variable and environmentally so stable, that it is possible practically to neglect comparisons in some characters (for instance, muzzle prints) which have to assume relatively great importance in those breeds showing little variation in hair color.

VI. Efficiency of Monozygotic Cattle Twins as Experimental Material

The superiority of monozygotic twins over ordinary animals in experimental work derives from the following conditions:

1. From twin experiment data it is possible to measure the extent to which observed variation in character is determined by heredity and environmental factors, respectively. This is obviously of very great importance in breeding investigations and in indicating the relative emphasis that should be placed on breeding and feeding programs in increasing productive efficiency.

2. The fact that in a treatment experiment using monozygotic twins the hereditary variation can be eliminated, permits a more precise estimate of the magnitude of treatment effects and their more efficient statistical comparisons. It is in this latter sense the term "efficiency" is applied in the following to the relative superiority of monozygotic twins over ordinary animals.

In order to clarify the methods of estimating twin efficiency values, the following introductory note seems desirable.

The variation observed in a group of animals in a given character can be ascribed to four sources:

1. Hereditary variation (as reflected by difference between animals of the same age kept under identical conditions).

2. Environmental variation (such as would arise when the same individual is subjected to different environmental conditions).

3. Variation due to environment $\times$ heredity interaction (*i.e.*, the differential effects of a given environment on different genotypes).

4. Variations due to "error" (these are assumed to be random and include, in addition to errors of the measurement, the specific effects on each animal of noncontrollable (extraexperimental) environmental factors).

In a non time-repeated experiment with ordinary animals, it is not possible to disentangle the hereditary variations from the "error" variance. As already indicated, however, the use of monozygotic twins permits the isolation and elimination of this source of variation. The consequent reduction of "error" in the analysis of experimental results increases the statistical efficiency or sensitivity of the treatment comparison.

Bonnier *et al.* (1946) first attempted to define a method whereby the relative efficiency of monozygotic twins could be estimated. They thus computed 'E' (the number of ordinary animals in each of two groups that one set of monozygotic twins can replace without loss of statistical precision) as the ratio

$$\frac{M_B}{M_W}, \tag{1}$$

where M_B is the mean square between sets of twins and M_W the mean square within sets of twins derived from a trial in which a number of twin sets have been treated in as nearly uniform a manner as practicable. Dick and Whittle (1951), however, pointed out that, instead of using the mean squares (variances), the respective variance components should be used in the ratio. Translated into mean squares, the formula they suggested for E became

$$\frac{1}{2}\left(\frac{M_B}{M_W} - 1\right). \tag{2}$$

In comparing the results obtainable by these formulas, it may be noted that the first formula gives values approximately twice as high as the second. As the use of the latter formula may provide twin efficiency values smaller than unity, Carter (1951) finally suggested that E should be estimated as

$$\frac{1}{2}\left(\frac{M_B}{M_W} + 1\right). \tag{3}$$

Dick and Whittle (1951) indicated that it was possible to estimate twin efficiency values not only from uniformity trials but also from data accumulated in experiments involving sets of twins with the members subjected to different treatments. It may be noted here that twin efficiency values derived from uniformity trial data are valid only if heredity × environment interactions are unimportant, whereas an efficiency value derived from a split-twin experiment presumably indicates accurately the relative efficiency gained by using twins in the particular experiment from which the value was derived.

Bonnier *et al.* (1946) deduced that the efficiency of monozygotic twins relative to ordinary animals decreases in trials which involve more than two treatments, and also estimated, on theoretical grounds, the extent to which the efficiency was reduced. However, in this they did not, as shown by Dick and Whittle (1951), take into account the fact that the most suitable experimental design in which twins may be used (incomplete randomized blocks) allows, in addition to direct, indirect treatment comparisons. In neglecting the indirect comparisons, the previous authors seriously underestimated the relative efficiency of monozygotic twins in trials involving several treatments. This value can be computed correctly, as suggested by Dick and Whittle (1951), with the aid of the general formula

$$\frac{nE}{2(n-1)},$$

where n = the number of treatments
E = the efficiency value for twins when used in a two-treatment trial.

These authors also considered fully various statistical designs suitable for trials involving twins as experimental material.

The efficiency of monozygotic twins is naturally connected with the reason for, and the magnitude of, differences within sets of twins, the members of which have been treated uniformly throughout their lives. Hancock (1950a) enumerated and discussed the following factors which may cause intra-set differences.

1. Chance.
2. Asymmetry reversal.
3. The cleavage process.
4. Uncontrollable environmental factors.
   a. Prenatal.
   b. Postnatal.

Little is known of the role chance (discussed first by Dahlberg, 1945) may play in this connection. The differences within sets caused by asymmetry reversal are obvious enough in man (Newman *et al.*, 1937), but in cattle it is uncertain whether they are of great importance. Hancock (1949b) suggested that some of the abnormal anatomical features seen in one member of some sets of monozygotic twins were due to damage suffered in the cleavage process. Intra-twin-set difference in birth weights were quoted as caused by differential prenatal nutrition. It was noted, however, that, from the point of view of the use of twins in experimental work, uncontrollable postnatal environmental factors were likely to be the most important causes of intra-set differences. The magnitude of these differences could be gauged from the results of "uniformity" trials—*i.e.*, from measurements of the performance of monozygotic twins which had been treated alike from birth onwards. As it seemed likely that the effect of uncontrollable factors would vary from character to character, it was considered important to determine the relative uniformity of twins in respect to all characters in which the experimentalist is interested. Accordingly, a series of uniformity trials was undertaken at the Ruakura Animal Research Station (Hancock, 1950a, 1950b, 1951c, 1952b; James, 1953), the results of which are summarized in Table 2.

The twin efficiency values given in Hancock's papers would all be one unit higher if computed according to Carter's (1951) method. Earlier attempts to estimate twin efficiency values were reported by Bonnier *et al.* (1946), who found $E = 50$ for growth between 30 and 180 days, and $E = 5$ for total calorific output of milk and growth. The latter figure

TABLE 2
Twin Efficiency Values

Reference	*Characteristic*	*Twin efficiency value*	*No. of sets used*
Hancock (1950a)	Milk	22	7
	Butterfat	54	7
	Casein	50	7
	Butterfat percentage	15	7
	Casein percentage	10	7
	Casein:Butterfat ratio	13	7
	Persistency of lactation	4	7
	Dairy merit	5	7
Hancock (1950b)	Grazing time	72	6
	Loafing time	6	6
	Lying down time	3	6
	Distances covered	3	6
	Number of defecations	8	6
	Number of urinations	1	6
	Number of drinks	5	6
Hancock (1951c)	Body weights	26	10
	Absolute growth rates	13	10
	Instantaneous relative growth rate	11	10
	Linear body measurements	5–30	10
Hancock (1952b)	Red cell count	4	12
	Red cell volume	8	10
	Hemoglobin content	13	10
	Red cell fragility	15	25
	Blood calcium	1	19
	Blood magnesium	19	19
	Blood phosphorus	15	19
	Blood sugar	1	18
	Total blood acetone bodies	1	19
	Body temperature	37	7
	Respiration rate	15	7
	Dry matter intake (pasture)	8	12
	Fecal nitrogen concentration	21	12
	Dry matter content of feces	5	12
	Milk fat Reichert-Meissl value	11	4
	Milk fat iodine value	4	4
	Milk fat softening point	1	4
	Milk fat saponification value	9	4
	Fecal egg count	2½	16
	Titer response to Br. Abortus vaccination	2	12
James (1953)	Sperm concentration	8	12
	Total sperm	8	12
	Service volume	2	12
	Reduction factor	4	12

was, however, derived from a split twin experiment. White (1951) concluded from a trial involving 4 monozygotic and 4 dizygotic twin-sets, that monozygotic twins had no advantage in experiments involving plasma chlorides, fragility, and red cell counts. Olson (1952) reported, without giving numerical data, a uniformity trial on various semen characters in monozygotic bull twins and triplets. Finally, from growth trials involving 21 sets of monozygotic twins, Thoele and Hervey (1952) computed twin efficiency values of 6–24 for body weight and 14–43 for height at withers.

Carter (1951) discussed the interpretation of twin efficiency values along the following lines:

1. The efficiency value determined from a limited sample is only an estimate of a hypothetical population quantity and is therefore subject to considerable sampling variation.

2. The sensitivity of statistical comparison of treatments depends not only on the "error" variance but also on the "degrees of freedom" available. For example, no test of significance at all would be possible with one split twin pair.

3. The possibility of real environment × heredity interaction when different treatments are applied cannot be discounted. This obviously necessitates the use of a reasonable genotypic range of experimental material (certainly more than one set) if dubious extrapolation is to be avoided. Since in a nonrepeated treatment-experiment, the treatment × heredity interaction is inevitably included in the "error," the estimate of error variation from a uniformity trial may well underestimate the error appropriate to treatment comparisons, with consequent overestimation of E.

4. It is probable that the genetic variability in a sample of monozygotic twins would be greater than that in a sample from a particular herd or farm. This would imply that the numerator used in the formula for "E" (*i.e.*, between-set variance) was in fact too high, leading again to an overestimate. On the other hand, the fact that such a twin sample was representative of a wide population should justify increased confidence in generalization of experimental results.

5. Problems of wastage also decrease the usefulness of monozygotic twins below the level indicated by the twin efficiency values. If one member of a set of twins dies or becomes useless for experimental purposes it is obvious that in most cases a co-twin must be excluded.

6. An efficiency value can only be related to a population which is clearly specified—in regard, for example, to age of animals and length of standardized lactation period.

With due regard to the above qualifications, the twin efficiency values

are nevertheless a useful concept in indicating, for most characters, the marked experimental superiority of twins over ordinary cows. It is important to recognize, however, that twins are more useful in some types of investigation than in others. To take an example: monozygotic twins would appear highly efficient in trials involving the measurements of the blood levels of magnesium or phosphorus but seem little superior to randomly selected animals in regard to investigations on calcium or sugar levels.

VII. Heritability Studies

The relative importance of inheritance and environment on the expression of any characteristic is conveniently represented by the coefficient of heritability (h^2), defined as the proportion of the total variance directly due to genetic differences between individuals in a specific population. As a measure of the accuracy with which the genotype of an animal can be identified from its phenotype, the value of h^2 plays an important part in predicting the merit of selection and breeding systems and the genetic progress expected.

In the past, practically all investigations on the genotype-environment complex in dairy cattle have been based on data obtained from field surveys. This approach has been necessitated by the lack of suitable experimental material. The collection of reasonable numbers of monozygotic cattle twins, however, has made possible a direct experimental approach to the problem. In "split-twin" experiments (as has already been mentioned on p. 164) the genetic variation can be eliminated from treatment comparisons, while the environment can be varied at will. The analysis of a twin experiment allows a partitioning of the total variation into the parts ascribable to genotype, to environment (treatment), and to genotype-environment interaction + error, respectively. The heritability coefficient derived from such an analysis differs from most previously calculated coefficients in that it relates to the total genetic variance—*i.e.*, it includes all the epistatic and dominance, as well as additive gene effects. A comparison of the two heritability values should therefore provide an indication of the importance of gene-interactions.

From the results of a growth trial using nine sets of monozygotic twins, Bonnier and Hansson (1946a) drew the conclusion that an animal has at any given time a maximal genetic capacity for growth which cannot be exceeded by increases in nutritional level. This "ceiling" concept, which is also applied to productive characters in dairy cattle, was further elaborated in later communications (Bonnier, 1946d; Bonnier and Hansson, 1948).

Bonnier *et al.* (1948) described two trials with monozygotic twins

which yielded data from which heritability coefficients for growth and production could be derived. The design of the trials is shown below in a simplified form:

		Level of Nutrition	
Trial	*Treatment Group*	*Before Calving*	*After Calving*
1	A	Normal	Normal
	a	Low	Normal
2	A	Normal	12½% above normal
	a	Low	12½% below normal

The average feed consumption from one month of age to calving and the body weights at 27 months of age were as follows:

Trial	*Treatment Group*	*Feed Units*	*% Difference*	*Body Wt.* (kg.)	*% Difference*
1	A	3705	48%	454	25%
	a	1928		342	
2	A	3486	37%	479	18%
	a	2196		394	

In Trial 2 the average feed consumption of the better-fed twins from one month of age to the end of the first lactation amounted to approximately 6250 feed units as compared with 4250 for the poorly fed groups. From this short summary it is clear that the environments to which the twins of the two groups A and a were subjected were widely different. It must also be noted that the genetic worth was probably not allowed to be expressed fully, in that each animal within the groups was given the same preset amount of feed irrespective of its innate ability to convert nutrients into growth and production. Data were presented for body weights and six different linear measurements at three ages (6, 18, and 27 months) before calving, and for milk yields (converted to a 4% fat basis) over the first 36 weeks of calving together with the butterfat, protein, and lactose percentages. Heritability coefficients, in the usual sense of the term, were not computed, but the contribution of heredity, environment, and interaction, were expressed as percentages of the total variation. There was a tendency, under the widely different nutritional conditions of Trial 1, for the hereditary portion of variation in body measurements to increase with age, indicating the decreasing power of environment to mold body proportion as the animal grows older. In general, the results indicated

that the anterior measurements of the body were more strongly under hereditary control than the posterior measurements. A comparison of the magnitude of the error terms derived from a separate uniformity trial, and those found in the Trials 1 and 2 described above, gave no indication that heredity × environment interactions were of importance. The relative contribution of heredity (between-set variation), environment (treatments), and their interaction (error) in the productive traits are shown below.

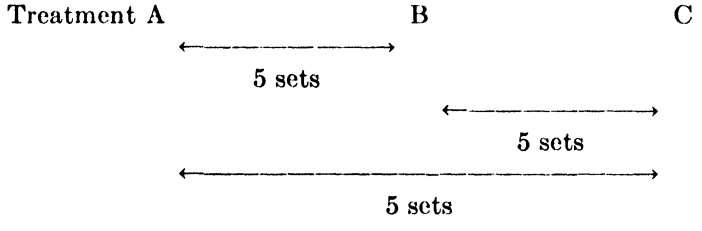
	Milk Yield (Ton. Cal.)	*Milk + Growth* (Ton. Cal.)	*Fat* (%)	*Protein* (%)	*Lactose* (%)	*No. of Lactations*
Trial 1						
Heredity	21	3	81	88	90	1
Environment	14	—	12	6	—	1
Interaction	65	97	7	6	10	1
Heredity	88	91	88	93	43	2
Environment	4	—	3	0	—	2
Interaction	8	9	9	7	57	2
Heredity	90	93	87	89	70	3
Environment	—	—	5	8	—	3
Interaction	10	7	8	3	30	3
Trial 2						
Heredity	70	50	83	82	33	1
Environment	—	12	2	7	—	1
Interaction	30	38	15	11	67	1

From these results the general conclusion was drawn that heredity plays a very prominent role in the expression of all productive characters, and in importance usually outweighs the effects of differences in feeding intensity, even when these differences are large. Especially such qualitative characters as the fat and protein percentages are very strongly inherited.

Robertson (1950) criticized some of these findings mainly on the grounds that the "hereditary" portion of variance (between-set differences) included also noninherited effects such as common prenatal environment, contemporaneity, and similar local environment. Recalculating the data given from Trial 1 (see above), he found that the twin-set × lactation interaction accounted for 66% of the total variation, leaving the between-set differences nonsignificant. By further reasoning, he concluded that it was most likely that the similarity between twin sets

within a lactation was due to the nongenetic causes listed and not to differences between sets in rate of approach to maturity.

Recently Hancock (1953b) concluded a trial in which 15 sets of twins were divided on three treatments in the following manner



As the rations were based mainly on growing grass, all treatments involved free-grazing experimental stock. Within a treatment the animals could thus (in varying degrees, depending on the severity of the treatment) determine their own nutritional levels. The treatments differed partly in that the grazing areas were unequal (in the proportion 10:10:6 for A, B, C), and partly in that the A cows had meal in addition to pasture. The trial lasted for three lactations. In the beginning of the second and third season, approximately one third of the twin sets were replaced. As the main result of this work, heritability coefficients of various productive traits were presented. The coefficients were computed as the between-set variance in percentage of the total variance after the treatment effects had been excluded. The average values for the three seasons were:

	Milk	*Butterfat*	*Casein*	*Butterfat* (%)	*Casein* (%)
Heritability Coefficient (%)	90.3	85.9	88.1	94.6	94.1

It was considered that the main reason for the high heritability values as compared with those found in previous work (Johansson, 1950) was mainly the strict control over environmental factors not common to all members of the herd. As the error term in general was very small, it was concluded that genotype × environment interactions were not important in causing variations in productive characters.

Thoele and Hervey (1952) estimated, from trials with monozygotic twin heifers, heritability coefficients of 86–96% for body weights, and 94–98% for height at withers.

From a uniformity trial involving 13 sets of monozygotic twins, Winzenried and Wanntorp (1948) found that genetic factors strongly influenced the vitamin A and carotene levels of the milk.

Jarl (1946) noted that although there was no significant difference in the ascorbic acid (Vitamin C) content between sets of twins, the differences within dizygotic twin sets were twice as great as the differences within sets of monozygotic twins. This was taken as evidence that ascorbic acid content of milk is to some extent genetically determined.

Bonnier and Hansson (1946b) concluded that the values of protein and of lactose attained at fixed fat percentages in milk are mainly determined by hereditary factors. This conclusion was based on investigations involving 19 sets of monozygotic and 9 sets of dizygotic twins in addition to 2 sets of twins of uncertain zygocity.

A trial, in which it was attempted to measure the effects of different feeding levels on the sexual strength of bulls, was conducted by Bane (1952) with 6 sets of monozygotic bull twins. It was concluded that intensity of feeding influenced principally the rate of physical development, but had only a slight effect on the total sperm production. As between-set variance was twelve times as great as the within-set variance, it was concluded that the sperm-producing capacity of bulls was determined by hereditary factors. Similar studies by James (1953), in which the effects of a great number of environmental factors were studied, yielded much lower estimates for the heritability of spermatic characters.

It is obvious that many trials from which heritability estimates have been attempted, suffer from certain weaknesses in design and scope which make conclusions drawn from them rather uncertain. The following few remarks on the analysis of twin experiments, from the point of view of extracting genetic information, will make this clear.

In uniformity trials (if not time-repeated) only two sources of variation can be distinguished: between-set differences and within-set differences. The between-set variance contains not only all genetic effects (additive as well as those due to dominance and epistasis) but also effects of different prenatal and other contemporary environments. This term also includes any genotype × environment interaction present. Heritability estimates based on uniformity trials are therefore likely to be too high.

In split-twin experiments it is, on the other hand, possible to distinguish between three sources of variation: between-set variance, treatment variance, and error. The first term contains, as in uniformity trials, all genetic variation and that due to common prenatal and contemporary environments. The genotype × environment interactions, however, are included in the error term. If the twin sets used are all of the same age and have been brought up together, it is possible, in a split-twin experiment, to separate from the hereditary portion of variance all other non-genetic influences except those due to difference in prenatal environment.

A comparison of the relative magnitude of within-set differences in uniformly treated mono- and dizygotic twins could provide an estimate of nonadditive gene effects. However, owing to the fetal anastomosis of cattle twins, it is possible that dizygotic twins are more alike than they should be on the basis of their common prenatal environment and the 50% common genes they possess. In view of these considerations, the ideal trial, designed to gauge the relative importance of the various factors which may cause variations in a character—*i.e.*, additive and nonadditive gene effects, pre- and postnatal environment, genotype × environment interactions, and not easily controllable environment (error)— would be planned on the basis of the use of adequate random samples of both monozygotic and dizygotic twin sets and an equal number of full sibs, with the members of the sets distributed over normally occurring environments, together with nonsplit monozygotic twin sets similarly distributed.

The importance of the above sources of variation can be estimated from the comparisons shown below:

Source of Variation	Estimated from
Environment	Systematic variations between members of split-twins and full sibs.
Total gene action + prenatal environment	Variations between monozygotic twin sets.
Additive gene action + prenatal environment	Variations between dizygotic twin sets.
Prenatal environment	Variations between sets of dizygotic twins versus full sib pairs.
Genotype × environment interaction	Nonsystematic variations within sets of split-twins versus variation within uniformly treated twin sets.

In a country such as New Zealand, where calving is strictly seasonal, it would not be impossible to locate contemporary twin sets of both kinds in sufficiently large numbers; suitable full sibs could be obtained from twin cows producing heifer calves in the same season. Whether a trial such as this will ever be carried out naturally depends on the importance of the issues involved.

VIII. Miscellaneous Twin Studies

In the last few years a number of publications have appeared describing work in which monozygotic twins were used as experimental material. Hansson (1946a) attempted to measure the loss of milk yield caused by the destruction of an udder quarter. In another paper (Hansson, 1946b) a case of mycotic intestinal inflammation in a set of monozygotic twins was studied. Hansson and Bonnier (1947) investigated the amount and composition of milk as affected by frequency of milking. The effect of

feeding excessive amounts of calcium and phosphorus to milking cows was the subject for a study by Hansson (1948a). He also investigated (Hansson, 1948b) the effect of premature parturition on udder development and milk secretion. Flux (1950) studied the effects of undernutrition before calving on two-year-old heifers. Hancock (1950c, 1953c, 1952c) summarized a number of investigations on grazing habits and grassland management, in which monozygotic twins had been used. Kelkar and Gullickson (1950) and Gullickson (1950) investigated growth gains in feeding trials with 2 pairs of female and 3 pairs of male monozygotic twins, respectively. Finally, Brundage and Petersen (1952) reported a study with twins on grassland management methods.

Many of the above studies suffered from the disadvantage that too few sets of twins were used.

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The Genetics of the Newer Human Blood Factors*

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I. Introduction

The recent contributions on individual differences in human blood were made possible by the discovery that isoimmunization may result not only from blood transfusion but more significantly also from placental transfer of fetal blood into the maternal circulation, resulting in hemolytic disease in the infant. Prior to 1940, the only blood factors available for genetic study were the four blood groups (OAB), the less well-defined subgroups of A (A_1A_2), the MN factors, P, and the secretor (Se–se) status of the OAB factors. Since 1939, the following blood factors were discovered, Rh–Hr and their variants, Kell–Cellano, S–s, Fy^a–Fy^b, Le^a–Le^b, Jk^a–Jk^b, Lu, and a series of "family" or "private" factors.

Any one of the terms blood factor, blood group, antigen, or more specifically agglutinogen refers to a property of the red blood cells which is produced by a gene capable of expressing itself either in single or double dose. The blood factor is demonstrable by agglutination, more rarely by hemolysis, of the red blood cells when allowed to react with a serum containing a specific antibody. A more detailed definition of blood factors in terms of their antibodies is given below.

A list of the better known human blood factors or antigens which determine at least nine fixed points on the chromosomes is shown in Table 1, which at the same time gives the possible number of combinations of both genotypes and phenotypes. The resulting individuality or multiplicity of blood group combinations is discussed further on page 220. For linkage studies several more points are available, *i.e.*, sex, ability to taste phenylthiocarbamide, sickling, and thalassemia. The "private" or "family" blood factors characterized either by absence or presence of the corresponding genes in members of only particular families are not included.

1. *Terminology of Blood Factors and Genes*

The discovery of all blood factors and their mode of heredity resulted from studies carried out by serologists who assumed the responsibility for naming not only the blood factors but also their genes, at times with insufficient evidence, as in the case of Le^a and Le^b of the Lewis system. Genetic usage was already violated in the names assigned to the four blood groups and the MN factors and their corresponding genes. A number of geneticists (Strandskov, 1948: Sinnott *et al.*, 1950) have recommended

terms to replace the obviously objectionable *O*, *A*, and *B*, by i^O, i^A, and i^B, the letter "i" representing "isoagglutinogen." The term "i," however, is not satisfactory, since all the newer blood factors which are genetically independent of A and B are also "isoagglutinogens." In view of the numerous new agglutinogens (factors), the terms Ag^M and Ag^N cannot be accepted as satisfactory substitutes for M and N.

TABLE 1
Individuality of Human Blood
Blood Factors Determining Nine Fixed Points on Human Chromosomes

Symbol	*Name*	*Phenotypes*	*Genotypes*
A_1, A_2, B, O	—	6	10
Se–se, Le^a–Le^b	Secretion, Lewis	3*	3*
M–N, S–s	—	9	10
Rh–Hr	—	18†	36†
K–k	Kell-Cellano	3	3
Fy^a–(Fy^b)	Duffy	2(3)	2(3)
Jk^a–(Jk^b)	Kidd	2(3)	2(3)
Lu^a	Lutheran	2	2(3)
P	—	2	2(3)
No. of possible different combinations:			
With available antibodies		139,968	518,400
Including rare antibodies		314,928	2,624,000

* Minimal values.

† d, and the variant D^u, C^w, and others not included.

If antibodies for factors in parentheses are available, the higher values may be applied. Genetic alleles of Lu^a and P have not yet been discovered.

Another source of confusion is the term S–s for the new blood factors related to the MN factors and the same letters for the secretor status of the O–A–B factors. In this review the latter property will be referred to as *Se* for the secretor gene and *se* for the nonsecretor gene; the letters "S" and "s" will be retained for the factors related to the MN system.

Since almost all of the new blood factors were discovered with the aid of agglutinins present in human sera, there was no other reference point for the identification of the blood factor except the name of the person who yielded the first specific antibody. As an example one may cite Lewis, Kell, Duffy, and Kidd. These names may serve to define the agglutinable blood factor, but they cannot readily be applied to their corresponding genes. In a number of instances such as the Rh and Kell factors, lower case and capital letters of the alphabet were employed, such as D–d, C–c, E–e, and K–k to designate genes later shown to have equal or almost equal dominance. In the naming of other factors and their

genes, this was later corrected by the use of superscripts, *e.g.*, Fy^a and Fy^b for the Duffy factors and Jk^a and Jk^b for the Kidd factors.

In the case of the Rh factor, the writer also used this term, first applied to a heterogenetic relationship of rhesus and human blood demonstrated by experimental animal sera (Landsteiner and Wiener, 1941), for the factor described with the aid of human isoagglutinins; serologically speaking, however, this relationship does not imply identity.

Although a uniform terminology is highly desirable, the corrective measures should be studied by an international committee of geneticists and serologists, perhaps under the auspices of the World Health Organization. The greatest difficulty lies in the genes for the OAB blood groups, since the phenotypes for the classification of recipient patient and donor are so well established for physicians and hospital personnel. It is largely for this reason that no changes will be suggested at present for the OAB blood groups and the MN factors.

2. *Blood Factors as Defined by Their Antibodies*

The several blood factors have varying degrees of importance in causing transfusion reactions or hemolytic disease by transplacental isoimmunization. For any given blood factor, the clinical importance is determined by several characteristics of the corresponding antibody. Genetic studies are simplified considerably if the antibody is potent, as indicated by a high titer, so that there is a sharp differentiation of bloods possessing or lacking the particular blood property. In general, selected isoantibodies produced either by antigenic stimulus of a transfusion or pregnancy with resulting hemolytic disease will be found to be satisfactory for genetic investigations. These have been classified as "warm" immune antibodies in contrast to physiologically occurring low-titered antibodies which do not cause hemolytic disease or transfusion reactions because their optimum activity occurs at 20°C or lower. The activity of the latter antibodies cannot be increased to any great degree by the antigenic stimuli of transfusions or pregnancies. (Although normal anti-A and anti-B react at 37°C all ABO studies, at least in the United States, are made with serum of donors who are immunized by injection of antigenic material.) In addition, there are antibodies intermediate between the two classes which, although initially of the naturally occurring variety, may become potent warm immune antibodies by antigenic stimulation (Levine, 1951b).

In general, blood factors demonstrable by warm immune isoagglutinins are the more useful for genetic studies because presence or absence is clear-cut and the blood factors are not subject to environmental influences. By and large, these factors have full expression in the newborn

and indeed in fetal blood, in striking contrast to those defined by antibodies active only at low room temperature.

The several differentiating features of these three varieties of antibodies and the factors defined are summarized in Table 2.

The reagents specific for the majority of the blood factors are derived from human sources, so that the supply of many of the antibodies is necessarily limited and in several instances only one or two examples of

TABLE 2
Properties of Intragroup Isoantibodies

	Naturally occurring	*Intermediate*	*Warm immune**
Optimum temperature	18°–20°C	20°–37°C	37°C
Titer	Low	Intermediate	High
Antigenic response	Weak, rare	Weak, occasional	Distinct
Positive antiglobulin test	Negative	Occasional	Always†
Clinical importance			
1. Transfusion	None	Rare	Important
2. Hemolytic disease	None	Not proved	Important
Factor in newborn blood	Not fully developed	Intermediate?	Fully developed
Use in paternity disputes	None	Selected sera only	Very useful
Blood factors	A_1–A_2, M, N, S, Le^a–Le^b, Lu^a, P	Le^a, M, N, S, Lu^a	Rh–Hr, K k, S–s, Fy^a–Fy^b, Jk^a–Jk^b, M, N (rare), Tj^a(?)

* Includes rare sera containing hemolysins.
† With incomplete antibodies.

the particular antibody are available. Unfortunately, none of the blood factors, aside from A, B, M, N, and very few others, is antigenic in the experimental animal.

3. *Gene Frequencies and Population Genetics*

With the discovery by Hirszfeld and Hirszfeld (1919) that the phenotype frequencies of ABO blood groups show striking differences in various racial groups, a new field of investigation was opened up. It is now evident that a more accurate description of these racial differences can be given in terms of the more fundamental gene frequencies, as first shown by Bernstein (1925). The earlier data on the gene frequencies *O*, *A*, *B*, and *M* and *N* in numerous population groups were summarized by Boyd (1939) and by Wiener (1946).

In a sense the various racial groups are Mendelian populations which in their reproductive life share a common gene pool. With a sufficient

number of genes it should be possible to accumulate data on movements of populations and to detect their admixtures. In his recent book, Boyd (1950) emphasizes the increasing importance of human genetics, which, by virtue of its extension to include population genetics, offers simpler and more accurate methods than were hitherto available to physical anthropologists, who were limited to the study of phenotypes determined by polygenes of uncertain heredity and subject to environmental influences.

A powerful stimulus for the comprehensive analysis of gene frequencies of all available racial groups was provided by the recent discovery of many new hereditary blood factors. A sufficient interval has not yet elapsed, but the very first exploratory and limited studies have already revealed striking differences for almost all blood group systems. In several instances, the genetic theory as derived from family studies was confirmed by analysis of gene frequencies in the several population areas showing difference in their gene frequencies. Many of these studies were carried out by Mourant and his co-workers, and the findings to date will be found in his forthcoming book (Mourant, 1954). Space does not permit more than brief reference to the more significant findings for each of the blood group systems.

II. The OAB Blood Groups and Their Variants

This discussion will be limited to the more recent findings which reveal a rather unexpected complexity of the OAB blood groups. From the outset it may be stated that the recent data do not invalidate the concept of three allelic genes *O*, *A*, and *B* which form six genotypes *OO*, *OA*, *AA*, *OB*, *BB*, and *AB* but only four phenotypes (blood groups). The gene frequencies of *O*, *A*, and *B* were determined by application of the Hardy-Weinberg law in the statistical analysis of a single generation of highly heterozygous populations. From the value of $(O + A + B)^2$, the incidence of the six genotypes may be derived as well as the frequency of the various matings and the offspring to be expected.

Reference will be made to attempts, as yet unsuccessful, to differentiate serologically *OA* from *AA* and *OB* from *BB* by means of an antibody believed to be specific for the *O* gene. In general, it may be stated that the newer developments deal more directly with detailed serologic findings obtained with agglutinins which are poorly reactive, but which are the only ones available. Nevertheless, these findings have rather significant genetic implications.

1. *The Subgroups of A*

The first observation on isoagglutinins which reacted with group O bloods were made in studies of the subgroups of A, called A_1 and A_2

(Landsteiner and Levine, 1929). Reagents for the differentiation of A_1 and A_2 are derived from rare atypical agglutinins in certain individuals of groups A_1 and A_1B whose serum contains an agglutinin originally called anti-A_2 and in others of groups A_2 or A_2B whose serum contains anti-A_1. (The latter antibody, which is almost always present along with anti-A in sera of groups O and B, may also be prepared by absorption with red cells of subgroup A_2.)

On testing bloods of all groups with anti-A_1 and anti-A_2, it was found that anti-A_2 agglutinates group O bloods even more distinctly than bloods of subgroup A_2 ($A_{2'}$ and A_2B). Furthermore, a small number of bloods which could not be readily classified as either A_1 or A_2 are intermediate in their reactions, as shown in Table 3, but the actual state of affairs is

TABLE 3
The Subgroup of Groups A and AB

		Tested at 18°C			
	Present in serum of subgroups	*Reactions with red blood cells*			
		A_1	Intermediate	A_2	O
Anti-A_1	A_2 (rare), A_2B	++	±	0	0
Anti-A_2*	A_1 (rare), A_1B	0	±	+±	++

* Formerly called anti-O, now known as anti-H.

far more complicated. A fuller discussion of this aspect is given by Wiener (1946) and Race and Sanger (1950).

2. *The Basic Substance H*

Subsequently, anti-A_2 was referred to in the literature as anti-O and more recently as anti-H, the letter "H" referring to heterogenetic material present in almost all human red blood cells but far more readily demonstrable in secretors independent of their ABO groups; it is also widely distributed in animals (*e.g.*, pig and horse gastric mucosa). The H substance is considered as a basic blood group property which is assumed to be altered in a stepwise fashion by a variety of transitional or mutant genes toward *A*, *B*, or *O* and with complete loss of H substance to "complete" A, B, or O, *i.e.*, *Ac*, *Bc*, or *Oc*. It is assumed that the action of most genes for A, B, and O results in the production of variable amounts of H substance. These views, first advanced by Hirszfeld and Amzel (1940a, b) and later modified by Morgan and Watkins (1948), are diagrammatically represented in Fig. 1, which indicates transitional forms of the A factor only.

Unexpected confirmation of these views was supplied by Bhende *et al.* (1952), who described three individuals, natives or residents of Bombay,

whose red blood cells belong to group O, but whose serum contained in addition to anti-A and anti-B a potent *naturally occurring* antibody, anti-H, which agglutinated every one of 2600 random group O bloods but failed to react with their own or with one another. It was concluded that the three individuals are genetically *OcOc* and that the occurrence of anti-H in their sera is just as normal for them as the normal distribution of anti-A, anti-B, and their simultaneous presence in individuals of group B, A, and O, respectively. In short, the complete absence of the basic substance H in bloods *OcOc*, their secretions, and the concomitant

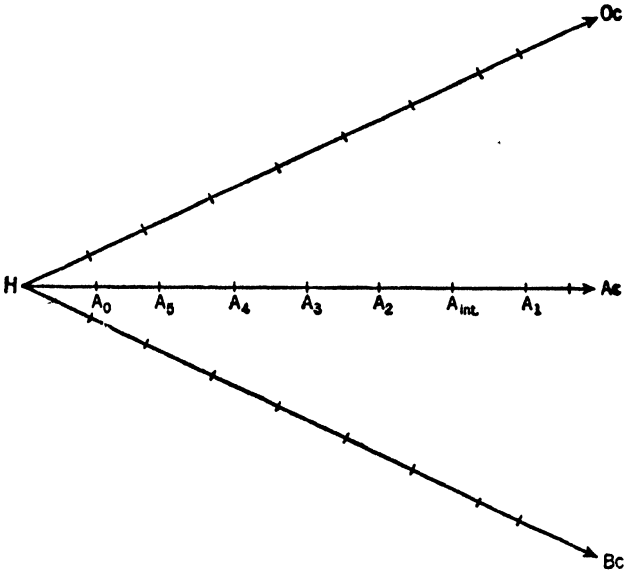


FIG. 1. Incomplete mutations from the common substance H. Modified after Morgan and Watkins (1948).

anti-H in their sera (specifically inhibited by H substance) are genetically controlled by a new allele *Oc*.

It is of interest that in one of the three Indians there was a history of cousin marriages so that the gene *Oc* was transmitted by each of the two parents, who may be assumed to be heterozygous for this gene. Apparently the heterozygous form is not serologically demonstrable. It is possible that this type of blood, though rare, may be indigenous to India, but it has never been observed elsewhere in thousands of bloods tested in the numerous blood transfusion services. As will be shown below (p. 217), blood *OcOc*, although superficially resembling another blood property Tj^a (Jay factor), is serologically different, since anti-Tj^a is not inhibited by H substance.

In addition to the occurrence of A_1, A_2, and the intermediates, further support for the concept of a series of transitional forms of the A factor is

found in the reports on still other varieties identified as A_3, A_4, A_5, which, as a group, may be classified as weaker forms of A_2, at least in terms of reactions with standard anti-A. Apparently the transitional varieties A_2 to A_5 have increasing amounts of the basic H substance. These relationships are presented in Table 4, which implies also the existence of similar

TABLE 4
Diagrammatic Representation of the Transitional Forms of A

	Tested at 18°C							
Human sera	A_1	*int.*	A_2	A_3	A_4	A_5	A_0	O
Anti-A_1	+++	+	0	0	0	0	0	0
Anti-H	0	±	+	+s	++	++	+++	+++

Tests with experimental anti-H produced in animals may be made at room temperature.
int. = intermediates.

transitional forms of the B factor, as indeed was suggested also in findings with more potent anti-H derived from animal sources (Grubb, 1949).

The genetic significance of these observations lies in the fact that the occurrence of the very rare forms A_3, A_4, A_5, and A_0 is limited to certain families (Estola and Elo, 1952; Dunsford, 1952; Grove-Rasmussen *et al.*, 1952). It is assumed that these several antigens are determined by a continuous series of allelomorphs which produce not only A but also variable amounts of the basic H substance.

The importance of these rather complicated relationships with anti-H and the recently revived anti-O depends upon their usefulness in the serologic detection of gene *O* in the differentiation of *OA* from *AA* and *OB* from *BB*. Anti-O was applied once more by Boorman *et al.* (1948) to an antibody which in contrast to anti-H is not neutralized by H substance, and was believed to be specific for gene *O*. Although the results were highly suggestive, the so-called anti-O as well as anti-H may also be claimed to be specific for genes A_2 and its variants. In short, there is at present no evidence for the existence of an antibody which will detect gene *O* by direct agglutination (Race and Sanger, 1950). As for the two varieties of antibodies with identical specificities, Sanger (1952) has shown that, by and large, anti-O and so-called anti-H are produced by secretors and nonsecretors of H substance, respectively.

3. *Genetics of the Subgroups*

In large part, these complicated relationships indicate that our knowledge of the OAB groups is far from complete. Nevertheless, those facts which are established, such as a new rare allele *Oc* found three times in Indians, and gene A_1 and A_2 instead of *A* do not invalidate the Bern-

stein theory. With only the frequent varieties taken into account, there are four genes, six phenotypes, and ten genotypes, as given below:

	Genotypes	
Phenotypes	Homozygous	Heterozygous
O	*OO*	
A_1	A_1A_1	A_1O and A_1A_2
A_2	A_2A_2	A_2O
B	*BB*	*BO*
A_1B		A_1B
A_2B		A_2B

An analysis of studies of 1068 families and 3134 offspring taken from the literature and analyzed by Wiener (1946) confirms the theory that genes A_1, A_2, and B are dominant over O, and A_1 is dominant over A_2. Exceptions to the rules of heredity were attributable to illegitimacy or to difficulties in classifying certain blood (intermediate) as A_1 or A_2.

The rare variants of A_2, *i.e.*, A_3, A_4, A_5, and A_o are also inherited and the occasional family studies indicate that their dominance is proportional to their degree of agglutinability with potent anti-A, the more sensitive forms being dominant over less reactive forms, *i.e.*, A_2, A_3, A_4, A_5 (Race and Sanger, 1950).

In performance of tests in cases of disputed paternity, an exclusion should be based only on violation of the three-gene theory O, A, and B. A decision of nonpaternity is generally not made if there is a violation of the heredity rule involving the A_1–A_2 relationship only. The reason for this lies in the existence of types intermediate between A_1 and A_2, in the failure of full expression of the A_1–A_2 variation in the newborn, and more specifically, in the fact that the reagents for OAB differentiation are far more potent and give much clearer definition than do those available for A_1–A_2 differentiation (Davidsohn *et al.*, 1952).

4. *Racial Distribution of ABO Genes*

In western Europeans the values for genes O and A are about 65% and 25%, respectively. Higher values for O, up to 75%, are found among peripheral peoples such as Basques, and Icelanders and also in parts of Ireland and Scotland. By and large, higher values for A are found in western Europe, but its distribution is patchy. Gene B is highest in parts of Asia and diminishes as one goes west, and it is assumed that the occurrence of B in western Europe is the result of admixture with Asiatics. It is of interest that among Europeans B is lowest in Basques, who apparently escaped admixtures with Asiatics.

The highest values of O, up to 99%, are found in American Indians, but another group (Blood and Blackfeet) as well as Eskimos have both

A and *O* genes, about 50% for each. Among Australian aborigines the values of *O* and *A* are 65% and 35%, respectively. Africans also have high frequencies of *O*, with moderate amounts of *A* and *B*. For a fuller discussion the reader is referred to Boyd (1950) and Mourant (1954).

The highest values for A_2 are found in Lapps of Norway and Sweden. A_2 and intermediates are also frequent in Africans and less so in all Europeans. Among Asiatics, American Indians, and Australians, A_2 is absent.

III. Secretor Variation Se–se and the Lewis Blood Factors

The A and B factors are present not only as properties of red blood cells, but they are demonstrable also in tissue cells such as spermatozoa and liver. In about 80% of all individuals of groups A, B, and AB, A and B substances are present in a soluble form in saliva, seminal fluid, gastric juice, the fluid of ovarian cysts, etc. These individuals are known as secretors "Se" in contrast to nonsecretors, "se," in whom the soluble A or B substances cannot be demonstrated. Although referred to in the literature as "S" or "s," the terms "Se" and "se" will be employed here to designate the secretor variation because subsequently the letters "S–s" were assigned to factors genetically related to M and N. Almost all the studies were carried out with saliva, which contains high concentrations of active substances and offers readily available material. More recently Morgan and Van Heyningen (1944) found still greater activity in pseudo-mucinous ovarian cyst fluid.

The unexpected observation was made by Grubb (1948) that the red blood cells of almost all nonsecretors react with a naturally occurring agglutinin which defines the Lewis blood factor. As was to be expected, this relationship indicated a close genetic relationship of Se–se variation and the Lewis factor.

1. *Se–se Variation of ABH Substances*

The early studies were limited to Se–se variation of the A factor in group A or AB and B factor in groups B and AB. With the availability of potent anti-H specific for bloods of group O (and A_2) derived either from normal eel serum or immune animal sera, it was possible to demonstrate the Se–se variation also in group O. As indicated above (p. 189), this reaction is specific for the H substance secreted in the saliva of group O individuals. Hence, the term ABH substance will be applied to the secretion variation in all four blood groups. It was soon established that the Se–se variation is independent of the A, B, and O factors in the red blood cells, the incidence of Se and se being about 80% and 20%, respectively, in each of the four blood groups.

The technique for the determination of ABH secretion consists of incubation of boiled and clarified saliva with graded doses of the respective antibody, *e.g.*, anti-A, followed by the addition of the specific test cell. For example, failure of agglutination of group A test cells in a mixture of anti-A and saliva of a group A individual indicates specific inhibition by the A substance in the saliva, and the individual is classified as a secretor. If, under the same conditions, the test cells are agglutinated, the A substance is not present in the saliva and the individual is then a nonsecretor.

The incidence of Se and se in several racial groups does not show any striking differences. Aside from one series of American Indians, all of whom were found to be secretors (Matson and Piper, 1947), ABH substances vary from 61% in Negroes of the United States to 74% in Denmark and 86% in Finland. The Se–se variation is inherited, as is shown in the combined study of seven authors comprising 282 families and 774 children (Table 5).

TABLE 5
Tests with Saliva
Heredity of the Secreting Type (Se–se)
Compiled from 7 studies cited by Race and Sanger (1950)

Matings	*No. of families*	*Children* Se	se
Se × Se	156	373	44
Se × se	100	187	99
se × se	26	0	71

These findings confirm the theory of Schiff and Sazaki (1932) that there are two genes *Se* and *se* and indicate the dominance of *Se* over *se*. The homozygosity of the nonsecretor type se is established by the results in the mating of se × se in which all 71 children were nonsecretors, all of whom are of the genotypes *sese*.

In the extensive studies of Hartman (1941), the se type was found in 26% and Se in 74%. Accordingly, the gene frequencies and the three genotypes are as follows:

$$\begin{aligned} se &= \sqrt{0.26} = 0.51 \\ Se &= 1 - 0.51 = 0.49 \\ SeSe + 2Sese + sese &= (0.49)^2 + 2(0.49)(0.51) + (0.51)^2 \\ &= 24 + 50 + 26\% \end{aligned}$$

In contrast to two-gene systems (M–N, S–s, or three Rh–Hr factors), the heterozygote type could not be detected directly in qualitative tests because only one reagent is available which detects both genotypes *SeSe*

and *Sese* as one class of secretors. There are indications, however, from the quantitative inhibition studies of Hartman (1941), that, as a group, the homozygous *SeSe* could be differentiated from the heterozygous *Sese*, the former showing greater concentrations of the secretion substance. In a sense, this recalls the double-dose effect of stronger activity by virtue of the additive effect of the two identical genes—a phenomenon which applies also for several genetic systems, MN, Ss, Rh–Hr, etc.

2. *The Lewis Blood Group System*

Mourant (1946) discovered two human sera which agglutinate about 20% of all (adult) bloods tested. The antibodies, which are probably of the naturally occurring variety, do not give maximal agglutination reactions and most of them react more distinctly at room temperature than at 37°C. In rapid succession numerous examples of this antibody were reported by workers in several countries, and it is undoubtedly identical with five examples studied, but not identified by name, by Landsteiner and Levine (1929).

The remarkable observation was made by Grubb (1948) that Lewis-positive (adult) individuals are, with rare exceptions, nonsecretors of ABH substances and apparently of genotype *sese*. It was further shown that individuals containing the Lewis factor in their red cells secrete the Lewis substance in their saliva. The heredity of the Lewis factors can be considered both in terms of the antigens in the red blood cells and, more accurately, in terms of the presence of the factor in saliva.

a. *The Lewis Factors in Red Blood Cells.* Andresen (1947) found that blood of the newborn gives almost 80% positive reactions with anti-Lewis (anti-Le^a) and that in the first few months of life many of these bloods lose their agglutinability until the age 1–2 years, when the adult incidence of 20% is attained. Apparently the red blood cells of the newborn exhibit the Lewis factor (Le^a) in the heterozygous form of Le^aLe', and adult bloods reacting with anti-Lewis are homozygous (Le^aLe^a). Andresen made the further significant observation that Lewis-negative parents may have Lewis-positive offspring. This was also noted by Landsteiner and Levine (1929), who failed to include this observation in their description of their unnamed blood factor.

Still a third observation made by Andresen (1948) was the discovery of an agglutinin which was specific for another blood factor (74% positive reactions) apparently genetically related to the Lewis factor. By agreement with a number of workers, the antibody was designated as anti-Le^b and the factor and gene as Le^b and Le^b, respectively. In tests with both sera only three types of bloods were noted, but a type reacting with both antibodies was not found in the Danish series. This type, however, was

found in a study of both whites and Negroes of the United States which revealed still other differences (Miller *et al.*, 1951). The findings in the three series are summarized in Table 6.

TABLE 6
Reaction of Red Blood Cells (Adults)

Anti-Le^a	*Anti-Le*^{b}	*White**	*White*†	*Negroid*†
+	+	0	1	1
+	0	20	22.3	23
0	+	74	72.3	60
0	0	6	4.3	16
Number of bloods tested		238	300	200
Selection of blood		Group O Danish	Group O U.S.A.	All groups U.S.A.

* Andresen (1948).
† Miller *et al.* (1951).

With certain anti-Le^b sera it was necessary to limit the studies to O and A_2 bloods, because A_1 bloods gave 42% instead of the expected 74%. This suppression of reactivity was attributed by Andresen to epistasis, since the factors A_1 and Le^b are genetically independent.

In the first studies on Le^a and Le^b in red blood cells, it was suspected that *Le*^{a} and *Le*^{b} may be allelic genes, as indicated by their terminology. Subsequent studies described below, based on the more fundamental property of secretion, revealed the presence of a type (about 2%) which lacked both substances. This seemed to exclude an allelic relationship, with the result that the allele of *Le*^{a} was termed *Le*^{l}.

The heredity of Le^a as shown by Andresen (1947) was confirmed in a study by Race *et al.* (1949a), and their findings are presented in Table 7.

TABLE 7
Heredity of the Lewis Blood Factor Le^a—149 Families
Studies of Race, Sanger, *et al.* (1949a) and Andresen *et al.* (1950)

Anti-Le^a	*No. of Matings*	*Children* +	0
+ × +	9	27	0
+ × 0	62	50	100
0 × 0	78	20	150

If Le^a positive individuals (adult) are homozygous, the incidence of gene $Le^a = \sqrt{22.04} = 0.4695$, and its genetic allele

$$Le^l = 1 - 0.4695 = 0.5305$$

Calculation of the frequency of six possible matings and the incidence of the expected children in each mating support Andresen's view that adult Le^a blood is indeed genetically Le^aLe^a.

The expected genotype frequencies would be:

$$Le^aLe^a = (0.4695)^2 = 0.2204;$$
$$Le^aLe' = 0.4695 \times 0.5305 \times 2 = 0.4981;$$
$$Le'Le' = (0.5305)^2 = 0.2814$$

With the use of only one serum, the anti-Le^a, the two types are represented as Le (a+) and Le (a−). In the cross of Le (a−) × Le (a−), there are in terms of genotypes three possible matings and in one of them, Le^{a}Le$'$ × Le^{a}Le$'$—the double heterozygous not detected serologically in adult bloods—one should expect 25% children who are Le^aLe^a and serologically detectable. If we assume a complete correlation of Le^a and *se* genes, the parallel mating of *Sese* × *Sese* is expected to yield 25% *sese* who are Le (a+), although both parents are Le (a−).

b. *The Lewis Antigens in Saliva.* So far as the Lewis factors are concerned, much greater importance should be given to saliva phenotypes than to red blood cell phenotypes. This holds true for both Le^a and Le^b. The striking discrepancy in the two sets of values illustrates the difficulty in presenting a genetic theory (Table 8).

TABLE 8
The Lewis Antigens in Red Blood Cells and Saliva
(After Grubb, 1951)

Anti-Le^a	*Anti-Le^b*	*Red blood cells agglutination*	*Saliva inhibition*
+	+	0	71.5
+	0	18	18.7
0	+	72	8.3
0	0	10	1.5

The saliva inhibition tests are based on 1000 specimens, but the tests with the red blood cells were limited to O and A_1. Of the 15 individuals whose saliva contained neither Le^a nor Le^b, 13 were nonsecretors of ABH substances, and 2 were secretors.

So far as the saliva phenotypes are concerned, the values given above cannot be explained in terms of a series of triple alleles such as Le^a, Le^b, and Le'. Calculation of gene frequencies by the Weinberg-Hardy law, as in the case of the four blood groups, shows that the sum of the three genes = 1.3601. Furthermore, values of 18.7% for Le^a and 8.3% for Le^b cannot possibly result in 71.5% containing both properties in the saliva.

The subsequent attempts to evolve a genetic theory taking into account saliva phenotypes of Se–se (ABH substance) and Le^a and Le^b

saliva phenotypes led to rather highly involved considerations discussed by Grubb and Morgan (1949), Grubb (1951), and Andresen *et al.* (1950). These workers are of the opinion that genes Le^b and *Se* are practically identical, but the existence of rare exceptional individuals of phenotypes Le (a−b−) whose saliva lacks all secretions makes it highly improbable that genes Le^a and *se* are identical. Accordingly, it is assumed that there are two, not necessarily linked loci, with genes Le^a and Le' on the one locus and *Se* (Le^b) and *se* on the other locus. This theory could be readily put to the test if potent anti-Le^a and anti-Le^b were available for sharply defined inhibition tests with saliva (*cf.* Table 2).

3. *The Chemical Nature of the ABH and Le^a Substance*

With the availability of soluble material such as saliva and particularly ovarian mucoid cyst, a number of workers (Morgan, 1950; Kabat and Meyer, 1948) obtained some significant results on the chemical nature of the group specific substances. The remarkable observation was made that A, B, H, and Le^a substances possess very similar physical, chemical, and immune properties. According to Morgan (1950), they are all complex mucopolysaccharides and each yields on mild hydrolysis about 13% methyl-pentose (fucose), and they contain similar amounts of amino acids and glucose in the form of *N* acetylglucosamine. Serologically, however, they are distinct in their specific inhibition with their antibodies, with less than 1% cross-reaction with heterologous substances.

It is not surprising that genes *A* and *B*, which occupy the same locus, determine the production of very similar chemical substance. Even though the H substance does not correspond to that produced by gene *O*, it is of interest that H substance is chemically almost identical to the A and B substances. The genes Le^a and Le^b (*Se*), however, are on different chromosomes or at least behave as such, and yet are responsible for the production of complex carbohydrates which differ remarkably little from the A and B substances. The specific action of the gene apparently is to induce slight changes in a particular portion of the complex polysaccharide which "has a common or basic framework" (Morgan, 1950).

IV. The MNSs Factors

Recent studies reveal that anti-M or anti-N may occur in human sera, but they are only rarely responsible for hemolytic disease or transfusion reactions. Almost all genetic and racial studies are carried out with experimental reagents prepared in animals which give clear-cut definitions of bloods as either possessing or lacking the factors (Landsteiner and Levine, 1928a).

In testing all bloods with anti-M and anti-N, three types are observed, corresponding to genotypes *MM*, *MN*, and *NN*. A type of blood lacking both M and N does not exist. The heredity of M and N factors is determined by two genes of equal dominance *M* and *N* (Landsteiner and Levine, 1928b). Given both reagents, the gene frequencies are determined by the serum of the homozygous plus one-half that of the heterozygous. If only one antibody is available, the gene frequencies may be determined by the square-root method. The double-dose effect was first observed by Landsteiner and Levine (1928a) with anti-N, since bloods of genotype *NN* gave stronger reactions than *MN*.

A weakly reacting form of N, known as N_2, has been described by Crome (1935), and Friedenreich (1936) found it several times in a particular family. Variants of M were described by Friedenreich and Lauridsen (1938) and Jakobowicz *et al.* (1950), but nothing is known of their relationship to each other. In general, these rare bloods are poorly reactive with some anti-M or anti-N sera, but the degree of their activity which is inherited is determined by rare alleles at the MN locus.

Nineteen years later Walsh and Montgomery (1947) discovered a new human isoagglutinin which revealed a specificity different from that of all other known agglutinins, and Sanger and Race (1947) further found that this new antibody split the MN types unevenly. The tendency of S to associate with M and the presumed s (S negative) with N is evident from a study of 364 English bloods cited by Race *et al.* (1949b) and summarized as follows:

	S+	S−
M	20.7	7.0
N	7.3	14.8

A total of 123 families were studied by Sanger *et al.* (1948) and Race *et al.* (1949b) in order to test the alternative theories that *S* is linked with *M* and *N* or that *S* is another allele at the MN locus. The authors adopted the symbols *MS*, *Ms*, *NS*, and *Ns* which may indicate either linked genes or multiple alleles. Although gene *s* was not identified at the time, its gene frequency could be determined by the square-root method. These heredity studies failed to show cross-overs in matings with 82 children capable of revealing it.

An antibody corresponding to the theoretical expectation of anti-s was described by Levine, Kuhmichel *et al.* (1951) in a serum of a mother whose infant had hemolytic disease. The results of parallel tests of bloods of American Caucasoids and Negroids of the United States with anti-S and anti-s are shown in Table 9.

An indicated above, the anti-s serum exhibited the double-dose effect

of the presence of both genes by stronger reactions given by *ss* than *Ss* bloods. To a weaker degree, the double-dose effect applies also for anti-S.

TABLE 9
Reactions with Anti-S and Anti-s
Based on the data of Levine, Kuhmichel *et al.* (1951)

Anti-S	*Anti-s*	*White* (%)	*Negroid* (%)	*Genotype*
+±	0	12.18	2.76	*SS*
+	+	46.46	28.96	*Ss*
0	++±	41.36	68.28	*ss*
	No. studied	(353)	(145)	

The double-dose effect for differentiation of the homozygous from the heterozygous was more distinct with anti-s than with anti-S.

The values of the gene frequencies in these two racial groups are given below:

	Caucasoid		Negroid
	English	U.S.	U.S.
Frequency of gene *S*	33.7	34.9	16.6
Frequency of gene *s*	66.3	64.3	82.7

The frequencies of the three genotypes in the English population are:

$$SS = 11.356$$
$$Ss = 44.685$$
$$ss = 43.960$$

From these values, it was possible to calculate, prior to the discovery of anti-s, the expected frequencies of the six possible matings and of the children to be expected in each mating. The matings are listed in Table 10, which serves as a model for the study of the heredity of blood factors

TABLE 10
The Expected Frequencies of the 6 Types of Matings and Children Expected After Race, Sanger, *et al.* (1949b)

Mating Type	Frequency	*Children* *SS*	*Ss*	*ss*
SS × *SS*	0.0129	0.0129		
SS × *Ss*	0.1015	0.05075	0.05075	
Ss × *Ss*	0.1997	0.04992	0.09985	0.04992
SS × *ss*	0.0999		0.0999	
Ss × *ss*	0.3929		0.19645	0.19645
ss × *ss*	0.1932			0.1932
	1.0001	0.11357	0.44695	0.43957

produced by two genes of equal dominance. The observed values in the later study of Sanger and Race (1951), which included tests with anti-s, are in complete agreement with the expected.

Considered together there are four genes or chromosomes and their incidence in the white population of England is as follows:

$$MS = 25.6 \qquad NS = 8.1$$
$$Ms = 27.9 \qquad Ns = 38.4$$

These four genes can combine to form 10 different genotypes with 55 different matings, *i.e.*, 10/2(10 + 1). Only 55 families and 123 children were studied with antibodies for four factors, and this limited material included 5 families capable of revealing crossovers, but none were found.

As already mentioned, the terminology of the genes at the M, N, S, s locus or loci is not satisfactory. The terminology suggested by Wiener (1952), which is based on the alternative theory of multiple alleles, can only add to the confusion. Another difficulty was the use of *S* to signify genes for the new factor related to MN and for the secretor of ABH substances. This source of confusion can be circumvented by assigning the terms "Se" and "se" for the secretor and nonsecretion genes.

It is of interest that the linkage relationship of S with M and N, which was readily detectable in an English or American white population, is not quite so evident in testing other racial groups, as shown by Miller *et al.* (1951).

The claim by Snyder *et al.* (1947) that the gene for sickle-cell disease is linked with those for M and N could not be substantiated in the studies by Waller *et al.* (1952), which included also tests for S. Although their findings confirmed linkage of S and MN, there was no evidence of linkage with sickling.

Still other blood factors, "Hunter" and "Henshaw," were found to be related to the MNSs system. The Hunter factor was discovered by Landsteiner *et al.* (1934) with the aid of agglutinins prepared in rabbits injected with the blood of a particular Negroid individual, "Hunter." The blood factor is associated mainly with Negroid bloods containing the factors Ns. Under similar experimental conditions the other blood property, Henshaw, was discovered by Ikin and Mourant (1951). Preliminary studies indicate that Henshaw occurs more frequently with NS. At this stage it appears that the genes for Hunter and Henshaw are each closely linked with MNSs, but it is not yet known whether or not they are allelic to each other (Chalmers *et al.*, 1953).

1. *Racial Distribution of MNSs*

The frequencies of *M* and *N* do not vary as widely as do those of *ABO* and *Rh*. In western Europe *M* has a frequency of about 53% and it

increases eastward with values of 60–65 in most of Asia. *M* is highest in Eskimos and American Indians (up to 90%) and is lowest in Australian aborigines (16–20%), Ainus, and Pacific Islanders.

Reference was already made to the tendency of *S* to be combined with *M* in the several European races studied. To a lesser degree this is true for Chinese and Negroes (Miller *et al.*, 1951). The reverse relationship holds for New Guinea natives with values for *MS* and *NS* as 5.2% and 8.4%, respectively. Although New Guinea natives and Australian aborigines have similar *M* frequencies (20–26%), the two populations differ strikingly in tests for *S*, which is entirely lacking in the Australian aborigines, whereas New Guinea natives have 13% *S* (Sanger *et al.*, 1951). Reference was already made to lower incidence of *S* in American Negroes as compared to whites, *i.e.*, 34% and 16%, respectively (*cf.* Neel and Hanig, 1951).

The genes for the new factors "Hunter" and "Henshaw" have been found in some West Africans and American Negroes, and they are very rare in Caucasoids (Mourant, 1954).

V. Rh–Hr Blood Factors

The clinical significance of the Rh factor (D or Rh_o) was established by the observation that more than 90% of mothers with infants suffering from hemolytic disease are Rh-negative in contrast to about 15% Rh-negative in the white population (Levine *et al.*, 1941). The lower incidence of hemolytic disease in Negroes and its almost complete absence in Chinese was shown to be directly correlated with the incidence of Rh-negative individuals in these two racial groups, *i.e.*, 5% and less than 1%, respectively (Levine, 1942b).

Because of its bearing on several borderline sciences, rapid strides were made in our knowledge of the Rh factor, particularly in its clinical and genetic implications. In the interval since the discoveries of the Rh factor itself and of the phenomenon of transplacental isoimmunization, thousands of publications on the subject have appeared in periodicals throughout the civilized world. In this survey the bibliography must necessarily be limited to selected original and essential contributions. For a complete and excellent review of the subject the reader is referred to Race and Sanger (1950).

1. *The Three Pairs of Rh–Hr Factors and Their Terminology*

The early studies of immune isoagglutinins produced mainly by Rh-positive mothers of affected infants revealed the serologic and by the same token the genetic complexity of the Rh factor. In this manner two other serologically different Rh factors were discovered as well as addi-

tional blood properties, Hr factors, which were obviously genetically related to the Rh factors. In short, there are three main Rh factors and three corresponding Hr factors, the term Hr indicating an allelic relationship to Rh (Levine, 1942a, 1946). The serologic relation of the three sets of blood factors as defined by their antibodies are given in Table 11, along with two terminologies in current use.

TABLE 11
The Three Pairs of Rh-Hr Factors

Alternate terminology		*% Reactions with red blood cells* +	0	*Incidence of elementary genes*	
Anti-D	Anti-Rh_0	83.2	16.8	*d*	41.03
Anti-d	Anti-Hr_0	65.2	34.8	*D*	58.97
Anti-C	Anti-rh′	67.8	32.2	*c*	56.73
Anti-c	Anti-hr′	81.3	18.7	*C*	43.27*
Anti-E	Anti-rh″	28.7	71.3	*e*	84.46
Anti-e	Anti-hr″	97.6	2.4	*E*	15.54

The above values based on a study of an English population by Race, Mourant, *et al.* (1948) were employed for determination of the Rh chromosome frequencies (given in Table 14). The incidence of positive and negative reactions does not differ significantly for a Caucasoid population in the U.S.
* Includes 1.29% of gene C^w, another allele at the *C* locus.

Each of the sets of factors is inherited on the basis of a pair of alleles which form three phenotypes corresponding to three genotypes. In the above table, the positive reactions include both the homozygous and heterozygous types. The two nonreacting types are each homozygous, so that the incidence of heterozygotes may be derived by the formula of 100 minus the sum of the two homozygous types.

Given only one of the antibodies, *e.g.*, anti-E, the frequency of gene *e* may be derived as the square root of the 71.3% nonreactors or 84.46% and the calculated value for gene *E* is 100% minus 84.6% or 15.54%. With the use of both anti-E and anti-e, the gene frequencies may be determined by the sum of the homozygotes plus one-half of the heterozygotes. These relationships are presented in Table 12, which illustrates also the double-dose effect, as indicated by stronger agglutination reactions given by homozygous bloods. The double-dose effect, which can be determined quantitatively by the scoring method of Lawler and Race (1951), is not always as distinct as represented in Table 12 for illustrative purposes (*cf.* Mourant, 1947a).

The two terminologies in current use reflect two different theories regarding the heredity of the Rh–Hr factors. Very early in the course of

the genetic studies, Wiener (1943) excluded linkage and suggested a series of multiple alleles at one locus, although at this time one Hr factor, c, was already known, and Fisher (*cf.* Race, 1944) predicted the existence of antibodies for the two remaining factors, d and e, which were later discovered by Haberman *et al.* (1948) (*cf.* Hill *et al.*, 1950) and Mourant (1945), respectively, with the expected frequency of 63% for anti-d and 97% for anti-e. Only two examples of anti-d were reported, but their

TABLE 12
Reactions with Anti-E and Anti-e*
The double-dose effect (diagrammatic)

Anti-E	*Anti-e*	*Genotype*	*Incidence*
++	0	*EE*	2.4
+	+	*Ee*	26.3
0	+++	*ee*	71.3
28.7	97.6	Incidence of positive reactions	

The double-dose effect is more readily demonstrable with anti-e than with anti-E, and also with anti-c than with anti-C (*cf.* Mourant, 1947a).
* Based on data of Table 11.

demonstration was made difficult because in each case the serum also contained anti-c.

In terms of available antibodies the stepwise development of the linkage theory is outlined below, and it is evident that as early as 1943 it was rather premature for final commitment to any genetic theory:

Landsteiner and Wiener	1941	D		
Levine *et al.*	1941	D	C c	
Wiener	1943	D	C	E
Race and Taylor	1943	D	c	
Race *et al.*	1943	D	c	E
Race *et al.*	1944	D	C c	E
Mourant	1945	D	C c	E e
Haberman *et al.*	1948	D d	C c	E e

The contrasting genetic theories are illustrated below:

Multiple alleles		Linkage
R^o, R^1, R^2, R^z	r, r', r'', r^y	*D* or *d* *C* or *c* *E* or *e*

The question at issue is that of multiple allelism or linkage (pseudo-allelism) and as in other examples in experimental genetics, the two theories are practically indistinguishable if the linkage is very close. In favor of the linkage theory is the reciprocal relationship of the anti-

bodies for D–d, C–c, and E–e, which is quite analogous to that existing in the MN factors with two genes M and N and three genotypes. The linkage theory requires that a single serologically detectable reaction indicates a blood factor with a single pair of alleles whereas in terms of multiple alleles several blood factors may be the product of changes (mutation) in a single gene.

2. *Rh Phenotypes and Genotypes*

On testing all bloods with any two anti-Rh sera such as anti-D and anti-C, the four possible types are observed, but the 83% containing the factor D are unequally split by anti-C into 67% and 16% and the 17% of bloods with factor d, into 2% and 15% (*cf.* Levine *et al.*, 1941). If all bloods are tested with the three anti-Rh sera, eight types result and on including tests with anti-c, the four types containing the C factor are subdivided into eight classes, thus yielding twelve types. With the added use of anti-e, there are an additional six types and the resulting eighteen types, their incidence, and the most likely genotype are given in Table 13.

TABLE 13
The Rh Genotypes of English People
(After Race, Mourant, *et al.*, 1948)

	Phenotype					*Incidence*	*Most likely genotype**	
	D	C	E	c	e		(Alternate terminologies)	
1.	+	+	0	+	+	34.89	*DCe/dce*	R^1r
2.	+	+	0	0	+	18.51	*DCe/DCe*	R^1R^1
3.	+	0	+	+	+	11.76	*DcE/dce*	R^2r
4.	+	0	+	+	0	2.33	*DcE/DcE*	R^2R^2
5.	+	+	+	+	+	13.34	*DCe/DcE*	R^1R^2
6.	+	+	+	+	0	0.07	*DCE/DcE*	R^zR^2
7.	+	+	+	0	+	0.21	*DCe/DCE*	R^1R^z
8.	+	+	+	0	0	0.00	*DCE/DCE*	R^zR^z
9.	+	0	0	+	+	2.06	*Dce/dce*	R^0r
10.	0	+	0	+	+	0.76	*dCe/dce*	$r'r$
11.	0	+	0	0	+	0.01	*dCe/dCe*	$r'r'$
12.	0	0	+	+	+	0.92	*dcE/dce*	$r''r$
13.	0	0	+	+	0	0.02	*dcE/dcE*	$r''r''$
14.	0	+	+	+	+	0.02	*dCe/dcE*	$r'r''$
15.	0	+	+	+	0	0.00	*dCE/dcE*	r^yr''
16.	0	+	+	0	+	0.00	*dCE/dCe*	r^yr'
17.	0	+	+	0	0	0.00	*dCE/dCE*	r^yr^y
18.	0	0	0	+	+	15.10	*dce/dce*	rr

* For a complete listing of all 55 genotypes based on the added use of anti-C^w and anti-d, *cf.* Table 25 in Race and Sanger (1950).

Using the above values and the most probable genotypes, Fisher (1946, 1947) calculated the "chromosome" frequencies with the aid of the complicated but accurate maximum likelihood method. A simple procedure based on the square-root method has been used by both Race and Sanger (1950) and by Wiener (1950). The chromosome frequencies based on a study of 2000 white individuals in England yielded the values given in Table 14.

TABLE 14
Rh Chromosome Frequencies in England
(After Race, Mourant, *et al.*, 1948)

Alternate Terminologies		*Per cent*
DCe	R^1	42.05*
dce	r	38.86
DcE	R^2	14.11
Dce	R^0	2.57
dcE	r''	1.19
dCe	r'	0.98
DCE	R^z	0.24
dCE	r^y	0.0

* Includes 1.29% of C^w.
Based on these values, the frequency of the 36 genotypes may be readily calculated.

Those who favor the linkage theory refer to the nest of three loci as the "chromosome" and at the same time employ an abbreviated form of eight variants of the letter "R" which corresponds to the eight alleles of the multiple-allelic theory. Another complication exists in the use of American workers of "R" and its variants for the four chromosomes containing "D" and "r" for those not containing "D," *i.e.*, for those with "d." Accordingly, the abbreviated terminology of the chromosomes has been altered to correspond to the names of the genes in the multiple allelic theory. The British workers employ the sequence CDE because of the alphabetic order and the earlier discovery of the C–c locus. The author prefers to use the order of DCE, since this corresponds to their probable arrangement on the chromosome as shown below, and because clinically the D–d variation is most significant in the identification of the blood as Rh-positive or Rh-negative.

It is evident that three of the eight chromosomes in white populations have high or moderately high frequencies which together account for 95% of the chromosomes, while the five rare chromosomes are responsible for the remaining 5%. Fisher and Race (1946) and Fisher (1947) suggested that three of the rarer chromosomes, r'', r', and R^z may result from a single crossing over from the common heterozygote. In each case the resulting genotype containing the rare chromosome carries with it

the R^0 chromosome so that the frequency of R^0 should be equal to the sum of the r', r'', and R^z, as shown below:

Common Heterozygote		Crossovers
DcE/dce		*Dce/dcE*
DCe/dce		*Dce/dCe*
DCe/DcE		*Dce/DCE*
Dce	= 2.57%	
dcE + dCe + DCE	= 2.41%	

The chromosome *dCE* (r^y) is rarest because it may result from a single crossover involving the infrequent heterozygous genotype containing the rare chromosome *dCe* (r'), *i.e.*, *DcE/dCe*. This results in the formation of *dCE* (r^y) and again *Dce* (R^0). Families containing one or more members with r^y have been reported by Van den Bosch (1948), Wiener and Hyman (1948), and others.

A second series of 1073 was included in the studies to determine the linear arrangement of the genes on the chromosomes. Fisher (1947) and Race, Mourant *et al.* (1948) believe that the order is *DCE*, as indicated by the ratio of the rare chromosome to the common genotype from which it is derived. In short, the distance of *D–E* is greater than *D–C* or *C–E* and in fact, the value for *D–E* is approximately equal to the sum of *D–C* plus *C–E*, at least for the second series of 1073 bloods. Furthermore, the concept is not contradicted in the first series of 927 bloods (Table 15).

TABLE 15
Evidence for Order on Chromosomes as *DCE*
Frequency Ratio of Crossover Chromosome to Its Derivative Genotype
(After Race, Mourant, *et al.*, 1948)

Rare chromosome	*Produced by crossover from*	*Number of bloods tested* 2000†	1073
dcE	*D–E*	0.108	0.074
dCe	*D–C*	0.030	0.034
DCE	*C–E*	0.021	0.038

† Includes 927 of the first series and 1073 of the second series (see text).

Race and Sanger (1950) point out that these relationships hold for other population groups studied: Mexican Indian, Basque, Japanese, and Australian aborigines. As an example, one may cite the Basques, in whom Chalmers *et al.* (1949) found the most frequent chromosomes to be *DCe* and *dce*. Crossing over from the most frequent genotype *DCe/dce* results in the formation of the rare chromosomes *Dce* and *dCe*, and both were found.

In apparent support of the linkage concept and also the order of the genes on the chromosome as *D*, *C*, and *E*, Race, Sanger, and Selwyn (1951) reported a blood of an unusual genotype which may be represented by *D*––/*D*–– to indicate what they believed to be a small deletion beyond the D locus. In short, the blood cells failed to react with antibodies for the C–c and the E–e factors, from which it was concluded that the C–c and E–e loci were missing. In this British case as well as in two American cases studied by Waller *et al.* (1953) and Levine *et al.* (1953), there was a history of cousin marriages, the parents being heterozygous *D*––. In the absence of evidence that the three individuals are derived from common ancestry, a deletion effect would seem to be excluded. Another objection to the deletion theory is the absence of anomalies frequently associated with deletions observed in experimental genetics. As an alternative, one may assume a mutation to a special allele of *D* which by virtue of its capacity to suppress the action at the C–c and E–e loci over-compensates by producing an excess of serologically demonstrable D antigen in the red blood cells. With the occurrence of several cases in apparently unrelated families, it would seem that D–– is not so rare and perhaps its incidence may be of the same order as that of the very rare *dCE* (r^v).

Wiener *et al.* (1952) cite the blood of a Negroid mother whose red cells failed to react with anti-E and anti-e, but the essential absorption tests and other pertinent data were not presented. The genotype could be represented as *Dc*–/*Dc*–, and simulates the deletion of the E–e locus. In terms of the multiple allelism, the effect was ascribed to a new gene R^{ou} which was present in double dose in the mother and transmitted in single dose to her two children. It is suggested that this gene may not be so rare in Negroids. The question arises whether or not this pseudodeletion determines a compensatory increase of the D or c factors or both. It is also of interest that this observation, if confirmed, is compatible with the order of arrangement of the genes on the chromosome as *D*, *C*, and *E*.

3. *Additional Alleles at D–d, C–c, and E–e Loci*

a. *D–d Locus.* At the D locus another gene, D^u, has been identified which is serologically expressed by variable degrees of weak direct reactions with some but not all anti-D sera. Tests of numerous D^u bloods with a battery of anti-D sera reveal a certain definite series of patterns which are genetically determined. Apparently, there is a continuous series of D^u types, some classified as low-grade and others as high-grade. By their association with other genes *C–c* and *E–e*, D^u has been classified as a variant of *D* rather than *d* so that D^u individuals have been considered as Rh-positive. Theoretically, at least, the lowest grades of D^u may be

considered as having less in common with D and increasing relationship to d (Wiener, 1944; Stratton and Renton, 1948; Race *et al.*, 1948a). In at least two instances, D^u bloods have been observed in patients who actually produced anti-D (Race, 1953; Argall *et al.*, 1953). The situation is such that it suggests a series of stepped-up or additive mutations analogous to those discussed above for the ABO locus. The D^u gene occurs more frequently in Negroids than in Caucasoids.

As already mentioned, the *D*-- chromosome with absence of the C–c and E–e loci may be considered as another allele at the D locus.

b. *C–c Locus.* Three variants have been observed at the C–c locus, C^w, C^u, and c^v. Of these, C^w is the most important both from the viewpoint of frequency and ease of demonstration (Callendar and Race, 1946). In contrast to D^u, an antibody for C^w, anti-C^w occurs, rarely by itself, with resulting hemolytic disease but more frequently along with anti-C. Apparently, the product of gene *C* is capable of inducing formation also of anti-C^w, which reacts with about 2% of all bloods. Pure anti-C^w was shown to exhibit the dosage effect of stronger reactions on C^wC^w than on C^wC, *i.e.*, in chromosomes DC^we/DC^we and DC^we/DCe, respectively (Callendar and Race, 1946).

C^u was identified in the bloods of two members of a family (mother and offspring), reacting with some but not all anti-C sera (Race and Sanger, 1951). The effect is quite analogous to that of D^u, and appears to be rare, but testing difficulties prevent determination of its true incidence.

Race *et al.* (1948b) also described the gene c^v in a father and son whose genotypes were assumed to be Dc^vE/dce. By the pattern of reactions with anti-C, both bloods reacting with the same three out of six anti-C sera, and both exhibiting the double-dose effect with anti-c, the new gene apparently is more related to *c* than to *C*. Another consideration is the greater frequency of *DcE* than *DCE*.

c. *E–e Locus.* In a manner analogous to that of D^u a poorly reacting E factor was found in bloods of 8 members of 3 generations of an Italian family (Ceppalini, 1950) and in 14 members of 3 generations of a Swiss family (Mourant *et al.*, 1952). The factor was called E^u and its gene E^u. As in the case of the D^u factor, an antibody specific for E^u does not exist. Because of its rarity, it is not known whether or not the E^u factor shows the same wide pattern of varying activity with a battery of anti-E sera as exhibited by D^u.

4. *Linkage vs. Multiple Alleles*

Reference was made above (p. 205) to the unequal division of the 85% D (Rh-positive) and 15% d (Rh-negative) in tests with anti-C. Wiener and Wexler (1952) reject the linkage concept for this reason: if

D and *C* were transmitted as separate genes on the same chromosome, then at genetic equilibrium the factor C should have the same incidence in the 85% Rh-positive and 15% Rh-negative groups. Although this concept is applicable to linkage with appreciable crossover rates, genetic equilibrium need not as yet have been attained if genes *D* and *C* are so tightly linked as to show no appreciable crossing over.

Although the analysis of chromosome frequencies in several racial groups offers a satisfactory theoretical explanation for the ocurrence of rare chromosome, crossovers have not been observed in the limited family studies—at least in those matings capable of revealing this effect. The absence of crossover types, however, may be due to the low crossover rate characteristic of closely linked genes (pseudoallelism). Even if such types were to be found in family studies, their occurrence could equally well be explained by mutations. In all instances it would be necessary to exclude nonpaternity as well as nonmaternity. Another difficulty presents itself, since the rate of crossovers may be very low and of the same order as the rate of mutations. It is these facts which led Race (1951) to suggest that the linkage may be so close that one may view the effect as due to three subloci within the boundary of one gene.

As indirect evidence in favor of linkage one may cite the ever-increasing examples of previously suspected multiple-allelic series in which further studies revealed pseudo-allelism (linkage) with very low crossover rates. Among these listed recently by Glass (1951) are the lozenge series and starasteroid loci in Drosophila with crossover rates of 0.06–0.15 and 0.01%, respectively, and also the alleles at the anthocyanin locus in maize, the R flower color locus of cotton, and probably two forms of color blindness in man. Still another example of at least 3 closely linked genes affecting the same developmental processes in the mouse was reported by Dunn and Caspari (1945).

From the viewpoint of multiple allelism, Wiener and Wexler (1952) suggested that, for example, gene R^1 determines the presence of the Rh_1 agglutinogen which consists of three separate blood factors, D, C, and e, which are genetically transmitted *in toto*. To lend credibility to the theory of multiple allelism, it became necessary to redefine blood factors as partial antigens within the same agglutinogen molecule.

Several analogies are discussed by Wiener and Wexler (1952), such as the components of the agglutinogen A_1 which consists of A, A_1, F_A (Forssman antigen), and a partial antigen common to all bloods and perhaps identical with the H substance. A similar state of affairs may be said to exist at the C–c locus for the several variants C^w, C^u, and c^v, and also at the D–d locus for the group collectively known as D^u and the D– effect. Indeed, all these observations as well as some of the findings in

cattle blood (Irwin, 1947) point to a mosaic structure of the agglutinogen, but only in terms of partial antigens which, as a rule, are poorly defined in terms of their antibodies.

These analogies hardly constitute proof that such well-defined blood factors as D–(d), C–c, and E–e can be incorporated as partial antigens defining a single agglutinogen. The fact that the genetic unit, whether it be called a gene or a chromosome, invariably or almost invariably, carries factors determining D or d, C or c, E or e, but never both members of any one pair, implies the existence of three systems of alleles within the complex genetic unit. In other words, the so-called partial antigens in Wiener's scheme follow a distinct and selective pattern so that, for example, the combinations *DCc* or *DEe* do not appear as agglutinogens produced by single genes. The double-dose effect as indicated by stronger reactions of, for example, bloods *ee* than *Ee* in tests with anti-e can be explained only in terms of separate loci.

In further support of their views, Wiener and Wexler (1952) find it necessary to deny the observation of Haberman *et al.* (1948) on the occurrence of anti-d. Even if anti-d were not discovered, this would not constitute proof against separate loci and the occurrence of anti-d on the theory of linked genes would then have to be assumed. Recent studies show that in several instances only one example of a particular antibody has so far been observed, *e.g.*, anti-Fy^b, anti-s, and anti-Jk^b.

Further complexity of the events at the Rh locus or loci is indicated by the very recent description of a new antibody, anti-f, which with rare exception detects chromosomes producing antigens c and e, *i.e.*, *Dce*, *D"ce*, and *dce* (Rosenfield *et al.*, 1953). All other chromosomes, again with some exceptions, have the presumed allele *F*. Sanger, Race *et al.* (1953) believe that the F-f antigens are produced by a fourth allelomorphic pair of Rh genes, *F* and *f*, at a locus beyond *E* so that the order of the genes is *D C E F*. The most frequent chromosomes are *DCeF*, *dcef*, and *DcEF*, and their recombinations after a single crossover from the common heterozygotes produce the exceptional chromosomes. An alternative explanation was offered by Richardson-Jones *et al.* (1953), who suggest that the behavior of the f antigen may be an expression of the so-called "position effect" in the component genes *c* and *e* in the pseudo-allelic series (*cf.* Lewis, 1951).

In conclusion, there is as yet insufficient evidence in support of either the linkage or the multiple-allele theory of the heredity of the Rh–Hr factors; thus there is no urgent need to adopt a single terminology. Indeed both terminologies serve useful purposes, particularly for the identification of the genes or chromosomes both in the abbreviated notations and in terms of the antigens present. For teaching purposes, however, it is

far simpler to present the subject in terms of close linkage at three loci. On the whole, the available genetic evidence along with the characteristic serologic findings points to a complex locus with at least three parts rather than to a single set of multiple alleles.

5. *Clinical Determination of the Genotype of the D Factor*

In favor of the linkage concept is the fact that the heredity of each of the three sets of blood factors may be considered separately. As an example, one may cite the findings in the family studies of the C–c factors, as given in Table 16.

TABLE 16

The Inheritance of Factors C and c in 800 Families Tested with Anti-C and Anti-c (Modified after Race and Sanger, 1950, from 11 studies by various authors)

Matings	*No. of families*	*Children*		
		CC	*Cc*	*cc*
CC × *CC*	26	55	0	0
CC × *Cc*	104	100	124	0
CC × *cc*	144	0	256	1*
Cc × *Cc*	134	69	143	70
Cc × *cc*	275	0	267	223
cc × *cc*	117	0	0	218

* Nonpaternity.

The excess of *Cc* children in the mating *Cc* × *cc* is due to selection of families in which hemolytic disease occurred.

A similar table has been compiled by Race and Sanger (1950) for the E–e factors, but this could not be done for the D–d factors because anti-d is not available. The results indicate that with rare exceptions obviously due to illegitimacy the factors E–e are inherited as single genes *E* and *e*, and in matings D negative × D negative (*dd* × *dd*) tested with anti-D only, all offspring are D negative or *dd*.

These relationships for C–c and E–e are useful for the determination of the genotype of the D factor in the husband of an Rh-negative woman. In the absence of anti-d, the C–c genotype is determined for all individuals of type Rh_1 (DCe), and the E–e genotype for all individuals of type Rh_2 (DcE). If an Rh_1 individual is homozygous for C, he is on a statistical basis also homozygous for D, since the genotype *DCe/DCe* is eight times more frequent than the genotype *DCe/dCe* (Tables 13 and 14). Similarly, if an Rh_2 individual is homozygous for E, his genotype is more apt to be *DcE/DcE* rather than the rare genotype *DcE/dcE*. In some instances an accurate determination of the husband's genotype may be made in tests of the children, if any, and/or the husband's parents and siblings.

The several possibilities of the genotype of a presumably Rh_1 heterozygous husband of an Rh-negative wife (dce/dce) are given below:

Choice	%	Genotype of *D*
1. *DCe/dce* (R^1r)	32.7	Heterozygous
2. *DCe/Dce* (R^1R^0)	2.2	Homozygous
3. *Dce/dCe* (R^0r')	0.05	Heterozygous

An Rh_1 blood containing the factors D, C, e, and c may be of any one of the three genotypes given above so that his chances for being heterozygous for D as well as for C are about 15:1. If one of the children is Rh-negative (*dce/dce*), the husband's genotype must be *DCe/dce*. If there are children of both types, DCe (Rh_1) and Dce (Rh_0), then the husband must be homozygous for D and of the genotype *DCe/Dce*.

If the husband of an *rr* woman is of type Rh_1Rh_2 whose blood contains the factors D, C, E, c, and e, he is on a statistical basis homozygous for D, and his genotype is made up of the frequent chromosomes *DCe/DcE*.

The genotype of husbands of Rh-positive mothers who may be immunized to other blood factors such as E, c, or rarely C and e may be accurately determined because the required antibodies are available.

6. *Racial Distribution of Rh Genes*

A higher incidence of *r* (gene or chromosome) is found in Basques of Argentina and Spain, with values up to 60% in contrast to 40% in most European countries (Mourant, 1947b). Lowest values of *r* are found in Asiatic peoples and American Indians, with as little as 1% or less.

The highest values of R^1 are found among Asiatic peoples and in the Pacific islands, whereas R^2 reaches its peak in American Indians and Polynesians.

The most striking racial difference involves R^0, which attains a value as high as 80% in Negroes in contrast to 1–2% in most European peoples. As shown by Mourant (1951) in a study of the Mediterranean areas those peoples who have been exposed to admixture with Negroes, such as the Egyptian, have intermediate values (16%). As a rule, all peoples with high R^0 have proportional amounts of D^u (D^uce), and this further supports the view that D^u is a mutation from *D* and not from *d*.

As is to be expected, the rare R^z is found in American Indians and Asiatic peoples who have high values for both R^1 and R^2, which may form R^z possibly by crossing over.

For a fuller discussion the reader is referred to Boyd (1950) and Mourant (1954).

VI. The Kell-Cellano Factors (K-k)

The Kell blood factor (K) was discovered by Coombs *et al.* (1946) with the aid of a new antibody which caused hemolytic disease. Anti-K agglutinated about 10% of all individuals tested. On the basis of gene frequencies, the theoretical anti-k was expected to give about 99% positive reactions. An antibody of this sort (anti-Cellano), also responsible for hemolytic disease, was observed by Levine *et al.* (1949), who in tests of 2500 random bloods found 5 which lacked the factor k. All 5 were shown to be of genotype *KK*.

Calculation of gene frequencies in terms of anti-k showed the following: *KK* = 0.2%; *Kk* = 8.6%; and *kk* = 91.2%. On testing five selected families with 23 children in which one of the offspring was *KK* (Cellano negative), it was found that in each case the mating was *Kk* × *Kk*, which has an incidence of 1:135. It was only because of the unusual selection for *KK* that the results in the offspring—*KK* 11, *Kk* 7, *kk* 5—varied from the 1:2:1 ratio expected (Levine, 1951a).

In another mating *KK* × *Kk*, the incidence of which is 1:3333, there were 3 children *Kk* and 3 *KK* in contrast to 0.2% *KK* in a random population. The six selected families would represent the yield from a study of 4000 random matings.

Anti-K has been observed many times in the several continents, but only two additional examples of anti-k were observed, once along with anti-D and therefore useless for genetic studies and more recently as the only antibody in the serum (Shapiro, 1952). Both anti-K and anti-k give the double-dose effect of stronger reactions with homozygous bloods.

From the point of view of hemolytic disease, the Kell factor is statistically just as important as factors E or c of the Rh-Hr system. With availability of anti-k, the genotype of the Kell positive husband could readily be determined.

Gene *K* has an incidence of about 5% in Caucasoids, 1.7% in American Negroids (Miller *et al.*, 1951) and 0.3% in South African Bantu (Shapiro, 1952); and it was not found once in a study of 200 Chinese living in New York.

VII. The Duffy Factors (Fy^a-Fy^b)

The Duffy factor, Fy^a, was discovered by Cutbush *et al.* (1950) with an antibody produced by the antigenic stimulus of repeated transfusions. The term "Fy^a" derives from the last two letters of the patient, Duffy, who produced the antibody. It agglutinated about 66% of all English bloods tested. Numerous examples of anti-Fy^a were subsequently found by workers throughout the world.

On the basis of reactions with anti-Fy^a, the gene frequency of the unknown allele, Fy^b, was derived from the square root of the nonreactors, 0.3401 or 58.32%, and the frequency of $Fy^a = 100 - 58.32$ or 41.68%. The frequency of the genotypes are readily calculated as follows:

$$\begin{aligned} Fy^aFy^a &= (0.4168)^2 &&= 17.37 \\ Fy^aFy^b &= 2(0.4168)(0.5832) &&= 48.62 \\ Fy^bFy^b &= (0.5832)^2 &&= 34.01 \end{aligned}$$

The sum of the calculated genotype frequencies of children in each of the six possible matings is identical with the values given above for the three genotypes in the random population of England (or the United States).

In a combined study of 137 families with anti-Fy^a, Race and Sanger (1952) provide proof that the presence or absence of Fy^a is determined by 2 alleles, one of which, Fy^a, in single or double dose produces the antigen. The expected antibody for Fy^b, which should give about 82.6% reactions, was discovered by Ikin *et al.* (1951) in the serum of a mother whose infant had hemolytic disease. The observed incidence of positive reactions with this antibody was 79% of 100 bloods. In parallel tests of 59 bloods with both antibodies, the three types were distributed as follows:

++	0	11	Fy^aFy^a
+	+	28	Fy^aFy^b
0	++	20	Fy^bFy^b

Both antibodies give the double dose effect as indicated by stronger reactions with the homozygotes. Only one example of anti-Fy^b has thus far been observed, but unfortunately the titer of the serum rapidly dropped to a level where it was no longer satisfactory for further studies.

Gene Fy^a is low in South American Indians and Africans and high in Lapps, Chinese, natives of India and North American Indians (Mourant, 1954).

VIII. The Kidd Factors (Jk^a Jk^b)

The two Kidd antibodies, anti-Jk^a and anti-Jk^b were discovered by Allen *et al.* (1951) and Plaut *et al.* (1953), respectively. In both cases, other antibodies were present, but, nevertheless, it was possible to demonstrate that anti-Jk^a and anti-Jk^b react with 77% and 73% of all Caucasoid bloods, respectively. From the incidence of the two genes, 52% for Jk^a and 48% for Jk^b, the following three genotypes are derived:

Jk^aJk^a	27%
Jk^aJk^b	50%
Jk^bJk^b	23%

Plaut *et al.* (1953) reported that anti-Jk^b exhibits the double-dose effect in blood homozygous for Jk^b. Since both antibodies occur very rarely, comprehensive data are not yet on hand. It is, nevertheless, established that both factors are independent of all other known blood group systems.

Jk^a, which has an incidence of 52% in Caucasoids, attained values of 38% in Amerindians of British Columbia (Chown and Lewis) and 78% in Africans (Ikin and Mourant, 1952). Only one heredity study was carried out with anti-Jk^a (Race, Sanger, Allen, *et al.*, 1951).

IX. Lutheran Factor (Lu^a)

Anti-Lu^a was discovered by Callendar and Race (1946) in a remarkable serum of a patient who produced, in addition to the already known anti-c and anti-N, antibodies for three new blood factors, C^w, Lu^a, and the "private" blood factor, Levay. The term "Lutheran" derives from the name of the donor whose blood stimulated the appearance of the antibody. Anti-Lu^a is probably a naturally occurring antibody which by stimulation may assume in part some of the properties of warm immune antibodies (see Table 2). As a rule, the titer is low and cannot be increased to a degree attained by "warm" immune antibodies.

The Lu^a factor is present in about 8% of all Caucasoids, but it can be readily differentiated from the Kell factor, which has the same incidence in Caucasoids. The heredity of the factor was analyzed by Race and Sanger (1950) in a combined study of several workers. In the absence of anti-Lu^b, the three sorts of matings yielded the expected proportion of offspring, as determined by the gene frequencies of $Lu^a = 3.9\%$ and $Lu^b = 96.1\%$. In 34 families in which both parents were Lu^a negative the factor was not present in any of the 77 children. Anti-Lu^b, which should react with 99.85% of all bloods, has not yet been discovered.

In their studies of random populations, Race and Sanger (1950), Bertinshaw *et al.* (1950) found no evidence of linkage of Lu^a with any of the known blood factors (*cf.* Holt *et al.*, 1952). Mohr (1951), however, reports linkage of Lu^a and Le^a (Lewis) in a study of 21 families in which one of the parents had the Lu^a factor. In view of the close genetic relationship of *Le^a* and *se* confirmatory studies should include tests for these two properties as well as Lu^a.

X. P Factor

The P factor, its heredity, and its greater incidence in Negroids of the United States was described by Landsteiner and Levine (1927, 1930). Although anti-P was known to be present in normal human and animal serum as a naturally occurring antibody, few, if any, studies were carried

out until the reawakening of interest of the 1940–1950 decade. The titer of anti-P, as a rule, is low and its activity is limited to low room temperature, with the result that the classification of bloods as positive and negative is not always definite. In most Caucasoids, it is present in 70–80% in contrast to about 97% in Negroids and 27% in Chinese (Miller *et al.*, 1951).

The P factor as defined by its several grades of activity is inherited as a dominant property. The genetic alleles have been designated as *P* and *p*, but an antibody specific for p (P-negative) has not yet been discovered. The P factor is independent of all other known blood group systems. As in the case of all naturally occurring antibodies, the blood property is not fully expressed in the blood of the newborn (Henningsen, 1949).

Using a more delicate technique, Henningsen (1949) demonstrated a more frequent occurrence of anti-P in P-negative individuals and with his more active antibodies, it was possible to classify individuals as P-strong, P-medium, P-weak, and P-negative. Family studies indicated that in a mating of P-positive × P-negative, the offspring do not have a P factor of greater receptor strength than that of the P-positive parent. Possibly there are a series of different genes which produce varying degrees of P activity, as in the case of the common substance H of the ABO system.

XI. Private or Family Blood Factors

These blood properties are characterized either by very high or very low incidences, the absence or presence of the blood factors respectively being limited to members of certain families. In some of the cases the antibody is produced by isoimmunization with resulting hemolytic disease, but in other instances it is not known whether the antibody is physiologic or immune. Because the antibodies occur rarely and the incidence of positive reactions is either exceedingly high or remarkably low, heredity and racial studies are necessarily limited.

1. *High-Incidence Factors*

Of these the most important one is the Jay factor (Tj^a) discovered under most unusual circumstances. The antibody, discovered in the serum of a 66 year old female patient suffering from gastric carcinoma, hemolyzed and agglutinated each one of 3000 random bloods tested except for that of her younger sibling. The propositus may be considered as homozygous for the genetic allele or Tj^bTj^b so that she could develop or produce anti-Tj^a. It was assumed that the antigenic stimulus for an apparently immune antibody was the tumor which seemed to absorb the

antibody. This would imply isoimmunization by antigenic material not introduced from the outside world in the form of a transfusion or pregnancy. If this line of reasoning is correct, it necessitates the assumption of a somatic mutation in the tumor cells which was responsible for the production of a blood property not transmitted by the patient's parents (Levine, Bobbitt, *et al.*, 1951).

Tests of the sibling's serum in 1951 failed to reveal the antibody, but, remarkably enough, the antibody was present in tests of the serum in 1953. In rapid succession, six other examples of the antibody were observed in a white patient living in South Africa, two sisters in Australia (Walsh and Kooptzoff, 1952), a Polish woman (Hirszfeld and Grabowska, 1952), and in a young woman, Mrs. E. E., and her brother, G. G., living in the United States. In the latter case, and also in the first pair of siblings, the parents are first cousins, each parent transmitting one gene Tj^b (Levine, 1954).

The effect superficially resembles that described by Bhende *et al.* (1952) in their three group O Bombay sera containing anti-H, but two of the anti-Jay sera belonged to group A. Furthermore, anti-Tj^a was not inhibited by H substance.

The essential features in the eight cases studied and diagnosed or confirmed by the author are summarized in Table 17.

TABLE 17
The Jay Factor (Tj^a)
Eight Examples of a High-Incidence Blood Factor

Individuals of genotype Tj^bTj^b	*Age*	*Blood group*	*Anti-*Tj^a	*Probable antigenic stimulus*	*Author*
1. D. J.†	68	O	Present	Tumor?	Levine, Bobbitt *et al.*, 1951
2. B. D.†	43	O	None 1951*	None	Levine, Bobbitt *et al.*, 1951
3. Mc	39	O	Present	4 Miscarriages	Zoutendyk and Levine, 1952
4. —	22	O	Present	1 Miscarriage	Walsh and Kooptzoff, 1952
5. —	19	O	Present	None	Walsh and Kooptzoff, 1952
6. E. E.†	30	O	Present	3 Miscarriages	Levine, 1954
7. G. G.†	36	A	Present	None	Levine, 1954
8. Za	23	A	Present	4 Miscarriages	Hirszfeld and Grabowska, 1952

Of the 8 caucasoid individuals, 4 reside in the U.S., 2 in Australia, 1 in South Africa, and 1 in Poland. With the exception of case #7, all are female. Cases 1 and 2, 4 and 5, 6 and 7 are siblings.
* Anti-Tj^a present in 1953.
† Siblings whose parents are first cousins.

In view of the fact that there is no obvious antigenic source for the antibody in at least four of the cases, 2, 4, 5, and 7, one may consider the possibility that anti-Tj^a may be a naturally occurring antibody, and that the occurrence of the tumor in the first case was probably incidental. There is additional and very recent evidence to support the view that the 12 miscarriages with no full-term pregnancies in the four young married women may be due to the action of anti-Tj^a.

At least two other high-incidence blood factors were found with the aid of antibodies produced by patients suffering from carcinoma, but in both cases there was an antigenic stimulus from transfusions. One of these, anti-Vel, agglutinated all but 4 random bloods out of 10,000 tested (Sussman and Miller, 1952). The second antibody studied by Levine and Derman (1953) hemolyzed each one of 200 bloods tested. The two blood factors seem to be independent of each other and are distinct from Tj^a.

2. *Low-Incidence Factors*

The first well-described hereditary and low-incidence factor, Levay, was reported by Callendar and Race (1946) with the aid of anti-Levay, one of the several antibodies produced by a particular patient. Anti-Levay reacted with the blood of the donor, Levay, and with that of his sibling and his father, but it failed to react with 350 random bloods.

The Gr factor was discovered by Graydon (1946) with the aid of a presumably naturally occurring antibody which agglutinated 5 out of 6 members of 3 generations of a particular family, but the antibody failed to react with 61 random Caucasoid bloods, 91 Papuans, and 39 Australian aborigines.

Of considerable interest are two blood factors, Mi^a (Miltenberger) and Be^a (Berrens), which produced isoimmunization with resulting hemolytic disease. The Mi^a factor was found in the blood of two siblings, their father, and paternal grandmother, but it was not present in 425 random bloods (Levine, Stock *et al.* 1951). The Be^a factor was present in the blood of one of two siblings, their father, paternal aunt, and paternal grandmother, but it could not be demonstrated in 428 random bloods (Davidsohn *et al.*, 1953). The antibodies for Mi^a and Be^a are not identical; thus these factors are genetically independent of each other. The factors Mi^a and Levay are not related to each other, but simultaneous tests of all these antibodies with the several blood factors are still to be done.

Other low-incidence factors were described by Wiener (1942b), Gilbey (1947), Elbel and Prokop (1951) and Wiener and Brancato (1953). As mentioned above, some of the finer differences of A_2 (such as A_3, A_4, A_5, or A_0), M_2 and N_2 which occur very rarely are limited to certain families, and, as such, may be considered as low-incidence factors.

XII. Individuality of Human Blood

Historically it is of interest that the concept of the individuality of blood in any given species was already evident from the work of Ehrlich and Morgenroth (1900) on isoimmunization by cross-transfusion in goats. The application of the same method led to successful demonstration of the individuality of red blood cells in cattle, chickens, rhesus monkeys, rabbits, and several other species (*cf.* Wiener, 1946).

The lag in demonstration of the individuality of human blood was overcome by the observation that isoimmunization may also result from the physiological effects of a pregnancy. Accordingly, a vast source of normal human material became available for the discovery of many new blood factors. As indicated in Table 1, the resulting individuality of human blood is now almost complete, and the concept is further supported by the occurrence of several different low-incidence and high-incidence factors. Undoubtedly, a number of human blood factors are still to be discovered (*cf.* Landsteiner, 1931).

As an example, one may cite the experience of Bertinshaw *et al.* (1950), who, in tests of 475 people in a London hospital with antibodies for the seven blood group systems, A_1A_2BO, MNS, P, Rh (D, C–c, E–e), Lutheran, Kell, and Lewis (Le^a), found 296 group combinations distributed as follows:

211 bloods occurred	once	211 bloods
45	twice	90
17	3 times	51
9	4	36
7	5	35
1	6	6
4	7	28
1	8	8
1	10	10
296		475

Thus, 44% of the bloods were individually specific, and this value as well as the number of combinations would undoubtedly be increased considerably on including antibodies for Duffy (Fy^a–Fy^b), Kidd (Jk^a–Jk^b), s, and some of the so-called "private" blood factors.

Fisher (1951) showed that the usefulness of any blood group system in differentiating 2 random bloods could be represented mathematically by the sum of the squares of the phenotype frequencies. The resulting

value indicates the percentage of failures in establishing individuality. The order of varying efficiency of the 9 different blood group systems is indicated below:

	Failures (%)
MNSs	16.35
Rh	19.5
A_1, A_2, B, O	32.8
Jk^a–Jk^b	37.6
Fy^a–Fy^b	38.5
P	61.5
Lewis (Le^a)	64.8
Kell	81.7
Lutheran	85.4

In the above analysis of 475 random bloods and in other family studies there was no evidence of linkage of any of the 9 blood group systems with each other or with sex (*cf.* Holt *et al.*, 1952). Linkage, however, does exist for the (1) three sets of Rh–Hr factors; (2) S–s and perhaps other factors (Henshaw, Hunter) with MN; and (3) Lewis-secretion factors. There is, in addition, one unsubstantiated report by Mohr (1951) of linkage of Lutheran and Lewis blood factors, but no studies were made with Lewis as a secretion phenotype. As of the present, there is no evidence of linkage of any of the markers on 10 human chromosomes (including sex) with hereditary conditions or diseases, but studies in terms of the more recently discovered blood factors are still to be made. Reference to the several statistical approaches for detection of linkage in either one or two generations of human families developed in the last fifteen years by Bernstein, Wiener, Haldane, Fisher, Finney, and others, is given by Boyd (1950).

1. *Some Practical Applications*

Blood factors offer peculiarly suitable material for the differentiation of monozygous from dizygous twins, and for rendering decisions in cases of disputed paternity and in cases of interchange of babies. Such studies should be limited to those blood factors listed in Table 1 which are clearly defined by potent antibodies and which are fully expressed in the newborn.

a. *Twin Studies.* Twins with differences in any of the blood factors are obviously dizygotic. Twins whose red cells are identical for the A_1, A_2, B, O, MNSs, and the Rh-Hr factors are probably monozygous, and their chance for monozygosity increases considerably if the identity extends to all the newer blood factors. The probability for monozygosity in any given case must be determined separately, and the value obtained will depend upon the number of differences in the factorial composition

of the parent's blood, and the probability of coincidence as determined by genotypes of parents.

A remarkable case of an individual whose red blood cells contained a mixture of O and A_1, *kk* (Kell-negative) and *Kk*, Jk (a+) and Jk (a−) was reported by Dunsford *et al.* (1953). For each of the three different blood group systems, the ratio of the contrasting kinds of blood factors was 61% and 39%, respectively. Evidence is presented that this individual was a member of dizygotic twins and as a result of vascular anastomosis "primordial red cells belonging to one twin take root in the other twin and continue . . . to produce red cells with genetically foreign antigens," in this case A_1, K, and Jk (a−).

b. *Use in Cases of Disputed Paternity.* For the past 20 years, the blood factors A and B, M and N have been successfully applied in cases of disputed paternity. In a number of states in the United States, statutory authority exists which compels the plaintiff, almost invariably the mother, her child, and the accused to submit to these tests, which are carried out by experts who report their findings to the court. The report is receivable in evidence, but only in cases where there is definite exclusion of the accused as the father of the child. With the availability of potent diagnostic antibodies for D, C, E, and c, the tests were extended to these factors and this resulted in greater chances for rendering decisions of nonpaternity.

Briefly the rules for nonpaternity (or nonmaternity) are as follows:

1. Any of the blood factors commonly employed, A, B, M, N, D, C, E, or c, cannot appear in the blood of a child unless it is present in the blood of one or both parents (rule of dominance).

2. The following combinations in terms of genotypes of any one parent and child indicate nonpaternity: $AB \rightleftarrows OO$; $MM \rightleftarrows NN$; $CC \rightleftarrows cc$; $EE \rightleftarrows ee$.

Similar rules may be formulated for the newer blood factors, but their practical application must be delayed until sufficient supplies of potent antibodies become available. The rule of dominance is not applicable to the Lewis blood factor, Le^a, but it does apply to Le^a as a secretion phenotype.

The chances for detection of nonpaternity for a population like that of England or the United States in terms of the given factors are as follows:

ABO, MN	34.32%	(Wiener, 1950)
ABO, MNS	40.2%	(Race and Sanger, 1950)
ABO, MN, Rh (D, C, C^w, c, e)	about 50%	(Wiener, 1950)
ABO, MNS, Rh (D, C, E, c)	55%	(Race and Sanger, 1950)

The inclusion of S–s in addition to M and N, Jk^a–Jk^b, and Fy^a–Fy^b will increase the chances to a still greater value. In a special report to

the American Medical Association, Davidsohn *et al.* (1952) recommend that for the present the tests be carried out with antibodies for ABO, MN, and D,C,E, and c. The limited usefulness of such blood properties as the subgroups A_1–A_2, Lewis-secretion, Lutheran, and P have been discussed above (Table 1). The subject is discussed more fully by Wiener (1946) and Race and Sanger (1950).

XIII. Fetal Death and Loss of Genes

Hemolytic disease in man is the first example in any species of fetal and neonatal morbidity attributable to a mechanism involving antigenic incompatibility of the mother's genes with the father's and affected infant's genes (Levine, 1946). The condition has since been observed under natural conditions in mules (Caroli and Bessis, 1947) and horses (Bruner *et al.*, 1948), and it will probably be found in still other species characterized by the sufficiently long periods of gestation required for antibody production. In species with short gestation periods as in rabbits (Kellner and Hedal, 1953) and dogs (Young *et al.*, 1951), the disease has been produced experimentally. In all instances, the affected young are necessarily heterozygous for the blood factors involved so that this loss of genes cannot be considered as due to lethal factors which affect individuals homozygous for a particular gene.

1. *Gene Loss of D and d Due to Hemolytic Disease*

Hemolytic disease in a population with about 15% Rh-negative individuals occurs about once in every 150 full-term pregnancies, and in the 13% incompatible matings (85% × 15%) about once in 19 full-term pregnancies. It is probable that the incidence in future generations will be still lower if the antigenic stimulus of indiscriminate transfusions will be controlled.

Since all affected infants are heterozygous, this involves the loss in each generation of one gene, *D*, derived from the father and the contrasting gene *d* from the mother. Accordingly, hemolytic disease has been considered as an example of selection against the heterozygote (Haldane, 1942). Reference was already made to the higher incidence of hemolytic disease in Caucasoids of Europe and the United States with 40% of gene *d* and its almost complete absence in Asiatics (and American Indians) who have less than 1% of gene *d*. With a more rapid loss of less frequent *d*, Haldane (1942) speculated that in about 600 generations there would be sufficient loss of *d* to approximate the low levels found in Asia.

In order to explain the varying incidence of Rh genes, *D* and *d* in several population areas, it was necessary to assume either repeated mutations (Hogben, 1943), or the existence of a people with predominance

of *d* and their admixture with Asiatics, who have a high frequency of *D* (Wiener, 1942a). As for the concept of mutation, this has been criticized on the ground that it would necessitate an unusually high mutation rate (Fisher *et al.*, 1944). Furthermore, it was shown that the Basques have the highest incidence of gene *d*, up to 60% (Etcheverry, 1945), and it could be assumed that in their prototype, the early Europeans, the incidence was still higher (Chalmers *et al.*, 1949).

Opposed to Haldane's view of uncompensated loss of *d* genes is the concept that the relative gene frequencies of a particular race tend to remain in stable equilibrium over long periods and that this stability is maintained by a balance of selective agencies (*cf.* Race and Sanger, 1950). If mutations cannot compensate for the loss of genes, an alternative explanation, suggested by Fisher (cited by Race, 1944) and later supported by data of Glass (1950), lies in the increased number of births in families with hemolytic disease. Even with a *DD* father, not all affected infants die and indeed many are now salvaged by therapy with immediate replacement transfusion.

2. *Loss of Other Genes Due to Hemolytic Disease*

The same considerations—higher birth rates—probably apply to the smaller group of hemolytic disease due to other blood factors such as C–c, E–e, and particularly K–k. In the latter system, the affected infant *Kk* suffers from the action of anti-K, whereas anti-k is produced only exceptionally. With gene frequencies of 5% for *K* and 95% for *k*, there is in each generation a much higher rate of loss of *K*.

Recent studies show that hemolytic disease due to ABO incompatibility occurs more frequently than suspected (Grumbach and Gasser, 1948), and it may account for as high as 10% of all affected infants. Most frequently the mother is in group O, and the infant is either *AO* or, more rarely, *BO*. In a Caucasoid population with about 70% *O*, 25% *A*, and 5% *B*, one would expect a more rapid loss of the less frequent *A* and *B* genes. The condition in the infant is, as a rule, milder than in Rh cases and furthermore, the recovery rate in the severely affected is now higher.

3. *Loss of A and O Genes from Other Causes*

About three decades ago, Hirszfeld and Zborowski (1925) observed that in their so-called "heterospecific" pregnancies there was a selective loss of group A offspring presumably due to "icterus gravis," now known as hemolytic disease. With the discovery of the pathogenesis of hemolytic disease due to isoimmunization by the Rh factor, Levine (1943) revived the Hirszfeld concept and defined compatible and incompatible matings

in terms of isoimmunization of the mother by dominant hereditary factors in fetal blood transmitted from the father. Thus, a mating O father × A mother was classified as a compatible mating and an A father × O mother, as incompatible. (Hirszfeld considered an A or B mother with an O child as a heterospecific or incompatible mating.) In further reference to the matings, the father's blood group will precede the mother's. A statistical analysis of 8 heredity studies revealed that in 7 of them there was indeed a deficiency of A children in the A × O mating as compared to the O × A mating, apparently owing to miscarriages or perhaps stillbirths (Levine, 1943).

The subject was investigated at greater length by Waterhouse and Hogben (1947) in a statistical analysis of 12 heredity studies which included 4 from the series of Levine (1943) and an additional 8 taken from the literature. The result indicated a loss of 25% of group A offspring in the A × O mating (Table 18). Since this mating contributes roughly

TABLE 18
Specific Loss of A in Incompatible Mating A × O
Summary of Twelve Genetic Studies of 453 Families with 1521 Children
(After Waterhouse and Hogben, 1947)

	Mating	*Number of families*	*Children*			
			O	A	Total	%A
Compatible	O × A	244	320	515	835	61.7
Incompatible	A × O	209	301	385	686	56.1
	Difference:			130		

33% of all group A children, the over-all loss is 25% × 33%, or roughly 8% of group A children. For a population of about 40% group A, this represents a total loss of 3% of all conceptions—a loss of genes far exceeding that resulting from hemolytic disease due to all blood factors combined.

The most likely cause of fetal death in these cases seems to be loss by miscarriages, but the published genetic studies carried out prior to 1945 did not take into account the outcome in each pregnancy. At present, there is no explanation for the mechanism which would compensate for the loss of 1.5% *O* and 1.5% *A* genes out of every 100 genes. For a more detailed presentation of this important subject the reader is referred to Levine (1943), Waterhouse and Hogben (1947), and particularly to Race and Sanger (1950), who discuss also the protective role of ABO incompatibility against isoimmunization by the Rh factor (*cf.* Kirk, *et al.*, 1953).

XIV. General Remarks

It is not generally recognized that modern genetics and that branch of immunology which deals with individual differences of human and animal blood share the same birth year, 1900. In the following four decades, the contributions of the numerous geneticists far outstripped those made by the very limited number of workers in this specialty of immunology, but this circumstance can be attributed to the wealth of favorable research material in the plant and animal world available to geneticists. Serologic investigations of individual differences, however, have been, and in large part still are, limited to red blood cells which, endowed by nature with a number of genetically determined antigenic factors, lend themselves to classical methods of observation.

The accelerated rate of discoveries of new blood factors in the last decade was stimulated by the causal relationship of some of them to hemolytic disease of the fetus and newborn, and their urgent application on a vast scale to blood transfusion during the war years. Among the numerous discoveries, those made by the British workers, Race, Mourant, and Sanger, are outstanding because their well-planned and comprehensive program was executed, as it were, under a unified command. At the same time they were fortunate in having at their disposal the guidance and skill of Professor R. A. Fisher.

An interval is now required to consolidate the gains acquired and to accumulate supplies of rare antibodies which will permit extensive heredity studies and world-wide tests of large population groups. Possibly many new blood factors remain to be discovered, but the indications are that those to be found with the aid of human antibodies will be classified either as high-incidence or low-incidence blood factors, having the same limitations for genetic and population investigations as those shown by gene-controlled anatomical anomalies or the metabolic disorders of alcaptonuria, cystinuria, pentosuria, etc.

It remains to be seen whether or not new blood factors can be discovered by more intensive studies of certain heterogenetic relationships of human and animal blood (*cf.* Landsteiner, 1945) and by wide-scale immunization in man or animal with non-European bloods. If it is assumed that red blood cells are exhausted as a source of genes which have simple relation to phenotypic differences, body fluids could be explored for the occurrence of serologically active substances such as the Lewis blood factors. Another possible source lies in the numerous individuality factors presumed to exist in skin and tissue cells which determine the success or failure of transplantation (Landsteiner, 1945; Loeb, 1945). This material, however, does not offer the advantages of a readily acces-

sible supply and comparatively simple technical procedures. Spermatozoa are the only other source of free cells in suspension, but serologic studies of these are complicated by their small size, asymmetry, and tendency to spontaneous agglutination.

With an ever-increasing number of hereditary antigenic blood factors, systematic studies are indicated to detect linkage with other normal or pathologic hereditary properties or correlation with susceptibility to specific diseases. Studies of numerous pathologic conditions limited to the ABO groups have failed to yield reproducible results, but a significantly greater incidence of group A in cancer of the stomach was recently reported by Aird and Bentall (1953) in a series of 1046 cases in northern England and also in 704 cases in Basle, Switzerland. This greater susceptibility to cancer does not result in specific loss of genes, the fertility of the affected individuals by virtue of their age not being involved; nevertheless, this observation if confirmed and extended gives further indication that the ABO genes are not neutral in selective value.

It has been generally agreed on the basis of heredity and population analysis that the genes determining the ABO groups are not located in the sex chromosomes. Unexpected results, however, were obtained by Sanghvi (1951) in a Bombay study of the sex ratio in combinations of O mothers-O infants, and B mothers–B infants. In both instances there was an excess of male infants. This was confirmed at least for the O–O combination in a New York study which showed also a reverse relationship, *i.e.*, an excess of females in the A–A combination. This differential in prenatal mortality and in gametic fertilization cannot be explained in terms of isoimmunization because the disturbed sex ratios were observed in pregnancies in which the mother and her infant belonged to the same ABO blood groups.

The recent observations on the greater susceptibility of group A to cancer and Sanghvi's findings on disturbed sex ratios in certain combinations of mother and infant, lend support to the significant remark by Race and Sanger (1950) that "it seems unlikely that selection would work only through maternal-fetal incompatibility."

For students of evolution, the implications from isoimmunization with resulting fetal and neonatal morbidity were most unexpected. It was generally assumed that heterozygotes with few exceptions are from the point of view of selection superior to the corresponding homozygotes. But in hemolytic disease and in the much larger group of selective fetal death due to ABO incompatibility, the selection is against heterozygotes. The significance of this loss of genes, its relation to the concept of gene equilibrium, and the roles of mutation and selection must be reserved for consideration by specialists in the field of evolution.

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Comparative Incompatibility in Angiosperms and Fungi

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I. Introduction

The term self-sterility was originally applied to a mating system in hermaphrodite plants, all individuals of which produce normally functioning male and female gametophytes and which fail to produce zygotes after self-fertilization. But the discovery that certain cross-pollinations also failed to produce zygotes, and the association of the term sterility with the lack or inviability of one or other of the two gametophytes led to the term incompatibility. This term has since been extended to include mating systems in certain groups of fungi.

In the flowering plants there is no danger of confusing incompatibility with separation of the sexes, dioecy, but in the fungi the distinction is more difficult and requires some clarification (*cf.* Catcheside, 1951). The

Ascomycetes is a group in which both sex organs, the ♂ antheridia and the ♀ oögonia, are produced on the same single spore culture. But antheridia will not fertilize oögonia on the same culture; for effective fertilization they must come from different cultures having different mating reactions. This bears a resemblance to the situation found in flowering plants and because of its main feature—the restriction on self- and certain cross-fertilizations—it is an incompatibility system. The *Basidiomycetes*, with the exception of the rust fungi, do not produce differentiated sex organs. Mycelial colonies which arise from basidiospores (the immediate products of meiosis) have one haploid nucleus per cell. They are monokaryons. The undifferentiated hyphae of the monokaryons fuse, nuclei are exchanged, and both cultures produce new mycelial growth which contains two different nuclei per cell—they are dikaryons. Both cultures then produce fruiting-bodies which result in meiosis and basidiospore formation. Thus every basidiospore colony has the potentiality of carrying out both the female function of receiving nuclei and producing a fruit body and the male function of giving nuclei. The successful fusion of hyphae and exchange of nuclei is controlled by an incompatibility system.

The *Mucorales* contain dioecious species having differentiated sex organs on different mycelia, and species having two mating types, which cannot be differentiated morphologically or functionally into two sexes. The product of fusion, the zygospore, is shared equally between the two mating types; there is no exchange of nuclei. Such a mating system is more correctly thought of as a primitive sexual differentiation than as incompatibility; however, the distinction between incompatibility and dioecy in these lower types is a fine one.

Our knowledge of the distribution of incompatibility in the plant kingdom is extremely fragmentary and much less complete than our knowledge of other breeding mechanisms, such as dioecy or the non-synchronous development of the male and female organs in a hermaphrodite. This is because incompatibility requires a breeding test for its detection and because, unlike dioecy and other morphological differences, it is not recorded in the course of taxonomical description.

East (1940), in his survey of the distribution of self-incompatibility throughout the families of flowering plants, estimated that self-incompatibility was present in more than three thousand species distributed among twenty families. This is probably a small fraction of the actual number of incompatible species. Bateman (1951), in a survey of the *Cruciferae*, found 43 self-compatible species and 41 incompatible.

The widespread distribution and high frequency of incompatibility in the higher plants and fungi indicate that it is one of the most impor-

tant outbreeding mechanisms in plants. It appears to take the place of dioecy in animals. The reason for this difference may be that, whereas animals are mobile and can adjust their sex separation without undue gametic wastage, plants because of their immobility have not evolved an efficient means of transferring pollen from the male to the female. Furthermore, a male animal has a useful function in the rearing of young, whereas a male plant can only occupy space which might have supported a seed-bearing female. Clearly dioecy in plants is a wasteful outbreeding system and hence its rarity (Mather, 1940).

If incompatibility in plants takes the place of dioecy in animals, it is not surprising that just as there are different genetic systems in sex control in animals, so there are in plants different systems of incompatibility. Also whole phyla in plants may have the same system, just as different sex mechanisms divide the animal kingdom. There is no doubt therefore of the antiquity of the systems.

Both in angiosperms and fungi there are three different systems. A comparison of these in relation to the life cycle of the organism is an illuminating study.

The genetics of the systems provide several points of interest in relation to gene action, such as pleiotropy, time of gene action, and complex gene interaction involving dominance and competitive interaction between alleles.

The origin of new alleles by mutation is a problem to which the incompatibility system is technically well suited; however, this system has yet failed to give a satisfactory demonstration that new gene material arises by mutation.

From the practical breeding aspect, incompatibility is important where it occurs in any commercial crop, particularly in vegetatively propagated fruit trees. It can also be used as a breeding tool in the production of F_1 hybrid seed.

II. Systems in Flowering Plants

There are three ways in which the different systems of incompatibility in flowering plants can be classified. They are based on the following: (1) flower morphology; (2) genetic control; and (3) the physiology of the mechanism.

1. The flower morphology classification comprises two well-defined groups: heteromorphic, which includes distyly and tristyly, and homomorphic, in which there are no floral morphological differences accompanying the incompatibility.

2. The genetic classification depends upon three criteria: the number of alleles, whether two or many; the control of the pollen, whether it is

sporophytic or gametophytic, and whether the alleles show dominance or individual action; and finally the control of the style, whether the alleles show dominance or individual action.

All combinations of these different gene actions, with one exception, are possible and indeed have been found. The exception is the combination of two alleles with gametophytic control and individual action, for in this combination all plants would be cross-incompatible, and hence the species would be sterile.

3. The physiological classification comprises two well-defined groups: *complementary* and *oppositional*. The complementary mechanism depends upon the combination of *unlike* gene products in pollen and style, producing either a necessary stimulant to pollen-tube growth or an antidote to an inhibitor always present in the pollen. The oppositional mechanism depends upon the combination of *like* gene products from pollen and style to produce an inhibitor of pollen-tube growth.

TABLE 1

Classification of Incompatibility in Flowering Plants Based on Three Different Criteria: Morphology, Genetics, and Physiology. Note the Approximate Correspondence between the Classifications

(Spor. = sporophytic; Gam. = gametophytic control)

Morphology	*Genetics*			*Physiology*
	Alleles	Control of Pollen	Style	
Heteromorphic	2	Spor. Dom.	Dom.	Complementary stimulant or Oppositional inhibitor
Homomorphic	Many	Spor. Dom. or Indiv.	Dom. or Indiv.	Oppositional inhibitor only
		Gam. Indiv.	Indiv.	

A comparison of the three methods of classification given in Table 1 has shown that although there is a certain correspondence between them there is some overlapping of the groups; thus no one classification is entirely satisfactory (*cf.* Lewis, 1944; Bateman, 1952b). This review will describe only systems which have actually been discovered, as other hypothetical systems have been described in some detail by Bateman (1952b).

1. *Heteromorphic*

a. *Distyly.* The classical examples of distylic species described by Darwin (1877) are found in the genus *Primula.* The two types of flowers—borne on different plants—are *thrum,* with short styles and high anthers, and *pin,* with long styles and low anthers. The short style is about half the length of the long style, and the heights of the two kinds of anthers correspond with the levels of the stigma in the other type of flower. Pollinations are compatible only between anthers and stigmas at the same height, *i.e.*, between pin × thrum and the reciprocal.

The difference between the pin and thrum flowers is a complex of six pairs of contrasting characters: the length of style, height of anthers, incompatibility reaction of style and pollen, the size of the pollen grains, and the size of the stigmatic cells. The high anthers of the thrum contain pollen grains which have twice the volume of the pollen in the low anthers of the pin, and the long style of pin has larger stigmatic cells than those of the short thrum style. This complex of characters is summarized in Table 2.

TABLE 2

Characters Found in Pin and Thrum Plants

Character	Pin	Thrum
Incompatability reaction of pollen	'Pin'	'Thrum'
Incompatability reaction of style	'Pin'	'Thrum'
Length of style	Long	Short
Size of stigmatic cells	Large	Small
Height of anthers	Low	High
Size of pollen	Small	Large

The genetic control of this character complex is by a single gene *S* with 2 alleles, the thrum being *Ss* and the pin *ss.* Although the incompatibility between these two types is essentially a reaction between haploid pollen tubes and diploid style, the pollen of thrum, despite the segregation of *S* and *s,* behaves as if it all had the *S* constitution. This is due to the sporophytic determination of the pollen's reaction.

A basically similar situation is found in several species in widely different families (*cf.* Lewis, 1949a). Although *Linum perenne* has the full distylic complex as in *Primula,* a variant of this system is found in *Linum grandiflorum,* in which there are long and short styles; however, the anthers are at approximately the same height in both types, and the pollen grains are of the same size.

In the *Plumbaginaceae* a whole series of variants of the distylic theme

have been described by Baker (1948 and unpubl.). *Limonium vulgare* has the same complex as *Primula*, *i.e.*, it is dimorphic in all characters: anther height, style length, pollen, and stigmatic surfaces. Many species of *Armeria* and *Limonium* are monomorphic in regard to anther height and style length, but the stigmatic surface and pollen are dimorphic. Finally many species of *Goniolimon* and *Acantholimon* are dimorphic only in the pollen character. All these species are self-incompatible; other species which show no dimorphism are self-compatible. Thus this interesting group shows a complete series in the evolutionary development of a full heterostyled system. Their genetics would be of great interest and would well repay a detailed study.

The one conclusion that can be drawn from these variants is that the morphological differences are not the cause of the self-incompatibility; rather, they are secondary elaborations accumulated around the primary effect—the prevention of self-fertilization by the control of pollen-tube growth.

A similar conclusion must be drawn from some abnormal types which have been found in *Primula* species as a result of a breakdown in the complex genic control. Ernst (1936) found exceptional plants of *Primula viscosa* to be homostyled; they had stigmas and anthers at the same level and were self-fertile, as would be expected if the incompatibility reactions of the pollen and style were conditioned by their positions. But a further breakdown was found in other plants: one plant had large pollen grains in low anthers and another had small pollen in high anthers, both being the reverse of the normal situation. The incompatibility reaction of this pollen was that to be expected from the pollen size and not the anther height. The evidence from the genetic control of these abnormal plants supports the hypothesis that the *S* gene is a super gene of at least three parts. These were represented by Ernst as: *G* which controls the style characters; *A*, for anther height; and *P*, for pollen size. But the *P* section is itself probably a complex of pollen size and incompatibility determiners; also the *G* part is probably a complex of three determiners, style length, stigmatic surface, and incompatibility reaction.

The full complex is illustrated in Fig. 1.

Thrum	Pin
$\frac{GSI_1I_2PA}{gsi_1i_2pa}$	$\frac{gsi_1i_2pa}{gsi_1i_2pa}$

FIG. 1. Hypothetical structure of the super gene controlling heterostyly. I_1 = stylar incompatibility, G = style length, S = stigmatic surface, A = anther height, P = pollen size, I_2 = pollen incompatibility.

The complex of characters is therefore controlled by a super gene: a rare crossover between *G* and *A* and between *A* and *P* could give the

abnormal homostyle plants found by Ernst, although Ernst suggests a mutational origin.

Homostyle plants in *Primula acaulis* have been found as large populations in the wild (Crosby, 1949). These plants are self-fertile and are controlled by an allele, or more precisely a pseudo-allele, at the *S*-locus. This pseudo-allele may have been the result of a rare crossover, as in *Primula viscosa*. The wild homostyled plants provide an interesting study in population genetics because the character involved is of major importance in the breeding structure of the species.

A breakdown of the character complex in *Primula sinensis* is due not to changes in the *S* super gene but to two independent loci which have pleiotropic effects. The mutant Primrose eye (*a*) shortens the style and affects the size of the stigmatic cells and the incompatibility reaction of the style. The mutant Fertile Double (*m*) raises the level of the anthers without altering the size or incompatibility reaction of the pollen (Beale, 1939; Mather, 1950). Mather and de Winton (1941) have shown that apart from these major genes there must be a polygenic complex balancing the whole incompatibility system; for horticultural selection by self-pollination has gradually reduced the degree of incompatibility over the past 100 years.

Primula obconica is rigorously pin and thrum with an incompatibility so strong that selfing produces seeds only as a great rarity. In the diploid strains which have been grown and examined in large numbers for seventy years no homostyle forms have been recorded. In the last ten years tetraploid horticultural strains of unknown origin have arisen. These tetraploids, examined by Dowrick and Lewis (unpublished), contain normal pins and thrums which show no breakdown due to polyploidy of the normal incompatibility mechanism. Furthermore the *S* allele is dominant over three doses of the *s* allele. However, both of the two different tetraploid strains examined contained about 5% of homostyle pins. These were fully self-fertile and are probably due to another pseudo-allele of the *S* super gene due presumably to unusual crossing over.

A self-fertile homostyle was found as a great rarity in the diploid *Fagopyrum esculentum* by Schoch-Bodmer (1934). Esser (1953) produced autotetraploids of this species by colchicine treatment, and in the limited number of plants in F_1 and F_2 generations he found three homostyles. Two were homostyle pins which were self-fertile, and one was a homostyle thrum which was self-sterile. The implication of tetraploid homostyles will be discussed after another example in a tristylic species has been described.

All the comparative and genetic evidence in distylic species points to the evolution of a complex of characters under the control of a super

gene. The remarkable feature of the system—and since many species in widely different families have been examined, it appears to be more than a coincidence—is that thrum is always dominant to pin.*

b. *Tristyly.* Examples of tristyly have been described in *Lythrum salicaria* and *Oxalis* species (Darwin, 1877). The styles are of three lengths, short, mid, and long, and the anthers are of three heights corresponding to the positions of the stigmas. Any one plant has one style length and two anther heights. As in the distylic species, only pollinations between stigmas and anthers at the same level are compatible. Thus each type of plant can effectively fertilize the other two types. The genetics of the system in *Lythrum* has received attention from several workers but was elucidated finally by Fisher and his collaborators. There are two independent loci, each with two alleles *Ss* and *Mm*. Plants with the dominant *S* are short-styled irrespective of their *Mm* constitution; plants with the recessive *s* and the dominant *M* are mid-styled; and the bottom recessive is long-styled. *Lythrum salicaria* is an autotetraploid forming some quadrivalents, and Fisher and Mather (1943) showed that the *Mm* locus is tetrasomic, and Fisher and Martin (1947) showed that the *Ss* locus is also tetrasomic.

The situation in wild populations of *Lythrum* is complex, for there are fifteen different genotypes in respect of the *M* and *S* loci.

In a diploid species *Oxalis valdiviensis* a similar but slightly different genetic situation exists (Fyfe, 1950). There are two loci *Ss* and *Mm*, each allele having a similar control of the heterostyly incompatibility complex as in *Lythrum*, but the loci are linked with 5.7% recombination.

Fisher (1949) has pointed out that two independent tetrasomic loci and two disomic loci with linkage would provide the ideal equilibrium of equality in numbers of the three types.

It should be noted that as in the distylic species the dominance in *Lythrum* and *Oxalis* goes from short to long style.

An abnormal plant in *Lythrum salicaria* which arose from selfing a mid plant had the short and long anthers of a mid plant but the style was long. It was a *semi-homostyle* and was fully self-fertile (Stout, 1925). Although this species is already a polyploid, a further chromosome doubling was achieved by colchicine treatment (Esser, 1953). Among the limited number of double autotetraploids a homostyle plant was found with long style, and long and short anthers, this plant was self-fertile as expected.

The appearance of homostyles in tetraploids of two distylic and one tristylic species appears to be more than a coincidence. The tetraploids

*Since writing, Baker (Proc 8th Int. Bot. Congress 1954) has reported that in **Armeria** the cob stigma, which is equivalent to "fin," is dominant. This appears to be an exception.

of *Primula obconica* are of unknown origin, and the homostyled plants may be the result of unconscious selection by the grower, who would select any spontaneous tetraploid for their large flower size. Since tetraploids would occur as isolated pin or thrum plants they could be effectively pollinated only by diploids, resulting in complete sterility. But if a rare homostyle—which as a diploid would be overlooked—became tetraploid, it would be self-fertile and the line could be continued. This mode of origin would require two rare events to occur in the same plant. Furthermore, if a homostyle plant became doubled it would be duplex for the new homostyle allele and also for *S* or *s*; thus it would segregate only two types of plants—homostyles and pins or thrums. All three types are found in tetraploid *P. obconica*, thus the possible origin of the tetraploids from one homostyle diploid plant is excluded. In view of the precisely known origin of homostyles in tetraploid *Fagopyrum* and *Lythrum* it is probable that the tetraploid condition actually induces the production of homostyles, as a result of a shift in the normal range of position of chiasmata by the novel pairing situation in a quadrivalent.

This would increase the chance of crossing over within the super gene. Darlington (1940) has shown that in a trivalent of a triploid the position of chiasmata was different from that in a bivalent. If linkages which are undesirable for breeding purposes can be broken down by tetraploidy, then it might be a tool for the plant breeder.

In *Narcissus triandrus* Bateman (1952a) has found that the tristyly is independent of the incompatibility. In this example however there are only two different anther heights and each of the three types of style length has the two same anther heights. The morphological differentiation into short, mid, and long is therefore different from that found in *Lythrum* and *Oxalis*. It is of interest that other species of *Narcissus* are self-incompatible and are homomorphic. The significance of the tristyly in *N. triandrus* is obscure, although it may represent a transitional stage in the production of a full tristylic system.

2. *Homomorphic*

a. *Gametophytic.* The gametophytic oppositional system was first found in *Nicotiana sanderae* by East and Mangelsdorf (1925) and has subsequently been found in several widely separated families (*cf.* Lewis, 1949a). The main features about this system are: (1) the single controlling gene *S* has a very large multiple-allele series; (2) the control of the pollen reaction is gametophytic; (3) the alleles have an individual action in the style without any interaction; (4) pollen tubes are unable to grow down a style in which both tube and style have the same *S* allele.

This system generates three main types of pollination: (1) fully incompatible—$S_{1,2} \times S_{1,2}$, in which both alleles are common; (2) half the

pollen is compatible—$S_{1.2} \times S_{1.3}$, in which one allele is different; (3) all the pollen is compatible—$S_{1.2} \times S_{3.4}$, in which both alleles differ. The progeny from type 2 pollination fall into two cross-compatible groups; both groups are compatible reciprocally with the female parent; with the male parent one group is incompatible and the other compatible. The progeny from type 3 pollination fall into four cross-compatible groups, all of which are compatible with both the male and female parents. The patterns of reaction of these two progenies are given in Fig. 2.

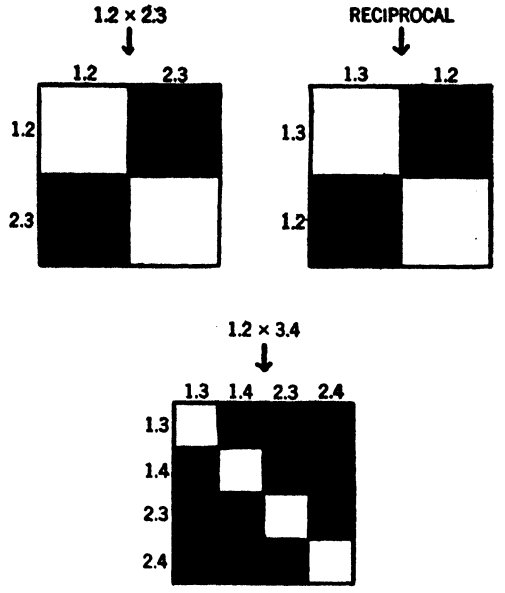


FIG. 2. The two patterns of inter-sib pollinations obtained with the gametophytic system: the numbers represent the different *S* alleles; black = compatibility; white = incompatibility; the female parent is placed at the left-hand side and the male at the top of the squares.

The genetic system as shown by these patterns of reaction behaves with extraordinary regularity. From studies of hybrids and backcrosses between a self-incompatible species *Petunia violacea* and the self-compatible *Petunia axillaris*, Mather (1943) showed that this regularity of behavior depends to a considerable extent upon a polygenic background, for *P. axillaris* has a genetic background which reduces the *Rigor* of the incompatibility system of *P. violacea*.

East (1934) showed that different *S* alleles in *Nicotiana sanderae* differed in their power to prevent self-fertilization. Watkin Williams (1951) from a detailed study of *pseudo*-fertility (selfed seed set) in the species *Trifolium hybridum* found a similar difference between alleles and found no evidence for segregation of polygenic modifiers. Thus once the system

is balanced by the polygenic background as shown in *Petunia*, it appears from *Trifolium* that these modifiers are more or less homozygous.

(i) *Breakdown with polyploidy.* In a normal diploid the stylar tissue carries two different S alleles, both of which have an individual and positive inhibiting effect only on pollen tubes carrying the corresponding allele. In an autotetraploid the style carries four alleles, which can include two, three, or four different ones, *e.g.*, $S_{1.1\ 2.2}$, $S_{1.1\ 2.3}$, $S_{1.2.3.4}$. Clearly the dosage relationships in the last two genotypes are abnormal for the diploid. In the haploid pollen grain of a diploid there is only one S allele; thus interaction of any kind at the S locus is excluded. In the diploid pollen of tetraploids, however, there are two S alleles; these may be either the same, *e.g.*, $S_{1.1}$, or different, $S_{1.2}$; the latter provides a new situation of gene interaction.

With the two new possibilities, novel interaction in the pollen and different dosage relationships in the style, we are in a position to review the findings in autotetraploids. Because of the unusually favorable material, the most revealing analysis has been with *Oenothera organensis*. This will be discussed first, although earlier hints at interactions with polyploidy came from a fruit tree *Pyrus commonis* (Crane and Lewis, 1942) and from *Petunia violacea* (Stout and Chandler, 1941). The fruit tree was quite unsuitable for further analysis and *Petunia* proved to be inferior to *Oenothera*. But it should be noted now that in both these species tetraploidy had an effect of completely breaking down the incompatibility system and producing self-fertile plants.

Oenothera organensis has an unusually strong incompatibility reaction, imposing a rigorous control of self-fertilization. The pollen tubes are inhibited in the top 10 mm of the style, which is 160 mm long (Emerson, 1938). Tetraploids of known S genotype were made by colchicine treatment and the interactions of four alleles were analyzed in all their combinations (Lewis, 1943a, 1947). A detailed analysis was possible because unlike *Pyrus* and *Petunia*, the breakdown was not complete, and the tetraploids were not fully self-fetile. This less drastic effect of polyploidy did not obscure, as it probably did in the other species, two different underlying causes of the breakdown.

Before any reliable interpretation can be made on the results of interpollinating tetraploids, the S genotype of individual plants has to be established beyond doubt. The constitution of an individual produced by somatic doubling is a matter of simple deduction, $S_{1.2} \rightarrow S_{1.1\ 2.2}$. But tetraploid individuals which are the progeny of sexual reproduction can only be known with certainty by their reactions with the pollen from diploid test plants or by laborious F_2 tests. For example, a plant which rejects all the pollen of $S_{1.2}$ and $S_{2.3}$ testers but not of $S_{3.4}$, $S_{2.4}$, and $S_{1.4}$

testers is carrying 1, 2, and 3 but not 4. Fortunately for this method the S alleles in the tetraploid style show no interactions or dosage effects. A tetraploid style $S_{1.2.3.4}$ will reject all pollen carrying any one of the four alleles. A style carrying two doses of one allele and one dose of two others also rejects all pollen carrying any one of the three alleles. Thus in the tetraploid style, as in the diploid, all alleles act individually and without any dosage or interaction effects. Lack of interaction is to be expected, since in the diploid there has been a rigorous selection for individual action of alleles in the style; for if dominance or other interaction occurred, with gametophytic pollen control one of the two pollen genotypes would not be inhibited and the plant would be self-fertile. The absence of dosage effects in the tetraploid cannot be accounted for in this way because in the diploids with only two different alleles present there has been no opportunity for them to occur. But strong selection for individual action would be expected to result in absence of dosage effects.

With the stylar action of the alleles determined it is possible to interpret the action of diploid pollen grains on different tetraploid styles. In self-pollination either of two results may occur according to the genotype: (1) All the pollen tubes are inhibited almost as strongly as in the diploid; (2) a proportion of the pollen tubes are not inhibited. The problem is to determine which of the pollen genotypes are not inhibited. It was possible to do this in diallelic plants by a process of discrimination. The two different results of polyploidy are listed for several genotypes in Table 3.

TABLE 3

Shows Two Classes of Tetraploids Based on Their Behavior on Selfing; by Excluding the Fully Inhibited Pollen Genotypes from the Left-Hand Column, the Noninhibited Genotypes on the Right Are the Heterogenic **2.6**, etc.

	All pollen tubes inhibited			*Proportion of pollen tubes not inhibited*		
Plant genotypes	$S_{2.2.3.3}$			$S_{2.2.6.6}$		
	$S_{2.2.4.4}$			$S_{3.3.4.4}$		
	$S_{4.4.6.6}$			$S_{3.3.6.6}$		
↓						
Pollen genotypes	2.2	2.3	3.3	2.2	**2.6**	6.6
	2.2	2.4	4.4	3.3	**3.4**	4.4
	4.4	4.6	6.6	3.3	**3.6**	6.6

From the left-hand column we know that all the pollen genotypes are inhibited by styles carrying the same alleles as those in the pollen. Hence all the homogenic pollen grains are fully inhibited; this leaves in the right-

hand column the three heterogenic pollen grains $S_{2.6}$, $S_{3.4}$, and $S_{3.6}$, which are not inhibited. Extensive data from triallelic and tetrallelic plants fully confirm this interpretation without exception. Thus we have in diploid pollen grains a new type of allelic interaction resulting in both alleles failing to produce their effect; this has been termed *competitive interaction.*

It will be noticed that not all heterogenic combinations show the competitive interaction. The next problem is how do the heterogenic pollen grains—both competing and noncompeting combinations—behave on styles having only one of the alleles present in the pollen? Do the alleles exhibit dominance in the pollen? It was found in *Oenothera* that this type of behavior could be measured by seed set. If competitive interaction had given a complete breakdown of the inhibition, resulting in seed set as it has done in *Pyrus* and *Petunia*, then this second type of behavior could not have been analyzed without extensive F_2 tests.

Information on dominance in the pollen was obtained by a comparison of the pollen genotypes in incompatible and compatible crosses in which some pollen genotypes would have one allele common and one different to the style. The general type of this comparison is given as:

Cross	Relevant Pollen Genotypes	Result
$S_{2.2.4.6} \times S_{2.2.3.6}$	**2.6**, 3.**6**	−
$S_{4.4.6.6} \times S_{3.4.6.6}$	3.**4**, 3.**6**	+

The heavy type indicates that this allele is present in the style. The incompatible nature of the first cross shows that **3.6** pollen is incompatible in a style containing S_6 and not S_3; thus in the second cross we are left with $S_{3.4}$ as the only compatible genotype and style containing S_4 and not S_3. By these methods dominance is shown when a pollen genotype, say, $S_{3.4}$, is compatible in a style $S_{4.4.6.6}$ but incompatible in $S_{3.3.6.6}$. In this example S_3 is dominant over S_4. A full confirmation of the results was also obtained by determining the genotypes of the progeny produced by the crosses.

The four alleles which have been analyzed in *Oenothera organensis* have been arranged in a linear order on a dominance competition scale, the distance apart of the alleles on the scale being determined by the degree of dominance as measured by the amount of seed produced in the critical crosses and by the degree of competition as measured by the length of self pollen-tube growth (see Fig. 3).

It will be seen from the scale that pairs of alleles which are close together on the scale, *e.g.*, S_2 and S_6, show competition and no dominance; pairs of alleles farther apart, S_6 and S_4, show dominance and no compe-

tition; pairs of alleles at an intermediate distance apart, S_3 and S_4, show dominance and competition; and alleles at the extremes, S_2 and S_4, have no interactions.

How do these two principles, dominance and competitive interaction in diploid pollen grains, account for the effects of tetraploidy known in other species? An autotetraploid pear, *Pyrus communis*, described by Crane and Lewis (1942) and Lewis and Modlibowska (1942) is fully self-fertile, and this effect resides in the diploid pollen and not in the style. Some of the pollen grains are fully incompatible. The self-fertility is most

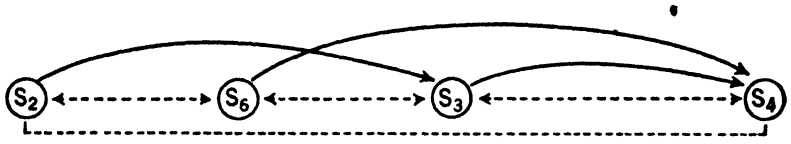


FIG. 3. Four S alleles in *Oenothera organensis* arranged in linear order according to their interactions, the distance apart being determined by the degree of competition and dominance: → dominance, ←···→ competition, ------ individual action with no interaction.

probably due, therefore, to competitive interaction in the heterogenic pollen.

In *Petunia*, Stout and Chandler (1941) found that autotetraploids are fully self-fertile and that all progeny of selfed tetraploids are self-fertile and inter-compatible. All these progeny are incompatible when crossed as females with their diploid parents, but are all compatible as males on to their parents. These facts prove that the offspring are all heterozygous for their S alleles, and that the self-fertility lies in the pollen and not in the style. Thus it has been proved that no homogenic pollen grains function in self-pollination, and the reasonable interpretation is that the breakdown is due to competitive interaction.

In autotetraploid *Trifolium repens*, Atwood (1944) showed that some tetraploid genotypes are self-fertile and others are self-sterile. These results were interpreted (Atwood and Brewbaker, 1950) on the basis of competitive interaction and dominance between different allele pairs, as in *Oenothera*.

A similar interpretation applies to *Antirrhinum glutinosum*, except that dominance was not demonstrated (Straub 1941).

The results in the different species with the gametophytic system in which autotetraploids have been produced are given in Table 4.

(ii) *Gene interaction and selection.* It is significant that the breakdown of the system due to competition in diploid pollen is so widespread, for it shows the importance of selection in determining gene action and interaction. The only breakdown in the genic system controlling incompatibil-

ity following tetraploidy is one which could not have been selected against; for diploid pollen grains are not a part of the normal breeding system. When the sporophytic oppositional type of incompatibility has been considered, we shall see further confirmation of this principle.

In the tetraploid forms of heterostyled plants described in the previous section the pollen is under sporophytic control and therefore is selected for gene interaction; here no breakdown occurred. The only breakdown in the system was probably due to an increase in crossing over

TABLE 4

Species with the Gametophytic Oppositional Incompatibility System, in Which Autotetraploids Have Been Examined, Showing the Effect on Incompatibility. It will Be Noticed That with One Possible Exception There Is a Breakdown of the System Which Is Due in Most Cases to Competitive Interaction in Diploid Pollen Grains

Species	*Effect of polyploidy*	*Competition, dominance*	*Author*
Antirrhinum glutinosum	Self-fertile	Comp.	Straub, 1941
*Campanula persicifolia**	Self-fertile	?	Gairdner, 1926
Oenothera organensis	Slightly self-fertile	Comp. Dom.	Lewis, 1943a, 1947
Oenothera rhombipetala	No effect?	?	Hecht, 1944
Petunia	Self-fertile	Comp.	Stout and Chandler, 1941
Pyrus communis	Self-fertile	Comp.	Crane and Lewis, 1942
Pyrus malus	Self-fertile	?	Johansson, 1945
Solanum 3 species	Self-fertile	?	Livermore and Johnstone, 1939
Trifolium pratense	Self-fertile	Comp.	Pandey, unpubl.
Trifolium repens	Self-fertile	Comp. Dom.	Atwood, 1944, 1950

* Incompatibility not known but probably gametophytic.

within the *S* super gene resulting from the pairing properties of a quadrivalent—a situation which would not have been subject to selection.

The main conclusion which must be drawn is that dominance, competition, and individual gene action can all be accidental primary properties of the gene, but the insurance that any one of these properties is a regular feature of a gene can only be obtained by selection.

b. *Sporophytic.* It is now known that Correns (1912) first described in *Cardamine pratensis* a system of incompatibility that belongs to the sporophytic oppositional type; however, his inappropriate terminology, which was due to the general lack of appreciation of the situation, delayed the realization of its full significance until the work of Hughes and Babcock (1950) on *Crepis foetida* and Gerstel (1950) on *Parthenium argentatum.* Three further examples have been analyzed subsequently: *Cosmos*

bipinnatus by Crowe (1954); *Iberis amara* by Bateman (1954); and *Theobroma cacao* by Rogers and Knight (1954). It should be noted that *Crepis*, *Parthenium*, and *Cosmos* are members of the Compositae.

The sporophytic system is similar to the gametophytic in having a single gene with multiple alleles but differs in its pollen control, which is sporophytic as in heterostyled plants. The alleles may show either dominance, individual action, or competition in the pollen and styles according

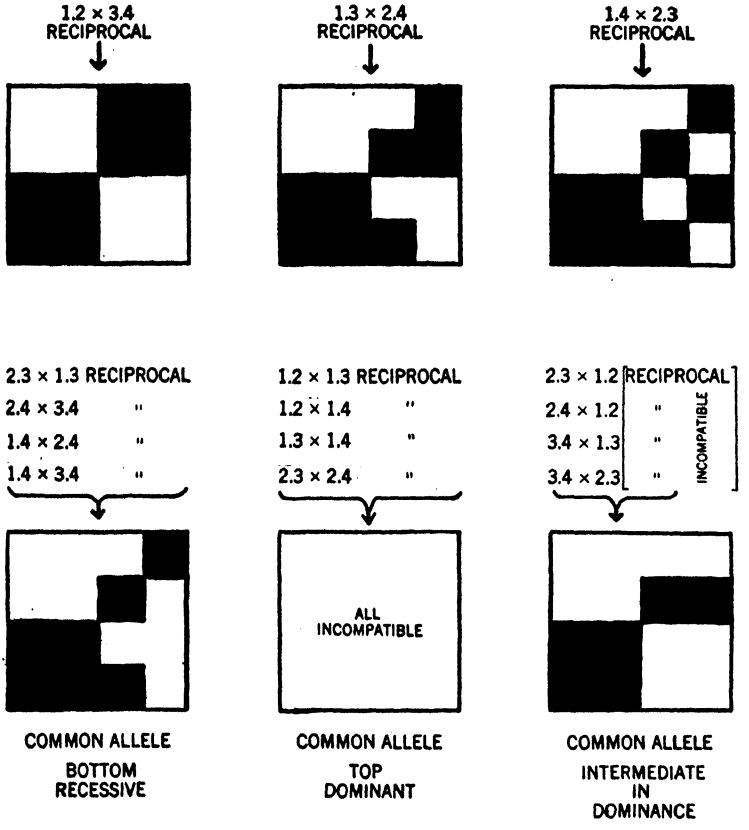
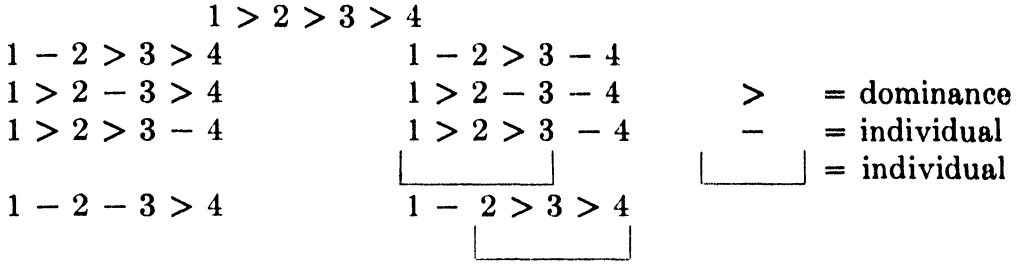


FIG. 4. The five patterns of inter-sib pollinations obtained with the sporophytic system and four alleles. The alleles have individual action in the style and a graded series of dominance in the pollen, 1 > 2 > 3 > 4, 1 being the top dominant and 4 the bottom recessive.

to the allelic combination. This variability of allele interaction leads to great complexity of incompatibility relationships. Instead of the two simple pollination patterns of the gametophytic system (Fig. 2) there are more than thirty-five different ones with sporophytic control and variable allele interaction. For example, if we take the simplest case of 4 alleles, all of which are individual in the style and in which the dominance relations in the pollen are 1 > 2 > 3 > 4, 1 being the top dominant and 4 the bottom recessive, then from intercrossing the 6 possible heterozygous

genotypes generated by the 4 alleles there are 18 compatible and 12 incompatible pollinations. The 18 compatible pollinations produce progeny which when interpollinated will give five different patterns. Three of these patterns are produced from the three pollinations in which there are no alleles common to the two parents, *e.g.*, 1.2 × 3.4. The fourth pattern results from pollinations in which one allele is common to both parents and this common allele is the bottom recessive of the three. The fifth pattern results when the common allele is intermediate in dominance of the three. As pollination patterns are important in the interpretation of results, the method of their generation for this simple case is given in Fig. 4.

The situation illustrated in Fig. 4 in which four alleles are individual in their stylar action and show a regular dominance sequence in the pollen may be modified in nine different ways:



A similar number of variants in the style makes a total of eighty-one different combinations. There is a further complication that homozygotes of all alleles except the top dominant are produced because of the sporophytic pollen determination which masks the effect of a recessive allele.

Although the general situation in the sporophytic system is complex owing to the number of variations, it becomes a simple matter to allocate a system of alleles with their interactions once two or three pollination patterns have been established.

Apart from the variety of patterns the main features of this system which distinguish it from the gametophytic system are:

1. There are frequent reciprocal differences.
2. Incompatibility can occur with the female parent.
3. A family can consist of 3 incompatibility groups.
4. Homozygotes are a normal part of the system.
5. An incompatible group may contain 2 genotypes.

In general the more individual allele action there is in operation, whether in pollen or style, the more cross-incompatibility there will be, whereas dominance will reduce it.

Turning from the general situation to the particular species we find

examples of all these characteristics. The results can best be summarized by dominance relationship diagrams, as in Fig. 5.

The known situations in the different species are arranged in order of complexity from top to bottom. In *Cardamine pratensis* the dominance

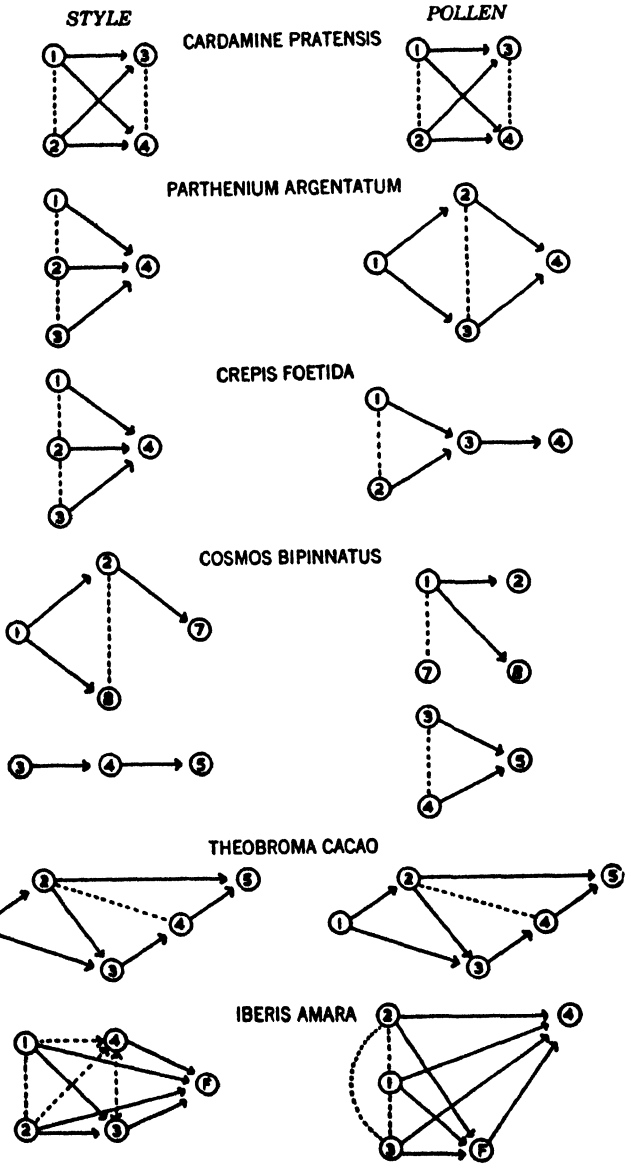


FIG. 5. Dominance relationship diagrams of sporophytic homomorphic systems: → full dominance, ⇢ weak dominance, ------ individual action, ⇠⇢ competition.

and individual action for allelic combination is the same in both pollen and style. There are two levels of dominance, and two alleles at each level. In *Parthenium* and *Crepis* the type of interaction between any pair of alleles is not always the same in both pollen and style.

In *Cosmos bipinnatus* eight different alleles have been identified, which show all the characteristics of the three previous species. There appears to be more dominance in the style than in the other species, but this may be a result of sampling error.

In *Theobroma cacao* the dominance relations of the five alleles is the same in the pollen and style as in *Cardamine*. There is, however, a relationship between three of the alleles which has not been found in the other species: 2 is dominant over 3, and 3 over 4, but 2 is individual with 4. Thus we know the dominance relationships between two of the pairs, but we cannot predict the third. This is a similar situation to that found in diploid pollen of the tetraploid *Oenothera organensis* (*cf.* Fig. 3).

In *Iberis amara* eight alleles have been identified, including an allele giving self-fertility. The relationships of six alleles have been established; the other two are probably recessive to the rest. A new feature in this species is the weak dominance between three pairs which gives about half the normal seed set. The self-fertility allele is dominant over one allele in the pollen and recessive to three others, and in the style it is recessive to at least four. This dominance of a self-fertility allele in a sporophytic system should not be confused with the so-called dominance of self-fertility alleles in the gametophytic system. For in this case actual dominance does not operate: the F_1 is all self-fertile because of gametophytic action and not dominance.

Most of the evidence on self-fertility alleles, which has come from the gametophytic systems, points to their being inactive alleles. Clearly the dominance of *SF* in *Iberis* shows that it has some activity in suppressing other alleles but that its final product is not an active incompatibility substance.

A unique system was postulated for *Capsella grandiflora* by Riley (1936): this was based on two loci each with two alleles exhibiting dominance and epistacy, as in the tristylic *Lythrum*, but *Capsella* is homomorphic. In view of the discoveries in the Compositae that all the known systems are sporophytic and of the fact that the same system is operating in *Iberis amara*, the question arises of an alternative explanation in *Capsella* which, like *Iberis* is a member of the *Cruciferae*.

In another Crucifer, *Brassica oleracea*, Kakizaki (1930) explained his results on a series of inhibiting *S* alleles and stimulating *T* alleles with gametophytic pollen control; again this unusual and unlikely explanation may have an alternative explanation in the sporophytic system.

Owen (1942) in *Beta vulgaris* postulated an unusual system, invoking two loci of *S* inhibitors with gametophytic control, to explain his pollination patterns. Each of his three families had patterns which are now known to be common features of the sporophytic system, but the inter-

relationship of the three families is difficult to explain on a sporophytic basis.

Further work in these exceptional species might substantiate a sporophytic system.

(i) *Effect of polyploidy.* Autotetraploid plants produced by colchicine treatment of the seed from a known cross of *Parthenium argentatum* resulted in four diallelic genotypes; these were all self-incompatible and behaved when interpollinated precisely as their diploid counterparts (Gerstel, 1950). With sporophytic control of the pollen, two alleles are normally present at the time of gene action. Thus there has been strong selection for the type of allele interaction operating in the pollen. It has been shown in the gametophytic system where only one allele is present at the time of pollen determination that a breakdown of allele activities occurs when two different alleles are present in the pollen of tetraploids. Clearly the negative effect of polyploidy in the sporophytic system is to be expected and confirms the conclusions obtained with the gametophytic system.

In several species of plants in which the incompatibility system has not been worked out, it has been found that polyploidy does not produce self-fertility. For example, in *Brassica* (Howard, 1942), *Raphanus sativus*, and *Secale cereale* (Lewis, unpublished), there is no effect. This negative evidence is a strong indication that these species may have the sporophytic system.

There will be possibilities of new dosage relationships in autopolyploids with the sporophytic system, and because weak and complete dominance are features of the system it is possible that such new dosage relationships will result in an occasional change in gene action.

III. Mutation of the Incompatibility Gene

Because of the extreme rarity of gene mutations, it is necessary for their profitable study to have an efficient means of selecting the mutants from the great number of normal individuals. In flowering plants this can be done with the *S* gene, for if a mutation occurs at this locus in the formation of the pollen, then the individual pollen grains carrying this mutation will have a different incompatibility reaction from the majority and will be rigorously selected to the exclusion of all the normal pollen grains in self-pollination. In some species it is possible to have several thousand pollen grains in effective contact with the stigma, so that the selection mechanism is highly efficient. The details of this method have been published only for *Oenothera organensis* and *Prunus avium* (Lewis,

1948, 1949b, 1951), but unpublished results obtained in these and other species will be included in this review.

One aspect of the mutation method of some importance is that the mutation, if it is to be selected, must have an immediate effect on the pollen grain: there must be no *phenotypic lag*. For example in *Oenothera* if X-rays are given to either mature or immature pollen grains any mutation that is induced is too late to have any effect on the pollen grain carrying it. The gene has already had its effect on the pollen before mutation. Thus the types of incompatibility in which the selective method would be expected to be most efficient are those in which the incompatibility allele does not have its effect until late in P.M.C. formation, *i.e.*, the types with gametophytic control, as in *Nicotiana* and *Oenothera*, etc. With sporophytic control the time of the *S* gene action is unknown, but it must be earlier than the tetrad stage of meiosis, and it is possible that the whole anther is determined in its initial stage of development. If this were so, the efficiency of the method would be reduced by a factor equal to the number of pollen grains in the anther. One further complication with the sporophytic system is that frequently the alleles show dominance in the pollen and individual action in the style. Thus if the recessive allele mutated, it would have no immediate effect and would not therefore be selected; and if the dominant allele mutated, it would have to retain its original dominance for its selection.

Before describing the kinds of mutations which have been obtained, I am making a distinction between *loss* and *constructive* mutations. Loss mutations are those which result in a loss of all or a part of the gene's function. They may include at the one extreme actual deletions of minute sections of chromosomes or at the other extreme only small molecular rearrangements.

Constructive mutations are those in which a new and positive function has resulted. With most genes this distinction is difficult if not impossible to apply, but with the incompatibility gene it has a real meaning, for the different alleles each produce a different and highly specific antigen- and antibody-like substance in the pollen and style. Thus a mutation from one normally active allele to another is evidence for mutation to a new specificity—a new antigen.

Among the loss mutations there are *permanent* and *revertible* mutations. The permanent ones, as the name implies, do not revert back to the old unmutated gene. The revertible mutations change back some divisions later.

The permanent loss mutations in *Oenothera organensis* have all been of one type. They show complete loss of the pollen-determining action

of the gene, whereas the activity of the part which is responsible for the stylar incompatibility determination is unchanged. This can be shown most simply by the crossing reactions given in Table 5.

TABLE 5
Reactions of Mutants Produced by X-Rays in *Oenothera organensis**

Mutation $S_1 \rightarrow S_1'$			
♀ *Style*		♂ *Pollen*	
S_1	×	S_1	−
S_1'	×	S_1'	+
S_1	×	S_1'	+
S_1'	×	S_1	−

* Note that the mutant allele S_1' is inactive only in the pollen; + = compatibility and − = incompatibility.

Thus the new allele S_1' does not produce an incompatibility reaction in the pollen but it does in the style, and a series of detailed tests have shown that it produces its style reaction as efficiently as the unmutated S_1 allele.

This has been interpreted as follows: the S gene has two functions which are possibly controlled by two parts of the gene, and the parts are mutationally distinct. All the ten permanent loss mutations in *O. organensis*, of which nine were produced by X-rays and one spontaneously, have been of this type. No mutations affecting the stylar function of the gene have been found. This does not imply that they do not occur, as we shall see later in *Prunus*, but it shows that the two parts in *Oenothera* do not often mutate simultaneously. For only mutants which affect the pollen part of the gene would be selected by the technique employed.

The revertible mutations in *Oenothera* are those which give full compatibility to the pollen grain in a style carrying the unmutated allele, but in the next generation the pollen has the old premutational reaction. The revertible mutations were found to be produced spontaneously and by X-rays. The evidence for the nature of the change producing revertible mutants has been discussed at some length (Lewis, 1951). The conclusion reached was that a true gene change was the most probable but that a cytoplasmic modification, although unlikely from the evidence, could not be excluded. Only new evidence will be given here.

In *Prunus avium*, Lewis and Crowe (1953) have found, apart from permanent loss and revertible mutations of the pollen part as in *Oenothera*, a remarkable mutant which proves not only that the revertible changes are genuine gene mutations which revert, but that the stylar part of the gene can mutate simultaneously with the pollen part. This mutation was pro-

duced by X-ray treatment of pollen-mother-cells. The action of this unique mutation is shown in Table 6.

TABLE 6

The Results of Test Crosses with a Mutant Seedling of *Prunus avium* Induced by X-Rays. The Incompatibility of the Mutant as Male on the Parent Shows that the Incompatibility Alleles S_3' and S_4' Are Active in the Pollen. The Self-Compatibility and the Compatibility as Female with the Parent Shows that One of the Alleles Is Inactive in the Style

Cross	*Result*	*Conclusion*
Parent $S_{3.4}$ × mutant $S_{3'.4'}$	−	Both alleles active in pollen
$S_{3.6}$ × mutant $S_{3'.4'}$	+	$S_{4'}$ pollen functions
$S_{4.5}$ × mutant $S_{3'.4'}$	+	$S_{3'}$ pollen functions
Mutant $S_{3'.4'}$ × parent $S_{3.4}$	+	$S_{3'}$ or $S_{4'}$ not active in style
$S_{3'.4'}$ selfed	+	$S_{3'}$ or $S_{4'}$ not active in style

The fact that the $S_{3'.4'}$ seedling fails as a male on the parent $S_{3.4}$ and is compatible as a male on $S_{3.6}$ and $S_{4.5}$ proves that the plant is not a homozygous $S_{3.3}$ or $S_{4.4}$ and is in fact a heterozygote. It also shows that both $S_{3'}$ and $S_{4'}$ are active in the pollen, but the compatibility of the cross using the parent as a male shows that either $S_{3'}$ or $S_{4'}$ is not active in the style. There seems to us to be one way in which the mutation could have arisen. A simultaneous mutation was induced in the two parts of the S gene, with the "stylar" mutation remaining permanent and the "pollen" mutation reverting to normal. The simultaneous occurrence after X-rays of a permanent "stylar mutation" and a revertible pollen mutation would require a high degree of improbability if their causes were independent, as they would be if the revertible changes were not due to gene mutation.

As with other genes, different S alleles differ in their mutation rates. In *Oenothera organensis* two of the four alleles tested have not mutated either spontaneously or with X-ray treatment in 80×10^6 gene divisions, whereas the two other alleles have given spontaneous mutation rates of 0.9 and 0.2 per 10^6 gene division and a correspondingly higher rate after X-rays. Furthermore, an allele can have an effect on the mutation rate of the accompanying allele. For example, alleles S_6 and S_4 give permanent loss mutation only when they are present with S_3, *i.e.*, in $S_{3.6}$ and $S_{3.4}$ plants. The genotype $S_{6.4}$ does not produce permanent loss mutations.

Mutation studies on another species, *Oenothera rhombipetala*, by Dowrick and Lewis (unpubl.) show similar differences and effects. Two alleles S_1 and S_2 have the same high mutation rate, whereas S_3 has not been known to mutate. When S_1 and S_2 are present with S_3 in $S_{1.3}$ and $S_{2.3}$

plants, the mutation rate of S_1 and S_2 is one-tenth of that obtained when they are present in the same individual, $S_{1.2}$.

Recent work on mutation in *Trifolium pratense* ($2n = 14$) and *Trifolium repens* ($2n = 32$) by Pandey (unpubl.) has revealed not only a difference between the mutabilities of different alleles within a species but also a difference between the two species. *Trifolium repens*, after X-rays, produces a high proportion of permanent loss mutations giving self-fertile plants, whereas *T. pratense* has produced only 1 in 9 of permanent mutations, the remainder being revertible mutants giving self-sterility like the parent.

The revertible *S* mutation produced by X-rays may be a more general phenomenon, and the reasons for their not being observed in other experimental material may be: (1) the inadequacy of the tests employed; or (2) revertible mutations may be induced by X-rays only when the chromosomes are in a certain stage of their reproductive cycle (such a stage may not be present in *Drosophila* sperm where revertible (mosaic) mutations have not been found *cf.* Lewis, 1951). Two other aspects of the *S* mutation work may be of general significance: (1) The most sensitive stage for mutation by X-rays coincides with 1st metaphase of meiosis; (2) the gene behaves towards X-rays as if it had reproduced at a stage in meiosis long before pachytene, when the chromosomes appear to reproduce (Lewis, 1951).

One further aspect of the mutation studies, and one of the most puzzling, is the lack of mutations of a positive constructive nature. With *Oenothera organensis*, *O. rhombipetala*, *O. pallida*, and *Prunus avium* no mutation from one operative *S* allele to another has been found out of a total of 350×10^6 tested. To explain the many alleles in the wild population, either we have to expect even lower mutation rates, or the present alleles have arisen in some different way from the type of mutation process we can study in the laboratory.

IV. Physiology and Biochemistry

Incompatibility is due to the failure of some process at any stage between pollination and fertilization. Sears (1937) has compared the pollen-tube growth after compatible and incompatible pollination in many species. In *Brassica oleraceae*, *Raphanus sativus*, and *Secale cereale*, the incompatible pollen usually fails to germinate, or if it does germinate, it does not penetrate the stigma. In some of these species, removal of the stigmatic surface removes the inhibition, showing that the reaction is localized in the stigma. In other species, *Nicotiana sanderae*, *Petunia violacea*, and *Oenothera organensis*, the pollen germinates and grows into

the style to a distance which varies not only with the species but with the environment and genetic constitution.

An extreme case was described by Sears in *Gasteria*, in which the incompatible pollen tubes grow at the same rate as the compatible tubes, reach the ovary, and fertilize the endosperm, but the egg nucleus is not fertilized. Unfortunately nothing is known about the incompatibility system in this species. A similar example of fertilization failure has been found in *Theobroma cacao* by Cope (1938) and Rogers and Knight (1954). In *cacao* the male nucleus fails to fuse with the polar nuclei to form the endosperm. The inhibition is between haploid nuclei, and as the pollen control is sporophytic, we have to postulate an influence on the male nucleus from the cytoplasm of the pollen and on the female nuclei from the embryo sac. It is also clear that without sporophytic control through the cytoplasm an incompatibility mechanism based on inhibition of nuclear fusion would not work, because segregation produces unlike pollen and egg nuclei, which with gametophytic control would be compatible.

Apart from the exceptional *Theobroma*, the incompatible reaction is between haploid pollen grains and diploid stylar tissue. If the incompatible pollen tubes are induced to travel the whole length of the style by suitable techniques, then there is no hindrance to the fusion of pollen and egg nuclei, as East (1934) first showed in *Nicotiana*. He obtained seeds from self-pollination in the bud stage and obtained homozygotes and heterozygotes for the *S* alleles in the expected proportions, and the *S* homozygotes were vigorous and fertile.

The physiological problem is how the pollen tubes are prevented from growing down the style.

1. *Theoretical*

There are two main theoretical possibilities for the physiological control of incompatibility. They are (1) that a positive inhibitor causes incompatibility and (2) that a *positive stimulant* results in compatibility. Both of these possible explanations have been given by some reviewers without any regard for the genetical evidence which supports or rejects them (Castle, 1940). There are, however, two aspects of the genetics which are of prime importance in defining the biochemical possibilities. These have been briefly defined by Lewis (1944, 1949a) but will now be given in more detail because of the additional experimental evidence at hand. This discussion of the genetical and biochemical controls of incompatibility is also justified for its wider application to the important problem of host-parasite relationships between fungal pathogens and higher plants. These have many features in common with the pollen-style relationship. The two basic biochemical controls are given in Table 7.

The genetical facts which can limit the choice between the possible biochemical controls outlined in Table 7 are obtained from the type of

TABLE 7

I. *Oppositional Inhibitor → Incompatibility*

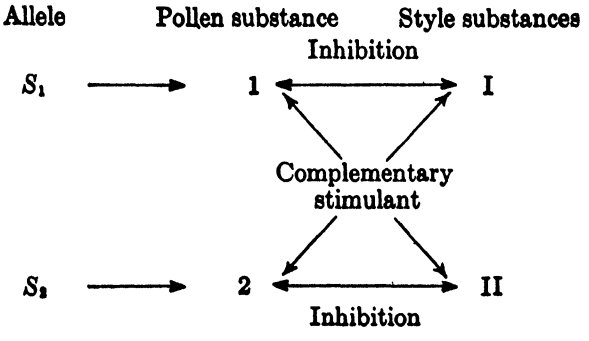
Reaction	*Mechanism*
between likes $S_1 \times S_1$ →	Inhibitor of pollen-tube growth: a. Inhibits ezyme or auxin necessary for growth; b. Blocks pollen-tube membrane; c. Inhibits enzyme necessary for penetration of the style

II. *Complementary Stimulant → Compatibility*

Reaction	*Mechanism*
between unlikes $S \times s$ →	a. Stimulant to pollen tubes; b. Antidote to an inhibitor normally present in pollen

genetic determination of the pollen reaction and the types of gene interaction in the style. If the pollen control is gametophytic, each plant must produce two pollen incompatibility substances; and similarly, if there is individual gene action in the style, each diploid style must produce two different substances. This is the situation found in the gametophytic oppositional system and an example is illustrated as follows:

$S_{1.2}$ Plant with Gametophytic System



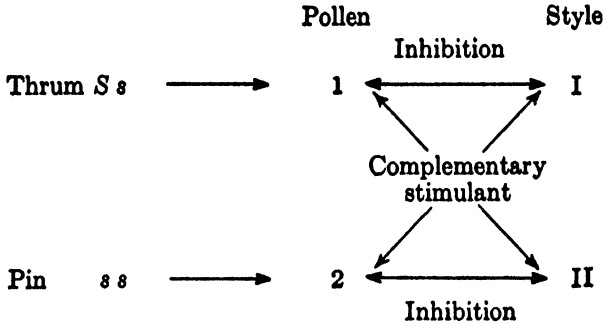
Clearly in this system if a complementary stimulant between unlike gene products is the cause of incompatibility—No. II possibility in Table 7—then the I stylar substance would stimulate 2 pollen and similarly with II style and 1 pollen. The plant would be self-compatible; hence, the only workable control is by an oppositional inhibitor between like gene products. A similar argument applies to sporophytic homomorphic systems because many allelic combinations show individual action in the style as well as sometimes in the pollen. Individual allele action with sporo-

phytic pollen control gives two different substances in the same pollen grain.

A further consideration is the number of alleles in the system. With a large number, as in the homomorphic systems, it is improbable that a whole series of different complementary stimulants could be in operation, because each mating group would have to have the complements to all the others but not to itself.

Where the number of alleles is limited to two, as in the heteromorphic system, a complementary stimulant is possible. Dominance of one allele in all its properties and *sporophytic pollen control*, results in one reaction in the style and the pollen of each of the two mating types; this is shown as follows:

Distylic System



With this system either an inhibition reaction between likes to produce incompatibility or a complementary stimulant to give compatibility would work equally well.

How the extreme limitation of the complementary-stimulation mechanism to two groups can be extended to three has been pointed out by Bateman (1952b). Bateman gives a mechanical model of a lock and key, the key of the pollen of one mating group fitting the lock of the style of the other. With three mating groups each type has to carry two keys in the pollen to fit the locks of the two others. The way in which a three-group system could work on a complementary basis is shown in the tristylic system. Each type has one style length (one lock) and two anther heights (two keys). As each anther height, from whichever type, is only compatible on one style, the parallel with the "lock and key" theory is complete. Each anther height corresponds to a single key "so that each form carries two keys" by virtue of its two anther levels. Apparently both "keys" cannot be borne in the same anther and one "key" cannot fit two "locks."

It should be emphasized that tristyly merely affords a hypothetical workable model of a complementary system with three groups, and it

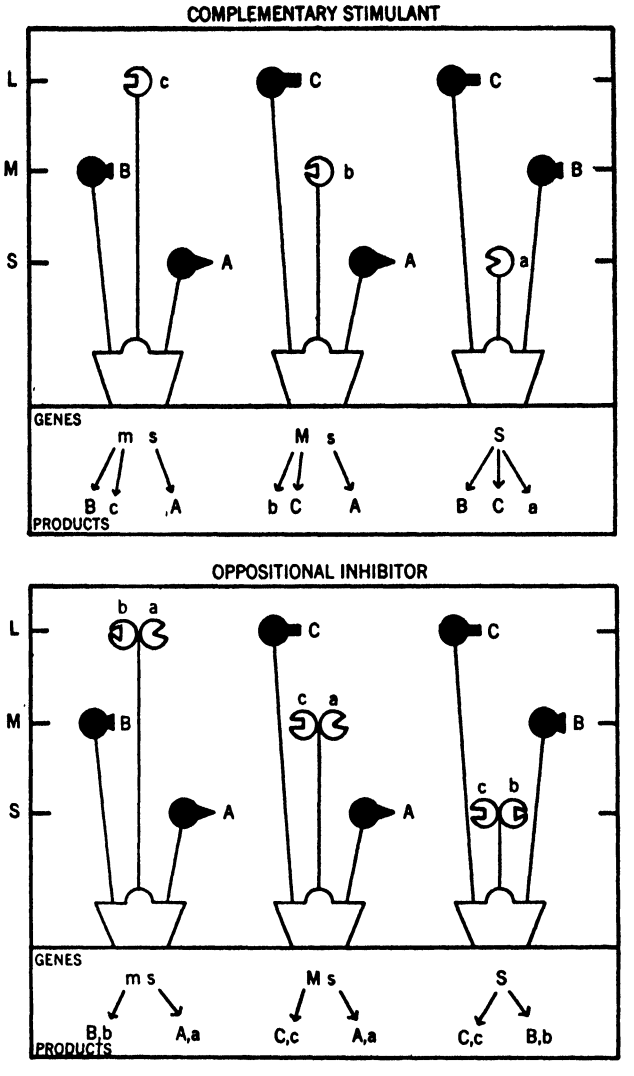


FIG. 6. Two physiological controls illustrated in tristylic *Lythrum salicaria*. Note that with the complementary control each flower produces three different specificities: each style (white) produces one substance represented by small letters, and the two different sets of anthers (black) produce two different substances (capital letters). With the oppositional control each plant produces only two specificities. Note the simpler gene action postulated with the oppositional control. (Complementary diagram by courtesy of A. J. Bateman.)

should not be supposed that a complementary system is the only one possible for tristylic species. In fact an oppositional inhibition system not only fits but is more economical in demands, especially where gene action is concerned. The two systems worked out for tristyly are given in Fig. 6.

The oppositional system demands that each style should have two different inhibitors; this is no more than is present in the style of a homomorphic species. Thus the three different mating groups have different

stylar reactions determined by the three different pairs obtained with three inhibitors. In each group there are two different pollen specificities which are the same as the two stylar specificities in the same group. These two pollen specificities must be kept separate, and this is obtained by having two different anther heights. This obviates the difficulty of two pollen specificities being produced with sporophytic pollen control—the control is sporophytic but under developmental influence.

When we consider the gene action in these two alternative physiological systems, we can see from Fig. 6 that with the complementary system the *S* allele has to produce all three different specificities or patterns of "lock" and "key," and the *M* and *m* alleles each have to produce two patterns. But in the oppositional scheme the *S* allele has to produce only two different inhibitors and the *M* and *m* alleles, only one.

It should be noted that the *S* allele produces both the products of *M* and of *m*, although a heterozygous *Mm* is unable to produce both. The *S* locus is epistatic to the *M* locus, and this interpretation of gene action in *Lythrum* affords a physiological model for epistacy of the type which produces a 12, AB, Ab :3 aB:1 ab ratio in the F_2.

From consideration of the theoretically possible physiological mechanisms and gene action we have seen that for both types of heteromorphic systems, distyly and tristyly, both physiological mechanisms are possible. For the gametophytic and sporophytic homomorphic systems only an oppositional inhibitor is possible. Let us see how this agrees with the data on physiological control.

2. *Effect of Temperature on Pollen-Tube Growth*

Pollen-tube studies at different temperatures (Lewis, 1942) have shown that the rate of growth in compatible styles increases with a rise in temperature. The Q_{10} for two gametophytic homostyled systems is 2.5 in *Oenothera organensis* and 2.0 in *Prunus avium;* for the heterostyled systems, the Q_{10} is 3.0 in *Primula sinensis* and 3.3 in *Primula obconica.* Thus the effect of temperature on compatible pollen-tube growth is similar to its effect on most growth processes and is an expression of a general increase in metabolic rates. The slightly higher figures for the two heterostyled species do not appear to have any significance.

Incompatible pollen-tube growth, on the other hand, is retarded by high temperature. Figure 7 shows results obtained in *O. organensis.* At 30°C growth ceases after 30 min. and 2 mm, whereas at lower temperatures growth may continue for more than 24 hours and 50 mm. This retarding effect has been found also in *Prunus avium* and in *Pyrus communis* and in *Pyrus malus* by Modlibowska (1945). The two heterostyled

species *P. obconica* and *sinensis* show the same effect. No effect, however, was found in the heterostyled *Linum grandiflorum.*

The best explanation of the growth-temperature curves is that the pollen tubes are positively inhibited in the style and that the inhibition reaction is faster at high temperatures; the products of the reaction accumulate as growth proceeds until a threshold is reached and the tubes stop growing. The temperature effect excludes the possibility of the alternative explanation of the complementary stimulation mechanism.

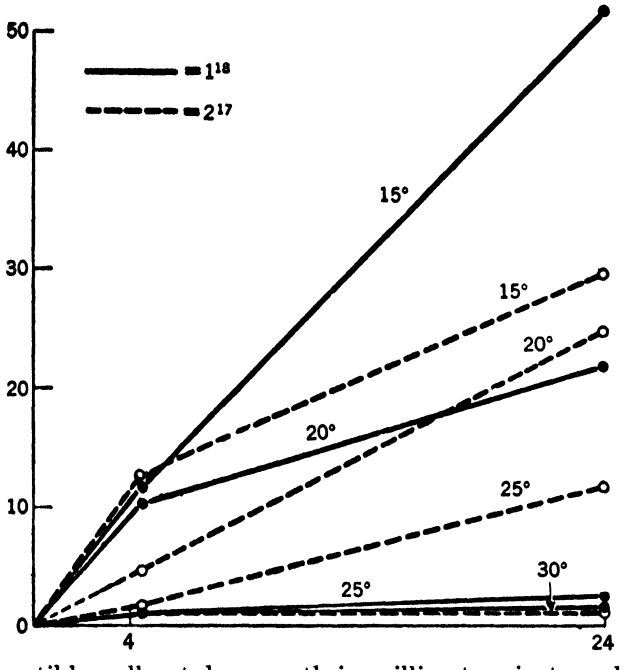


FIG. 7. Incompatible pollen-tube growth in millimeters in two clones, 1^{18} and 2^{17}, of *Oenothera organensis* at different temperatures: measurements were made at 4 and 24 hours.

The lack of a temperature effect does not, however, exclude the possibility of an inhibition reaction, because differential effects of temperature on different growth and incompatibility processes might cancel one another out, or the inhibition reaction might be absolute at all temperatures.

3. *Heteromorphic Species*

Linum grandiflorum, a distylic species, affords an example of a simple stimulatory physiological mechanism (Lewis, 1943c). The styles of the two types have different osmotic pressures, the Thrum being equivalent to 11% sucrose and the Pin to 20%. There is also a difference in the osmotic pressure of the pollen grains, thrum pollen being equivalent to 80% sucrose and pin to 50%. In the two compatible pollinations the O.P. ratio of pollen:style is 4:1, and pollen-tube growth is normal. The incompatible, thrum × thrum, mating has a ratio of 7:1, and the pollen

tubes burst on entering the style. The incompatible, pin × pin, mating has a ratio of 5:2 and the pollen grains fail to imbibe water and do not germinate. This simple stimulatory system is illustrated in Fig. 8, which shows not only the relative growth of the pollen tubes and the O.P. ratios but the bending of the styles as a result of withdrawal of water from the style in compatible and in thrum × thrum pollinations.

Forsythia is a genus of distylic species which has the normal pin and thrum differences that are found in *Primula*. One member of the genus,

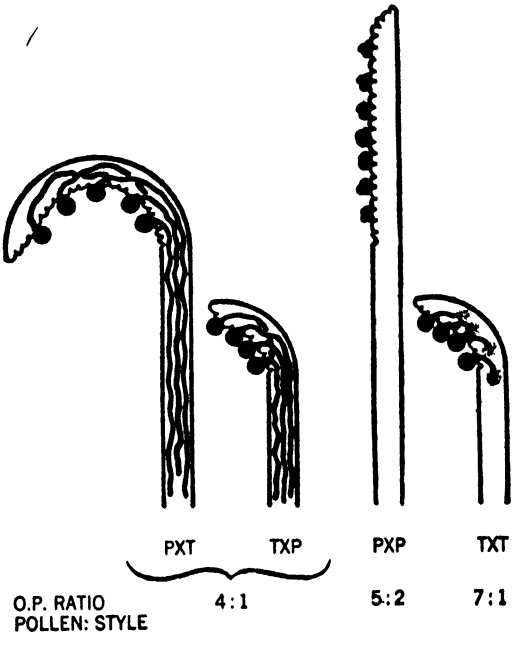


FIG. 8. The relationship between the osmotic pressure and the behavior of pollen and styles in *Linum grandiflorum*. Note that when the osmotic pressure ratio of pollen style is below 4:1 the pollen does not germinate; when it is higher, the pollen tubes burst.

Forsythia intermedia, is a commonly grown garden hybrid between *F. suspensa* × *F. viridissima*. This is of special interest because a biochemical explanation for its incompatibility has been postulated, which at present affords the only known example of the mechanism II(*b*) given in Table 7, *i.e.*, of a complementary action between products of unlike genes to give an antidote to an inhibitor normally present in the pollen.

Moewus (1950) has shown by chemical analysis, by paper chromatography, and by biological assay with *Chlamydomonas* that the pollen of the long-styled form contains the flavonol quercitrin, which is a 3-rhamnoside of quercetin. The pollen of the short-styled form does not contain quercitrin but has rutin, a 3-rhamno-glucoside of quercetin. He has also shown that quercitrin and rutin at a dilution of $1:10^6$ are

inhibitors of pollen germination in sugar solution and that this inhibition is counteracted by 0.01% boric acid. Boric acid had been known for some time to combine with flavonols.

When incompatible matings are made with mature pollen, no seed is produced, but when the pollen is applied to the stigma in 0.01% boric acid solution, a full set is obtained.

Other experiments using extracts of styles in sugar solution showed that the thrum extract would allow the germination of only the pin pollen, whereas the pin extract would allow the germination of the thrum pollen.

These elegant experiments are interpreted by Moewus in the following way. The thrum pollen contains the pollen inhibitor rutin, which is destroyed by a specific enzyme present only in a pin style. The pin pollen contains the inhibitor quercitrin, which is destroyed by another specific enzyme present only in the thrum style.

These experiments were carried out on two plants of *F. intermedia* growing in the Botanic Garden of Heidelberg University, and in view of the great interest of this example it seemed to be important to establish whether it was of general application to other species of the genus.

T. O. Dayton, working in this laboratory, has made chemical tests on pollen of a number of different plants from two species, including *Forsythia intermedia* var. *spectabilis*. In none of the pollen from either pin or thrum plants could quercitrin be detected, and all contained rutin. I have tested the effect of boric acid on pollen-tube growth on incompatible stigmas but with negative results.

From this survey it would appear that the rutin-quercitrin mechanism is not common to the genus. Unfortunately, we have been unable to examine the two parental species of *F. intermedia*. One of these species may contain quercitrin; this, if we assume segregation in later generations of the hybrid, would explain the presence of quercitrin in the one plant examined by Moewus.

Lythrum salicaria. A tristylic species offers a unique opportunity for testing, in a simple way, the type of physiological control in operation. The test is possible because each of the three types of pollen is incompatible in two types of styles. Thus, if the control is by a complementary stimulation to give compatibility then each type of pollen in both incompatible styles, because it merely lacks a stimulant, should grow equally in both styles. If, on the other hand, the control is by an oppositional inhibitor to give incompatibility, then it is possible for each type of pollen to be inhibited to a different degree in the two incompatible styles.

Esser's (1953) measurements of pollen-tube growth in *Lythrum salicaria* show that the growth of the same type of pollen is quite different in the two types of incompatible styles (see Fig. 9). It will be noted that

the mid pollen grows to 5 mm in a long style but only to 0.6 mm in a short style. Similarly short pollen grows to 3 mm in a long style but only to 0.8 mm in a mid style.

Clearly, without complicated additions to the assumptions, a complementary stimulatory control is excluded by these results, and they strongly suggest that a oppositional inhibitor is operating.

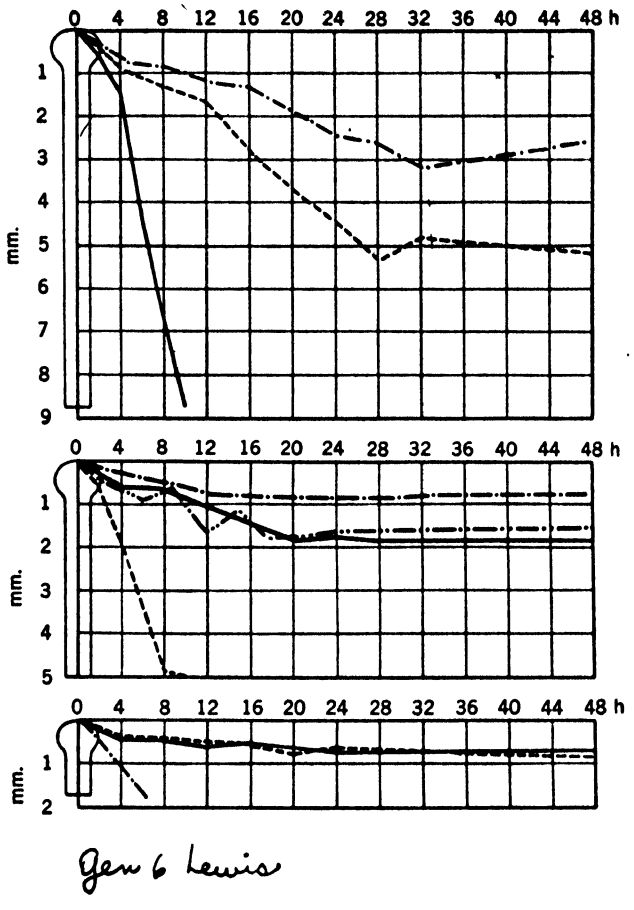


FIG. 9. Pollen-tube growth curves in long, mid, and short styles of *Lythrum salicaria:* pollen, —— from high anthers; – – – mid anthers; – · – low anthers; – · · – from high anthers treated with boric acid. Note that the same pollen is inhibited to different degrees in different styles (courtesy of Dr. Karl Esser).

Esser (1953) has concluded that an inhibitor is operating in the distylic species *Fagopyrum esculentum* from pollen-tube studies in diploids and tetraploids. This evidence does not, however, appear to exclude the alternative explanation.

4. *Homomorphic Species*

East (1929) first advanced the theory, which has been generally accepted but only recently proved, that the incompatibility in homomorphic

species is due to a reaction between an antigen in the pollen with an antibody in the style.

The physiological control in the gametophytic system in *Oenothera organensis* has been investigated by serological methods (Lewis, 1952). Pollen of four incompatibility genotypes were injected into rabbits. The different antisera gave precipitin reactions with all pollen extracts, but the homologous extracts gave stronger reactions. Furthermore, antisera

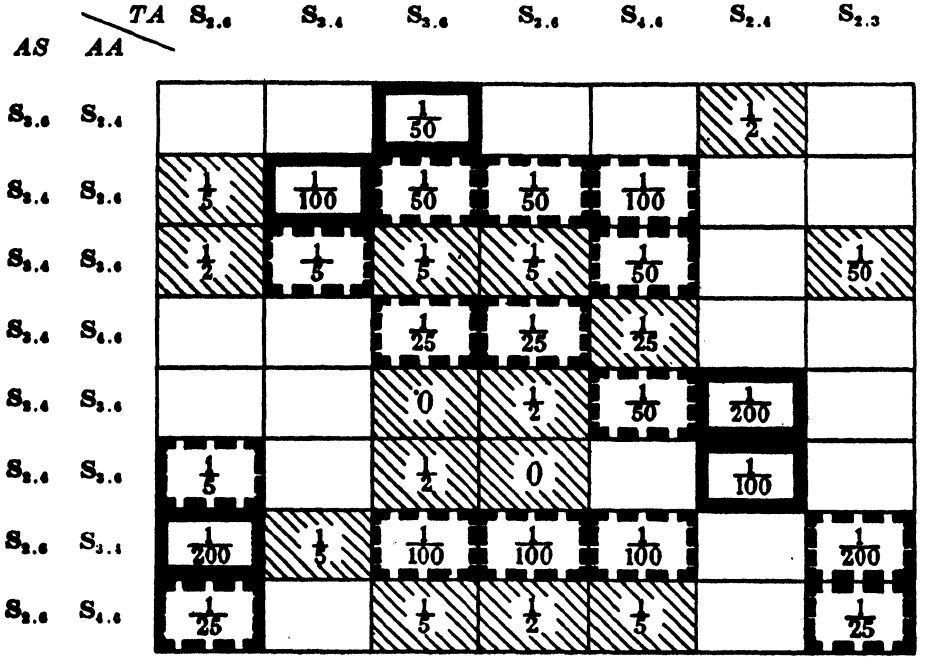


FIG. 10. The results of serological tests with pollen of *Oenothera organensis*. Titers from precipitin tests with absorbed antisera. AS, antiserum; AA, absorbing antigen (pollen extract); TA, test antigen (pollen extract).

Heterologous antigen, *i.e.*, no *S* alleles common to test antigen and absorbed sera.

Completely homologous, *i.e.*, both *S* alleles common to test antigen and absorbed sera.

Half-homologous, *i.e.*, on *S* allele common to test antigen and absorbed sera.

which had been fully absorbed with pollen extracts which were heterologous in respect of the *S* alleles gave reactions only with extracts which had *S* alleles in common with the absorbed antisera. The results of these tests are given in Fig. 10.

The good agreement between the serological behavior of the pollen and its *S* constitution shows that the *S* alleles are producing proteins or polysaccharides which act as antigens.

Although, through technical difficulties, it has been impossible to prove the presence of corresponding antibodies in the style, it is a reason-

able assumption to make. In *Oenothera organensis* the antibodies in the style are formed without the stimulation of the pollen antigen; this has been proved by double pollination. The first pollination with incompatible pollen does not enhance the inhibition of a second pollination.

The reaction between pollen and style in *Oenothera* is one of extreme rapidity. At 30°C the reaction takes not longer than 30 minutes after the application of the pollen and when only 1–2 mm of growth have occurred. Furthermore, this inhibition is irreversible (Lewis, 1952).

In *Petunia violacea*, Straub (1947) has shown by double pollination that the antibody in the style is preformed and does not have to be stimulated by the presence of the pollen antigen.

Linskens (1953) has used the method of electrophoresis on extracts of styles of *Petunia* which have been either compatibly or incompatibly pollinated. He has shown that after incompatible pollination a protein fraction was present which did not occur in unpollinated or compatibly pollinated styles. This new protein fraction he reasonably assumes to be the antigen-antibody complex.

The two complementary pieces of evidence in *Oenothera* and *Petunia* prove the antigen-antibody natures of the incompatibility reaction.

V. The Fungi

I have differentiated incompatibility from sexuality (dioecy) in the fungi by the ability of a single monospore culture to function in both male and female capacities. When the sexual organs, or if these are absent, the functions of both sexes, are produced by all mating types of monospore cultures, and if development of fruit bodies does not occur unless different mating-type monospore cultures have fused, then, incompatibility is at work.

1. *Ascomycetes*

Heterothallic species in the *Ascomycetes*, including the well-known *Neurospora crassa*, have an incompatibility system in which there are only two mating types, + and − (*cf.* Lederberg, 1948; Catcheside, 1951). Several other species appear to be homothallic, *i.e.*, a monospore culture produces normal fruit bodies. They are self-fertile. Such species include *Neurospora tetrasperma* (Dodge, 1942) and *Pleurage anserina* (Dowding, 1931; Rizet and Engelmann, 1949). In these species and probably in all other homothallic species with four-spored asci, each ascospore normally contains two nuclei, and these two nuclei are of opposite mating types. They are therefore really heterothallic (*secondarily homothallic*), but the association of the two types of nuclei occurs immediately after meiosis.

They are in fact permanent heterozygotes, which are genetically similar to the permanent heterozygotes of the ring chromosome system in the flowering plants. Rizet and Engelmann's explanation in *Pleurage anserina* for the high frequency of spores containing the + and − nuclei is by the regular occurrence of one chiasmata between the mating locus and the centromere, thus ensuring second-division (post-reduction) segregation; there is also a bias for symmetrical segregation. This is followed by a side by side synchronous mitosis after meiosis which gives alternate + and − nuclei in the ascus. In *Neurospora tetrasperma* (Dodge, 1927) the production of spores with + and − nuclei appears to be obtained by regular first-division segregation of the locus. Thus by quite different means from ring-forming flowering plants a result has been obtained with similar genetical consequences. *Pleurage*, apart from being an interesting permanent heterozygote, has provided good evidence for the kind of physiological mechanism controlling the + − mating reaction in the *Ascomycetes*.

Rizet and Engelmann have shown that as a rarity *Pleurage* produces small uninucleate ascospores which contained either a + or a − nucleus. By using two marker genes they showed that if a self-fertile heterokaryotic colony from a binucleate + − ascospore was in contact with a + or − homokaryotic colony, a line of perithecia would form at the meeting of the colonies. These perithecia segregated ascospores carrying marker genes which could only have come from the contiguous homokaryon and the complementary nucleus of the heterokaryon. This experiment proves that nuclei can enter a mycelium despite the presence of some incompatible nuclei and can perform the normal compatible function of stimulating and entering into the production of fruit bodies. It proves therefore that the physiological mechanism causing incompatibility is not an oppositional inhibitor produced by like gene products. It leaves the alternative mechanism, which is a complementary stimulant between unlike-gene products. This is the view taken by Hawker (1950), who states that "the difference in strain" (+ or −) "must be that each contributes some specific substance essential for fruiting which is not produced by the other."

That this complementary mechanism is probably the only one in the *ascomycetes* is supported by the fact that the two mating types in *Neurospora tetrasperma* are compatible with only the complementary mating types in *Neurospora crassa* (Catcheside, 1951).

From our knowledge of the systems in flowering plants we know that a complementary stimulant control of mating can produce only two, or with the complication of tristyly possibly three, different mating types. Thus in the *Ascomycetes*, we have a close parallel with the complementary systems found in some distylic plants. For the restriction to two mating

types in the *Ascomycetes* would be brought about by the type of physiological control adopted. In distylic flowering plants there are two possible causes for restriction to two groups; the flower morphological differentiation and the physiological mechanism. Either will restrict the number of mating groups. In the *Ascomycetes* there is no morphological differentiation which has to be maintained between the two groups, thus leaving the complementary mechanism as the sole restraint.

The type of physiological incompatibility mechanism in fungi is being considered in some detail because it may have implications beyond the immediate problem, and I hope to show that in certain groups it may be connected with parasitism.

2. *Ustilaginales*

The *Ustilaginales*, the smuts, are a group of plant parasites which do not have differentiated sex organs. The product of meiosis, the monokaryotic sporidia fuse in pairs, the nucleus of one passes into the other, and a dikaryotic mycelium is produced. Fusion occurs only between different mating types, and Holton (1942) has shown that the migration of the sporidial nuclei after fusion is conditioned not by the mating type but by the state of nutrition of the sporidia. Thus on our definition the control of mating is by incompatibility and not by sexual differentiation.

The incompatibility has been investigated in many species of smut fungi, and is commonly of the two groups +, − type. Whitehouse (1951) has reviewed the subject in detail and has concluded that the few reported exceptions to the two-group system are due to faulty interpretation. With the reservation that more work on the reported exceptions is desirable, I am accepting this conclusion because it not only clarifies the problem of incompatibility but also of parasitism in this order, for the incompatibility system is closely bound up with parasitism. This is shown by the following: (1) only + − dikaryons can infect the host plant, although monokaryons can be grown on artificial medium (Christensen and Rodenhiser, 1940); (2) dikaryotic lines of *Ustilago Zeae* which had become nonpathogenic gave monosporidial cultures which had lost their power to mate; (3) the sporidia of some species fail to conjugate on artificial media even when they are of compatible types (*cf.* Whitehouse, 1951).

Thus not only is parasitism dependent upon the conjugation of the two mating types, but conjugation is, in some species, dependent upon parasitism. This interdependence probably means either that two complementary gene products which are necessary for parasitic growth are each produced by a gene or genes situated on different homologous chromosomes and linked with the incompatibility gene, or that the two + −

incompatibility alleles not only supply the complementary stimulus for establishing the dikaryon but also that necessary for parasitism. Complementary nutritional requirements are suggested by the different nutritional needs of the + − types of *Ustilago violacea* in artificial media (Bauch, 1922).

With a two-group incompatibility system in a fungus with an extended dikaryon, this parallel evolution of incompatibility and a function such as parasitism is to be expected. A considerable section of the chromosome carrying the incompatibility gene will be permanently heterozygous; thus, complementary genes necessary for the dikaryon will accumulate on this segment. Since there are only two different segments, the + and −, there will eventually be only one heterozygote, and this will contain a balanced system of growth genes. With the more than two groups which would be present with a multi-group system, it seems unlikely that such a balanced heterozygote system would arise.

3. *Uredinales*

The *Uredinales*, the rusts, are a group of obligate plant parasites. Monokaryotic cultures produce male and female organs, but fusion and sexual reproduction only occur between + and − cultures, and there are only two groups, as in the *Ustilaginales* and *Ascomycetes*. In the rusts as in the *Ustilaginales* there is no direct evidence for the type of physiological mechanism, but it seems safe to assume that a complementary stimulant is at work.

In this order there is a similar but less rigorous dependence of parasitism on the establishment of the dikaryon. Many of the species have two different host species, the monokaryon infecting one and the dikaryon the other. Thus the ability to parasitize one of the hosts is dependent upon the presence of + and − nuclei in the same mycelium. This involves the same principle as in the *Ustilaginales:* viz., that with a two-group system, selection of a single balanced complex heterozygote linked with the +, − gene would become essential for growth in the environment which differs from that required by the monokaryon. An interesting exception is that the dikaryon of the heteroecious rust *Gymnosporangium Juniperi-virgininae* can parasitize the normal monokaryon host (Hotson and Cutter, 1951), but no reports of the reverse situation have been made. Since the dikaryon has the genes of both monokaryons, it is to be expected that it would be able to carry out the parasitic processes of the monokaryon.

In the many rusts with only one host (autoecious) both mono- and dikaryons would be parasitically similar.

4. *Hymenomycetes*

The *Hymenomycetes* and *Gasteromycetes* are devoid of any traces of sex organs. The heterothallic species have monokaryotic and dikaryotic phases, both of which are extended. When two compatible monokaryotic cultures meet, they exchange nuclei, become dikaryotic, and produce fruit bodies.

The incompatibility systems have been investigated in a large number of species; they have been reviewed by Whitehouse (1949a) and Quintanilha and Pinto-Lopes (1950). There are two systems: (1) the *bipolar*, which is controlled by a single incompatibility locus; and (2) the *tetrapolar*, which is controlled by two loci. Dikaryons are produced only in matings between monokaryons which differ in their alleles at the one locus in bipolar systems and at both loci in the tetrapolar systems.

Whitehouse estimates that 10% of the species are *homothallic*, *i.e.*, self-fertile, and that of the heterothallic species, 39% are bipolar and 61% tetrapolar. Quintanilha and Pinto-Lopes list 314 tested species, 28 (8%) homothallic, 121 tetrapolar, and 75 dipolar, with the remainder unknown. Most of the homothallic species are permanent heterozygotes, two basidiospores are formed instead of four, and each spore receives two different nuclei.

A striking constant feature of the incompatibility system throughout these groups is the large number of alleles. In the *Hymenomycetes* there are not less than 100 alleles per locus in a natural population. In the *Nidulariales*, which have restricted spore dispersal, the number of alleles is approximately 10 per natural population (Whitehouse, 1949a).

There is one possible exception in *Solenia anomala* (Greis, 1942), which appears to have one multiple-allele locus and a two-allele locus. The alternating pair of alleles at the two-allele locus also controls colony size, one allele producing a large colony and the other a small. Even when the incompatibility alleles at the multi-allele locus are different, compatible mating occurs only between large and small colonies, and even then nuclei pass only one way, from small to large.

The *Hymenomycetes* differ, therefore, from all other fungi in their incompatibility mechanism by having a multiple-allele system.

5. *Physiology of Hymenomycetes Incompatibility*

On *a priori* grounds, which have been discussed in the section on the physiological mechanism in flowering plants, a multiple-allele system implies a physiological mechanism of the oppositional inhibition type. In the *Hymenomycetes* it is possible to test this hypothesis by mating certain dikaryons and heterokaryons with monokaryons. The di-mon mat-

ings were first analyzed by Buller in 1931 and have been discussed by Quintanilha (1939) and Buller (1941), but the most complete and recent analysis, which is by Papazian (1950) in the tetrapolar species *Schizophyllum commune*, will serve as an illustration. In *Schizophyllum* illegitimate heterokaryons can be formed in which there is a common allele at one of the two loci and different alleles at the other locus, *e.g.*, $A_1B_2 + A_2B_2$. This heterokaryon has a mycelium which is distinct and different from that of a dikaryon. When it is mated with monokaryon testers, although both the A and B alleles of the monokaryon may differ from those in one of the heterokaryon nuclei, the heterokaryon is not dikaryotized. Thus an A_1B_1 or an A_2B_1 monokaryon cannot establish a dikaryon in the heterokaryon $A_1B_2 + A_2B_2$. If the physiological control is by a complementary stimulant between unlike gene products to give compatibility, then the A_1B_1 nucleus from a monokaryon should find its complement in the A_2B_2 nucleus, and similarly with the other monokaryon A_2B_1 and the other nucleus A_1B_2 from the dikaryon. Thus the experiment excludes a complementary stimulant and is in full accord with an oppositional inhibitor.

Other results in the same species based on the *Buller phenomenon* are difficult to interpret. The Buller phenomenon is the dikaryotization of a monokaryon by a dikaryon containing nuclei neither of which taken separately is compatible with the monokaryon. This can be illustrated by a dikaryon of the general type $A_1B_2 + A_2B_1$ and a monokaryon A_1B_1. The way in which the dikaryotization occurs has been the subject of speculation for some years. Buller (1941) and others supported the view that both nuclei of the dikaryon migrate into the monokaryon, supplant the original nucleus, and form the dikaryon. Quintanihla's hypothesis invokes the formation of a new nucleus which is compatible with the monokaryon by somatic nuclear fusion and reassortment of chromosomes in the dikaryon. The new nucleus and the monokaryon nucleus form the dikaryon. The evidence of extensive somatic crossing over in other fungi given by Pontecorvo (1953) gives some support to this hypothesis.

In *Schizophyllum* Papazian has made the most complete analysis of this phenomenon and his tests show that in 6 cases there was a migration of both nuclei of the dikaryon into the monokaryon and in 2 cases a new nucleus was constituted in the dikaryon. How this new nucleus was formed—whether by chromosome re-assortment or mutation—cannot be decided from the data.

But the migration of the two nuclei of the dikaryon into a monokaryon containing a nucleus which is incompatible with both of the dikaryon nuclei contradicts the evidence from the reaction of a heterokaryon and a monokaryon.

This contradiction is shown in Fig. 11. If this double migration is one cause of the Buller phenomenon, and Papazian's evidence seems to prove this, then we must conclude that the repulsion or inhibition of nuclei with like alleles is less between a dikaryon and a monokaryon than between two monokaryons. The nature of the forces operating between incompatible and compatible pairs of nuclei is extremely obscure. Quintanilha (1939) has concluded that it is "ces facteurs de stérilité eux

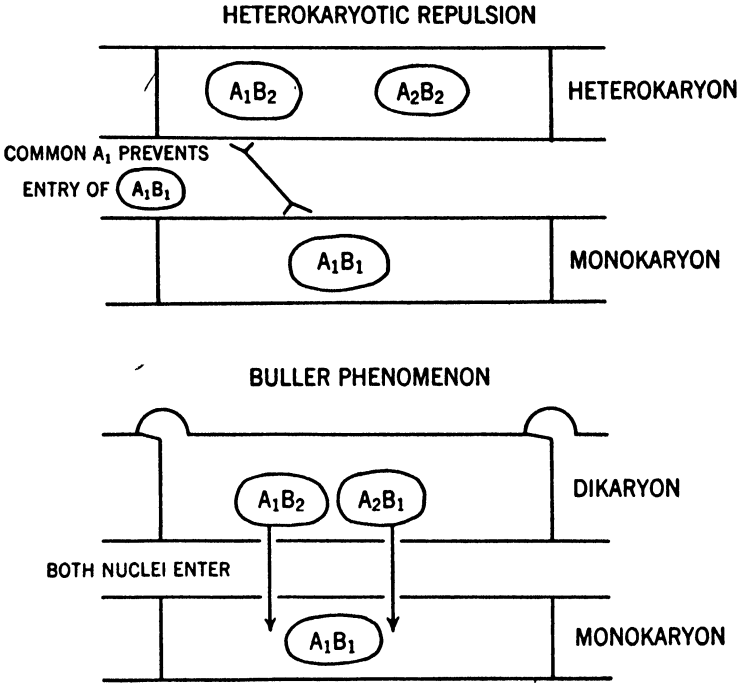


FIG. 11. Illustrates the reactions between a heterokaryon and a dikaryon with a monokaryon in *Schizophyllum*. In the Buller phenomenon shown below the two nuclei of the dikaryon can enter and form a dikaryon in the monokaryon. But the monokaryon cannot establish a dikaryon in the heterokaryon shown above.

meme qui jouent un double role, de répulsion entre facteurs communs et d'attraction entre facteurs différentes." This view, I think, is inescapable if we are to accept the findings on the Buller phenomenon and the heterokaryotic repulsion.

If we accept this double role of attraction and repulsion, then in the Buller phenomenon it is as if the forces of attraction and repulsion have been neutralized by the act of conjugation into a dikaryon. A crude analogy is found in + and − electric charges: like charges (alleles) repel, unlike attract and a conjugate +, − is neutral. It is significant for this speculation that the process of dikaryotization of the monokaryon in the Buller phenomenon is extremely patchy and slow, suggesting that there is some residual repulsion.

The force of attraction between different compatible nuclei would also explain the regular production in bispored homothallic species of spores with two compatible nuclei (*cf.* Whitehouse, 1949b). It must be assumed that in heterothallic species the forces of attraction binding compatible nuclei in the dikaryophase are in abeyance in the basidium, but that in the homothallic species these forces persist throughout the basidium stage.

Further work with other species on the Buller phenomenon and with heterokaryons might contribute greatly to the elucidation of the forces operating between the different nuclei and cytoplasm.

One other outstanding and baffling problem in the *Hymenomycetes*, as in the flowering plants, is the origin and genetic relationship of the many alleles. Whitehouse (1949a) and Papazian (1951) have reviewed the literature on mutation of the A & B loci and have concluded that except in *Schizophyllum commune* mutation is extremely rare. In *Schizophyllum* Kniep (1929) and Papazian (1951) have shown that mutation of both loci is common—of the order of 1%. Papazian has provided evidence from analysis of tetrads containing an aberrant mating type nucleus that the *A* and possibly the *B* locus are really complexes of two genes with about 4% crossing over between them. Although this finding explains the origin of some new alleles, it has given rise to new problems of gene action in which two different genes at 4 units apart are able to collaborate to produce the unique specificity of a single mating reaction.

In the *Ustilaginales* and *Uredinales* it was shown that the genetic control of the + − two-group incompatibility system was bound up with the determination of other characters, such as parasitism of the dikaryon. In the *Hymenomycetes* with a multi-allele system we should not expect this relationship. Evidence on the ability of monokaryons and dikaryons in the *Hymenomycetes* to perform similar growth, enzymatic, or parasitic functions is extremely scanty. But the reports on the ability to destroy wood by *Coriolus abietinus*, *Stereum purpureum*, and *Stereum rogosum* (Robak, 1936) have proved that the monokaryon is fully competent. Further work on the enzymatic and growth activities of monokaryons and dikaryons would be highly instructive.

VI. Incompatibility as an Outbreeding Mechanism

The relative efficiencies of the different systems of incompatibility in flowering plants and fungi have been described by Mather (1942) and Whitehouse (1949b). Mather pointed out the important effect of the intervention of the diploid stylar tissue between the male and female gametes in the angiosperms. This completely prevents self-fertilization with all incompatibility systems. In the fungi which lack a structure

analogous to a style, no system of incompatibility completely prevents self-fertilization.

Important as this aspect of the subject is, for it is the *raison d'être* of incompatibility, it will be referred to briefly in relation to the main problems discussed in previous sections because of the adequate treatment it has received in the past.

In considering the efficiency of the systems it is necessary to consider:

1. The amount of self-fertilization permitted.
2. The amount of incompatible cross matings in the population.
3. The amount of incompatible sib mating in the population; this is of far less importance than the other two.

The outbreeding characteristics of the main systems in angiosperms and fungi are given in Fig. 12, omitting the sib mating factor for the sake of simplicity.

All that need be said about sib mating is that, with the di-allelic systems in the heteromorphic angiosperms and in the *Ascomycetes*, a half of the sib matings are compatible; this is the same value for non-sib matings in the population. Therefore, there is no restriction on sib-fertilization. With multiple-allelic systems, on the other hand, the compatibility of sib matings relative to non-sib matings is reduced as the number of alleles increases. This difference between the two genic controls merely adds to the main differences in outbreeding efficiency shown in the table. In the second column will be seen the striking difference between the angiosperms and the fungi in self-fertilization due to the intervention of the stylar tissue and to the greater efficiency of the tetrapolar (2 loci) as compared with the bipolar (1 locus) in the *Hymenomycetes*.

The third column shows the effect on general cross-compatibility. With the di-allelic control only half the cross matings are compatible, whereas with the multi-allelic systems the amount of cross-compatibility rises with the number of alleles. The different multi-allelic controls give slight differences, but these are only of importance when the number of alleles is low—as it might be at the start of a system's evolution. For example, the gametophytic system in angiosperms with three alleles imposes less restraint on general cross mating than the sporophytic system. In the *Hymenomycetes* the bipolar system which is less efficient as a restraint on self-fertilization imposes less general cross-incompatibility than does the tetrapolar.

These differences within the multi-allelic are, however, trivial as compared with the great difference in efficiency between the di- and multi-controls.

It was first pointed out by Sewall Wright (1939) and since emphasized by several writers that any new allele that arises will have the advantage

of being compatible with all others. This results in a strong selection for a high number of alleles apart from the added efficiency it gives to the system.

The question one may ask is why have whole groups of plants without exception adopted the di-allelic system?

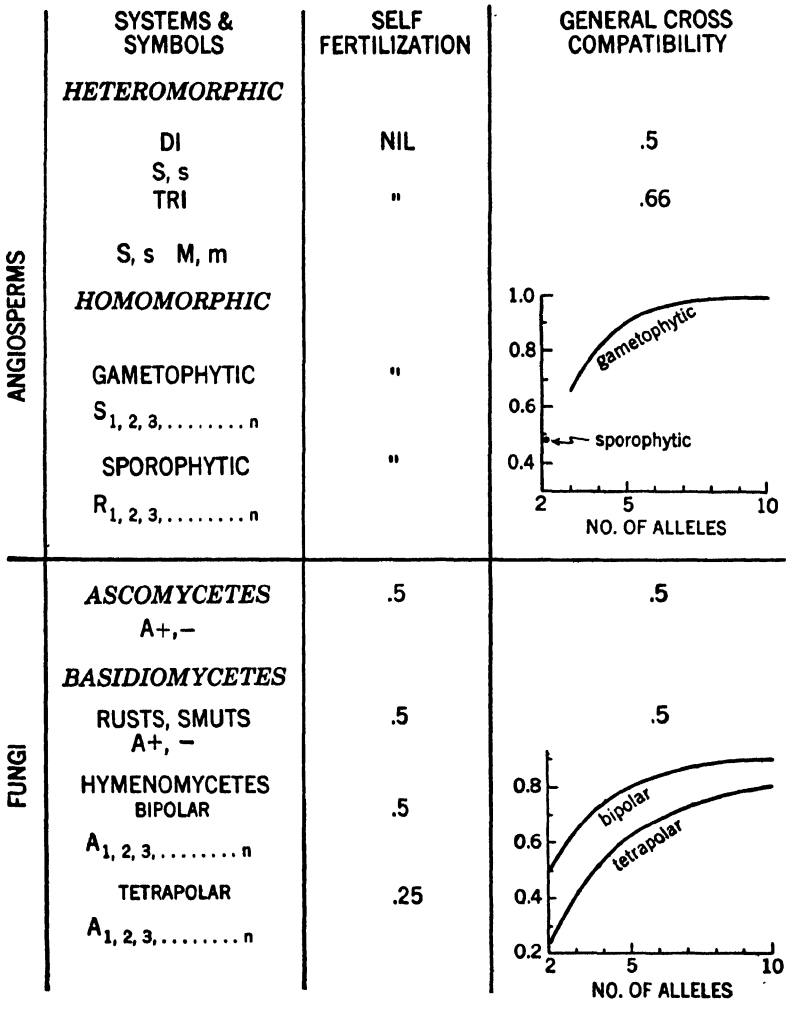


FIG. 12. The effect of the different systems in angiosperms and fungi on outbreeding. The term self-fertilization includes the fusion of products of the same zygote. Note the effect of the style in reducing self-fertilization to zero and the effect of increased number of alleles on the general cross-compatibility within the population.

The answer lies in the canalization caused by the restriction placed upon variation by morphological differentiation in the heteromorphic plants, and by the type of physiological control—a complementary stimulant—on the di-allelic flowering plants and fungi. Once a system has become established on a complementary basis, it will accumulate further complementary effects which will become so complex that the

establishment of a third complement would be as seriously handicapped as the development of a third sex in unisexual organisms (*cf.* Fisher, 1930).

VII. Incompatibility and Plant Breeding

The practical importance of incompatibility in clonally reproduced crop plants has been realized for more than fifty years and the fertility relationship of all well-known varieties of fruit trees have been determined with the detail which is necessary for the correct planning of fruit orchards (Crane and Lawrence, 1947).

But the study of the mutation of the incompatibility gene has provided new methods of plant breeding. The production of fully self-compatible varieties of all the sorts of fruit trees is now possible. Mutations of the *S* gene are produced by irradiating the pollen mother cells of a fruit tree and by using the mature pollen derived from the treated cells either in self-pollination or on another variety of the same incompatibility group. As a result, in *Prunus avium* 2% of the pollinated flowers set fruit as opposed to 0.1% without irradiation. Many of the seedlings obtained from the 2% of seeds produced are fully self-fertile.

By crossing these seedlings on to good established varieties many progeny have been obtained which carry the self-fertile allele, S_F. Thus it will soon be possible to select a range of varieties which are fully self-fertile.

Self-fertility in fruit trees has the obvious economic advantage derived from large block plantings of one variety. It also may have a considerable advantage where regularity of cropping is considered. Evidence for this comes from cultivated plums which because of their hybrid origin already contain self-fertile and self-sterile varieties. All the European self-fertile varieties of plums are regular and heavy croppers, but many of the self-sterile varieties are irregular croppers. The reason for this is that frequent cold weather during pollination results in inadequate bee activity. Self-pollination is effectively carried out by a small number of bee visits, but cross pollination between trees requires a high bee concentration.

One other application has been to produce triploids in apples and pears (Lewis, 1943b). By heat-treating pollen mother cells a small percentage of unreduced pollen grains are produced. If this pollen is placed on a compatible stigma, only the haploid pollen grains function. If it is placed on an incompatible stigma, only unreduced pollen grains function owing to competitive interaction in the pollen, thus giving triploid offspring. Since about 20% of our cultivated varieties are spontaneous triploids, this method has possibilities for fruit improvement.

In self-sterile crop plants which are propagated sexually, such as

clover, cabbages, rye, self-fertility would so seriously upset the genetic structure of the population that it would probably lead to inbreeding depression. But in these crops incompatibility can be put to good use to produce F_1 hybrid seed, as advocated by Odland and Noll (1950).

If two lines can be made homogeneous for their *S* alleles, then by interplanting the two lines only hybrid seed will be produced. This can of course be done by vegetatively cloning up an individual plant of each line or by producing plants homozygous for their *S* genes. To do this usually means resorting to self-pollination, and this may result in increased self-fertility, which must be avoided. The likelihood of selecting for self-fertility can be reduced either by making the incompatibility sieve less rigorous by pollinating in the bud before the incompatibility substances are fully formed (East, 1934) or at low temperatures (Lewis, 1942). Odland and Noll have successfully used both these methods in *Brassica*. In many other plants homozygotes are more difficult to obtain, but here they can be produced by X-ray-induced revertible mutations, as described in a previous section.

When homozygotes have been obtained, they can be used in two ways:

1. *A first cross* Line 1 $S_{1.1} \times$ Line 2 $S_{2.2} \rightarrow S_{1.2}$ (hybrid seed)

This method would produce an F_1 which would be sterile; this is an advantage where the crop is not the seed because the seed producer has full control of his F_1.

2. *A second cross* Line 1, $S_{1.1} \times$ Line 2, $S_{2.2} \rightarrow$ Hybrid $S_{1.2}$
Line 3, $S_{3.3} \times$ Line 4, $S_{4.4} \rightarrow$ Hybrid $S_{3.4}$

$$S_{1.2} \times S_{3.4} \rightarrow \text{all 2nd Hybrids } S_{1.3}, S_{1.4}, S_{2.3}, S_{2.4}$$

This hybrid from the heterozygotes will be fertile.

As a method of plant improvement there is little doubt that F_1 hybrids are going to play a big part in the future, and with genetic male sterility, incompatibility is another possible tool for their economic production.

If autotetraploids are being utilized for a commercial crop which is self-incompatible and where polyploidy does not break the system down, as in rye, it would appear to be necessary to introduce a large number of *S* alleles into the tetraploid strains. If the number is limited there will be sterility owing to lack of compatible pollen for tetrallelic ($S_{1.2.3.4}$) plants (*cf.* Lewis, 1943a).

If the mechanism is broken down by polyploidy as in *Trifolium* the danger with autotetraploidy for commercial crops may be the self-fertility, which could result in inbreeding depression.

VIII. Summary

1. Incompatibility in angiosperms is easily distinguished from other outbreeding mechanisms, but in fungi the distinction between separation of the sexes (dioecy) and incompatibility is a fine one. An incompatibility system is operating when all mating groups have the attributes and potentialities of both sexes.

2. The most satisfactory method of classifying the different systems in angiosperms and fungi, in our present state of knowledge, is by the genetic control—di-allelic or multi-allelic. With one possible exception this division coincides with a morphological classification, heterohomomorphic in angiosperms, and with systematic orders in the fungi.

3. The di-allelic system has developed a super gene which controls secondary characters such as morphological differences in angiosperms and nutritional or pathological characters in fungi. The di-allelic fungi with two monokaryons and one permanently heterozygous dikaryon develop a system of complementary genes, linked with the incompatibility gene, which are necessary for the dikaryon. The multi-allelic systems do not develop such secondary characters; this is a consequence of having a multiplicity of different heterozygotes.

4. The biochemical control is of two types: an oppositional inhibitor produced by an antigen-antibody reaction between like gene products to give incompatibility, and a complementary stimulant to give compatibility. Only the first mechanism is possible and has been found in multi-allelic systems, but both are possible and have been found in di-allelic systems.

5. The breakdown of the genetical control in angiosperms by autotetraploidy is due to creating new situations where natural selection could not have operated, such as the presence of two alleles instead of the normal one in a pollen grain. This type of breakdown has revealed that alleles can frequently have such properties as dominance, competitive interaction, and individual action as accidental primary properties of the alleles, and that only continual natural selection insures the certainty of any one of these properties.

6. Mutation—spontaneous or X-ray-induced—of the incompatibility gene in angiosperms has disclosed only loss mutations. Revertible mutations, in which the mutant alleles changes back to the normal some divisions later, are produced by X-rays. The origin of the vast number of alleles in these systems is one of the major problems to be solved.

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Cytoplasmic Inheritance in Epilobium and Its Theoretical Significance[1]

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I. Introduction

Investigations on cytoplasmic inheritance were begun when most geneticists still credited the nucleus with an exclusive monopoly of hereditary transmission. As early as 1918, Lehmann discovered the existence of clear-cut differences between hybrids from reciprocal crosses in Epilobium. The question of how to explain these and other reciprocal differences arose. Lehmann (1918) first thought that heterogamy, analogous to the conditions pertaining in Oenothera, might be involved. Later, he postulated a delayed nuclear effect resulting from the transmission of gene-produced hormones (1928, 1932) or of self-reproducing cytoplasmic products of the nuclear genes (1936). Renner and Kupper (1921), following the example set by Jones (1912) in his Digitalis experiments, assumed that the reciprocal differences noticed in Epilobium crosses might be explained by a hereditary cytoplasm, the "cytoplasmon" (Renner, 1929).

The present author began his experiments with Epilobium in order to clear up this controversy. In order to differentiate between cytoplasmic inheritance and indirect action of the cytoplasm through nuclear differences, the nucleus of one Epilobium species or strain was transferred to the cytoplasm of another species. Merogony (Boveri, 1918, etc.) and defect experiments (Harder, 1927) are not possible in higher plants. The exchange of nuclei can be obtained only by backcrossing the hybrids to the male parent for many generations—an experiment which was first performed by Correns (1916) with Cirsium crosses.

The first results of the sixth backcross generation of *Epilobium luteum* ♀ × *hirsutum* ♂ hybrids were published in 1929 and have since been confirmed and amplified through many other experiments with Epilobium species and strains (Michaelis, 1933, 1938; Michaelis and Michaelis, 1948). As soon as the first proofs of cytoplasmic inheritance in higher plants had been obtained, the relation between nucleus and cytoplasmon and the physiological significance of cytoplasm were investigated. The discovery in 1940 of clearly definable alterations of dissimilar hybrids from reciprocal crosses permitted a more exact examination of the alteration of the cytoplasm than had been possible in the first experiments. Thus it became possible to recognize the significant principles of cytoplasmic recombination. These experiments will be reviewed in this paper.

It is not proposed here to give a complete survey of all experiments or of the literature pertaining to experiments on cytoplasmic inheritance. For this I refer the reader to the review of Caspari (1948), Lehmann (1941b, 1941c, 1932, 1925), and Lehmann-Düppel (1950). The older experiments will be discussed only insofar as is necessary for an understanding of the latter experiments and for a grasp of the general importance of cytoplasmic inheritance. Tables and figures will be avoided in most cases, but references will be given to the original papers that contain them. Experiments which are not yet concluded, or which have to be repeated on a larger scale, will be mentioned briefly, insofar as they are important for the planning of new experiments.

As regards the theoretical aspect, it will not be possible to discuss all the problems of cytoplasmic inheritance completely and with reference to all the relevant literature. Only a few new and important trends of thought will be reviewed. An extensive discussion must be reserved for separate publications.

II. Terminology

1. *Plasmagenes*

Darlington (1939) and Wright (1941) introduced the plasmagene concept. I myself try to avoid using this concept in experimental investigations, in order to discourage any comparison with the nuclear gene. In most experiments on cytoplasmic inheritance we cannot distinguish between manifestations of single plasmagene units, but are limited to a recognition of the effect of all those hereditary components of the cell that do not show Mendelian segregation—or of the genetic effect of the total protoplasm of the egg cell. We cannot predict with any degree of certainty which cytoplasmic components have the capacity to be identically reproduced and therefore can be said to possess hereditary potency. Furthermore, we can assume that the cytoplasm is not simply the sum of

cytoplasmic units but a complicated hereditary system in which these units participate—in various, still unknown ways—in the composition and structure of the cytoplasm. We are therefore not justified in referring to the properties of single cytoplasmic units as if they were the properties of the whole cytoplasmic system. It may, for example, be possible to change the genetic properties of the cytoplasm not only through mutation or production of plasmagenes but also through an alteration of the whole system. It is for these reasons that I propose to use the term plasmagene, in theoretical considerations, only with reference to the ultimate genetical units, and in experimental work only when the effect of these units can actually be recognized.

2. *The Plasmon and the Cytoplasmic System of the Cell*

In all experiments on extranuclear heredity I propose to use the term "plasmon," introduced by von Wettstein. Von Wettstein defines the plasmon as the genetic element of the cytoplasm (1926, p. 259). Later geneticists tried to distinguish between plasmon and plastidom or plastom (Renner, 1929), but the difficulties inherent in any attempt to distinguish between the two were underestimated. If the behavior of cytoplasm and plastids is the same during fertilization and cell division—that is, if after transmission of male plastids through the pollen tube the paternal plastids and paternal cytoplasm remain together—then it is very difficult to separate and analyze their respective effects. The occurrence of sectors with plastids lacking chlorophyll, after the transfer of paternal plastids into the egg cell, is not necessarily conditioned by the genetic make-up of the plastids; an effect of the specific cytoplasm may also be involved. We encounter even greater difficulties in any attempt to distinguish between the effects of cytoplasm and chondriosomes, and so forth.

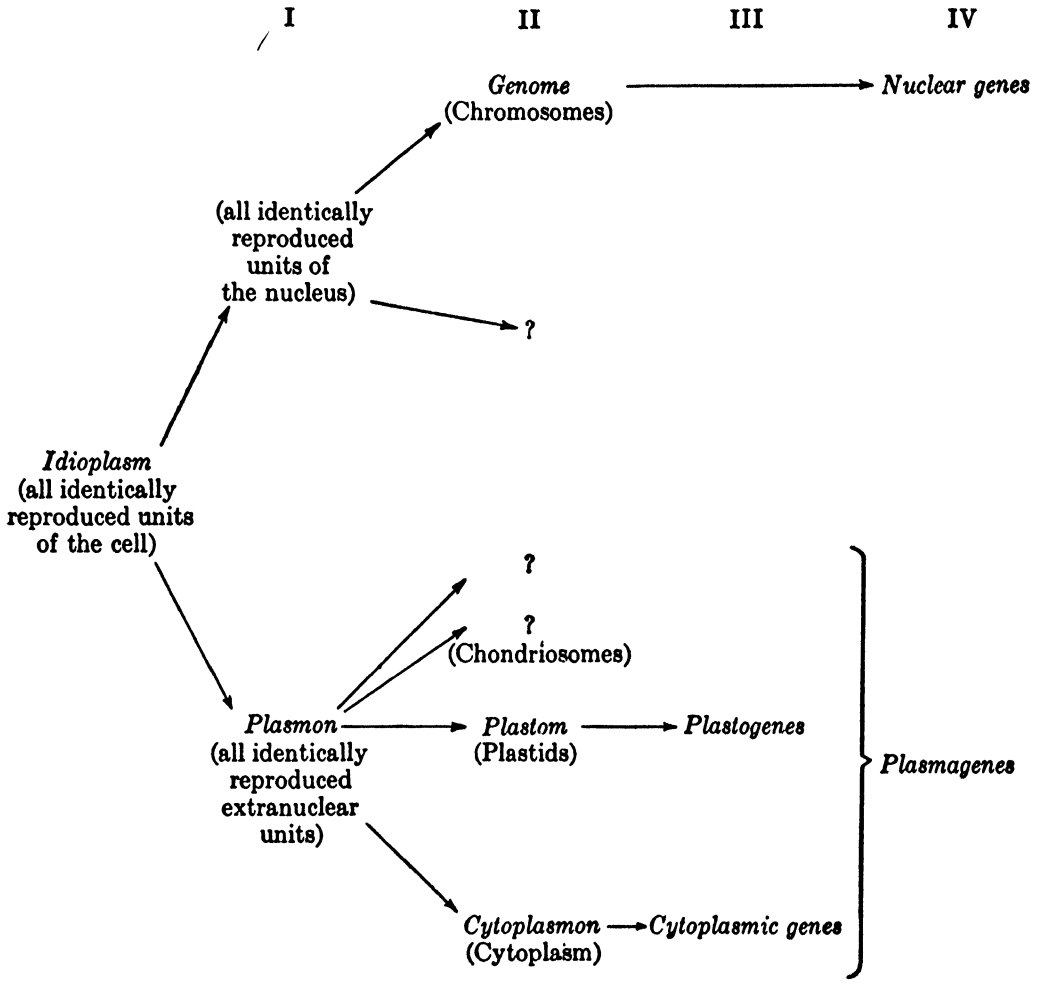
Since clear-cut distinction of the different cytoplasmic units is not possible in the majority of experiments, I propose (Michaelis, 1949a) with Renner (1929) to include all extranuclear hereditary elements of the cell in the term *plasmon*, and to subdivide this into (1) the cytoplasmon, that is, the elements of the cytoplasm, and (2) the plastom, that is, the hereditary elements of the plastids, etc. The diagram (Table 1) suggests a scheme for the classification of all the genetic components of the cell.

If we adopt the above-proposed terminology, it is possible to make *a priori* predictions about the mechanism and constancy of cytoplasmic inheritance, provided the behavior of the cytoplasm and its components during fertilization and cell division is known. The behavior of the nucleus during meiosis and fertilization results in Mendelian segregation and recombination. The behavior of the cytoplasm during fertilization causes

the presence or absence of maternal inheritance. Organisms with extreme oögamy would be expected to show a constant maternal inheritance. Among the widely distributed heterogamous organisms, maternal inheritance that is maintained for many generations can be expected only as an exceptional and irregular occurrence. In isogamous organisms, maternal inheritance will be absent altogether.

TABLE 1

Scheme for Classification of the Genetic Components of the Cell



The behavior of the nucleus during mitosis determines the equal distribution of the nuclear hereditary elements of the organism to all of its cells. If, on the other hand, the plasmagenes are not so equally distributed as the chromosomes, it follows that the cells of a given organism may differ as to content of plasmagenes.

Thus, whereas, on the cellular level, heredity depends on the behavior of the cell components during fertilization and mitosis, on the cytoplasmic

or secondary level, heredity depends on the structure of the cell components involved. Just as the laws of crossing over reflect the structure of the chromosomes, it should be possible to infer the submicroscopic structure of the cytoplasm and its components from the mode of cytoplasmic inheritance. It remains to be seen what similarities of action will be found to exist between nucleus and cytoplasm as a result of their common nucleoprotein content, and what specific differences arise from the fact that the chromosomes contain thymonucleic acids and the cytoplasm contains ribose nucleic acids.

3. *Plasmon-Sensitive Genes*

It has long been known that there are interactions between genes and cytoplasm. Renner and Kupper (1921) assumed that in dissimilar hybrids from reciprocal crosses the foreign cytoplasm provides an unsuitable substrate for the paternal genes. Lehmann (1932) assumed an alteration of the cytoplasm through the action of inhibitor genes.

In the analysis of the gene-cytoplasm interaction, I have called all those genes plasmon-sensitive which manifest different effects in different cytoplasmic environments. This idea is based on the assumption that, in the normal cell, nucleus and cytoplasm are so attuned to each other that reciprocal differences can arise only where a somewhat altered cytoplasm affects certain gene reactions sensitive to just such a cytoplasmic alteration (Michaelis, 1940a, p. 219; Michaelis and v. Dellingshausen, 1942, p. 374).

4. *Symbols*

The Epilobium species will be abbreviated according to their initial letters: *hirsutum, h; luteum, l; parviflorum, p; roseum, r; montanum, m.* The different strains will be designated by the names of the localities in which they were collected.

Von Wettstein (Correns and v. Wettstein, 1937) suggested that one should denote the theoretical composition of the plants in the following manner: the cytoplasm should be indicated by Greek letters; the haploid genome, by Latin symbols. Thus, *hirsutum*, *ιhh; luteum*, *λll;* F_1 hybrids, *ιhl* and *λhl*; and *hirsutum* with *luteum* cytoplasm, *λhh* (see also Caspari, 1948).

Since experiments have shown that plants with identical theoretical composition derived from different origins are not always alike, I prefer in experimental investigations to denote the origin of the plants by abbreviations. The maternal plant is listed first. If the same plant is used for the production of more than one generation, then the number

of generations is given as an index. Parentheses can be used for the presentation of very complicated crosses (Michaelis, 1940b). Plants of the theoretical composition $\lambda h_{\text{Jena}} h_{\text{Jena}}$, for example, were obtained by means of the cross $lh_{\text{Jena}}{}^{20}$, that is, by backcrossing the l ♀ × h ♂ hybrid with the same male h_{Jena} plant for 19 generations, as well as by the cross $lh_{\text{I}}{}^{12} \times h_{\text{Jena}}{}^{8}$, in which a *hirsutum* plant that arose by backcrossing the l ♀ × h_{I} ♂ hybrid to the same male h_{I} plant for 11 generations was further backcrossed with pollen of h_{Jena} for 8 generations (Michaelis, 1940b).

[Editor's Note: In this review article, for the sake of typographical efficiency, the species (or species symbol) and strain (or strain abbreviation) will be written together on the same line, separated by a hyphen (*e.g.*, *h*-Jena).]

III. Cytology and Genetics of Epilobium

1. *Cytology*

According to the investigations of Håkansson (1924), Schwemmle (1924), and Michaelis (1925), all Epilobium species contain the same number of chromosomes: $n = 18$. This facilitates the use of species crosses for the analysis of cytoplasmic inheritance. The chromosomes are small and morphologically similar, and fixation is difficult. These disadvantages, however, are not too important. The haploid pollen of the Onagraceae contains three large germ pores, all of which lie in one plane, whereas the hypohaploid pollen contains only two or one germ pores, and hyperhaploid pollen contains more than three germ pores. Diploid pollen, aside from its size, can be recognized by its four regular germ pores. Pollen grains with more than one nucleus, which arise from a whole pollen mother cell, contain many germ pores, distributed over the surface of the sphere. Single, scattered chromosomes result in dead and dwarfed pollen (Michaelis, 1926, see table on p. 573).

It is also important to recall that in Epilobium, with the exception of the subgenus Chamaenerion, the pollen is united in tetrads. A closer examination of these pollen tetrads, with reference to their arrangement of pollen grains and number of germ pores, permits one to recognize meiotic disturbances. Such examinations were performed on all the more important plants and on the parental generations of some of the crosses carried out. Occasionally, they were supplemented with cytological tests. Disturbances of meiosis were found in only three *Epilobium hirsutum* strain crosses, in which the *hirsutum* strain Munich participated (Michaelis and Bakker, 1948, p. 411).

2. *Polyploidy*

Wild polyploid strains have not been found so far, even though large samples of the most important species have been collected. Under experimental conditions only a few heteroploids have been found from *luteum-hirsutum* and *hirsutum* strain hybrids. Artificial polyploid plants were produced in some strains and species, first by low-temperature treatment during meiosis (Michaelis, 1926, 1928) and later by colchicine treatment. These plants were subsequently used in crosses (Michaelis, 1942a; Douwes, 1948). Haploid plants arose with relative frequency in species crosses. In *E. hirsutum* × *luteum* crosses these appeared with a frequency of 0.05% (Michaelis, unpublished), and in *E. hirsutum* interstrain crosses with a frequency of 0.18% to 0.67% (Michaelis 1948b, pp. 199, 200). Haploid plants were available for the most important strains, and in several cases secondary diploid plants, highly homozygotic, were obtained by treatment of these haploids with colchicine (Michaelis and Michaelis, 1948; Kisch, 1941).

3. *Gene Analysis*

A complete genetic analysis in Epilobium is not yet possible. A number of genes have been found, however, in *E. hirsutum* (Stubbe, 1935) and *E. parviflorum* (Michaelis and Kaplan, 1950) in mutation experiments. Correns (1901) investigated the gene for pollen and flower color in *E. angustifolium*. Stomps (1913) and Oehlkers (1935) analyzed the cruciatum character. The gene villosum (long hair on ovary) is linked with the gene albiflorum (Michaelis, 1939a) and with candidum (plants without anthocyanin, Michaelis and Bakker, 1948). All the important crosses were carried out with strains marked with certain genes. Thus in most cases cytoplasmic inheritance as well as nuclear segregation was investigated

4. *Elimination of Gones and Zygotes*

In order to recognize and avoid errors it is important to pay attention to competition and elimination of gones and zygotes. Competition between gones was avoided by weak and repeated pollination. Examination of the pollen tetrads permitted easy recognition of pollen lethality. The ovules are arranged in the ovary in rows of four and develop dispersal hairs even in the absence of fertilization. Lethality of egg cells and zygotes can therefore be recognized easily. The formation of pollen tetrads, and the parthenogenetic development of seed hairs allows determination of the number of haploid lethal mutations and their relation to the viable mutations in mutation experiments. In important crosses it is possible to pollinate the stigma with single tetrads, when fruit development is stimulated by afterpollination with strains that produce easily recognizable

hybrids. In such experiments the elimination of gones or zygotes can be detected with certainty (Michaelis, 1949b), and analysis of tetrads is possible (Michaelis, 1931a).

5. *Behavior of Cytoplasm during Fertilization and Cell Division*

The behavior of the cytoplasm during fertilization and cell division is basic for the laws of cytoplasmic inheritance. Unfortunately, investigations on this important but difficult problem are almost completely lacking in this as well as in other organisms. It is probable, however, not only that different organisms will behave differently but also that the same organism under different conditions will show differences of behavior and reactions. The behavior of plastids illustrates this point.

In older cytological investigations (Håkansson, 1924; Michaelis, 1924, unpubl.) the sperm nucleus of the pollen tube was never observed to be surrounded by the cytoplasm of the sperm cell. One should not accord too much importance to these older investigations, however, for often they were carried out with faulty fixatives (Finn, 1941; Schnarf, 1941). Investigations with more modern methods are very much needed.

6. *Transmission of Plastids to the Egg Cell through the Pollen Tube*

Certain inferences about the behavior of the cytoplasm during fertilization can be based on observations of plastids of paralbomaculate plants. Unfortunately, such plants occur in Epilobium very rarely. A cross of normal green ♀ × albomaculate ♂ could only be performed three times.

TABLE 2

Results of Crosses between Normal Green ♀ and Albomaculate ♂

		Number of Plants			
Year	*Species*	Total	Green	Albomaculate sectors	
1934	*E. hirsutum*	1774	1770	4	Michaelis, 1935a
1946	*E. parviflorum*	247	247	0	Michaelis, 1949b
1950	*E. parviflorum*	128	128	0	Michaelis, unpubl.

The data in Table 2 show that migration of paternal plastids into the egg cell occurs extremely rarely. It is quite probable that the frequency of transmission of paternal cytoplasm to the egg is of similar magnitude.

7. *Experiments with Plastid Mutants*

In order to increase the number of available albomaculate plants for these experiments, we tried to increase the plastid mutation rate. Treat-

ment of seeds and seedlings with X-rays (2000 r–9000 r) has so far been unsuccessful (Brücher, cited by Michaelis, 1949b; Michaelis, 1948–1950, unpubl.). This result is contrary to that reported by Knudson (1940) and Maly (1951) for ferns. It can perhaps be explained on the basis of sorting out of mutated plastids in the vegetation points; but in the investigations of Knudson and Maly it cannot be decided whether the plastid alterations result from plastid mutation or from nuclear-gene mutation.

The fact that albomaculate plants arising by transmission of plastids through the pollen tube show a different spotting pattern from that shown by spontaneously occurring albomaculate plants (Michaelis, 1935a, Figs. 2, 5) indicates that sorting out of plastids plays an important part here.

IV. Demonstration of the Plasmon

1. *E. hirsutum* × *luteum Hybrid*

See crosses of *E. hirsutum* × *luteum* (Michaelis, 1929, 1931b, 1932, 1933, 1934, 1937, 1938; Michaelis and Michaelis, 1948).

The hypothesis has been advanced that the nuclear genes transmit only the traits of the species, whereas the cytoplasm passes on the traits of the genus or family (Loeb, 1916; Winkler, 1924). We therefore investigated the Epilobium hybrids that showed the greatest amount of reciprocal difference after crossing of the most distantly related species (Table 3). Comparison of these and other reciprocal differences with both

TABLE 3
Reciprocal Differences after Crossing the Subgenera Lysimachion and Chrysonerion

	E. luteum ♀ × *hirsutum* ♂	*E. hirsutum* ♀ × *luteum* ♂
Development	Normal	Somewhat disturbed
Growth	Branched, decumbent	Erect, pyramidal
Leaves	Broad, oval, 59 × 23 mm	62 × 21 mm
Color of leaves	Normal green	Slightly yellow-green mottled
Buds	Normal, broad	More slender
Corolla	Normal, broad, 10.6 × 9.2 mm	4.6 × 3.4 mm
Anthers	Normal, yellow	Reduced to little green scales
Pollen	15%–20% viable, remainder without cytoplasm	Completely disorganized in open flowers; dies off after meiosis

parents is meaningful only in exceptional cases. The terms matrocliny and patrocliny are not applicable in the *E. luteum* × *hirsutum* hybrids, because their properties are determined not by maternal inheritance but by disturbances of nuclear-cytoplasmic interactions.

2. *Backcrosses of E. luteum × hirsutum Hybrid ♀ to hirsutum ♂*

In order to find out whether the reciprocal differences of the F_1 plants arise as delayed maternal effects of nuclear-produced substances or whether they arise through the action of a plasmon, an attempt was made to introduce the nucleus of *E. hirsutum* into the cytoplasm of *E. luteum* by backcrossing the *luteum* ♀ × *hirsutum* ♂ hybrid with pollen of *E. hirsutum* for many generations. Such a procedure is valid if the egg cell supplies nucleus and cytoplasm, and the pollen only a nucleus without cytoplasm, and if neither competition nor lethality prevents the origin of homozygotic plants.

a. *Method.* Backcrossing of *lh* (= hybrid of *luteum* ♀ × *hirsutum* ♂) with *h* is difficult and is achieved only exceptionally. Nine plants were obtained in 1924 from such a cross. Only five of them could be used for further backcrosses. In each subsequent backcross line, those plants most resembling the father were used for new backcrosses. In this way 50–90 parallel-running backcross lines were established. After the lh^8 generation the various backcross lines were alike, and selection experiments for 3 years showed negative results. In 1936 the lh^{14} backcross lines were reduced to four, to which were added two lines that arose through diploidization of two haploid lh^{12} plants (Kisch, 1941). These backcrosses were continued until 1945, when the lh^{24} plants were lost as a result of World War II.

In order to avoid errors caused by heterozygosity of male backcross parents, only a vegetatively multiplying clone of *hirsutum* was used during 23 years. All parent plants of the 24 lh^n backcrosses were preserved until 1945, and were used for measurement of flower size, leaf size, and pollen fertility. So far, only the results on pollen fertility have been published. The other measurements showed similar results.

b. *The lh^n Backcross Generations.* All the plants of the later backcross generations are typical *hirsutum* plants and indistinguishable at first sight from the paternal plants. A few minor differences are mentioned in Section VI, 1. The first few generations give rise also to a number of complicated segregants expected in species heterozygotes, as well as to plants with varying degrees of pollen sterility. These differences in fertility are tied up with the interaction between certain genes and the plasmon. Those plants with a high degree of fertility can be maintained only through self-pollination. In backcrosses of highly pollen-fertile plants, pollen-sterile plants are always segregated, and the more advanced backcrosses give almost 100% pollen-sterile plants (for exceptions, see Section VII). The genome of *E. hirsutum* together with *luteum* plasmon produces pollen sterility.

c. *Additional Crosses with E. luteum.* The cytoplasm of lh^n plants, derived from maternal *E. luteum* plants, affects most strikingly only pollen sterility. For the purpose of determining whether reciprocal differences of F_1 hybrids actually originate through cytoplasmic inheritance, we crossed the lh^n plants again with *E. luteum*. The first crosses of this kind were carried out in the fifth backcross generation. In order to avoid errors due to heterozygosity of the compared plants, the lh^4 plants, individually, were reciprocally crossed with *E. hirsutum*. Greater numbers of these two hybrids were then crossed with *E. luteum* (Table 4; Michaelis, 1929).

TABLE 4

		Width of leaves	*Size of flowers*	*Pollen fertility*
$h \times l$	*hirsutum* plasmon	20.8	4.6 × 3.4	Pollen disorganized
$(h \times lh^4) \times l$		20.8	4.6 × 3.5	
$(lh^4 \times h) \times l$	*luteum* plasmon	23.4	9.6 × 7.8	22.3%
$l \times h$		23.7	10.7 × 9.2	14.6%

These results show clearly that the *luteum* hybrids of the backcrosses resembled in all of their properties the l ♀ × h ♂ hybrids and not the h ♀ × l ♂ hybrids. The reciprocal hybrids lh^n ♀ × l ♂ and l ♀ × lh^n ♂ were both fertile and had large flowers. They showed no greater reciprocal differences. Subsequently, all the lh^n plants used for backcrosses were simultaneously crossed with *E. luteum* as a control procedure. In all cases, pollination of *hirsutum*-resembling lh^n-plants with *luteum* yielded hybrids that resembled the *luteum* ♀ × *hirsutum* ♂ plants.

These experiments not only demonstrate, as in other cases of maternal inheritance, that the reciprocal differences are determined by the cytoplasm; but also they constitute the first clear-cut demonstration—since von Wettstein's proofs (1926) of cytoplasmic inheritance in mosses—of independent cytoplasmic hereditary constituents among higher plants. These cytoplasmic constituents maintain their reaction norm even while subject to the influence of a foreign nucleus for as long as 24 generations.

d. *Crosses with E. montanum and E. roseum (Michaelis and Wertz, 1935).* These experiments can be confirmed by crossing *E. montanum* and *E. roseum*, in place of *E. luteum*, into the lh^n plants. The reciprocal crosses between *E. luteum* and *E. montanum* or *roseum* are alike. Apparently the cytoplasms of these three species are so similar that the nuclei of the hybrids can operate equally well with each plasmon. On the contrary, hybrids of reciprocal crosses between *E. hirsutum* and *E. roseum* differ

according to the strains used (Geith, 1924; Schwemmle, 1927; Schnitzler, 1933). The hybrid *hirsutum* × *montanum*-Jena is not viable when *hirsutum* cytoplasm is present. Other *hirsutum-montanum* hybrids with *hirsutum* cytoplasm are more or less stunted. If, however, we employ *hirsutum* with *luteum* cytoplasm, instead of *hirsutum* with *hirsutum* cytoplasm, both reciprocal hybrids are viable and completely or nearly normal.

e. *Crosses of Nuclei of Different E. hirsutum Strains into E. luteum Cytoplasm.* The experiments of Schnitzler (1933) showed that the extent of the reciprocal differences varies with the *hirsutum* race used. This fact led Lehmann to postulate (1932, 1936) that the nuclear genes of these races form substances that reproduce themselves in the cytoplasm and are passed on with the cytoplasm in heredity. This resembles the hypothesis of plasmagenes of nuclear origin proposed by Spiegelman (1946) and Sonneborn (1946). To test this possibility, not only were the first employed nuclei of the *hirsutum* strains I–IV introduced into the *luteum* cytoplasm, but also the nuclei of six other *h* strains, which produce *l* hybrids with very different reciprocal differences (Michaelis, 1938). The cross was made with the F_1 hybrid of this strain; and also the new strain was crossed into the *h* plants with *l* cytoplasm (*lh*-I^{12}). In the first case the differences between the two species must be overcome; in the second case, only the differences between the two *h* strains. The backcrosses were always carried out with one and the same clone in several parallel lines over 10 generations (Michaelis and Michaelis, 1948). In all six experiments the backcross plants resembled the paternal plant; but in all these examples the *lh*-x^{10} × *l* hybrids or the (*lh*-I^{12} × *h*-x^{10}) × *l* hybrids resembled the *l* × *h*-x and not the *h*-x × *l* hybrid, quite independently of the extent of the F_1 reciprocal differences.

3. *Backcross of E. hirsutum* ♀ × *luteum* ♂ *with luteum* ♂

Attempts to introduce the *luteum* nucleus into the cytoplasm of the *hirsutum* strain I (Michaelis, 1933) and into the *hirsutum* strain Munich (Michaelis and Michaelis, 1948) failed because of the lethality of the third backcross generation in the former case and because of high sterility and flower reduction in the latter. The nucleus of these two strains is not viable in the *luteum* cytoplasm. It is not unlikely, however, that the combination of *luteum* nucleus with the cytoplasm of other *hirsutum* strains may yet succeed. In a case involving *luteum* pollination of a triploid plant which contained (probably through double fertilization) *hirsutum*-Jena cytoplasm, two genomes of *hirsutum*, and one genome of *luteum*, the offspring included some diploid plants that resembled *E. luteum* to a high degree and showed no developmental disturbances.

These plants probably arose as a result of elimination of the *hirsutum* chromosomes (Michaelis, 1942a).

4. *Investigation of Interstrain hirsutum Crosses*

a. *Material.* It was found that reciprocal differences in crosses within or between geographical strains of *hirsutum* surpassed those of interspecies crosses, and therefore the investigations were extended in that direction. The nuclei of 40 different strains were introduced by backcrossing into the cytoplasms of 32 strains, in 92 combinations. These backcrosses were carried out in parallel lines for 3–11 generations. In the first few generations the number of plants involved varied between 150 and 500; in the later generations it was at least 40. All backcrosses were carried out with the same clone, parental and offspring plants were preserved as vegetatively propagated clones, and examinations with reference to pollen formation and gone elimination were scrupulously carried out. The results are summarized in Table 5.

TABLE 5

No. of backcross generations	*No. of nucleus-cytoplasm combinations*	*Totals*
3	9	
4	17	
5	6	32
6	10	
7	15	
8	15	
9	19	
10	11	70
11	2	
16	2	
22	1	
24	1	6
		108

The following types of behavior during backcrossing are to be distinguished:

b. *Transmission of Maternal Properties.* In some cases the transmission of properties characteristic of the maternal plants was observed. These maternal qualities were in all cases only minor quantitative differences,

as for example in height, growth, relative width of leaves, and intensity of leaf color. They could be determined only by measurement of a great many plants. It seemed, however, that all plants of the first few backcross generations possessed the maternal traits. All plants of the combination *h*-(Stuttgart × Jena[4]), for example, were a somewhat darker green color that those of the comparable cross *h*-(Jena × Stuttgart) × Jena[3]. The *h* strain Jena has a lighter green leaf color; the strain Stuttgart has a darker green leaf color; and the reciprocal F_1 hybrids also are different. Transmission of such maternal characteristics could be followed through only about 4–6 backcross generations. The author hesitates to decide whether a purely maternal inheritance phenomenon or interaction between cytoplasm and nuclear genes is involved, until these observations are repeated with material that can be better analyzed.

c. *Disturbances of Interaction between Genes and Cytoplasm.* In all reciprocal differences that can be easily observed, segregation of these differences takes place because of heterozygosity of plasmon-sensitive genes.

(i) *Backcrosses of dissimilar hybrids from reciprocal crosses.* In the crosses of different strains of *E. hirsutum*, we observed three groups of reciprocal differences that were produced by disturbances of interaction between nuclear genes and cytoplasm: (1) Differences in pollen fertility, as in the crosses with the Greek *h* strain Xanthe (Michaelis and Bakker, 1948). These arise because of the higher sensitivity of the haploid male gones, but they can have a secondary influence on flower size. (2) Differences in flower malformation, whereby the buds do not open normally. Such malformations frequently occur in crosses of the *h* strain Kew with South German strains, and in several other crosses (Michaelis, unpubl.). (3) Differences of inhibition of vegetative development. This can take the form of a stimulative augmentation of growth (heterosis), as in the cross *h*-Jena × Afghanistan (Michaelis, 1940a, 1951b), or a slight decrease in growth, as in the cross *h*-Jena × Tübingen. Many crosses with *h*-Jena cytoplasm are stunted or dwarfed (Michaelis, 1940a, 1940c), or die as embryos, as in crosses of *h*-Jena with South African strains (Michaelis and von Dellingshausen, 1942). The last-mentioned case seems important, as cytoplasm-induced embryo lethality is probably frequent in Epilobium and other organisms.

The various reciprocal differences can occur side by side in the same F_1 combination, but they need not behave in a similar manner in the F_n generations. The results in the group of stunted and dwarfed hybrids are especially clear. In the cross *h*-Munich ♀ × Jena ♂ with *h*-Munich cytoplasm, all the F_1 and F_n plants show normal growth. The F_1 of the reciprocal cross *h*-Jena ♀ × Munich ♂ are stunted to a high degree.

The offspring of the various F_2 crosses include plants that are stunted and dwarfed to different degrees; the backcross with *h*-Munich is especially stunted. The *h*-Jena × Munich[9] backcross contains plants that uniformly show the same degree of stunting as the F_1 plants. Thus the reaction norm of the maternal cytoplasm is retained over many backcross generations. The effect of plasmon-sensitive genes can be observed

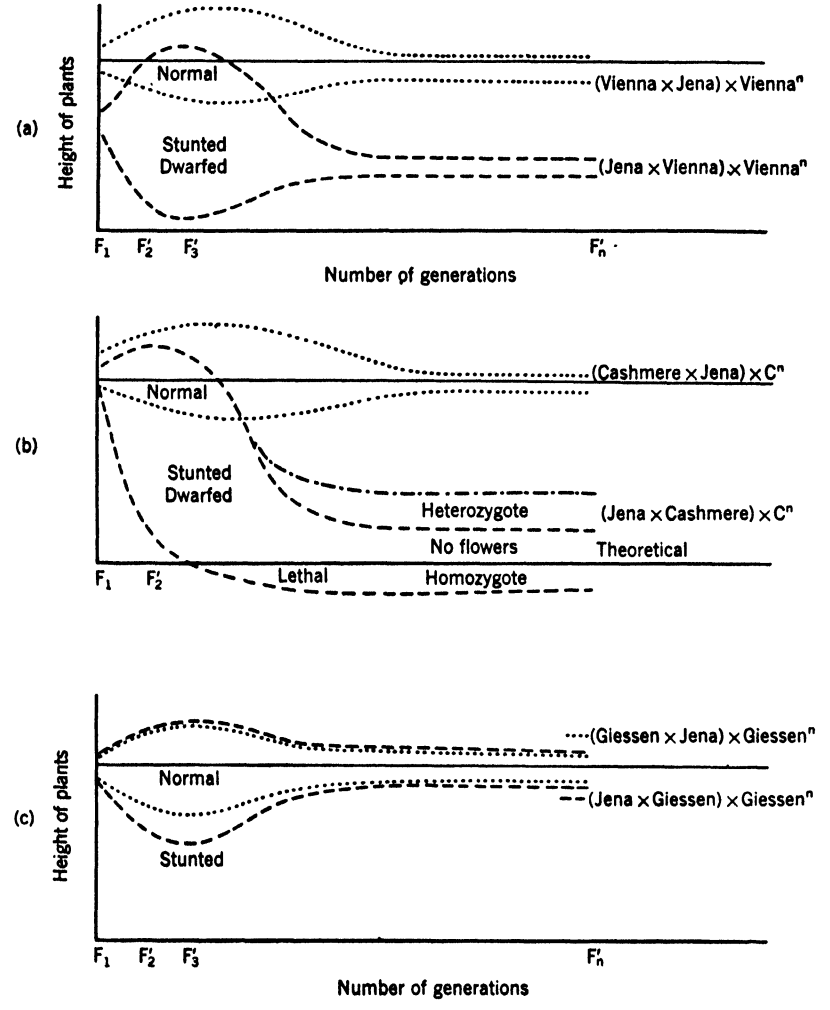


FIG. 1. Diagram showing variations during backcrossing.

only in the first heterozygote generations. In most of the other strain combinations, growth inhibition increases with additional backcrossing, until in the end the degree of growth is determined by the plasmon-sensitive genes of the nucleus of the male parent (see Fig. 1*a* and Michaelis and Michaelis, 1948, Fig. 4).

In many combinations the developmental disturbances increase to such a point during backcrossing that the introduction of foreign nuclei

into the cytoplasm becomes impossible. Especially interesting are four combinations in which all later backcross generations remain heterozygous. The homozygous nucleus of the Cashmere strain, for example, produces in the Jena cytoplasm dwarfed nonflowering plants, and backcrossing is possible only on the flowering heterozygous plants (see Fig. 1*b*; and Michaelis and Michaelis, 1948, Fig. 5).

A slight decrease of developmental inhibition occurs rarely. It was noted in the cross Jena × Seelow[9]. In this case the decrease occurred among the heterozygous generations at a time when elimination of maternal genes took place. After this elimination the reaction norm remained constant. The degree of stunting in the later backcrosses was determined by the plasmon-sensitive genes of the introduced nucleus.

(ii) *Backcrosses of similar hybrids from reciprocal crosses.* Similar hybrids from reciprocal crosses can arise under several different conditions: (1) The cytoplasms may be alike, or else the nuclear genes may not react to their differences. Example: *h*-Jena × Insel candidum (Michaelis and Michaelis, 1948). (2) The cytoplasms may be different, but the quality or quantity of plasmon-sensitive genes may not be sufficient in the F_1 or backcross plants for manifestation of these differences. (3) The cytoplasms may be different, but the F_1 hybrids of two strains (A and B) may be alike because the plasmon-sensitive genes of strain A react with the B cytoplasm in the same way as the B genes with the A cytoplasm. More exact measurements show mostly small quantitative differences. This explains the pollen sterility of both reciprocal F_1 hybrids of *h*-Xanthe × Erlangen (Michaelis and Bakker, 1948).

The second case deserves a more detailed description. In most cases the genes of one strain are sensitive to the cytoplasm of the other. If the plasmon-sensitive genes are recessive, the F_1 hybrids of the reciprocal crosses are similar. In many cases backcrosses with the paternal plants yield offspring with developmental disturbances. The cross Caen × Insel candidum provides an example. No differences in pollen fertility were observed among the F_1 hybrids of reciprocal crosses. In the crosses with cytoplasm of the strain Insel candidum, (I × C) self-crossed and (I × C) × C, some pollen-sterile plants arose; there were none in the crosses (C × I) self-crossed and (C × I) × C. All plants of the later, more advanced backcross generations with cytoplasm of Insel candidum were pollen-sterile (Michaelis and Bakker, 1948). In selfing and backcrosses of similar hybrids from reciprocal crosses of Vienna × Kew albiflorum in combinations with Vienna cytoplasm, 25% and 50% of the plants, respectively, had deformed flowers. All Vienna × Kew *alb*[10] plants had deformed flowers (Michaelis, 1939a; Michaelis and Michaelis, 1948). In the similar hybrids of the reciprocal cross *hirsutum* Jena × Cashmere, stunted plants

arose in all F_2 crosses with *hirsutum* Jena cytoplasm. The families arising from backcrosses continued to segregate, as mentioned above.

Several other examples have been found in which the F_1 hybrids of reciprocal crosses are similar, but in the F_2–F_4 backcross generations there occur only a few plants with inhibited development, and these disappear in the later backcross generations. These disturbed plants can be maintained only by selfing. Such stunted plants were found in the crosses Jena × Giessen, Jena × cruciatum, etc. (Fig. 1*c*; Michaelis, 1940c, Fig. 13; Michaelis and Michaelis, 1948); plants with deformed flowers in the cross Munich × Vienna (Michaelis and Michaelis, 1948, Fig. 11); and single pollen-sterile plants in the cross Fall × Jena (Michaelis and Michaelis, 1948, p. 495).

Such cases can easily be overlooked, or mistaken for pollen-sterile plants that are produced as a result of chromosomal disturbances or segregation of normal genes for pollen sterility. The fact is, however, that such plants occur only in a specific cytoplasm. They are produced by combination of several plasmon-sensitive genes derived from both parents. In the later backcross generations the cytoplasm seems to lose its effect. If a definite number of plasmon-sensitive genes is introduced, however, the specific reaction norm of the cytoplasm will manifest itself in an unaltered manner.

TABLE 6

Crosses	*Normal plants*	*Plants with disturbances characteristic for Jena cytoplasm*
Jena × Erlangen	100%	—
Jena × Erlangen^n	100%	—
(Jena × Erlangen) × Lisbon	1.3%	98.7%
(Jena × Erlangen^n) × Lisbon	—	100%
([*luteum* × Jena^9] × Erlangen) × Lisbon	100%	—

Lastly, many cases were discovered in which introduction of nuclei into cytoplasms of known characteristic disturbances showed no effect either in the F_1 or in later generations. Here it appears again, as in the previously described cases, as though the cytoplasm changes its reaction norm under the influence of the newly introduced nucleus. Actually, all that happens is that the effect of the sensitive genes fails to reach the threshhold in the later generations. Introduction of plasmon-sensitive genes at this stage will easily bring forth the expected disturbance (Table 6). The application of such test genomes has no effect, of course, if the specific cytoplasm is missing. Table 6 shows, for example, that the char-

acteristic disturbances of the Jena cytoplasm fail to appear if the mother is a Jena plant with *luteum* cytoplasm.

5. *Summary*

These backcross experiments demonstrate with certainty (Michaelis, 1953b) that the cytoplasm does not transmit gene-dependent reactions or substances during a limited time, as could be assumed from the results of earlier experiments. The cytoplasm contains autonomous hereditary factors, which, to be sure, interact closely with the nucleus to induce the reactions in the cell, but which cannot be changed by the nucleus in a direct manner. This theory corresponds with the well-known fact that the cytoplasm is an autonomous substance, which can be increased only by self-reproduction and cannot be formed *de novo* by the nucleus.

The results of the backcross experiments also allow us to reach certain conclusions about the nature of the interactions between nuclear genes and cytoplasmic units; there will be discussed in detail in the following section.

V. Interactions between Nuclear Genes and Cytoplasmic Hereditary Units

1. *Crosses of Different hirsutum Strains*

Lehmann and his co-workers found that the reciprocal differences of Epilobium hybrids varied if different strains of the species were used for the crosses; and they emphasized the importance of the nuclear genes in the origin of these differences. The author discovered very different cytoplasms among the various *hirsutum* strains. In order to clarify these separate findings, as many different nuclei as possible were introduced into some of the more striking types of cytoplasm, first in F_1 crosses and later in backcrosses.

The cytoplasm of the *hirsutum* strain Jena was most thoroughly investigated. It characteristically produces disturbances of vegetative development (Michaelis, 1940–1948). From an assortment containing 630 different *hirsutum* strains gathered from the whole area of their occurrence, 390 strains were chosen for reciprocal crosses with *h*-Jena (Michaelis, 1947a). The results are summarized in Table 7.

Deviations from normal growth occurred exclusively in one of the reciprocal crosses with Jena cytoplasm. The other reciprocal hybrid was quite normal, showing only deviations of pollen fertility. It is true that in the same *h*-Jena cytoplasm different degrees of reciprocal differences were observed, but when larger and more noticeable differences occurred they were always similar in nature.

The cytoplasms of the *h* strains Xanthe and Kew albiflorum were investigated as representative for producing disturbances of pollen and flower development. Eleven of 186 reciprocal combinations of the strain Kew albiflorum in the Kew albiflorum cytoplasm showed various degrees of pollen sterility. Especially, crosses with Mediterranean, Atlantic, and African strains were pollen-sterile. Ten crosses with strains from Southwest Germany produced sterile flowers whose buds could not open normally (Michaelis, 1942c). On the other hand, disturbances of vegetative development were completely lacking in these crosses.

TABLE 7

Reciprocal Hybrids of 390 Different Strains of *hirsutum* with *h*-Jena Cytoplasm

		Per cent
Normal	Without reciprocal differences	42
+ Heterosis	Quantitative reciprocal differences	4
− Heterosis		26
Stunted	Qualitative reciprocal differences of vegetative growth	22
Dwarfed		3
Lethal		3

Forty-four reciprocal combinations of the Greek strain Xanthe and twenty-two combinations with the strain Attica showed differences in degree of pollen sterility, whereas flower disturbances and, in the F_1, vegetative disturbances, also, were completely lacking (Michaelis and Bakker, 1948).

A survey of all these F_1 combinations indicates two important facts. There are different cytoplasms in which all crosses display similar deviations of development, if reciprocal differences arise at all. But the degree of deviation differs greatly in the separate crosses. It seems likely that the cytoplasm determines the kind of reciprocal differences, and that the nuclear genes determine their degree.

This assumption is further confirmed by the results of the F_2 and backcross generations described in Section IV. The segregation of the F_2 and early backcross generations always yielded genotypes which were already represented among the various F_1 crosses. In the Jena cytoplasm, for example, degree of deviations of development segregated in various ways, but the kind of deviations was always similar.

Thus the problem of reciprocal differences has two aspects: one involving the action of the nuclear genes; the other, the effect of the cytoplasm. In spite of their interactions, the effects of cytoplasm and genes will be discussed separately.

2. *The Action of the Plasmon*

a. *Plasmon of the hirsutum Strain Jena.* The large number of F_1 crosses and backcrosses shows that the various cytoplasms are not alike and that their differences can be transmitted over an indefinite number of generations. It is not always easy to denote these differences. One cannot associate a certain cytoplasm with a certain phenotype. With one cytoplasm, for instance, that of *hirsutum* Jena, the plants can be normal, heterotic, or a little smaller; they can also be compressed or stunted, or pulviniform and dwarfed. The leaves can be crippled, curved, and wrinkled, narrow or needle-shaped, with white or yellow spotting, fine mottling, or a dark green lamina without anthocyanin. The flowers may turn green, or be malformed or reduced; or the plant may die as an embryo (Michaelis, 1940a, 1940c). In spite of these many effects, all the diversities can be considered as expressions of a single norm of reaction. The various phenotypes can be conceived as belonging to a sequence in which, with increasing disturbance of the gene-cytoplasm interaction, certain deviations from the normal developmental pathway occur. Slight disharmonies between nucleus and cytoplasm may stimulate heterosis (Michaelis, 1951b); greater disharmonies may weaken the plant; and with increasing disturbances of the gene-cytoplasm balance, a whole complex of developmental deviations may arise, which will result in crippling, dwarfing, and finally lethality of the plant.

The assumption that the various phenotypes of the F_1 and F_n plants belong to a uniform developmental sequence is confirmed by the following observations:

1. Among the offspring of the various F_1 hybrids, all stages of the observed sequence of disturbances appear. Among the offspring of normal or heterotic parents, plants with strong disturbances can be found; whereas among the offspring of the variously stunted parents appear plants that are normal or only slightly disturbed. As a rule the whole sequence of deviations will be represented among the offspring of variously disturbed plants, but the number and degree of their disturbances increase directly with the degree of abnormality manifest in the parent plant.

2. The investigations of Ross (1939, 1941, 1948b) lead to a hypothesis that permits a uniform interpretation of all the diverse disturbances in the Jena cytoplasm. Ross assumes that disharmonious interaction between gene and cytoplasm may disturb the enzyme balance in varying degrees. This may be brought about as a result of alteration of protoplasmic hydration, as is the case in mechanical cell damage. Thus oxidative inactivation of growth substances such as auxin is followed by stunt-

ing of plants or crippling of leaves (Ross, 1939); activation of oxidative enzymes can make the anthocyanins disappear; and intensification of oxidases can change carbohydrate balance, etc. (Ross, 1948b).

3. The degree of developmental damage is highly dependent upon environmental conditions and can easily be altered by experimental means. Ross's hypothesis provides the clue to the means of altering or eliminating the disturbances. Treatment with heteroauxin, reduction of respiration by low temperatures (Ross, 1948a), cultivation under short-day conditions, surplus carbohydrate concentration in runners and rosettes, all tend to diminish the developmental disturbances, whereas culturing in high temperature or with long-day conditions tends to increase them. It has thus become possible to diminish lethality (Michaelis and von Dellingshausen, 1942) or disturbances (Michaelis, 1940a), or to make stunted plants into normal or heterotic ones.

4. During the normal development from winter to summer, under different conditions of temperature, day length, and concentration of starch in the runners, the same plant can undergo, one after another, all the degrees of developmental disturbances that are represented in the series of F_1 or F_2 phenotypes (Ross, 1942b, 1943, 1948a).

5. In order to investigate whether this series of disturbances can be assigned to a specific effect of the Jena cytoplasm, an attempt was made to alter the pattern and mode of developmental deviations by introducing a variety of different nuclei. Three hundred and ninety nuclei of *hirsutum* strains, taken from different ecologic and geographic localities, were crossed into the Jena cytoplasm. In addition to these, 111 strains of 15 other Epilobium species were introduced into the Jena cytoplasm. If reciprocal differences arose, all these different crosses fitted into the pattern, and the hybrids of different origin could scarcely be distinguished, provided that they had the same degree of disturbance in development (Michaelis, 1947a, Fig. 2).

b. *Plasmon of Other Epilobium Strains.* The results of these investigations differ from those of other experiments performed with different cytoplasms. As mentioned before, many nuclei were transferred into the cytoplasm of *E. luteum* as well as *E. hirsutum* Kew albiflorum. In part, those nuclei were used which stimulate the most pronounced disturbances in the *h*-Jena cytoplasm. In these crosses, all those disturbances which are characteristic for the *h*-Jena plasm were completely lacking. Pollen sterility was the only thing observed; and the cytoplasm in these cases is not marked by a clear-cut fertility gradient. The various F_1 crosses and F_2 combinations showed different grades of fertility, and it was found that environmental conditions can influence pollen fertility to a high degree and in very specific ways (Michaelis and von Dellingshausen, 1935).

These and other investigations show that in cases of disturbances of proper interaction of nucleus and cytoplasm, the manner in which the norm of development deviates is characteristic for the type of cytoplasm involved. The differences in reaction norm are difficult to recognize, since very different phenotypes can arise, depending on the degree of developmental disturbance.

c. *Geographical Distribution of Different Plasmons.* Although reciprocal differences in pollen fertility are frequent and common among crosses between strains of different geographical origin, cytoplasmic effects like those of the Jena strain are rare.

Since the Jena strain was taken from a botanical garden, it was at first suspected of having acquired the cytoplasm of another species by way of a backcross according to the scheme spec. $\times h^n$. A comparison of the most frequent species with the *h* strain Jena through reciprocal crosses showed cytoplasmic differences in every case (Michaelis, 1943). The introduction of the very disturbing nuclei of *E. luteum* and *hirsutum* Kew albiflorum into 391 strains of the *hirsutum* assortment yielded plants whose cytoplasms resembled the Jena cytoplasm. These were found eleven times in naturally occurring strains and three times in strains taken from botanical gardens. In the environs of Jena and in Thuringia the frequency of cytoplasms resembling that of Jena is higher (41%) than in other localities (1.6%) (Michaelis, 1948a).

d. *Comparison of Similar Plasmons.* Those cytoplasms causing vegetative developmental disturbances, as well as those causing differences in degree of pollen fertility, were more closely compared. Reciprocally different F_1 hybrids between *h* strains whose cytoplasm is similar to the *h*-Jena cytoplasm were obtained only in crosses between strains from different localities. Most of the F_1 hybrids from reciprocal crosses between strains from the same area were similar. But cytoplasmic differences were detectable whenever the disturbances between nucleus and cytoplasm could be increased by crossing into the cytoplasm an intensifier genome (*Verstärker-Genom*), according to the following scheme:

$$(A\ ♀ \times B\ ♂)\ ♀ \times G_{Verst}\ ♂ \text{ and } (B\ ♀ \times A\ ♂)\ ♀ \times G_{Verst}\ ♂$$

Under these conditions differences were found even among strains that lived distances of only 0.5 to 3 kilometers apart. The cytoplasms of strains that lived in the same geographical area but in different ecological niches—for example, in dry ground or moist ground—turned out to be different (Michaelis, 1948a).

All hybrids belonged to the same series of developmental disturbances, but the degree varied with the plasmon even in the presence of the same genome, or if many plants of the cross $(A\ ♀ \times B\ ♂)\ ♀ \times G_{Verst}\ ♂$ and

of the cross (B ♀ × A ♂) ♀ × G_{Verst} ♂ were compared. In some cases qualitative distinctions were added to the quantitative differences. For example, a comparison of equally disturbed plants with Lobeda cytoplasm and Fuchsturm cytoplasm (both these localities are in the vicinity of Jena) showed that the plants with Lobeda cytoplasm had much slenderer growth, owing to weaker branching, and thicker, rougher, narrower leaves of a lighter green color. The cytoplasms of the different strains produce disturbances that are only superficially alike; more precise examination discloses wider differences in the cytoplasmic reaction norm.

Investigations of cytoplasms that produce only differences of pollen fertility (Michaelis and Bakker, 1948) are much more difficult to undertake, because it is not easy to detect specific disturbances of pollen development. In the cytoplasm of *E. luteum* vegetative disturbances never occur, even if strong intensified genomes are used. The cytoplasms of the Greek strains Xanthe and Attica are reminiscent of the Jena cytoplasm, but in the Jena cytoplasm pollen sterility is probably only a consequence of vegetative disturbances in strongly disturbed plants. In the Xanthe and Attica cytoplasms pollen sterility is brought about by even slight disturbances of the interactions between nucleus and cytoplasm. In these cytoplasms, vegetative developmental disturbances in addition to pollen sterility can be induced, but only by the introduction of intensifier genomes which in the Jena cytoplasm produce lethality. In both Xanthe and Attica cytoplasms, the sequence of developmental disturbances is quite different from that in Jena.

Our findings on the investigated Epilobium strains can be summarized by the conclusion that different plasmons can be demonstrated in all cases by introduction of suitable test genomes. The cytoplasm determines differences of quantity, quality, and sequence of processes that interfere with or divert the course of normal development. In spite of these clearly demonstrable differences, which occur also in plants possessing the same nucleus in different cytoplasms, it is hardly possible to establish the true properties and reactions of the various hereditary cytoplasms; for the nucleus is involved in all these reciprocal differences, and the cytoplasmic differences can be recognized only indirectly.

3. *Plasmon-Sensitive Genes*

a. *Inhibitor Genes.* Any discussion relating to the influence of the nucleus on reciprocal differences must distinguish clearly between plasmon-sensitive genes, which manifest themselves differently in different cytoplasms, and nonsensitive genes, which can induce developmental disturbances similar to those caused by plasmon-sensitive genes but which do not react differently in different hybrids of reciprocal crosses. For the

latter category of genes, Lehmann used the term "inhibitor genes." Such inhibitor genes are frequently found in Epilobium crosses. Lehmann and his collaborators (Schwemmle, 1927; see Lehmann and Düppel, 1950) investigated them in detail in different hybrids, and Michaelis in *luteum-hirsutum* hybrids (Michaelis, 1938) and *hirsutum-parviflorum* crosses (Michaelis, 1943). They alter growth and pollen fertility in a number of ways. In the F_2 generations relatively simple segregations can be observed, which are similar in both reciprocal combinations and which give similar phenotypes. These genes cannot be used for the investigation of plasmon differences (Brücher, 1938), since they are not sensitive to the compared cytoplasms. It is not impossible, nevertheless, that the plasmon does interact with these genes, without its being perceptible to our experimental techniques. The fact that developmental disturbances induced by inhibitor genes can be alleviated by changes of the plasmon (Michaelis, 1949g) might fit such an interpretation (see Section VII, 4).

b. *Number of Plasmon-Sensitive Genes.* It is not easy to analyze the genes that are involved in bringing about reciprocal differences through interaction with the cytoplasm. In some organisms reciprocal differences are caused by single plasmon-sensitive genes. In Epilobium this is seldom the case. Only in the *E. hirsutum* strain Kew albiflorum has a case been reported of one gene, deformatum, which causes deformed flowers in some cytoplasms (Vienna, Giessen) in the following proportions: 25% after self-pollination, and 50% after backcrossing with Kew albiflorum. The F_1 cross is normal, and the action of the gene in the Kew albiflorum cytoplasm is unknown. The gene is linked with the genes albiflorum and villosum (Michaelis, 1939a). The degree of flower malformation and pollen sterility is modified partly by the cytoplasm and partly by whatever modifier genes are present (Michaelis and Michaelis, 1948). This deformatum gene is somewhat reminiscent of the gene described for Streptocarpus by Oehlkers (1938, 1942), and of some other examples of pollen sterility caused by interaction between a single plasmon-sensitive gene and the cytoplasm. In these other cases, however, the hereditary nature of cytoplasmic differences has not been so thoroughly investigated as in Epilobium and Streptocarpus.

In all other cases of reciprocal differences in Epilobium many plasmon-sensitive genes cooperate with the cytoplasm (Michaelis, 1940c; Michaelis and Michaelis, 1948). The segregation follows polygenic expectations. The analysis of the segregation is made difficult by the fact that expression of the sensitive genes is influenced to an unusually high degree by environmental conditions. The slightest variations in nutrition, temperature, or light increase the variability of particular genotypes, despite all precautions. Differences of a few days in germination or transplanting

make comparison impossible; and when experiments are repeated in different years, identical genotypes will yield quite different phenotypes. All calculations relating to exact gene composition are therefore subject to uncertainty; and for this reason formulations of genic constitution have not been attempted.

The coincidental presence of genes that react differently in different cytoplasms can lead to situations that are difficult to analyze. The *luteum* crosses with the different *hirsutum* strains can be divided into several groups, according to their growth characteristics (Michaelis, 1938). The genes influencing the growth of the *luteum* hybrids do not manifest themselves in crosses of *hirsutum* strains with each other. The *luteum* crosses following the scheme (*h*-1 × *h*-2) × *l* segregate mostly in the ratio 1:1. In most crosses the cytoplasmic differences influence exclusively pollen fertility and flower size, without altering growth differences of the hybrids. Only in the Jena cytoplasm is the growth of the plants also reduced, but in this cytoplasm the unifactorial segregation was not found. Each plant was characterized by a different degree of growth inhibition. In the Jena cytoplasm the growth of plants is influenced by many plasmon-sensitive

TABLE 8

Cross	*Height of plants* (cm)			*Size of flowers* (mm)			*Anthers*
	Segregation	*Genotype* Jena	cruc	Segregation	*Genotype* Jena	cruc	
l ♀ × *h*-Jena ♂	—	31	—	—	16.2	—	Fertile
h-Jena ♀ × *l* ♂	—	16	—	—	1.6	—	Degen.
l ♀ × *h*-cruc ♂	—	—	14	—	—	10.8	Fertile
h-cruc ♀ × *l* ♂	—	—	14	—	—	6.3	Sterile
l ♀ × (*h*-Jena ♀ × *h*-cruc ♂) ♂	Monog.	22–32	10–18	Monog.	14–16	9–11	Fertile
(*h*-cruc ♀ × *h*-Jena ♂) ♀ × *l* ♂	Monog.	22–32	9–16	Monog.	10–12	5–6	Sterile
(*h*-Jena ♀ × *h*-cruc ♂) ♀ × *l* ♂	Polyg.	2–18		Polyg.	2		Degen.

genes. Table 8 shows the influence of the cytoplasm on segregation of the cross (*h*-Jena × *h*-cruciatum) × *luteum*.

c. *"Plasmafremde" and "Plasmaeigene" Sensitive Genes.*[2] A comparison of the F_2 generation derived by selfing with the two backcrosses to

[2] Editor's note: The terms *plasmafremde* and *plasmaeigene* have no precise English equivalents. *Plasmaeigene* genes can be defined as genes derived from the parent strain from which the cytoplasm was derived, and *plasmafremde* genes as genes derived from the other parent strain, from which the cytoplasm was not derived.

paternal and maternal parents shows, as a rule, that backcrossing with the maternal parent diminishes the number and degree of disturbed plants, whereas backcrossing with the father increases them (see Table 9).

TABLE 9
Pollen Fertility of the *h*-Xanthe × Jena F_2 Crosses
Michaelis and Bakker, 1948

Combination		*Segregation*	*Degenerated* (%)	*Dead* (%)	*Viable* (%) 1–40%	41%–80%	81%–100%
(Jena × Xanthe)	Selfed	Normal	0	1.4	2.8	5.5	90.3
	× Xanthe		0	0.6	1.2	1.9	96.3
	× Jena		0	0	1.8	7.0	91.2
(Xanthe × Jena)	Selfed	Of plasmon-sensitive genes	45.6	53.8	0.6	0	0
	× Jena		97.8	2.2	0	0	0
	× Xanthe		0.7	67.9	15.8	9.5	6.1

The F_1 of the cross Jena × Xanthe has 98.2% living pollen; the backcross Jena × Xanthe[8] is fertile; the cross Xanthe × Jena F_1, like the Xanthe × Jena[6] backcross, has only degenerated pollen. The genes of *h*-Jena become plasmon-sensitive in the "foreign" Xanthe cytoplasm. This applies not only to genes influencing pollen fertility, but also to those causing vegetative disturbances or flower malformation.

In some cases we have observed combinations of different plasmon-sensitive genes from both parents. In the combinations *h*-Jena × cruciatum and *h*-I × cruciatum, for example, stunted plants appeared in the F_2 with a frequency of less than 3%; and similar frequencies of plants showing flower deformation were found in the F_2 of the combination *h*-Munich × Vienna. Abnormal plants were absent in reciprocal crosses of cruciatum and Vienna cytoplasm. The abnormal plants resulting from the crosses showed great variability. They disappeared after backcrossing with both parents for one or two generations. Whenever selfing was possible the segregation followed polygenic ratios (Michaelis, 1940c; Michaelis and Michaelis, 1948). In these and other cases, combinations between *plasmaeigene* and *plasmafremde* genes became plasmon-sensitive.

Finally, in crosses with the *h*-Jena strain it was observed that *plasmaeigene* genes participated in the inhibition; but it was only after the introduction of intensifier genomes that the inhibiting reaction of these

genes crossed the threshold of observability. Here too, with one exception (Michaelis, 1942b), the segregation followed a polygenic ratio.

d. *Influence of Quantity of Plasmon-Sensitive Genes.* The degree of reciprocal differences can vary greatly among polyploid hybrids. Von Wettstein (1926) and his collaborators (Schwanitz, 1932) investigated this problem thoroughly in polyploid mosses. Figure 2 shows measurements (Douwes, 1948) of stomata number per unit surface among hybrids of the cross *h*-Jena × Algiers. The offspring containing Jena cytoplasm

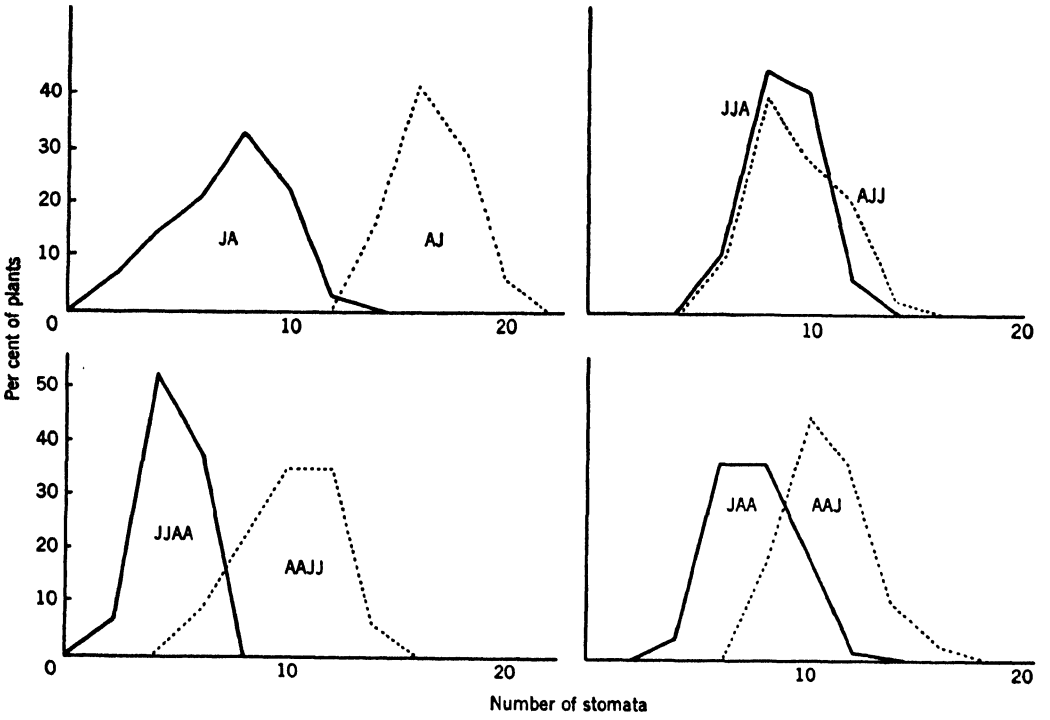


FIG. 2. Number of stomata per unit area in polyploid *E. hirsutum* Jena × Algiers hybrids.

are crippled. In a strongly affected hybrid having *h*-Jena cytoplasm, the developmental disturbances cannot be eliminated by changes in the genome ratios. The height of stunted plants is greater among triploids than among tetraploids, and it is increased to a greater degree in the Jena-Jena-Algiers than in the Jena-Algiers-Algiers combination. Among the four triploid combinations, the reciprocal differences in stomata number disappear when the *h*-Jena genome is doubled; whereas with a doubling of the Algiers genome the reciprocal differences remain (Douwes, 1948).

In one triploid *hirsutum-luteum* hybrid, in which *h*-Jena cytoplasm was combined (probably by double fertilization) with one *h*-Jena, one *h*-I, and one *luteum* genome, most of the inhibitory disturbances charac-

teristic of *h*-Jena cytoplasm were lacking. Table 10 shows the effects of various genome combinations with *h*-Jena cytoplasm (Michaelis, 1942a).

These findings are supported by Schlösser's work (1949) on polyploid sugar beets, which also shows that polyploidy can affect the nature and degree of reciprocal differences.

TABLE 10
Inhibition of Polyploids with *h*-Jena Cytoplasm

Genomes in the Jena cytoplasm	*Origin*	*Crippled growth*	*Mottling of leaves*
h-Jena (1*n*, 2*n*, 3*n*, 4*n*)	Parthenogenesis, 2*n* × 4*n* (colchicine)	0	0
Jena + *luteum* (2*n*)	*h*-Jena ♀ × *luteum* ♂	+++	+++
Jena + Jena + *l* (3*n*)	*h*-Jena 4*n* ♀ × *l* ♂	++	+++
Jena + I + *l* (3*n*)	*h*-Jena ♀ × *h*-I ♂ × *l* ♂ (double fertilization)	0	+
I + *l* (2*n*)	*h* (J × [*lh*-I^n]^n) ♀ × *l* ♂	++	++
Jena + I (2*n*)	*h*-Jena ♀ × *h*-I ♂	0	0
h-I (1*n*, 2*n*, 3*n*, 4*n*)	*h*-Jena ♀ × (*lh*-I^n) ♂^{n} = 2*n* Parthenogenetic haploid, 2*n* × 4*n*, colchicine-4*n*	0	+

e. *Analysis of the Lethal Combination hirsutum Jena × hirsutum Parys.* The morphology and physiology of the South African strain *h*-Parys are quite different from those of the European and Asiatic strains. The F_1 embryos of the cross Jena ♀ × Parys ♂ die after a few cell divisions, whereas the reciprocal hybrid with Parys cytoplasm develops normally and segregates in typical fashion in the F_2. In order to analyze this case further, F_1 plants from the above-indicated cross, and numerous F_2 segregants derived from the same cross utilizing the Parys cytoplasm, were crossed with *h*-Jena ♀. The combinations with certain F_2 segregants were not successful, because the growth of the pollen tube is hindered in the styles of *h*-Jena plants. Here we have an interesting case of sterility involving interaction of pollen nucleus with the cytoplasm of the tissues of the styles. Other combinations resembled the F_1 hybrids in yielding only dead embryos. Those combinations which were viable turned out to be disturbed to a greater or lesser degree in their vegetative growth or pollen fertility. Only a few plants were completely normal. It is of great interest to note that those few normal plants were indistinguishable from the *h*-Jena strain as such. Observations on the inhibited plants showed that the disappearance of the traits and properties of the Parys strain in the hybrids could be correlated with a proportional reduction of developmental disturbances. It follows that the *plasmafremde* Parys genes

strengthened the disturbances. Therefore the plants that were more or less disturbed resembled the Parys strain more; but with a further increase in the degree of disturbance the properties of both parents were replaced by new qualities, and the dwarfed plants no longer showed any resemblance to *E. hirsutum* strains (Michaelis and von Dellingshausen, 1942).

The conclusion is warranted that most of the Parys genes become plasmon-sensitive in the *h*-Jena cytoplasm. Plasmon sensitivity is probably not a distinctive attribute limited to only a few genes. Most genes—probably all—interact with the cytoplasm, and their phenotypic effects can be altered as a consequence of cytoplasmic changes. To the same extent that the interactions between cytoplasm and genes are disturbed, the genes seem to lose their customary effect, and all the different genes cooperate in releasing a new tendency in development, which at first sight seems to be dependent on the cytoplasm.

f. *Geographical Distribution of Plasmon-Sensitive Genes.* Crosses of *h*-Jena strains with the numerous other strains of the *hirsutum* collection point to a definite geographical distribution of the genes that are sensitive to Jena cytoplasm. Of course, very sharp lines of demarcation cannot be expected among plants whose seeds fly and float easily. Nevertheless, *h*-Jena crosses with strains from the northern areas of Europe are seldom accompanied by reciprocal difference. Large reciprocal differences have been noted in crosses of Jena with strains from the Atlantic and Mediterranean regions. All crosses of *h*-Jena with strains from North and South Africa are either lethal or sublethal (Michaelis, 1942c). Smaller series of crosses with the cytoplasm of other Central European strains have given similar results. In the cytoplasm of *h*-Jena latent limits of species formation seem to become visible, which cannot be recognized otherwise. All efforts to find a similar geographical distribution of morphological variations have so far been unsuccessful.

It seems evident that the crippled and dwarfed plants with Jena cytoplasm have a negative selective value; but this does not hold true under all conditions. Slight disturbances of the interaction between genes and cytoplasm may lead to heterotic plants; and the stunted and pulviniform types can become superior to the normals under certain circumstances, especially in winter climate conditions. As will be shown later, plants with plasmon alterations can also assume a positive selective value.

g. *Mutation Experiments.* In all these crossing experiments with natural strains it was impossible to ascertain the effect of the plasmon-sensitive genes in their normal cytoplasmic environment. Therefore an attempt was made to induce gene mutations and to analyze the plasmon sensitivity of the induced mutants. These experiments were carried out on

Epilobium parviflorum Tübingen rather than on *hirsutum* strains. The treated seedlings (= F_1) were selfed in the usual manner; and at the same time nuclei of these same plants were transferred into a strongly disturbing cytoplasm-gene combination by means of the cross *h*-Essen ♀ × *p*-Tübingen (treated) ♂. The experiments have not yet been completed (Michaelis and Kaplan, 1950). The mutants found so far are listed in Table 11.

Most of the mutants are normally recessive and therefore do not express themselves in the *h* × *p* hybrid. In rare cases the mutants that are recessive in selfed *parviflorum* express themselves in the hybrid as a result of change in dominance.

Other mutants can be detected only in the cross with *hirsutum* Essen cytoplasm. In the *parviflorum* or *hirsutum* Essen selfed the mut/mut and +/mut plants cannot be distinguished from the +/+ plants. In the hybrid *parviflorum* +/mut ♀ × *hirsutum* +/+ ♂ or *parviflorum* +/+ ♀ × *hirsutum* mut/+ ♂, also, the +/mut plants cannot be distinguished; in the reciprocal cross with *hirsutum* cytoplasm, 50% mut/+ plants have a changed degree of disturbances.

In these experiments the surprising fact was discovered that the *parviflorum* clone used for all experiments was very heterozygous. This heterozygosity cannot be detected in self crosses or in the *parviflorum* ♀ × *hirsutum* ♂ crosses. In the reciprocal hybrid, cultivated under normal light conditions, only the greatest gene differences can be observed, whereas with cultivation in a shaded greenhouse most of the combinations demonstrate a high heterozygosity and large differences in degree of growth disturbances. Conclusions on the effect of single plasmon-sensitive genes are possible only in crosses of *hirsutum* mutants with a *parviflorum* plant that gives a uniform *h* × *p* hybrid.

These experiments reinforce the hypothesis derived from the investigations with crosses between strains, according to which the cytoplasm can alter dominance and expression of the genes.

4. *Theory of the Genetic System*

The experiments pertaining to the origin of reciprocal differences have in most cases demonstrated interactions between cytoplasmic units and chromosomal genes. Two alternatives are therefore available for any investigation of the problem; and the older experiments can be classified in two categories. In the first, reciprocal hybrids were investigated and maternal transmission was explained on the basis of cytoplasmic inheritance alone, without reference to the F_2 segregations. In the second category, Mendelian segregation was investigated, and gene-determined sterility and lethality were observed in some cytoplasms. In these cases

TABLE 11
Mutation Experiments (preliminary data)

	E. parviflorum	*E. parviflorum* Treated with		*E. hirsutum*	
		Radioactive P^{32} and S^{35}			
	3000 r–9000 r of X-rays 1951	0.12–3.00 μC 1952	Control and ineffectual concentr. 1952	0.12–3.00 μC 1952	Control and ineffectual concentr. 1952
No. of plants treated (= F_1)	1954	132	93	329	152
% of F_1 plants with 50% lethal pollen or egg cells	12.4%	27.3%	2.1%	7.7%	0
% of F_1 plants with alterations	2.4%	1.5%	0	19.1%	0.7%
No. of F_2 self-crossing combinations	1067	116	30	175	44
% of F_1 plants with 1 mutant in F_2	5.8%	12.9%	0	23.4%	0
% of F_1 plants with 2 mutants in F_2	0	0.9%	0	9.1%	0
% of F_1 plants with more than 2 mutants in F_2	0	0	0	4.6%	0
No. of crosses of F_1 plants with *hirsutum* Essen ♀ × *parviflorum* ♂	825	116*	30*	175	44
% of crosses where the +/mut plants are not recognizable	7.2%			28.0%	0
% of crosses with mutants (+/mut) where segregations are recognizable	0.48%			0.6%	0
% of crosses where segregations occur only in the *h* × *p* hybrid, not in self-crossing	0.12%			1.1%	0

* Most crosses grown in a shaded greenhouse were different from one another and heterozygous.

the cytoplasm was assigned the role only of an unspecific substrate. Specific cytoplasmic differences could not be recognized because only one deviating cytoplasm was used in these investigations. The experiments on Epilobium combined both approaches, because both genic and cytoplasmic differences were examined. These experiments have demonstrated that the hypothesis of an unspecific cytoplasmic substrate is not correct.

a. *The Specificity of Different Plasmons.* The hypothesis of an unspecific cytoplasmic substrate is most easily contradicted by the fact that it has not been possible to find identical cytoplasms among the different *h* strains. Some plasmons have been found in which primarily pollen sterility occurs, and others in which inhibitions of vegetative growth are most common. Among the latter, some produce dwarfed plants with broad leaves and others dwarfed plants with needle-shaped leaves (Michaelis, 1948a). Even among cytoplasms that lead to the same kind of reciprocal differences (either pollen sterility or growth disturbances), differences are detected if suitable genomes are introduced. The degree of reciprocal differences also varies when the same nucleus is present. There can be no doubt that the strains and species of Epilobium do have specifically different plasmons.

b. *The Specificity of Reciprocal Differences.* It is noteworthy that although plasmons differ in many ways, the types of reciprocal differences observed are very similar. This fact has impressed other investigators also (Lehmann, 1932; Lehmann and Düppel, 1950, etc.), and led to the assumption of an unspecific cytoplasmic substrate. At first I believed that I would have to assume at least two types of reciprocal differences: differences in pollen sterility, and disturbances of growth. But when it proved possible to induce growth disturbances, by the use of especially strong plasmon-sensitive genes, in those cytoplasms that normally are characterized by pollen sterility (Michaelis and Bakker, 1948), the hypothesis of specific kinds of reciprocal differences could no longer be maintained. It is quite feasible that no fundamental differences exist at all. The haploid pollen may have a different sensitivity from that of the diploid plant. The same cause of disturbance may have different effects in haploids and diploids, in different organs, or with reference to different reactions—leading to different sequences and degrees of disturbance in different hybrids. The original attempt to characterize each cytoplasm by specific reciprocal differences has failed, and can succeed only in the case of those reactions, difficult to detect, which proceed without any disturbance of the interaction between genes and cytoplasm.

Neither has it been possible to explain the characteristic reciprocal differences on the basis of *plasmaeigene* or *plasmafremde* genes. For ex-

ample, it is impossible to distinguish phenotypically among the dwarfed plants that arise from crosses of Jena cytoplasm with the plasmon-sensitive genes of entirely different strains and species (Michaelis, 1947a, Fig. 2). Neither the particular plasmons nor the different genes can alter the specificity of the disturbed developmental tendencies. They can only induce them.

c. *Theory of the Genetic System (Michaelis, 1947a).* The developmental disturbances of reciprocal Epilobium hybrids belong to a group of traits that seem to be independent, as far as their specificity is concerned, of both genes and plasmons, but which nevertheless are inherited. Of all the different possible theses to explain this fact, the following hypothesis seems to possess the highest degree of probability and the greatest explanatory significance for biology.

Not only do the various components of the cells form a living system, in which the capacity to live, react, and reproduce is dependent on the interactions of all the members of the system; but this living system is identical with the genetic system. The form of life is determined not only by the specific nature of the hereditary units but also by the structure and arrangement of the system. The whole system is more than the sum of its parts, and the effect of each of the components depends on and is influenced by all previous reactions, whose sequence is in turn determined by the whole idiotype.

The author assumes that in such a system the individual links in the reaction are so adjusted to one another that a deviation from the normal range of the reaction is possible only in exceptional cases. In Epilobium this reaction system seems to be so little specialized that at certain points a transgression of the limits of the reaction is tolerated and several developmental pathways become available. The location of these points and the nature of the new developmental possibilities depend not only on the altered reaction links but also, essentially, on the sum total of chain reactions, and are therefore determined by the system.

d. *First Attempts to Analyze the Genetic System.* The analysis of such a system is of course attended by many methodological difficulties. Some inferences regarding interaction of cytoplasmic and chromosomal components can be based on a comparison of 11 *h* strains, which possess cytoplasms resembling those of *h*-Jena and which for the most part were found in the environs of Jena. The differences between the plasmons and the plasmon-sensitive genes become obvious only when the introduction of a test genome containing many plasmon-sensitive genes results in growth disturbances. These 11 strains were compared in twenty reciprocal combinations and after introduction of four different test genomes (Michaelis, 1948a). Figure 3 shows the comparison between *h*-Jena and

h-Klausenburg after introduction of the *h*-Munich test genome. The degree of inhibition was measured by the height of the plants.

Comparison of plasmons is possible through comparison of the crosses (*h*-K ♀ × *h*-J ♂) ♀ × *h*-M ♂ and (*h*-J ♀ × *h*-K ♂) ♀ × *h*-M ♂. This comparison indicates that the Klausenburg cytoplasm is far more disturbing than the Jena cytoplasm. The influence of plasmon-sensitive genes can be investigated by comparison of the crosses *h*-J ♀ × *h*-M ♂ with (*h*-J ♀ × *h*-K ♂) ♀ × *h*-M ♂, and *h*-K ♀ × *h*-M ♂ with (*h*-K ♀ × *h*-J ♂) ♀ × *h*-M ♂. The introduction of *h*-Klausenburg genes diminishes the growth inhibitions, whereas introducing of *h*-Jena

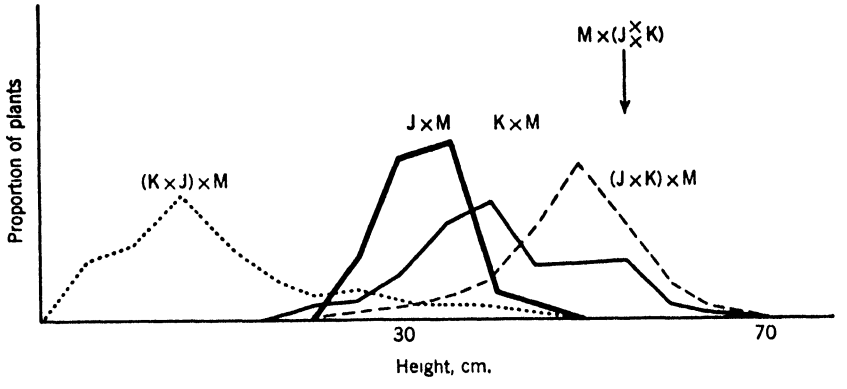


FIG. 3. Comparison of the plasmons and plasmon-sensitive genes of the *hirsutum* strains Jena and Klausenburg. The arrow indicates the average height of plants with normal cytoplasm (*h*-Munich). (Michaelis, 1948a.)

genes increases them. Thus the Klausenburg strain contains weak plasmon-sensitive genes in a strong cytoplasm, and the Jena strain has strong genes in a weak cytoplasm. All comparisons of strains with distinct plasmon differences showed the same result. The cytoplasm and the plasmon-sensitive genes are contrasting in intensity. These experimental findings make it seem probable that normal development results from an equilibrium between plasmon and genome, and that both factors influence certain processes in the reaction system in contrasting directions. Developmental disturbances seem to arise when the equilibrium is upset, by the combination of unbalanced reaction quantities of nucleus and cytoplasm, to such an extent that the subsequent reactions are altered or interfered with. Physiological studies are needed to clarify the question of what kind of equilibrium is disturbed in the dissimilar hybrids of reciprocal crosses in Epilobium.

5. *Summary*

These investigations have led to the hypothesis that nuclear genes and plasmon form a genetic system, which is identical with the life system

of the cell. Genes and cytoplasm act upon each other, probably in opposite directions. If the balance between the two is upset, there arise specific alterations in development, whose nature depends not only on the genetic properties of the genic and cytopalsmic units but also on the particular structure of the system as a whole. Gene dominance and expression can be altered by changes in the strcuture of the system.

VI. Physiological Investigations of Reciprocal Differences Induced by Exchange of Cytoplasm

1. *Comparison of E. hirsutum Plants with hirsutum and with luteum Cytoplasm*

The physiological investigation of the lh^n plants is of greater interest, because these plants are pollen-sterile but show no inhibition of vegetative growth. The lh^n plants resemble typical *hirsutum*, which makes it easier to investigate cytoplasmic differences as such rather than differences of the whole genetic system.

a. *Fertility of Pollen.* The most outstanding characteristic of the lh^n plants is their reduced pollen fertility. Degree of pollen fertility varies with the number and kind of plasmon-sensitive genes employed. Reduced growth of petals is correlated with sterility. Aside from sterility, pollen of lh^n plants has been observed to have an altered sensitivity disturbing influences. Observations of lower pollen fertility following a sudden change in the weather were recorded for lh^n and *h* plants (Michaelis, 1933). Plants with "foreign" cytoplasm were also more sensitive to low-temperature treatment, whereas increases in temperature, changes in the source or the amount of light, and artificial induction of withering had no differential effect. Both cytoplasms were susceptible to intracellular poisons, but plants with the foreign *l* cytoplasm were more sensitive to treatment with mercuric chloride, copper sulfate, zinc sulfate, nickel sulfate, and chloral hydrate. The pollen damage takes place after meiosis, during the formation of the pollen kernels. The period of pollen damage is followed by a phase of stimulation, during which sterile plants are capable of forming transitory pollen (Michaelis and von Dellingshausen, 1935). In parallel investigations of permeability and cytoplasmic resistance, plants with their own cytoplasm tolerated a higher concentration of chloral hydrate. Treatment with sublethal concentrations of that substance was followed by a lowering of permeability, which in turn resulted in increase of resistance. Plants with *l* cytoplasm (but *hirsutum* genes) showed the reverse effect. Permeability was increased and cytoplasm viscosity was decreased (von Dellingshausen, 1935, 1936).

b. *Growth.* Growth progress can differ in plants having their own or

foreign cytoplasm, under different circumstances, with resulting differences in height, leaf shape, and density of branching. The plants with *l* cytoplasm develop better under conditions of starvation and limited space than plants with *h* cytoplasm (Michaelis, 1935b, 1935c). In liquid synthetic culture medium, differences occur with different concentrations of the various nutrient salts and particularly with different proportions of potassium (Fürtauer, 1940). The ratio of mono- to disaccharides has been altered in this way in plants with *luteum* cytoplasm (Knodel, in Fürtauer, 1940).

Temperature, length of daylight, and vernalization can influence the growth progress of plants with their own and foreign cytoplasm in very different ways. The growth of seedlings having *l* cytoplasm is improved by higher temperature, high light intensity, and longer periods of daylight (Fürtauer, 1940). In vernalization experiments the following optima were recorded:

	Optimum Day Length	*1st Temp. Optimum*	*2nd Temp. Optimum*
hh^n	17–24 hr	3°–6°C	20°C
lh^n	<16 hr	3°–6°C	—
ll^n	16 hr	10°C	—

The values recorded for the response of the lh^n plants show a correspondence to those of the cytoplasmic constitution of the plants (at any rate as far as daylight is concerned). Owing to the differences in optimum conditions, under specified conditions the *h* or lh^n plants will be superior to all others and the reciprocal differences can be reversed (Michaelis 1938, p. 439; 1939b). Cuttings of *h* plants form better roots than do those of lh^n plants (Fürtauer, 1940). The embryological development is better, too. A shortened period of seed ripening products, in lh^n-plants, more seeds that give seedlings without vegetation points because of fatty degeneration between the cotyledons (Michaelis, 1929, 1934).

The *lh* and *h* strains show a considerable difference in resistance to *Erysiphe graminis*. *Luteum* plants are completely resistant, lh^n plants very slightly susceptible, and *h* plants very highly susceptible (Michaelis, 1935b, 1935c).

c. *Cell-Physiological Investigations of the Cytoplasms.* A serological analysis, of the kind planned by Professor Moritz of Kiel in 1934, could not be undertaken because of the incompatibility of the experimental animals to the Epilobium protein.

Cytoplasmic viscosity was investigated by means of centrifuge experiments and observations on the shape of plasmolyzed cells. In all cases

the *l* cytoplasm was shown to have a lower viscosity (von Dellingshausen, 1936). Permeability was tested with a number of substances of varying molecular size and lipoid solubility. The *l* cytoplasm was more permeable to KCl, glycerine, and succinimid—a sign of a different size of cytoplasm pores and a different composition of cytoplasm lipoids (von Dellingshausen, 1935). These experiments, together with those pertaining to alteration of cytoplasm constants by chloral hydrate, and those on water permeability and dye absorption, which demonstrate different values for the isoelectric points of the two compared cytoplasms (von Dellingshausen, unpublished), prove that the chemical constitution and structural composition of the *h* and *l* cytoplasms are different.

Furthermore, experiments show that in plants with *l* cytoplasm the starch of the plastids disappears more rapidly when the plants are kept in darkness (von Dellingshausen, 1936, p. 296).

A comparison of the *l* cytoplasm of *l* and lh^n plants points to a somewhat higher permeability in the *l* plants. The value for lh^n plants falls between those for *l* and *h* plants. One can reasonably assume that the cytoplasmic properties of the maternal plant are retained to a considerable degree throughout all the backcross generations. One should be careful, however, not to reach any hasty conclusion. It must be remembered that the *l* cells contain a different genome, and that any physiological reaction will be influenced by the growth differences between the two species. It is a fact, however, that cytoplasmic differences have been found which were maintained over twelve backcross generations, and which could not be altered by the foreign nucleus. Most of the differences between *h* and lh^n plants discussed in Section VI, 1, a and b (this section) can be explained in terms of the observed cell-physiological differences of the cytoplasms. It is not certain that these experiments demonstrated differences of the cytoplasmic hereditary units themselves; but we can assume that physiologically determined cytoplasm differences in the reaction chain are very close to the primary reactions of these hereditary units, and that they are only slightly influenced by the genetic system.

2. *The Physiology of Developmental Disturbances in Plants with Jena Cytoplasm*

The inhibited plants with *h*-Jena cytoplasm differ from the reciprocal hybrids in most of their traits. The physiological analysis of those traits that can be altered in such a way that the reciprocal differences are reduced is of greatest interest. Examples of these are auxin and carbohydrate metabolism. Another group of interesting physiological reactions comprises those that are identical in disturbed and normal plants with

Jena cytoplasm, because here the influence of the whole system is not so great.

a. *Modifiability.* All disturbed plants with Jena cytoplasm—and, for that matter, all instances of cytoplasmic inheritance—are characterized by a high degree of variability, quite unusual in the more customary genetic experiments. The smallest differences in composition and structure of soil, or in temperature, light, and moisture, alter the plants to a high degree. This great modifiability increases the difficulty of experimentation and requires special precautions. Repetition of physiological experiments is quite impossible without constant and controlled temperature and artificial lighting. This fact, confirmed by all investigators (Lehmann, 1941b), makes it probable that reciprocal differences occur frequently only in those cases where the physiological reactions have a high degree of variability and where deviations from the normal reaction are tolerated.

b. *Auxin Metabolism.* Lehmann advanced the hypothesis (Lehmann, 1936) that reciprocal differences arise as the end products of gene-produced hormones which are transferred by the cytoplasm. He initiated analysis of the auxin content of dissimilar hybrids from reciprocal crosses; this led to the discovery of reciprocal differences in auxin content (Hinderer, 1936; Graze and Schlenker, 1936). In hybrids with *h*-Jena cytoplasm which displayed an intermediate degree of inhibition and were distinctly stunted, Ross (1939) was able to equalize the reciprocal differences in growth by an artificial supply of heteroauxin to the vegetation point. Only differences in longitudinal growth were eliminated by this method, however; other reciprocal differences were unaffected. It seems, therefore, that differences of auxin content are not the primary causes of reciprocal differences. The differences might be related not to differential production of auxin, but to differential oxidation of auxins (Ross, 1941). Reciprocal hybrids, if grafted together, do not influence each other in their auxin content. If auxin is transferred at all, it is destroyed in conformance with the idiotype of the cell.

c. *The Effect of Temperature and Light.* In all experiments on plants with *h*-Jena cytoplasm, all conspicuous reciprocal differences disappear upon cultivation under conditions of wintry climate, after autumn sowing, and in runners and rosettes that survive the winter. In particular, growth disturbances of dwarfed plants disappear, whereas the less clearly defined and very fine differences of melanization are retained. By cultivating in a low temperature and with artificial lighting it is possible to effect, in hybrids that show 20% to 50% of the growth of the reciprocal cross, a reversal of the reciprocal differences. Such hybrids may actually flower and produce fruit (Michaelis, 1940a; Ross, 1948a). The degenera-

tion of embryos in the *h*-Jena × *h*-Parys hybrids can be retarded by subjecting the ripening seeds to cooler temperature (Michaelis and von Dellingshausen, 1942), and the degree of inhibition of the whole following period of growth can be diminished by such treatment of seeds (Michaelis, 1948d, Fig. 2) and by vernalization. In some cases the inhibition of one of the two reciprocal hybrids is strongest under conditions that are optimal for the other reciprocal cross (Fürtauer, 1940).

d. *Carbohydrate Metabolism.* Under conditions of low temperature and limited light exposure (short day) *E. hirsutum* plants form dorsiventral runners in the axils of leaves of rosettes; and in such runners large amounts of starch are stored. If these starch-containing runners form premature offshoots during the summer, the inhibitions of growth normally found are lost to a degree proportional to the amount of starch accumulated. Thus on the same plant different offshoots may show quite different degrees of inhibition, which otherwise can be observed only in different crosses (Michaelis, 1940a, 1948d; Ross, 1942a, 1948a, 1948b). The winter rosettes behave similarly. Also, offshoots of disturbed hybrids grow normally until the starch reserves are exhausted, after which again the typical degree of disturbance reappears. Likewise, the inhibitions disappear at all places where the transport of carbohydrate is blocked (Ross, 1948a). All inhibited hybrids having *h*-Jena cytoplasm are characterized by a reduced starch content, which may be explained as a result of increased respiration and catalase activity. At the same time, disappearance of anthocyanin and accumulation of tannin can be observed (Ross, 1941).

e. *Redox Potential.* Ross (1941, 1943, 1948b) investigated peroxydase activity through colorimetric measurements of the Nadi reaction. The activity shows periodic variations: it is low during flowering and high in the rosette stage of the "long-day" strains. Peroxydase activity increases with disturbances of the interaction between nucleus and cytoplasm, but reciprocal differences in peroxydase activity are independent of the morphological manifestations of the gene-cytoplasm relationship. The high activity remains even during the winter when the reciprocal hybrids develop identically, or when the starch-rich runners show heterotic growth. These observations lead to the assumption that the differences in peroxydase activity constitute a cause rather than an effect of the disturbances of development, and that they occupy a relatively primary position in the chain of reactions between genetic induction and manifestation.

f. *Hypothesis Regarding the Cause of Reciprocal Differences (Ross, 1941, 1948b).* The observations and experiments reported so far provide credibility to the hypothesis that the redox potential becomes displaced in the

direction of oxidation as a result of disturbances of gene-cytoplasm interaction. Increased respiration by means of activation of the respiratory enzymes can induce heterotic growth, if sufficient reserves of carbohydrates are present, but it can also use up the building material of the developing organism and thus cause dwarfing and other growth inhibitions. Low temperature inhibits respiration more than it does assimilation, and therefore the influence of temperature and light on carbohydrate metabolism can explain the behavior of the hybrids having *h*-Jena cytoplasm. It is also possible to interpret anthocyanin loss along similar lines, for the anthocyanin and flavone also form a redox system. According to the experiments of Thimann (1934), van Overbeek (1938), etc., inactivation of auxins by means of oxidation is possible. The assumption of an alteration in the reduction-oxidation potential not only enables one to explain all the various physiological differences from a single primary assumption, but also is in agreement with the genetic experiments. It is not difficult to understand that many genes become plasmon-sensitive, since many genes undoubtedly either directly or indirectly affect the redox system of the cell or auxin and carbohydrate metabolism, once the limiting threshold value is surpassed. It is of course not certain that the redox system and the other reaction equilibriums dependent upon it are the only systems that can be altered through disharmonious interaction of genes and cytoplasm, but they certainly provide a good model for such interrelations. The mechanisms involved in the alteration of the redox potential are not yet known. Ross assumes that a differential hydration of cytoplasmic protein colloids may be involved.

3. *Summary*

Reciprocal differences arise: (1) by inheritance through the cytoplasm of certain maternal cytoplasmic reaction constants such as viscosity, permeability, and sensitivity to temperature, light, or cell poisons; (2) by alteration of reaction systems as a consequence of disharmonious interaction between nuclear genes and cytoplasm. One of the most important observations made on Epilobium hybrids characterized by reciprocal growth differences is the displacement of the redox potential, which influences auxin and carbohydrate metabolism and which is influenced by a large number of genes either directly or indirectly.

VII. Alterations of Cytoplasmic Inheritance

Section IV described detailed experiments which demonstrated the constancy of plasmon, even when the cytoplasm constituents are exposed to the influence of different genomes and environments. In discussing

alterations of cytoplasmic inheritance, therefore, it must be emphasized that in general, in all experiments, the constancy of plasmon has been well established. Alterations of reciprocal differences have been observed only in single, exceptional plants or in quite definite genome-plasmon combinations.

Furthermore, we must always bear in mind the theoretical consideration that alteration of reciprocal differences may come about either through mutation of plasmon-sensitive genes or through plasmon alteration, and that the latter can be caused not only by mutation of cytoplasmic units but also by a change in combination of these units within the structure of the cytoplasmic system. If the cytoplasm consists of many homogeneous units, in most cases mutation of one unit cannot become manifest until after a segregation and separation of the different units has taken place. Mutation may or may not be the cause of such segregation; but since so little is known so far about the laws governing segregation and recombination of cytoplasmic units, the term cytoplasmic mutation is avoided in the subsequent discussion and the neutral expression "alteration of plasmon" is used in preference.

1. *Alterations in hirsutum Plants with luteum Cytoplasm*

The differences between *h* and *lh*^{n} plants are small and in most cases can be demonstrated only through measurement of a great many plants. Investigations of single cytoplasmic alterations are therefore difficult, and the results are not so certain as those obtained with the *hirsutum* × *parviflorum* hybrids. They are, however, of interest in connection with the experiments discussed below.

a. *Alterations of Pollen Fertility in hirsutum Plants with luteum Cytoplasm.* Normally, *lh*^{n} plants are pollen-sterile. Pollen-fertile plants or sectors sometimes occur in lines that have been pollen-sterile for many generations. The frequency of these pollen-fertile deviations varies in the different lines, but among the later backcross generations such fertile plants appear with somewhat greater frequency. In most lines no pollen-fertile plants appear at all throughout crosses involving many generations and thousands of plants. In other lines, pollen-fertile plants may occur with a frequency as high as 10% or more. Most of the offspring of the pollen-fertile plants are fertile, whereas most of the offspring of the sterile plants of the same family are sterile. Mendelian segregation cannot be demonstrated (Michaelis, 1933). This irregular segregation was not interrupted when, in the descendants of a fertile sector there arose a haploid plant which became diploid and probably homozygous by treatment with colchicine (Michaelis and Michaelis, 1948). Continued selection for sterility or fertility produces lines in which plants having the opposite degree

of fertility very rarely occur. Reciprocal crosses between these lines show maternal inheritance of the different stages of fertility.

Besides the above-described plants with fertile sectors, some plants showed irregular variations of pollen fertility, which could not be ascribed to induction by environmental factors. In these, the offspring of different flowers of the same plant were characterized by different degrees of fertility, corresponding to the degree of fertility of the maternal flower (Michaelis, 1935a).

On the basis of the material at hand the author prefers not to give a definite interpretation of these observations of variations in fertility. Plasmon alteration seems the most probable mechanism. This may come about as a consequence of transfer of cytoplasm through the pollen tube and subsequent reassortment of mixed cytoplasmic units.

b. *Alterations in luteum-hirsutum Hybrids.* Exact measurements of often repeated crosses of lh^n × *luteum* plants show little change in pollen fertility and other properties in the course of the backcross generations (Michaelis, 1932, 1933, 1938). In general, pollen fertility decreases, as does flower size, and the leaves become broader (Table 12). The hybrids

TABLE 12
Measurements of *luteum* hybrids of various lh^n backcross generations
Michaelis, 1932

	Leaf		*Corolla*					
	Length (mm)	Width (mm)	Length (mm)	t	Width (mm)	t	*Living pollen* %	t
luteum × *hirsutum*	53.2	18.4	11.1		8.9		26.4	
$lh^4 \times l$	49.4	18.1	10.0		8.1			
				7.6		4.9		
$lh^5 \times l$	53.0	19.4	9.6		7.4		21.9	
				10.6		7.8		1.1
$lh^6 \times l$	60.4	22.4	8.3		6.5		21.3	
				2.1		2.6		8.8
$lh^7 \times l$	60.2	22.1	8.6		7.0		17.3	
hirsutum × *luteum*	57.6	20.1	4.9		3.4		Disorganized	

of the later backcross generations come to resemble the *hirsutum* × *luteum* hybrids somewhat. This change stops after 10 to 15 backcross generations, without having reached the values recorded for *hirsutum* × *luteum* hybrids. The *luteum* hybrids of lh^{20-24} plants still contain living

pollen, whereas the *hirsutum* × *luteum* pollen is disorganized. The same behavior can be observed with different backcross lines and also in hybrids of crosses of *montanum* or *roseum* × lh^n plants (Michaelis and Wertz, 1935).

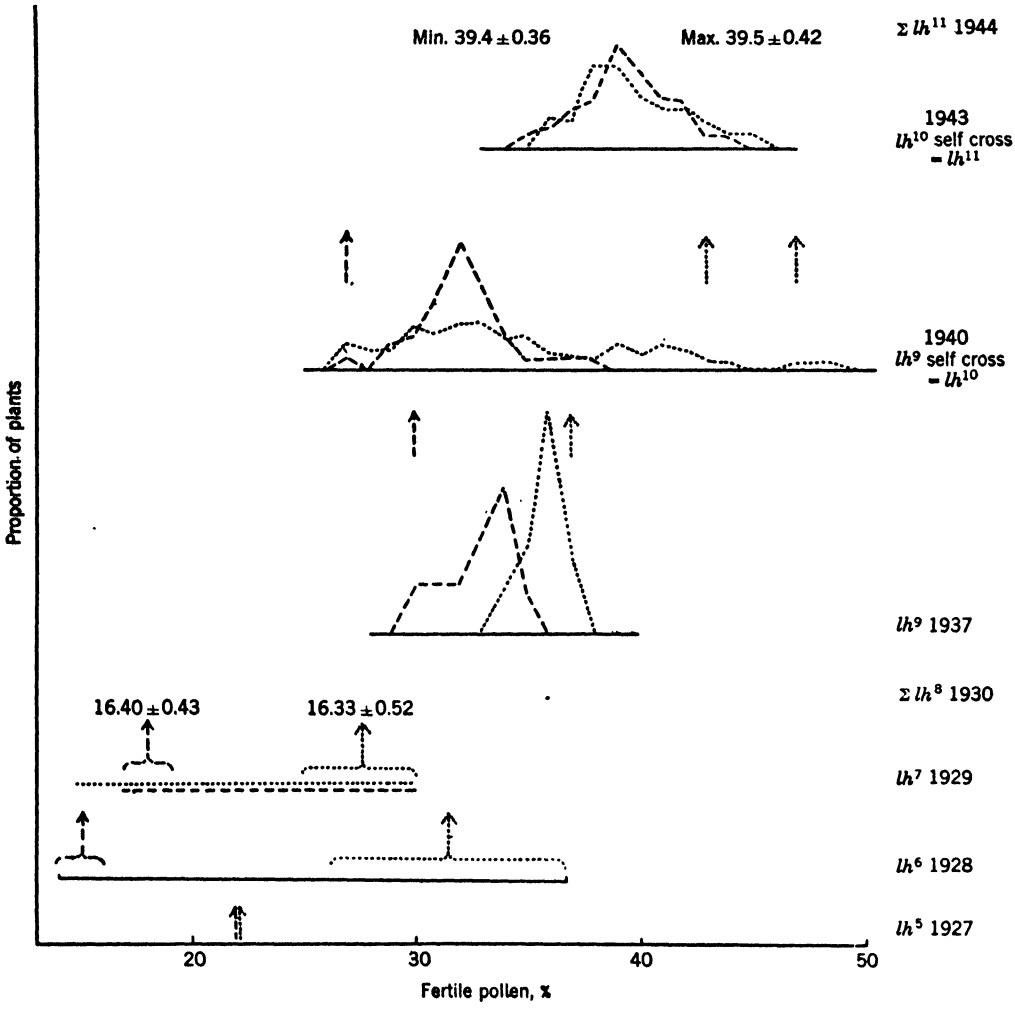


FIG. 4. Selection experiments, lh^6–lh^{11}. The values for the years 1927–1930 are taken from Michaelis (1938), since the detailed protocols for these years were lost as a result of the war. Values for the different years cannot be compared. Broken lines represent selection towards lower fertility; dotted lines, selection towards higher fertility. Each value was obtained by measurement of 20 to 60 lh^n × *l* hybrids from one lh^n plant.

c. *Selection Experiments.* The first backcross generations are heterozygous, and therefore the question arose whether the observed changes had occurred through elimination of the maternal genes or through cytoplasmic changes resulting from the introduction of the male nucleus or from transfer of male cytoplasm.

In 1934, from an lh^2 backcross family the plants most resembling the *luteum* parent and the plants most resembling the *hirsutum* parent were selected and each of these two groups crossed with *luteum*. The hybrids of *hirsutum*-resembling plants showed significantly higher pollen fertility. In other words, the elimination of the *luteum* genes was accompanied by an increase and not a decrease of pollen fertility (Michaelis, 1938). A repetition of this experiment with backcross generations of two other *hirsutum* strains yielded the same results (Michaelis, unpublished).

If the alteration of pollen fertility of the *luteum* hybrids arose as a result of heterozygosity, then it should be possible to select lh^n lines from which *luteum* hybrids with higher and with lower pollen fertility could be obtained. Three selection experiments were undertaken with the 5th, 10th, and 13th lh^n backcross generations. Those lh^n plants that in the preceding year had produced *luteum* hybrids with the highest or the lowest pollen fertility were used for backcrossing (Michaelis, 1938). After 1 to 4 backcross generations, 2 selfed generations were included to speed up selection, although the alterations of fertility occurred only during backcrossing (Fig. 4, Table 13).

TABLE 13
Final Results of Selection Experiments

Selection series		*Pollen fertility of luteum hybrids* Minimum	Maximum
lh^5–lh^9 backcross lh^9–lh^{11} selfing	1943	39.4 ± 0.36	39.5 ± 0.42
lh^{10}–lh^{11} backcross lh^{11}–lh^{13} selfing	1942	36.3 ± 0.13	36.2 ± 0.12
lh^{13}–lh^{14} backcross lh^{14}–lh^{16} selfing	1942	37.4 ± 0.23	37.7 ± 0.23

World War II prevented experiments intended to compare the different series. From the results obtained, however, alteration of pollen fertility of *luteum* hybrids cannot be ascribed to heterozygosity and elimination of *luteum* genes.

d. *Are the Alterations Caused by Transmission of Male Cytoplasm?* By means of individual reciprocal crosses it can be demonstrated that alteration of pollen fertility is brought about by a change of cytoplasmic inheritance. The reciprocal cross $lh^n \times l$ shows that the cytoplasm of the lh^n plants resembles that of the l plants, but is not identical with it. The *luteum* cytoplasm of the lh^n plants is altered during the backcross generation (see Table 14).

This cytoplasmic alteration could be accounted for in many different ways, for example: (1) by transfer of male cytoplasm through the pollen tube to the egg cell; (2) by recombination; (3) by quantitative differences

of reproduction of cytoplasmic units under the influence of the newly introduced nucleus (Michaelis, 1933, 1935a); (4) by nuclear-produced plasmagenes; (5) by mutation of cytoplasmic units; and so forth. A choice among these alternatives is scarcely possible on the basis of present knowledge.

TABLE 14
Alteration of Cytoplasm during Backcrossing

Cross	*Pollen fertility*	t
$l \times lh^7$	36.42%	4.96
$lh^7 \times l$	18.24%	
$(lh^8 \times lh^{18}) \times l$	26.2 ± 0.34%	5.59
$(lh^{18} \times lh^8) \times l$	23.7 ± 0.29%	

It has been feasible, however, to examine experimentally the possibility of transfer of cytoplasm through the pollen tube. The idea gains probability by the fact that the distribution of fertile sectors on the lh^n plants resembles the distribution of pale-green sectors after transfer of plastids through the pollen tube. If we assume that the pollen-fertile sectors arise in the lh^n plants through transfer of *h* cytoplasm, then we must expect the *luteum* hybrids of the fertile lh^n plants to resemble the $h \times l$ offspring more than they do the $l \times h$ offspring, and to show a lower degree of pollen fertility than the *l* hybrids of the sterile lh^n plants. No such relationships were observed, however (Michaelis, 1933, 1938).

Whenever *h* plants with mutated plastids (paralbomaculate plants) were found, the cross $lh^n \times h$-alb was carried out. All seedlings obtained from the cross *h*-alb × *h*-green were white, and died. Among 1774 offspring from the cross lh^n-green × *h*-alb 0.23% variegated plants were found. Unfortunately these plants, unlike the paternal plant, had no clear white sectors, but only irregular white spots, which were lost at the time of flower formation. No changes in pollen fertility were observed (Michaelis, 1935a). The question of the origin of pollen fertility remained unsettled.

2. *Alterations in hirsutum-Essen × parviflorum Hybrids*

Other changes involving reciprocal differences besides pollen sterility were observed among plants with developmental deviations. Some of these provide better material for investigation, since they can be observed throughout the whole period of growth without extensive measurements. Such alterations were first observed by Sirks (1937, 1938) in Phaseolus and Brücher (1940) in the *h*-Essen × *p*-Tübingen hybrid. I myself have

found alterations among hybrids of two *hirsutum* strains, and relatively frequent alterations in several *hirsutum* × *montanum* and *hirsutum* × *parviflorum* hybrids (Michaelis and Ross, 1943; Michaelis, 1947b, 1948b–d, and 1949b, 1949g). All these cases have in common the occurrence of one or more branches, on otherwise growth-inhibited plants, which show more or less normal growth and development and resemble those branches in which inhibitions are removed as a result of low-temperature treatment or carbohydrate reserves. But these experimentally altered branches retain typically disturbed growth under normal conditions and after vegetative and generative multiplication. The *h*-Essen × *p*-Tübingen hybrid was investigated more extensively, because it germinates and grows well and because its alterations do not undergo further changes so easily as do those of other hybrids.

a. *The Typical F_1 Hybrid and Its Modifiability.* The *h*-Essen cytoplasm resembles the *h*-Jena cytoplasm in genetical and physiological behavior. The hybrids with *h*-Essen cytoplasm have only a slightly lower degree of developmental interference than the *h*-Jena hybrids.

Under normal conditions the F_1 of the cross *h*-Essen × *p*-Tübingen is a compactly ramified dwarf, approximately 1.5–5 cm high, which cannot flower. The reciprocal hybrid is 50–80 cm tall, and its flowers contain approximately 50% viable pollen. In cold years some of the developmental disturbances resulting in growth inhibition can be eliminated and the *h* × *p* dwarf can grow to a height of 20–40 cm and form pollen-sterile flowers. In experiments in which *h* × *p* hybrids in a climate room were exposed to low temperatures for 10 weeks, either continuously or at night, they approached the height of plants of the reciprocal cross (Michaelis, 1948d, Fig. 3; 1949b), either the lateral branches or the whole plant losing the effects of inhibition. During such phenotypic normalization, the branches reach or grow through different degrees of disturbance which cannot be distinguished from plasmotypes arising from plasmon alterations. However, when these modified plants, or cuttings from them, are returned to normal conditions the typical degree of developmental disturbance reappears. During the first period of growth, hereditary alterations were never observed in the years 1941–1951, although during this period more than 37,000 hybrids were cultivated under very different circumstances.

b. *Alterations of Reciprocal Differences.* Hereditary alterations can arise after the autumn of the first year in basal buds that survive the winter, and in other parts of the plant from spring of the second year on. These altered shoots lose their developmental disturbances partly or wholly, and displace and overgrow the other branches of the plant. Each of the weakly growing *h* × *p* plants that survives the winter is capable

of forming such altered shoots, and no plant has been found which in several years of cultivation did not give rise to alterations. The typical growth can be preserved only by the removal of all alterations.

Each plant can form several different alterations, which can be isolated by cuttings. Most of the physiological and morphological properties can be altered, including intensity of growth, degree of stunting, kind of ramification, denticulation and hirsuteness of leaves, rate of transpiration, content of anthocyanin and chlorophyll, size, shape, and color of flowers, fertility, and so forth. The altered shoots, when isolated by cuttings, can maintain their changed pattern of development and growth for several years, but they can also form new alterations. Among the great variety of alterations exhibited by these shoots one can distinguish certain constantly recurring and distinctly characterized groups, among which there is a quite definite mutual relation (Fig. 5). For example, within a period of 5 years the alteration called transformatum arose 55 times from stenophyllum plants, but never from plants of the plasmotype irregulare; whereas the alteration adaequatum arose 70 times from irregulare plants, but never from stenophyllum. In adaequatum and transformatum no further alterations were observed. A more detailed examination permits a further division of these groups. All the adaequatum and irregulare clones which had arisen in different ways were found after exact measurement to be similar but not identical in their properties and behavior.

Although cytoplasmic alterations resemble phenotypic modifications morphologically, they are nevertheless distinguishable from them by the circumstance that they are irreversible. Only the sequence irregulare $\leftrightarrow$ argilloides $\leftrightarrow$ poliogramma is an exception to this rule. Although several hundred alterations have been observed yearly—1863 different ones in the period 1941–1947 alone, some of them in many hundreds of cuttings and under different culture conditions—in no case has restoration of the former state been observed. Furthermore, the plasmon alterations differ from the modifications and from one another in their behavior during vegetative and generative multiplication.

c. *Maternal Inheritance of the Alterations.* Experiments involving crosses were difficult to carry out at first, and crosses with the original dwarfs were impossible, except that in 1947 some crosses of modified (normalized) hybrids were successful (Michaelis, 1949b, Table 20). Some alterations did not flower; others were pollen-sterile. The few selfings on the more fertile alterations, and the $p \times h$ hybrids that did succeed, showed the complicated segregation of a species hybrid. More favorable results for our purpose were obtained from crosses of the alterations with *E. luteum* and with strains of *E. hirsutum*. These crosses showed relatively simple segregations, which were different for different alterations,

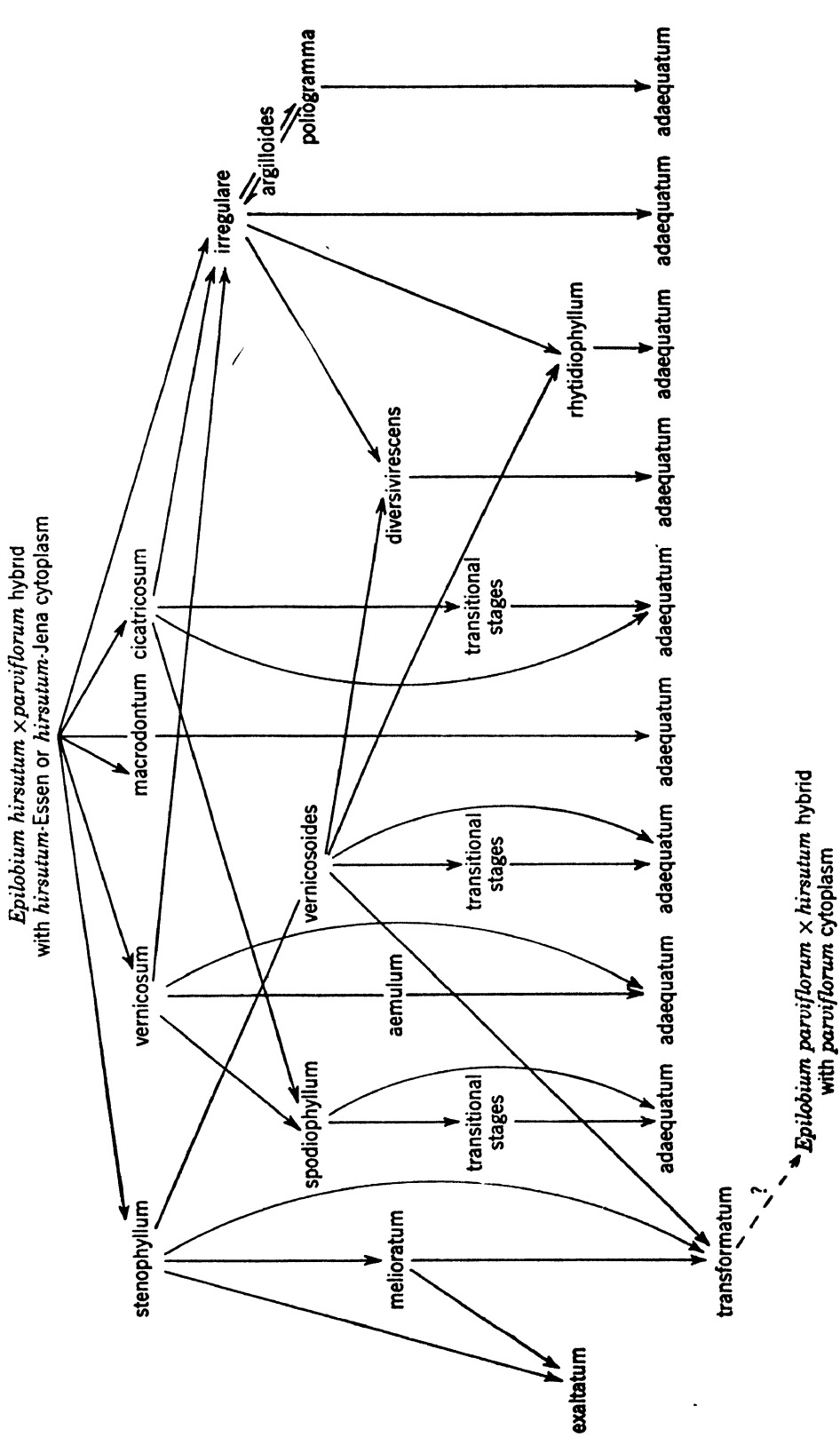


FIG. 5. Cytoplasmic alteration of $h \times p$ hybrids. Arrows indicate the directions of the observed alterations.

even if these alterations were cuttings of a single plant (Michaelis, 1947b; 1949b, Tables 17–19). Repeated backcrossing with *h* strains replaced the original *h* × *p* genome with more or less homozygous genomes of the *h* strains. The cytoplasmic alterations irregulare and rhytidiophyllum, which had formed originally on one plant of the *h*-Essen × *p*-Tübingen cross, were backcrossed with *h*-Munich for 5–6 generations, giving rise to plants that all resembled *h*-Munich but possessed the irregulare properties to an even greater degree (Michaelis, 1949b, Table 24; and Michaelis, unpublished).

The inheritance of the alterations could be cleared up in an unequivocal manner when we found some *h* strains which, when introduced into the crosses, gave rise to perfectly pollen-fertile plants that in all other respects possessed the properties characteristic of the alterations. Here the decisive reciprocal crosses could be made. For six of the alterations investigated definite maternal inheritance was found, both in reciprocal crosses of alterations × *h* strains and in crosses among the alterations themselves (Michaelis, 1949b, Tables 22–23).

d. *Examination of Possible Sources of Error.* In cytological examinations of meiosis and mitosis (Brücher, 1940; Douwes, unpublished) and of pollen tetrads, no abnormalities showed up. From crosses of tetraploid, triploid, and heteroploid plants of *h*-Essen with *parviflorum*, no descendants were found which resembled any of the alterations noted. Thus there are no grounds for explaining the alterations on the basis of chromosomal irregularities. Attempts to demonstrate virus infection by such methods as grafting and inoculation of sap from altered shoots and branches were unsuccessful (Michaelis, 1949b, Table 16). The explanation of the inheritance of these alterations through elimination of gametes or zygotes also does not fit the facts. Reciprocal pollination with single tetrads was carried out. For the pollination *h* ♀ × alteration ♂, pollen of irregulare flowers was used, which in the cross altered flowers ♀ × *h* ♂ produced nearly 100% irregulare plants. In some cases all four plants of one tetrad pollination were obtained. All plants of the tetrad pollination *h* ♀ × alteration ♂ were normal (Michaelis, 1949b, Table 25). These experiments prove that the investigated alterations, and probably others related to them, actually resulted from changes in the plasmon.

e. *Behavior of Some of the Plasmon Alterations during Sexual Reproduction.* Among the different alterations, various categories could be distinguished on the basis of their reproductive behavior. The alterations irregulare—argilloides—poliogramma, which are connected by reversible steps of vegetative change, cannot be distinguished in crossing experiments, though they are morphologically very distinct (Michaelis, 1949b, Figs. 12–16). Likewise the progeny of the transitional stages—

which are close to vernicosoides, spodiophyllum, etc.—are similar to these.

The alterations rhytidiophyllum and irregulare show quantitative differences among their descendants. Comparable crosses between both alterations contain equal numbers of rhytidiophyllum plants. Irregulare plants occur rarely among the offspring of rhytidiophyllum (1%–10%), but more frequently among the offspring of weak (10%–40%) and strong (75%–90%) irregulare plants. The irregulare plants have spots and areas of different tissues on leaves and flowers. Therefore, flowers from the axils of weak or strong irregulare leaves of the same plant give very different offspring (Michaelis, 1949b, Table 6). There is a clear correlation between the degree of these irregulare abnormalities and the number, and degree of abnormality, of irregulare offspring. In a similar manner the more or less sterile adaequatum alteration differs from the medium-fertile transformatum alteration. Among the transformatum offspring the frequency of fully fertile and medium-fertile plants is far greater than among the offspring of adaequatum. Among the transformatum offspring there are no plants that cast off their buds (Michaelis, 1949b, Table 18). The offspring of cicatricosum resemble those of vernicosum, but include more irregulare plants (Michaelis, unpublished).

Comparison of the very different plasmon variants shows qualitative differences as well. From crosses of irregulare only the following plasmotypes are obtained: irregulare, rhytidiophyllum, poliogramma, diversivirescens, and adaequatum. The same plasmotypes occur also among the vernicosum crosses, although less frequently. Instead, one finds stunted plants with the humped, glossy leaves characteristic of vernicosum (Fig. 6*b*). Spodiophyllum crosses, on the other hand, produce only stunted plants; the plasmotypes of the irregulare complex are not included among their offspring. The adaequatum and transformatum crosses give only vegetatively normal plants with different degrees of fertility (Fig. 6*a*). The original, cytoplasmically unaltered $h \times p$ hybrid in crosses gives only stunted plasmotypes (Michaelis, 1949b, Tables 17–23).

The manifestation of the different plasmotypes can be greatly influenced by the kind of nucleus that is introduced. After introduction of the *h*-Jena nucleus, the disturbances characteristic of the altered cytoplasm are weak, whereas they are intensified after introduction of the *h*-Munich or *h*-Coimbra nucleus.

In heterozygous hybrid combinations the segregation of plasmon-sensitive genes is polygenous, as in other examples of cytoplasmic inheritance. But the differences in the segregations between reciprocal crosses of two alterations of a single plant, and the percentage of different plants in the descendants of homozygous backcross plants (for example, irregu-

lare × *h*-Munich[6]), are non-Mendelian. The frequencies in these segregations are closely correlated with the frequency with which the alterations arise *de novo*, and can be explained by new plasmon alterations in the germ cells.



FIG. 6*a*.

FIG. 6. The influence of plasmon on gene manifestation. (*a*) Segregation of the cross (*h*-Essen × *p*-Tübingen) × *h* in normal plasmons. *Upper row*, segregation with *p* cytoplasm; *lower row*, segregation with *h*-adaequatum cytoplasm. The plants photographed were those showing the greatest differences. (*b*) A small selection from among

f. *Behavior of Different Cytoplasmic Alterations during Vegetative Reproduction*. Alterations that occur in the *h*-Essen × *p*-Tübingen hybrids during the first winter retain their peculiar growth pattern even if cultured under different environmental conditions. Only the deviation irregulare, which is composed of different types of tissue, changes more often. Probably the cytoplasm of the meristematic cells is constant also in irregulare plants; but we can assume that during biogenetic develop-

ment and differentiation the irregulare cytoplasm changes frequently, and that as a result of this the leaves and archespores consist of a mosaic of different tissues. Altered branches arise preferably from the callus or dormant buds. In the course of several years new alterations become more



FIG. 6*b*.

the different unusual plants of the comparable segregation with *h*-vernicosum cytoplasm. Adaequatum and vernicosum are plasmon alterations of one individual of the *h*-Essen × *p*-Tübingen hybrid. Scale more enlarged than in (*a*).

frequent, and after 3–5 years it becomes more and more difficult to retain the primary alteration (see Table 15).

Those alterations that are characterized by the most extreme growth inhibition have the highest frequency of subsequent changes. Secondary

TABLE 15
Frequency of Altered Shoots Recorded on Seven Clones during the Course of Several Years

	stenophyllum		*vernicosum*		*vernicosoides*		*irregulare*		*spodiophyllum*		*adaequatum transformatum*		
Year	Cuttings*	% Alteration	Cuttings*	% Alteration	Cuttings*	% Alteration	Cuttings*	% Alteration	Cuttings*	% Alteration	Cuttings*	% Alteration	%
1941	4	0	5	0	2	0	10	10.0	1	0	8	0	3.3
1942	124	0.8	44	0	16	25.0	51	31.4	8	0	46	0	7.3
1943	34	30.2	23	13.0	19	21.1	103	47.6	6	0	32	0	31.8
1944	46	110.9	30	170.0	31	96.8	101	71.6	6	0	24	0	85.7

* Each cutting gave rise to several shoots.

alterations have never been observed among the heterotic alterations exaltatum and spodiophyllum, nor among adaequatum and transformatum, which have the growth pattern of the normal reciprocal $p \times h$ hybrid. This probably indicates that selection within the cells and tissues plays a significant role. Only in slowly growing tissues do the new alterations have positive selective value.

The type and nature of secondary alterations that may occur are in agreement with the different categories of descendants arising from the same alterations. The offspring of the vernicosum clone, for example, included, besides 50%–100% stunted vernicosum-like plants, 0–5% normal plants, and 1%–15% irregulare plants (Michaelis, 1949b, Table 19). On the same vernicosum clone nine normal shoots and thirteen irregulare shoots were noted (Michaelis, 1949b, Table 11). The alteration cicatricosum, which was discovered in 1947, resembles vernicosum but shows simultaneous irregulare disturbances on most vernicosum leaves, and tends toward secondary alterations in the direction of adaequatum, spodiophyllum, and irregulare. Among the offspring of some flowers all plants were normal, as in adaequatum crosses. In other crosses the offspring resembled those from spodiophyllum, with 30%–100% stunted plants (Table 16). Other flowers yielded preponderantly irregulare-like plants.

TABLE 16
Descendants of Different Flowers of One Cicatricosum Inflorescence Which Altered to Adaequatum

Sequence of flowers	*n*	*% Stunted plants = spodiophyllum*	*% Normal plants = adaequatum*
2	53	—	100%
4 and 6	54	55.6%	—
7, 14–18	203	—	100%
22	13	30.8%	—
23 and 24	76	—	100%

In addition to such qualitative relationships, there are also quantitative relations between appearance, vegetative behavior, and segregation. The following example will illustrate what is meant. In 1941 a number of irregulare shoots were isolated. One of these, referred to as E2, showed relatively weak irregulare disturbances. E2 gave rise to 23.2% adaequatum offshoots during four years. The offspring of a cross of E2 $\times$ *h*-Munich included 15.3% normal plants. Another clone, E7, showed strong and frequent irregulare disturbances. This clone gave rise to only 6.7% adaequatum shoots, and none of the offspring included normal plants.

Such observations indicate that the same causes may affect vegetative alteration and sexual segregation. The results of all these experiments and observations are summarized in Fig. 5. It shows that the original $h \times p$ hybrid possessed various possibilities for alteration, and that in each of the ensuing alterations these possibilities were limited in a different manner. Only in the normally growing plants can this limitation be produced through a decrease in frequency of alterations as a consequence of unfavorable selection conditions for the altered plasmotypes. In the retarded plants such an explanation is unlikely; instead, as a working hypothesis, we can assume the occurrence of a quantitative or qualitative elimination of cytoplasmic units and thereby a different plasmon composition. Thus the failure of stenophyllum to form irregulare alterations and the failure of irregulare to form stunted plants might be due to their respective lack of the corresponding cytoplasmic units.

g. *Alteration as a Directed Process.* With the exception of the step irregulare $\leftrightarrow$ poliogramma, the alteration process is irreversible. In the scheme as outlined in Fig. 5 the arrows show the direction in which new alterations were observed (Michaelis, 1948c, Table 1). Certain sequences of alteration are recognizable. Slower growing plants always give rise to better growing ones. The opposite process—that is, the origin of stunted plants from well-developed and normally growing ones—has never been observed. The sequence of the stenophyllum alterations provides an illuminating example. The original $h \times p$ hybrid grows 4–6 cm tall; stenophyllum grows 30–50 cm tall; melioratum grows to 150 cm in height but still has curved and abnormal leaves, indicating growth disturbances; exaltatum is heterotic and reaches a height of 210 cm; and transformatum is indistinguishable from the reciprocal hybrid and, like the latter, grows 120 cm high. Alterations occur frequently in the $h \times p$ hybrid and in the stenophyllum alteration, but only rarely in melioratum. The transformatum alteration and the exaltatum alteration never change.

Up to the year 1947 a total of 1863 vegetative alterations had been observed, and each year after that additional hundreds of new alterations have been recorded. In all these cases there has been observed no deviation from the rule that the process of alteration is directed toward an elimination of developmental disturbances. In my opinion, this rule is to be explained not by directed mutation but by competition and selection, within individuals, among the different plasmotypes.

In a few rare cases, deviations from this rule have been noted during sexual reproduction. Among the 2807 offspring of the irregulare alteration, four plants were found that had a sector resembling the vernicosum alteration. Such cases occur only when crosses are made shortly after the origin of the irregulare alteration. Crosses between plants separated by many

generations (either vegetatively or sexually) from the parent originally giving rise to the primary hybrid never produce any vernicosum plants or sectors. The exceptions noted above can be explained by assuming that shortly after the occurrence of the alteration the plasmon components are not yet completely separated, and that selection among archespore cells has a lower coefficient than in growing vegetation points. In spite of these exceptions, the fact of directed alteration cannot be doubted.

h. *The Formation of Altered Shoots.* Altered shoots usually arise during the winter from basal buds (turions). The shoots are altered either as a whole or in a sector, and remain constant thereafter. The process of alteration must take place in the dormant buds, which are formed in the axils of rosette leaves. In these cases the alterations appear quite suddenly and spontaneously.

More rarely, alterations arise during summer growth. Usually, these appear as clear and distinct sectors, similar to the winter alterations. If they occur in quickly growing meristem tissue, however, the alterations lack clear, sharp boundaries. They have a more gradual, "flowing" character, and are connected with the original type by a series of transitional stages, which take in only a few leaf whorls or a longer shoot (Michaelis, 1948c). Such alterations closely resemble environmentally induced modifications from which they can be distinguished only by their offspring and by their behavior during vegetative multiplication.

In a few cases it was possible to cross the flowers of such transitional alterations, but owing to faulty germination the results were too poor to yield statistically significant data. Occasionally a transitional alteration was divided in several cuttings. In four cases in which the alteration vernicosum changed to adaequatum, the cutting of the altered shoots did not stop the process of alteration, and all the cuttings were transformed to adaequatum. In two cases, however, the different transitional types remained constant in the cuttings and could be isolated and retained as such. One of these cases has been described in detail (Michaelis, 1948c). In the other case also a different inheritance of the cuttings of one transitional alteration could be demonstrated.

i. *Chimeras.* As a consequence of alterations which in different parts of the same plant proceed in different directions and at different rates of speed, complicated chimeras may arise, the interpretation of which is not always easy by means of anatomical and genetical investigations or regeneration experiments. The analysis of these chimeras is of particular interest, however, because their composition permits certain inferences about the causes and origins of the alterations. Not only may sectors of different plasmotypes arise, but the same leaf may contain sectors in which the same alteration shows different degrees of expression. For

example, the small striae characteristic of the poliogramma leaves may be arranged in varying degrees of density (Michaelis, 1950a, Fig. 7). The offspring of flowers of a single irregulare inflorescence may contain different frequencies of irregulare plants among them (Michaelis, 1949b, Table 6).

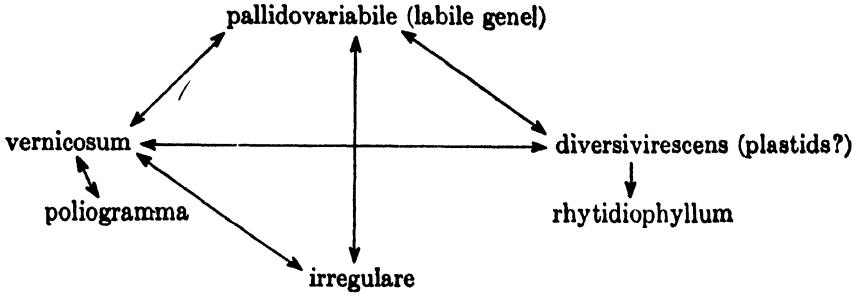
Sectional chimeras occur very frequently. The light-green alteration diversivirescens forms a sectional chimera only for a short time and then quickly and regularly forms periclinal chimeras with different numbers of cell layers. Such behavior has been noted frequently among plants with different plastids (Renner, 1936). Among other cytoplasmic variants, periclinal chimeras can be identified very rarely. It is difficult to decide whether we have failed to recognize periclinal chimeras or whether the behavior of the cytoplasmic units that condition these alterations is quite different from that of the plastids during cell division.

The complicated chimera composition is characteristic of the irregulare alteration. The appearance of irregulare leaves and shoots is caused by the composition of tissues of differing growth rates. During the slow growth of winter, sorting out and isolation of differing plasmotypes takes place, which permits the occurrence of complicated chimeras. One leaf may consist of 3–5 different plasmotypes, and often the diversivirescens part forms a periclinal chimera, whereas in other areas of the leaf no periclinal structures can be demonstrated (Michaelis, 1949b, Figs. 4–7; Michaelis, 1950a, Figs. 5–7). During the summer, such definite sorting out in altered shoots is rare, although in some cells, such as gametes, it is significant. A feasible hypothesis is that the plasmon units composing irregulare are united in a regular manner in the apical cells of the growing point, and that this structure is reproduced regularly. During differentiation of the cell outside the growing point, the bond between the plasmon components seems to be loosened to a variable degree. Under summer conditions, the number of cell divisions is sufficient for distinct separation only in the cells and tissues of the leaves, not in the whole shoot. This type of behavior may provide us with an interesting model of differentiation, from meristem cells to specialized cells.

Similar processes can be discovered among other plasmotypes as well as among the original $h \times p$ hybrids. The leaves of the $h \times p$ hybrids are very finely mottled. Occasionally some larger spots arise, especially following X-ray treatment of leaves after the stage of cell division and during the stage of cell extension. These spots look very much like those observed in the irregulare alteration types. Similarly, in the mottled leaves of the unaltered *h*-Jena hybrids, cells have occasionally been found which contained plastids of different sizes (Michaelis, 1940a, Fig. 18).

It seems possible that in these cases the leaves contain cell complexes that differ from one another by their plasmotypes.

k. *Overlapping and Penetration of Different Sectors in Chimeras and Their Significance.* It is a significant fact that once diverse tissues have arisen as the result of plasmon alteration these tissues not only can overlap but also sometimes penetrate one another. Such cases of penetration have been noted among the following alterations.



No reciprocal influence of different layers of tissue on one another has yet been demonstrated, either after grafting, or at the sharp edges of sectors, or when differently altered layers overlap.

Penetration of different types of alteration in one layer of tissue is possible only if different cell constituents are concerned in the formation of the alterations, and if these cell constituents behave differently during cell division. Pallidovariabile is caused by a labile gene; diversivirescens may be caused by the reaction of different plastids. In such a case, penetration is made possible as the consequence of the different behavior of chromosomes and plastids during cell division. Penetration of the other alterations must depend on similar assumptions. On the basis of these considerations, we must assume the existence of several distinct plasmon constituents in addition to the plastids and the nuclear genes. Further conclusions regarding the coordination of plasmon alterations and cytoplasmic constituents must await detailed cytological analysis, which under the particular circumstances has not so far been possible.

l. *Summary.* Numerous alterations which are passed on through maternal inheritance can arise among the $h \times p$ hybrids. They are very probably caused by sorting out of several cytoplasmic constituents within the individual. Some of these alterations, which can appear either suddenly or by a series of transitional changes, resemble environmentally induced modifications. Through selection processes within the plant, these alterations proceed in the direction of elimination of developmental disturbances. The investigation of cytoplasmic recombination in chimeras is a method for analysis of plasmon, corresponding to the Mendelian analysis for the nuclear genes (publication in preparation).

3. *Alterations in hirsutum-Jena × parviflorum Hybrids (Michaelis and Ross, 1943; Michaelis, 1949b)*

In the course of investigations concerned mainly with the effect of the *hirsutum*-Jena cytoplasm (see Section V, 2, a), the *hirsutum* strain Jena was crossed with 36 different *parviflorum* strains. The resulting $h \times p$ hybrids with *h*-Jena cytoplasm showed wide variation in the degree of typical growth disturbances induced as a result of cytoplasm-gene interaction (Michaelis, 1943). Alterations arose in only 7 of the 36 combinations, but in these they occurred in large numbers. In 1600 hybrid plants, more than 1596 primary and secondary alterations arose during the years 1941–1945. These seven types of $h \times p$ hybrids were all characterized by a similar high degree of growth disturbances. The weakly disturbed hybrids, and those hybrids that did not show any reciprocal differences, never gave rise to alterations. The same was true of the extremely inhibited (scarcely growing) plants. It seems as though the establishment of cytoplasmic alterations requires (1) that the plant undergo a sufficient number of cell divisions to permit sorting out of plasmon units, and (2) that the new plasmotypes possess a selective advantage in growth rate over the original unaltered phenotype. In this way plasmon-sensitive genes, by determining the degree of inhibition, can at the same time influence the formation of alterations. In addition to this, however, the parviflorum genome seems to be essential to the alteration process, for alterations of the same *h*-Jena cytoplasm have never been observed in *hirsutum* interstrain crosses, even though developmental disturbances are quite similar in degree in the $h \times h$ and $h \times p$ hybrids (Michaelis, 1947a, Fig. 2; 1954b).

The single plasmon variants arising in the Jena $\times$ *p* hybrids all resemble the variants of the *h*-Essen $\times$ *p* hybrid in morphological appearance, though the *h*-Jena cytoplasm can be clearly distinguished from the *h*-Essen cytoplasm in crosses, and though different parviflorum strains are used as male parents. The frequencies of the different alterations are different, however, in the two types of cytoplasm. For example, diversivirescens occurs more frequently in the Jena cytoplasm, and vernicosum more rarely.

In crosses with parviflorum, the stability of the *h*-Jena cytoplasm is lower than that of the *h*-Essen cytoplasm. The number of altered shoots is greater and the alterations can be induced more easily with low temperature. After autumn sowing, altered shoots appear as early as June of the next year (Michaelis and Ross, 1943, Fig. 1). In the *h*-Essen cytoplasm, alterations do not appear after autumn sowing.

The stability of the altered shoots is also low. More transitional stages

can be observed, and the isolation of constant alterations is far more difficult. For example, adaequatum shoots of normal appearance which had arisen from irregulare plants were investigated by crosses. The offspring of some of these consisted of only normal-looking plants. The offspring of others included different percentages of irregulare plants (Michaelis, 1949b, Table 21), which were lower, however, than the percentage found in offspring of irregulare parents. If we accept the hypothesis of a sorting out of cytoplasmic units, such behavior can be explained by assuming that the irregulare-inducing units must be present in a certain minimum concentration in order to produce the irregulare disturbances, and that these units were sorted out very slowly. In the *h*-Jena cytoplasm a stable balance in the quantity of cytoplasmic units seems to be more difficult to maintain than in the *h*-Essen cytoplasm. Therefore the two cytoplasms differ in the degree of their suitability for certain kinds of investigation, the *h*-Essen cytoplasm being more suitable for studies of the stability of alterations and the *h*-Jena cytoplasm for studies of the actual process of alteration.

As for the rest, no essential differences in the formation of alterations could be observed between crosses of *h*-Jena × *pariflorum* and *h*-Essen × *parviflorum* or between crosses with different *parviflorum* strains. The maternal inheritance is also identical.

4. *Alterations in Similar Hybrids from Reciprocal hirsutum × parviflorum Crosses*

In addition to *h*-Jena, *h*-Munich and *h*-Coimbra also were crossed with different *parviflorum* strains. All the resulting hybrids were alike in the reciprocal crosses. In a few cases some stunted dwarfs appeared among the hybrids. But such dwarfs can easily be distinguished, by form and structure of leaves, from the dwarfs with *h*-Jena cytoplasm. They originate through the action of inhibitor genes. These hybrids also gave rise to alterations of a kind that tend to eliminate growth disturbances. The manner of their origin is the same as was described for alterations of dissimilar hybrids from reciprocal crosses. Investigations of their inheritance have not yet been made (Michaelis and Ross, 1943).

The hybrid between *h*-Jena and *parviflorum*-Oberleinleiter is of particular interest (Michaelis, 1949g). The cross *h*-J ♀ × *p*-O ♂ showed the typical disturbances of the Jena cytoplasm; and the reciprocal cross *p*-O ♀ × *h*-J ♂ showed the disturbances characteristic of the action of inhibitor genes. The *h*-J ♀ × *p*-O ♂ hybrid gave rise to an alteration that eliminated the cytoplasmic disturbances. After this alteration most of the shoots of the plants were indistinguishable from those of the reciprocal cross, except that one shoot showed a transitional change. In the follow-

ing year this shoot gave rise to a new alteration, which was completely normal. Here also the stunting and dwarfing induced by inhibitor genes were eliminated during the formation of typically cytoplasmic alterations. Therefore it seems probable that alterations of the similar hybrids from reciprocal crosses are identical with the cytoplasmic alterations of the *h*-Jena × *p*-Oberleinleiter hybrid.

5. *Alterations in Two hirsutum Strain Crosses*

Although a large amount of material from *hirsutum* strain crosses was examined, only two cases of alterations were observed (Michaelis, 1948b).

a. *Alterations in hirsutum-Wittmund × hirsutum-Jena Hybrids.* The reciprocal cross *h*-Jena × *h*-Wittmund was repeatedly performed. The *h*-Jena ♀ × *h*-Wittmund ♂ hybrid has the typical stunted growth of the *h*-Jena cytoplasm. The *h*-W ♀ × *h*-J ♂ hybrid is quite normal. Many seedlings of one fruit had somewhat paler greenish sectors on the primary leaves. These sectors also showed the fine, hazy spotting characteristic of the Jena cytoplasm. It is possible that these sectors came about by transfer of *h*-Jena plastids and cytoplasm through the pollen tube, because similar but nonspotted sectors were observed in crosses of the normal-green *h* strains with the pale-green *h*-Kew albiflorum. Probably the *h*-Jena plastids cannot develop the normal green color in the strange environment.

In the following summer four plants of these seedlings (cross *h*-Wittmund ♀ × *h*-Jena ♂) gave rise to altered shoots. All together, nine different categories of alterations could be distinguished, of which as many as four categories occurred in the same plant; and different plants gave rise to similar categories. One shoot was a periclinal chimera and resembled completely the diversivirescens alteration of the *h*-Jena ♀ × *parviflorum* ♂ hybrids. In regeneration experiments normal-green and light-green clones were isolated. The pale-green clones were completely sterile and failed to show the fine spotting observed among the first sectors. Several other shoots showed pale-green color on all leaves, and differed in their growth and in flower and leaf shape (Michaelis, 1948b, Figs. 2–13). These alterations remained constant during four years of extensive vegetative propagation, except for a possible phenotypic change in pollen fertility between the first and second years. Two plants gave rise to shoots that showed the stunted growth typical of the reciprocal cross with *h*-Jena cytoplasm. The clones of these two were not constant, and in the course of the years sectors of most of the shoots became normal. Sterility prevented large-scale crossing of the alterations, but the offspring of the crosses that could be performed with different alterations were similar and entirely normal.

The results of the crossing experiments make the interpretation of the alterations difficult. The alterations might be compared to dauermodifications. There is a certain degree of likelihood in the hypothesis that the alterations are caused by transmission of cytoplasm through the pollen tube and subsequent sorting out of the different cytoplasm constituents. But this hypothesis alone cannot explain the results of the crossing experiments. It is possible that, as with the alteration step irregulare-poliogramma in the $h \times p$ hybrid, the alterations differ quantitatively and that these differences are reversed during fertilization or gamete formation. No chromosomal disturbances or virus infections could be demonstrated.

b. *Alterations in hirsutum-Jena × hirsutum-Seelow Hybrids.* The *h*-Jena × *h*-Seelow hybrid with *h*-Jena cytoplasm shows weak growth disturbances only if grown under appropriate weather conditions. In one among many hybrid plants, a shoot was found with strikingly wavy and faded leaves (Michaelis, 1948b, Figs. 14–20). Subsequent vegetative reproduction of this lomatophyllum clone produced occasional shoots that lost the altered appearance and became normal, either suddenly or gradually. Such normalized shoots may resemble the typical *h*-J × *h*-S hybrid, or they may lose the disturbances of the Jena cytoplasm and become heterotic.

The offspring of all the different crosses differed in no way from the crosses of the typical *h*-J × *h*-S hybrid. Here, too, neither chromosomal rearrangements nor virus infections could be demonstrated.

6. *Investigations on the Origin of the Alterations in the Different hirsutum-parviflorum Hybrids*

a. *Results of Experiments, and the Different Possibilities for Alteration.* The experiments with the $h \times p$ hybrids demonstrate with certainty the cytoplasmic nature of most of the alterations. In all probability the cytoplasm is the sum of different hereditary constituents, and the alterations that we have observed differ from one another by differences in the quantitative or qualitative combination of these constituents. It is furthermore very probable that during the formation of the alterations there is a sorting out of these cytoplasmic constituents, in which the direction of the alteration process may be determined by selective influences affecting the rate of reproduction and of growth. Such an assumption can explain all the observations and experimental results.

From this point of view, there are several alternative explanations of the origin of cytoplasmic alterations to be considered:

1. It has not been possible to demonstrate experimentally an invasion of infectious particles (virus) or foreign nucleoproteins (Michaelis, 1949b).

2. The formation of plasmagenes *de novo* by the nucleus is improbable in Epilobium. This hypothesis will be discussed in Section IX.

3. All alterations might be conceived as plasmon mutations, in analogy to chromosome mutations; but this is not useful. The possibility of mutations of plastids or other cell constituents cannot be denied. The observation of different kinds of plastids in one leaf of an *h*-Jena hybrid (Michaelis, 1940a) and the frequent origin of diversivirescens periclinal chimeras might be interpreted in this manner. On the other hand, we cannot eliminate the possibility that in the alterations different kinds of proplastids, which normally remain in a mitochondria- or chondriosome-like stage (Guilliermond, 1941) develop into plastids. No definite case of mutation of cytoplasmic units or plasmagenes is known, and none can be observed directly when the number of similar plasmagenes is large. Mutations of cytoplasmic units can be expressed only after several cell divisions or after a number of divisions of the constituents, when these comprise several units. In most cases, the mutation of cytoplasmic units cannot be successfully investigated until their sorting out during cell division can be comprehended.

4. Cytoplasmic differences can arise as the result of transfer of male cytoplasm through the pollen tube and subsequent sorting out of the different cytoplasmic units. Such an assumption provides a useful hypothesis to explain the alteration of pollen sterility in the lh^n plants (Section VII, 1) and especially the alterations in the *h*-Wittmund × *h*-Jena hybrid (Section VII, 5).

5. Finally, recombination of cytoplasmic units seems to be possible without mutation or addition of units only if the stability of the genetic system is lost or disturbed by change of genotype or environment.

These possibilities of cytoplasmic alteration are not the only ones, but they are the most important, and must be investigated as thoroughly as possible.

b. *Role of the Pollen Cytoplasm in the Alteration Process of the h × p Hybrids.* Transmission of the contents of the pollen tube has been demonstrated in Epilobium (Section III, 6). The frequency of such transfer might conceivably vary among the different kinds of crosses. In the hybrids derived from the cross *h*-Wittmund × *h*-Jena alterations arise, presumably as the result of transfer of male cytoplasm, which resemble some of the alterations of the *h*-Jena × *p* hybrids.

If we assume a transfer of male *p* cytoplasm into the *h* egg cell, and if we assume a sorting out of paternal and maternal cytoplasm, then it must be possible to associate some alterations with the *h* cytoplasm and others with the *p* cytoplasm. As a rule, introduction of *plasmafremde* genes results in an intensification of disturbances between gene and cyto-

plasm, whereas introduction of *plasmaeigene* genes results in a decrease of these disturbances.

The different alterations of one $h \times p$ hybrid plant were crossed with h and p. The stunted growth of vernicosum and spodiophyllum, as well as the sterility of adaequatum, all of which resemble the disturbances caused by the h cytoplasm, did decrease among the offspring after h was introduced by crossing, and increase after the introduction of p. Therefore it seems possible to associate these alterations with the cytoplasm of the mother *hirsutum*. On the contrary, the alterations of the irregulare complex disappeared after introduction of p and increased after introduction of h. Thus the irregulare cytoplasm seems to work better with the p genome. The irregulare defects have not been found so far in *hirsutum* strains and interstrain crosses. In 1953 the first distinct irregulare plant was found in h-Essen self-crossed. It is not known whether they arise in the *parviflorum* cytoplasm, since during the last few years no *parviflorum* strains have been found that showed reciprocal differences in crosses between strains (Michaelis, 1949b). These observations, therefore, do not contradict the assumption that the male *parviflorum* cytoplasm is involved in the origin of alteration.

In many experiments we have tried to find or produce albomaculate *parviflorum* plants, which are very rare. All together, only five flowers were found on white or pale-green sectors. Among the offspring of the cross h-Essen green $\times$ p white, no white sectors were found. Obviously, the observed alterations are much more frequent than the white sectors originating through plastid transmission. This contradicts the hypothesis that the alterations of the $h \times p$ hybrids arise through transmission of paternal p cytoplasm, unless we assume that the transmission of cytoplasm is more frequent than the transmission of plastids, or that the p plastids are not viable in the h-Essen cytoplasm. Thus the experiments correspond less to the hypothesis of cytoplasmic transfer (Michaelis, 1954b).

c. *Mutation Experiments on the $h \times p$ Hybrids.* The problem of mutation of cytoplasmic units has been attacked in some preliminary experiments by treating plants with cellular poisons (Section VII, 6, f) or with X-rays (Michaelis and Kaplan, unpublished). The h-Essen and h-Jena cytoplasms were irradiated in different stages, since sorting out of cytoplasmic units is of prime importance. Both h-Essen and h-Jena maternal plants were irradiated as seedlings, before bud formation, and during bud and flower development. The treated plants were then crossed with *parviflorum*. The $h \times p$ hybrids were treated as embryos on the mother plant, as seedlings, during the first summer, and also during the stages of alteration in the springtime of the second year. In most experiments relatively few plants were treated, but the few treated plants were increased nu-

merically by cuttings, because in mutation experiments on cytoplasm probably not only the number of plants but also the number of cell divisions is important. The doses used varied from 1000 r to a sublethal dose. In addition, plants were treated for three weeks with X-rays of a thorium preparation, irradiated daily for five weeks with X-rays, or fed with radioactive phosphorus and sulfur. In all of these experiments, which are being continued, no unquestionable cytoplasmic alterations have been demonstrated except the spontaneous alteration. The following phenotypic effects have been noted: (1) Growth stimulation at low doses. (2) Fine spotting of the leaves (if treated during cell growth and cell stretching). These spots become more prominent in strong sunlight and resemble the spotted leaves of the stunted hybrids. (3) Anthocyanin formation in the vegetative zone but not in the inflorescence. (4) Transformation of flower buds to vegetative buds if treated prior to carpel formation. Leaves which undergo cell division during or after treatment have defects in some sectors, probably as the result of gene mutation or chromosomal rearrangement. Among the *h* plants, flowers of abnormal size and color have appeared, but the shoots and offspring of such plants are normal. Changes in frequency of the $h \times p$ alteration have been noted when radiation treatment was given while the actual process of alteration was in progress, probably because the alterations are less sensitive to the treatment than is the dwarfed $h \times p$ hybrid (see also Section VII, 6, g below).

These negative findings correspond to the results of experiments in which an attempt was made to increase the frequency of plastid mutations by means of X-ray treatment.

From the experiments it cannot be decided whether the cytoplasmic units differ in their sensitivity to mutagenic agents or in mutability as such, whether mutations among a large number of similar units fail to manifest themselves, or whether the mutated units are too rapidly eliminated. Neither do the experiments lead to any explanation of the observed cytoplasmic alterations (new results, Michaelis, 1954a).

d. *Induction of Alterations under Conditions of Winter Climate.* The alterations of the $h \times p$ hybrids do not commence before the end of the first vegetation period and the beginning of winter, but then they appear in large numbers. The possibility was considered that low temperatures and short days are essential factors in the origin of plasmon recombinations. To test this hypothesis, seedlings of the cross *h*-Essen $\times$ *p* were grown with three different day lengths and under various conditions of temperatures—that is, some in normal temperatures, some in a cooled greenhouse, and some in normal temperatures during the daytime and in a 0°C cold room at night. The height of the longitudinal axis was used as a measure of the degree of inhibition.

The inhibitions were strongest with long-day culturing. The treatment was followed by numerous normalizations, which occurred the more frequently when the plants were more strongly inhibited. A slow, gradual normalization of the main axis occurred only when growth was not inhibited during the night. All these normalizations were indistinguishable from cytoplasmic alterations, but all cuttings from the normalized plants showed the typical growth disturbances in the following year. Cytoplasmically inherited alterations occurred only in the following winter, the lower temperature promoting the formation of alterations. It was not possible to advance the time of alteration arbitrarily (Table 17; Michaelis, 1949b).

TABLE 17
Normalization of *h*-Essen × *parviflorum* hybrids

Treatment					*Modifications*		
Length of day	Temperature (C) during day	Temperature (C) at night	*n*	*Gradient of inhibition measured by height in cm*	Sudden normalization of lateral axis	Gradual normalization of main axis	Cytoplasmic alterations in the next year
	17°–34°		311	11.4	32.5%	0%	7.5%
17 hours	10°–17°		298	5.7	58.7%	12.1%	3.3%
	17°–34°	0–2°	212	4.9	93.9%	0%	13.2%
	17°–34°		334	21.8	11.7%	17.7%	7.5%
12 hours	10°–17°		320	11.0	28.1%	38.1%	4.3%
	17°–34°	0–2°	196	24.2	20.4%	0%	24.4%

Neither could the time of alteration be advanced by sowing in the fall and by letting the rosettes winter without the use of greenhouses. In such experiments the *h*-Jena × *p* hybrids gave rise to alterations in June following the winter (Michaelis and Ross, 1943). In new experiments (Michaelis, 1954b) the frequency of the alterations as well as their kind could be changed by variation of temperature conditions (see Table 18).

We did succeed in these experiments, however, in inducing modified normalizations that resembled the alterations, as well as in increasing the number of alterations; but the induction of alteration at will was not possible through low-temperature or short-day treatment; this implies that they cannot be the decisive factors in the origin of alterations.

e. *The Influence of Age.* Alterations do not usually occur until a developmental period of a certain length has been completed. The number and frequency of secondary alterations increases with the age of the clone

TABLE 18

The Influence of Environmental Factors and of Genotype on the Plasmatic Alterations of the *hirsutum* × *parviflorum* Hybrid (experiments 1952–1953, unpublished)

hirsutum-Essen ♀ crossed with:	Clone: *parviflorum*-Tübingen, 1-8-1947							Gene mutations of *parviflorum*-Tübingen													Clone 1-8-1947	Total 1952–1953
								Hybrid higher than control				Recessive mutations. Growth of hybrid exactly same as control										
								resistens (dominant mutant)	normalisatum*		flavoviride	aureum	pallidum	albidum	♀ lethal₂	♀ lethal₁	mut₁*	mut₂*	mut₃*	Control	
									+/+	+/n											
Treatment with:	Radioactive isotope				Streptomycin	Temperature		Wintering		Uniform wintering in unheated greenhouse												
	Control	S^{35} 0.01–0.2 μC	S^{35} 0.21–0.5 μC	P^{32} 0.02–2.16 μC		28°–35° May–June, 1952	28°–35° May–October, 1952	Cold	Warm													
Number of plants	139	143	115	123	308	65	27	23	35	519	326	293	692	335	191	217	432	168	521	555	1261	8915
% of altered plants	52.5	60.1	68.7	58 5	50.7	*87.7*	*0*	*100 0*	*2 9*	*54.7*	*74 7*	72.0	45.9	37 3	39.3	25.7	33 8	53 6	27 6	31 9	36.7	—
Number of alterations	83	261	184	149	374	85	0	28	3	396	240	153	330	108	103	172	217	119	155	209	554	5177
contractum†	20.5	18.0	20.7	14.1	15 2	2.4	—	*78 6*	—	*10.1*	*72 1*	0	0 3	8.3	6 8	9.4	6.0	1.7	1 9	2 4	6 1	648
nervatum†	1.2	0.4	0	0 7	0.8	0	—	0	—	0	0	0	0.3	0	0	0	0	0	0	0	0.4	13
lanceifolium†	0	0	0	0.7	*1.6*	0	—	0 3	0.4	0	0	0	0	0	0	0	0	0	0	0	0 2	10
stenophyllum	0	0	0	1.3	1.3	0	—	0	—	5 3	1.7	2.6	1.5	0	*5.8*	0	2.8	3.4	4.5	4 3	2 9	113
melioratum	0	0.8	1.1	0.7	1.6	4.7	—	0	—	1.5	0	0.7	0.3	0 9	0	0	0	0	0 6	1.0	1.3	40
macrodentatum†	4.8	*3.4*	1.1	8.7	12.3	15.3	—	0	—	16 2	4.6	*2.6*	17.3	18.5	15 5	*19 7*	18.9	16 0	12 9	8 1	17 7	676
vernicosum	9.6	7.7	3.8	2.7	7 2	2.2	—	10.7	—	21 7	3.8	23.6	18.5	19.5	*8 7*	15 2	18 9	15 9	*25.9*	14 8	18 6	742
cicatricosum	3.6	5.7	3.8	6.7	7.0	7.1	—	10.7	—	6 3	*1 2*	13.1	11 8	11.1	12 6	*16 3*	10.1	16 0	12.3	11.0	8.7	451
irregulare	12.0	13.0	6.5	7.4	8.8	18.8	—	0	—	13.6	5 8	12.4	7 6	6.5	10.7	7.6	9 2	11.8	9.7	9 6	10 3	510
rhytidiophyllum	4.8	6.5	2.7	2.7	6.7	3.5	—	0	—	4.5	1.7	4.6	9.4	4.6	9.7	5.8	6 9	5.0	2.6	9.1	4.0	272
diversivirescens	0	2.7	0	1.3	0.5	2.4	—	0	—	2.0	0.4	0 7	1.2	0	2.9	2.3	1.8	1.7	1.9	1.0	1.6	71
longifolium†	4.8	8.0	2.7	3.3	*14.0*	7.1	—	0	—	6.3	3 3	11.1	5.8	3.7	9 7	9.9	6.9	5.0	9.0	*15.8*	8.1	417
cuneifolium†	2.4	0	5.4	*24.2*	—	0	—	0	—	0	0	0	0	0	0	0	0	0	0	0	0	37
ovalifolium†	1.2	0	1.1	*4.7*	1.8	0	—	0	—	0	0	0	0	0	0	0.5	0	0	0	0	0.2	45
serratifolium†	—	8.0	*12.0*	—	—	0	—	0	—	0	0	0	0	0	0	0	0	0	0	0	0	46
angustifolium†	2.4	8.4	*19.6*	2.0	5.1	0	—	0	—	0	0	0	0	0	0	0	0	0	0	0	0	90
adaequatum	31.4	16.5	*9.8*	15.4	14.4	34.1	—	0	—	11 9	4 6	*28 8*	24 5	26 9	15.5	13.4	*2.8*	21.8	20 6	22.5	19.1	901
transformatum	—	—	—	—	—	—	—	0	—	0	0	0	1 2	0	1.9	0	*15.8*	0	0	0	0.5	41
New alterations†	1.2	0.8	*9.8*	3.3	1.6	2.4	—	0	—	0.3	0.4	0	0.3	0	0	0	2.3	1.7	0	0.5	0.4	54
Combinations of several alterations	14.5	21.1	*29.3*	*29.5*	16.6	12.9	—	21.4	—	8.1	1 2	2.6	5 1	3.7	8.7	9.9	9.2	10.9	6.4	4.0	9.4	554

% of alterations

* Gene mutations that are rarely or never manifested in *parviflorum*.

† New alterations, probably caused by plasmon recombination, although genetical analysis has not been completed.

of the primary alteration. Most of the alterations arise in dormant buds. These three observations imply that age may influence the alteration process.

To investigate the nature of this influence, several plants were divided into numerous cuttings. Each shoot that grew to a length of two internodes was used as a cutting, one node being used for root formation and the other for bud formation. In the course of one year 10–70 cuttings could be obtained from a dwarfed plant normally 3–5 cm in height. This treatment forced each bud to grow until autumn, after which the plants could recover from the treatment. These plants gave rise to no alterations, and most of them died during the winter. Most of the untreated hybrids were very sensitive, too, and died during the winter if no alterations occurred. But it cannot be stated definitely whether plants died as a consequence of treatment or because of failure to form alterations. The experiments demonstrated that the dormant buds were not altered primarily. The alterations were formed in the course of time.

Attempts to inhibit growth with plaster of Paris and to force differentiated cells to regenerate, are not yet sufficiently extensive. So far, no alterations have resulted in these experiments. In 1952, for the first time, alterations appeared in the first vegetation period as a result of starvation in pure sand (Michaelis, unpublished).

f. *Experiments on the Change of Cytoplasmic Constants and on Selective Damage of Cytoplasm Constituents.* It is possible that lower temperature promotes the sorting out of cytoplasmic constituents through increasing the viscosity of the cell and by retarding cytoplasmic streaming. We tried, therefore, to change the viscosity by heat treatment (thermostat bath three times weekly at 40°–43°C for 10 minutes). Treatment with narcotics was also used (alcohol, chloroform, and chloral hydrate). We also tried to displace cytoplasm constituents through centrifugation and to damage them selectively. Experiments were carried out with aluminum chloride, potassium cyanide, lithium sulfate, various bases and acids, formaldehyde, streptomycin, carcinogens, and other substances (Michaelis, unpublished). These experiments complement the mutation experiments, and were carried out with $h \times p$ hybrids and with vernicosum and irregulare clones. The substances were introduced either through the roots, or by infiltration of the vegetation points, or through intercellular gas diffusion. Different concentrations, mostly sublethal, were used. Attention was concentrated on possible alterations as well as on changes in resistance. So far, treatments with streptomycin and with radioactive sulfur and phosphorus (Michaelis, 1954a) have produced various frequencies of different alterations and a number of new alterations (see Table 18); this must be investigated more intensively. In other experi-

ments, no hereditary alterations or dauermodifications have been observed (Michaelis, unpublished).

g. *Influence of Nuclear Genes on Plasmon Alteration.* As noted previously, alterations of cytoplasm arose frequently in the *hirsutum* × *parviflorum* gene combination. After the introduction of various mutations of *parviflorum* into this plasmon-genome combination, there was a change in the kind and frequency of alterations, even though the recessive mutations were not expressed morphologically in the hybrids (see Table 18) (Michaelis, 1954b).

h. *Summary.* Most of the experiments do not permit any final conclusion as to causes of the formation of alterations. The alterations occurring among *hirsutum*-strain hybrids are probably caused by transfer and sorting out of male cytoplasm. In the $h \times p$ hybrids the number and kind of alterations can be changed, but scarcely the time of origin. It is of course possible that the suitable agents, concentrations, and methods for effecting this have not yet been tried. The experiments are being continued. In view of the expectation expressed by several authors that directed environmentally induced alteration of cytoplasmic inheritance should be readily possible, the failure of our experiments in this direction is noteworthy. At the present time it must be assumed that the phenomenon of alteration is a very complicated process, in which internal factors, like genotype, are more important than external conditions, which probably exert a less direct influence. Further experiments are necessary to clarify the relationships.

VIII. The Influence of Cytoplasm on Gene Mutation

Stubbe (1935) gave X-ray treatment to pollen of *hirsutum* having its own cytoplasm and *hirsutum* having *luteum* cytoplasm, and found mutation rates of 2.08 ± 0.48 for the *h* cytoplasm and 4.76 ± 0.84 for the *l* cytoplasm. The difference is not great, and not quite significant. Another case of cytoplasmic influence was observed in the vernicosum alteration. Among the offspring of vernicosum plants a varying percentage of pallidovariabile (pall) plants was found. These plants are pale green or white with normal-green spots and sectors. Very infrequently, single white dots appear in these normal-green sectors. Both reciprocal crosses of pall with *hirsutum* strains or with different $h \times p$ alterations yielded various percentages of pall plants, in which the number of specks—that is, the frequency of mutations of the pall gene—was the same in the different cytoplasms. The only exception was in the reciprocal crosses with the alteration irregulare, where no definite examples of pall were found in irregulare cytoplasm, for pall cannot manifest itself in irregulare plants.

In this case maternal inheritance is simulated, and a reciprocal difference results indirectly through a blocking of gene manifestation as a consequence of cytoplasmic disturbances of development.

Pall is a dominant, labile gene, lethal in homozygous conditions, which is of interest because in this case the origin of the labile allele could be observed (Michaelis, 1949d, 1953a). In 1947, which had a long, cold spring, the *h*-Essen × *p* hybrids formed flowers, and crosses could for once be made. The offspring of 79 plants included no pall. The following year, the alterations that had arisen from the same *h*-Essen × *p* hybrid plants could be investigated anew by crossings. Now 24% pall plants were found among the offspring of vernicosum shoots, and none among the offspring of other alterations of the same plants. In several cases, numerous flowers of one vernicosum inflorescence were crossed, but pall plants appeared only in the offspring of some single flowers.

The labile pall allele arises especially in inflorescences with vernicosum cytoplasm, but the mutation rate back to normal green is independent of the cytoplasm. (The reciprocal influence of the genotype on plasmon alteration is discussed in Section VII, 6.)

IX. General Discussion

1. *The Material Basis of Cytoplasmic Inheritance*

a. *Correlation of Cytoplasmic Differences with Cytoplasmic Constituents.* Maternal inheritance as found in Epilobium and other organisms permits us to distinguish only between nuclear inheritance and cytoplasmic inheritance; it does not allow any differentiation among the various hereditary constituents of the cytoplasm. To draw inferences concerning the localization of plasmagenes from the transmitted characters of the organisms or of the cytoplasmic constituents is feasible only in a very limited sense. The inheritance of chlorophyll content, for example, permits no conclusions about the localization of the responsible factors, because the formation of chlorophyll in plastids depends not only on the genetical nature of the plastids but equally on nuclear genes and cytoplasm. Positive localization of cytoplasmic factors is possible only if the behavior of the cytoplasmic constituents differs during multiplication of constituents within the cell, during cell division, and during the alternation of generations, and if this behavior corresponds with the inheritance. Such correct localization has so far been accomplished only by Preer (1950) in Paramecium. In Epilobium it is only certain that the plasmon as a whole does not correspond to one gene—a possibility discussed in earlier times (von Wettstein, 1937; Winkler, 1924). The behavior of chimeras shows that several constituents are involved in maternal

inheritance. At most, only one of them can be associated with the plastids; the others must belong to the cytoplasm outside the plastids. Observation of chimeras of one individual provides a method for the analysis of plasmon corresponding to the Mendelian analysis of the genome.

Regarding the localization of the plasmotypes found in this way, we have only hints at present. The two alterations vernicosum and cuneifolium, unlike most plasmon alterations, very frequently disappear in a gradual, "flowing" manner. The alterations diversivirescens and longifolium regularly form periclinal chimeras and come to the external layers of the vegetation point. Such observations must demonstrate specific behavior on the part of some particular cytoplasmic constituents. The occurrence of specific alterations after treatment with radioactive isotopes can also be utilized in localization.

b. *Cytoplasmic Inheritance and Identical Reproduction.* Although localization of the experimentally observed cytoplasmic hereditary factors is not possible in Epilobium, a theoretical consideration as to which constituents of the cytoplasm are likely sites of plasmagenes may be of value. In earlier works complete *autonomy* was postulated for hereditary units. The experiments on Epilobium demonstrate that we cannot distinguish between properties inherited through the cytoplasm and those inherited through the nuclear genes. All the essential properties of dissimilar hybrids from reciprocal crosses originate through interactions between nucleus and cytoplasm. This is probably a general rule in other cases as well. Autonomy of genes can be expected to apply only to the first step in the reaction chain, not to the final characteristic produced by this chain of reactions.

Similar conditions can be assumed to apply in identical reproduction, which is the most important prerequisite of a living and hereditary unit. The close relations between the constituents of the hereditary system, not only during physiological reactions but also during their multiplication, makes it seem probable that the reproduction of the genetic elements is a cyclic process, in which most of the various hereditary constituents provide important links in the reaction chain. The different hereditary constituents seem to be autonomous only with regard to their *identity* in reproduction. From this point of view, the hereditary elements are identically reproduced by a strictly organized hereditary system, in which the specific autocatalytic capacities of each unit are utilized. Virus also acquires the capacity of "being identically reproduced" only after it inserts itself into a suitable genetical system.

For the hereditary elements of the cytoplasm only the *capacity* to be identically reproduced must be present. This capacity can be realized to various extents, and it can be lost altogether if the genetic system under-

goes change. Such change may be induced by crossing, as Sonneborn found in Paramecium, and also by environmental influences, and by interactions between different cells and organs. These possibilities seem to be important in connection with problems of differentiation and determination during ontogeny. Probably cytoplasmic constituents having the capacity for identical reproduction can be subjected to reversible or irreversible changes in form and function, and can be utilized in the formation of such cytoplasmic structures as flagellae, cytoplasmic membranes, and other alloplasmic substances. In many cases such a change may be accompanied by loss of the capacity for reproduction, in a manner similar to that found in the nuclei of gland cells. An extreme example of such a possibility is the vacuoles. Although it is obvious that vacuoles, which may arise *de novo*, cannot be hereditary units, it is an open question whether the submicroscopic precursors of vacuoles in meristematic cells have the capacity for identical reproduction, in which case they would be hereditary units. Other examples are provided by some cytoplasmic structures of protozoa.

c. *Cytoplasmic Systems.* Furthermore, it must be taken into consideration that in most cases the plasmagenes are not isolated but are probably reversibly or irreversibly united to form cytoplasmic systems. For example, it is an open question whether the plastids correspond to a single gene or are composed of several genes in accordance with their submicroscopic composition of many discs. Especially in many cryptogams having intricately formed plastids with pyrenoids, in which fragmentation of the plastids is possible, the plastids might be composed of many, perhaps even of different kinds of plasmagenes. Similar problems arise in connection with chondriosomes and other cytoplasmic components, in the event that they are genetic constituents. Even in optically empty cytoplasm we probably have to assume regular structure and organization at the submicroscopic level (Michaelis, 1954c).

This association of the plasmagenes into systems having regular structure can influence not only their physiological effect—in a manner analogous to the position effect in nuclear genes—but also the possibility and frequency of exchange of cytoplasmic units, the rhythm of their reproduction, and their distribution during division of cytoplasmic components or cell division. Therefore it has great significance for the whole problem of genetics of cytoplasm. Confusion of cytoplasmic units and systems, and premature generalizations, can lead to misunderstanding and error—and have already done so. On the other hand, experiments performed primarily with the aim of clearing up the mechanism of cytoplasmic inheritance may contribute toward further elucidation of the cytoplasmic structure. The genetics of cytoplasm can advisedly be used

in conjunction with morphological cytology as a method for the investigation of cytoplasmic structures.

A preliminary outline of the most important members of the genetic system is given in Table 1. In this scheme the plasmon is a deeply articulated system. At each level of this system definite hereditary rules apply following from the nature, behavior, and number of the components. At the molecular level the nature of the genes determines stability and mutability. It is not certain whether the differences between the desoxyribose nucleoproteins of the nucleus and the ribose nucleoproteins of the cytoplasm have any great influence on mutation, and whether other proteins in addition to the nucleoproteins can be identically reproduced. To bring mutated plasmagenes to visible manifestation, higher levels of the system have to come into play. The next submicroscopic level determines arrangement and exchange. These vary greatly in kind and frequency among the various cytoplasmic components, and perhaps play a role in intraindividual plasmon alteration. Linear arrangement of plasmagenes is unlikely in the case of most cytoplasmic constituents. At the microscopic level no structures can be found in the cytoplasm which are analogous to nuclear structures. This is one of the most important differences that account for the absence of synchronous division of plasmagenes, of equal distribution during cell division, and of Mendelian segregation. On the ultimate level of the cell, oögamy or isogamy determines the occurrence of maternal inheritance and the mixing of different cytoplasms. All of these relationships, with their genetic consequences, may differ profoundly among the members of the cytoplasmic system. Maternal inheritance, for example, is not a characteristic of cytoplasmic inheritance but only a consequence of oögamy.

d. *Functional Importance of Number of Cytoplasmic Constituents.* At all these levels of the system, the variable number of equal constituents is important for genetic behavior. The detection of alternative differences between genes becomes more difficult with increasing number. This is also a factor contributing to the absence of distinct segregations, the difficulty of observing mutations, and many peculiarities of plasmon alterations. The large number of plasmagenes was instrumental in giving rise to the opinion that the cytoplasm constitutes only an unspecific substrate for the nuclear genes. This point of view is no more justifiable than would be a denial of the existence of nuclear genes because they are difficult to analyze in cases of polygenic inheritance. For these reasons cytoplasmic inheritance shows some parallels with the genetics of virus and bacterial populations, or of the multinucleate organisms, and in many respects can be better compared with these than with normal alternative inheritance by nuclear genes. It should be pointed out again, however,

that the differences mentioned here are not differences between nuclear and cytoplasmic inheritance as such, but are essentially a function of the number of hereditary units. For example, precision of distribution during cell division can be increased, in chondriosomes, through the concentration of single chondriosomes into higher units (chondriokinesis), and in plastids through a decrease in their number. The plastids of some archegoniates can even be included in the distribution mechanism of mitosis. In contrast to plasmon mutations, it is easier to observe mutations of plastids, which are present in relatively small numbers in the cell. All these arguments are of necessity incomplete, but they indicate the importance of considering the material bases of cytoplasmic inheritance in theoretical discussions and of investigating them in more detail.

e. *Cytoplasmic Constituents and the Bacterial Nucleus.* As long as the theory was current that schizomycetes had no nucleus, bacterial genetics could be regarded as a branch of cytoplasmic inheritance. For the question as to the bacterial nucleus, comparison of nuclear and cytoplasmic constituents at the different levels of the genetical system is important. In many cases an answer to this question has been proposed without consideration of the various possibilities. Observations that demonstrated equivalents of nuclear genes or chromosomes were advanced as proof of the existence of a complete nucleus. In other cases, it was not taken into account that the observed behavior could also be expected of cytoplasmic constituents, even though, as a consequence of neglect of cytoplasmic inheritance, it had not previously been observed. If, for example, the bacterial genes corresponding to nuclear genes in higher organisms are organized into chromosomes equivalents but not into a nuclear system, and if consequently there is no precise mechansim of distribution during cell division, corresponding to that of mitosis, this could result in genetic behavior like that found in cytoplasmic inheritance. If genes homologous to the plasmagenes are present in small numbers, then their genetic behavior could in many respects resemble that of the nuclear genes. These examples may serve to induce greater caution in resolving the question of the bacterial nucleus. The fact that recent mutation experiments with bacteria have in several cases yielded multiple-hit curves may also suggest a need for caution.

f. *Cytoplasmic Inheritance and Virus.* In Paramecium (Sonneborn) and Drosophila (L'Héritier), cytoplasmic inheritance has been related to infectious, virus-like particles. In plants no example of cytoplasmic inheritance through infectious particles can be cited. It is true that in most cases no special investigation regarding virus participation has been made; but in Epilobium it has not been possible, in spite of extensive investigations carried out on the various hybrids and alterations, to demonstrate

virus-like particles that might be responsible for cytoplasmic inheritance. Therefore a comparison of the above-mentioned examples in animals with cytoplasmic inheritance in plants is only partially possible.

In two exceptional cases in Epilobium we found branches infected with virus (Ross, 1941; Michaelis, unpublished), which could not be identified with any of the known viruses. The origin and effect of such virus infections would be of great interest. The properties induced by one of these two virus infections resembled in many ways the disturbances induced by disharmonious interaction between nucleus and cytoplasm. The similar effect of virus and plasmon might be considered an indication that the various characters are not specific for a particular gene, plasmon, or virus, but are determined by the structure of the genetical system, which reacts in similar ways to disturbances originating from different sources (see Section IX, 3, c).

For the interpretation of relations between cytoplasmic inheritance and virus, an important question is whether, in certain viruses, endogenous formation *de novo* may be assumed (Doerr, 1923; etc.). If such a possibility exists, then a mutation of plasmagenes to greater stability, which would permit their isolation from the living protoplasm without irreversible loss of their ability to multiply, seems a more probable explanation than origin from nuclear genes (Lindegren, 1938). In this case, virus inheritance can be included in cytoplasmic inheritance. If virus originated by reduction from other organisms, then, theoretically, the transmission of virus must be distinguished from cytoplasmic inheritance in the same way as the transmission of parasites and symbionts. The fact that in a system of polyphyletic origin relations arise between host and symbiont that are necessary for the maintenance of both is no argument against their exclusion from cytoplasmic inheritance. But in any case virus is a useful model for the study of reproduction, mutation, and—especially—alteration, in populations composed of numerous units.

2. *Alterations of the Plasmon*

Considerations of the material basis of cytoplasmic inheritance give rise to several possibilities for the interpretation of plasmon alterations. These will be discussed both in relation to the Epilobium experiments and in general.

a. *Symbionts or Parasites.* Invasion by units belonging to a different species, having the capacity for identical reproduction, can be excluded in Epilobium, and need not be discussed here.

b. *Gene-Produced Plasmagenes.* Spiegelman (1946) postulates that the nuclear genes produce more or less complete replicas of themselves,

which diffuse into the cytoplasm as plasmagenes and enzyme precursors, and which retain the capacity of self duplication. Sonneborn (1946) represents a similar line of reasoning when he assumes that the origin of cytoplasmic factors is to be traced ultimately to the nuclear genes. The present author will not deny the possibility of nuclear-produced plasmagenes, but cannot believe that all plasmagenes arise in such a way and that this hypothesis has general significance in all cases of cytoplasmic inheritance. The fact that the nucleus cannot reform cytoplasm after it has been removed, and that in most cases it cannot change unsuitable cytoplasm, even if the nucleus is represented in polyploid number (von Wettstein) or is retained in the cytoplasm for many backcross generations, cannot be reconciled with the hypothesis of gene-produced plasmagenes. The observation of a balance in natural populations between genes and cytoplasm that act in opposite directions (see Section V, 4, d) is scarcely comprehensible in conjunction with this theory; nor is the sorting out of cytoplasmic constituents in chimeras which contain the same nucleus in all cells.

Although in the experiments on alterations in the $h \times p$ hybrids the p nucleus was found to be one of the prerequisites for the formation of alterations, it seems to act only as an indirect, releasing agent, exerting little influence on the course of the alterations. The fact that in some exceptional cases the cytoplasmic reaction norm was modified in the direction of the reaction norm of the introduced nucleus (see Section VII, 1, b), and that in some experiments the maternal characteristics tended to disappear during backcrossing, can be explained just as easily by selective influence of the nucleus as by production of plasmagenes. The nucleus can indeed produce substances which influence the reproduction of factors already long present in the cytoplasm, but they are not identical with plasmagenes and need not possess the capacity of self reproduction. The nucleus can also influence the multiplication of plasmagenes in a less direct manner, such as by exertion of selective influence in favor of those plasmagenes that can interact with it more or less harmoniously. In the case of yeasts, even if the interpretations of Lindegren and Lindegren (1946) and Spiegelman (1946) can be maintained in view of the criticism of Winge and Roberts (1948), the author sees little possibility of a decision between the concept of nuclear-produced plasmagenes and that of a directive nuclear influence on the reproduction of *plasmaeigene* genes. In Epilobium, and in many other examples of cytoplasmic inheritance, the hypothesis of nuclear-produced plasmagenes could be applied only in extremely rare instances, and is essentially less probable than the assumption that cytoplasmic alteration comes about through selective influence, particularly since this assumption must be postulated for the explanation

of a number of different facts. This hypothesis, moreover, is in agreement with all the other observations made on this organism.

c. *Mutation of Plasmagenes.* The following section will consider mutation as a possible explanation of the alterations in Epilobium hybrids, but the discussion will be limited to mutation of plasmagenes. Changes affecting the aggregate of the plasmagenes, the plasmon, might be designated mutations, in analogy to chromosome or genome mutations, but such a procedure does not seem profitable.

The question that presents itself is whether or not the alterations of the Epilobium hybrids are brought about through mutation. The alterations in Epilobium show several peculiarities that contrast with the known laws of gene mutation. These distinctive features include the following: (1) There is a high rate of independent alterations. Once the process of alteration has been set in motion, in most individuals the different alterations arise almost explosively. (2) In different individuals and in different hybrids that have widely differing genomes and plasmons, a limited number of similar alterations occur. (3) The alterations are directed toward an elimination of developmental disturbances, and in the process normal development can come about in various ways. (4) The alterations can occur gradually, through a series of different stages. (5) Similar-appearing changes can arise either as modifications or as hereditary plasmon alterations, and, moreover, there are intermediate stages and transitions between the two (dauermodifications). (6) The usual agents for increasing mutation rate have no effect on plasmon alterations.

If one were to think of these observations in terms of plasmagene mutation, they would mean that the differences between the thymonucleoproteins of the chromosomes and the ribonucleoproteins of the cytoplasm result in fundamentally different rules of mutation, and that all our known concepts of mutation would have to be reversed. Such behavior of plasmagenes is improbable, and therefore such assumptions are justifiable only if there are no alternative possibilities for interpretation. This is not the case. On the basis of three assumptions, which are in accord with the structure and behavior of the cytoplasm, all the observations relating to alterations in Epilobium can be explained unreservedly and can be fitted in with our knowledge of genetics.

1. The first of these assumptions, that the plasmon is the sum of various constituents, most of which are represented in large numbers in the cell, is consistent with the findings of cytoplasmic cytology and with theoretical conclusions regarding the material basis of cytoplasmic inheritance. The assumption that the optically clear cytoplasm contains a sum of plasmagenes can be derived logically from the fact that during fertilization combination and addition of different cytoplasms can take place.

2. The second assumption is that under certain conditions reproduction and distribution of diverse cytoplasmic units may be subject to specific differences, even during ontogenetic development. Sonneborn and Preer have demonstrated the influence of nuclear genes and of environmental factors on multiplication of the killer factor. Similar effects are known to exist in plastids of some protophytes. Differences in distribution during cell division can be deduced from the observations on chimeras having different plastids. Moreover, differences in distribution of plastids or chondriosomes, and of nonliving or foreign cytoplasmic particles such as starch or bacteria, can be observed cytologically. This unequal distribution plays a significant role in differentiation and determination in plants and animals. In most organisms there is no known mechanism that can guarantee such precise distribution during cell division as does mitosis for the distribution of chromosomes. Even cytoplasmic streaming in combination with variations in viscosity can produce no such accuracy. The efficiency of chondriokinesis is unknown. Even if in many cases the large number of cytoplasmic units, and the possibility of restoring the original number through multiplication, may offset the inequalities of distribution, the possibility of intraindividual alteration of the sum total of the plasmon cannot be disputed. Under such circumstances, essentially all the known facts relating to the behavior of genes in populations and generations could apply in the plasmon of one individual organism. The high frequency of alterations and chimeras, the origin of like plasmotype in different hybrids, the transitional alterations, and many other peculiarities of Epilobium alterations are easily understood on the basis of these two assumptions. The only factors that are still unknown are those which, on the one hand, preserve the constancy of the plasmon and, on the other hand, permit sorting out and recombination in the cytoplasm. It is surprising that the alterations noted in Epilobium had not been expected long ago, on the basis of cytoplasmic behavior.

3. In order to explain several other peculiarities of Epilobium alterations, and in particular the phenomenon of directed alteration and the parallels between modifications and hereditary alterations, a third assumption is necessary, as follows: The various kinds of plasmotypes have different selective values, and selection can operate effectively on an intraorganismic level. Competition between different plasmon constituents within the cell is only a likely hypothesis; but competition between cells and branches of an individual plant can frequently be observed in Epilobium.

These three assumptions are consistent with all the biological facts. They suffice to explain all the experimental observations on Epilobium

and many other organisms without recourse to auxiliary hypotheses. Therefore the opinion seems justified that recombination of plasmon constituents, and not mutation of plasmagenes, is the principal cause of alterations. The occurrence of mutations is of course not excluded, but in every case they will be obscured by recombinations. The observations on Epilobium permit no conclusions about mutation of plasmagenes.

The question remains to be answered, why are mutations of plasmagenes scarcely known up to this time, and why have mutation experiments met with so little success? It is not probable that plasmagenes never mutate. Plastid mutations are known, and likewise mutations of virus, whose ribose nucleoprotein content is reminiscent of the plasmagenes. Even in the absence of investigations, it remains probable that plasmagene mutation differs little from that of nuclear genes. The following explanations for the absence of plasmagene mutants are more comprehensible:

1. The sensitivity of plasmagenes may be so slight that cell-lethal doses of radiation would not significantly affect their mutation rate. This interpretation does not seem very probable, and might at best prove applicable only to single plasmon constituents.

2. The most effective agents and methods may not have been found yet.

3. The plasmagenes may be organized into systems of a sort that require several hits in order to be changed.

The following possibilities seem to be especially important:

4. In cases involving a large number of similar units, mutation of one unit can be manifested only after multiplication and concentration, or sorting out, of the mutated unit. Neither the amount of time necessary for sorting out nor the conditions favorable to it are known. It is possible that the number of cell divisions in the vegetation points of one generation is not sufficient for the separation of mutated units, and that too slow sorting out would be overlooked.

5. In a genetic system whose members are all harmoniously related to one another, most mutations will have a more or less negative selective value within the system. Unless the system is altered, mutated members will be eliminated, or at least will not be concentrated. The cytoplasm is the vital substance in which all important reactions take place; and a disturbance of these reactions by mutation is not "desirable." In the large number of similar plasmagenes nature has evidently found a mechanism capable of preventing the occurrence of the most disadvantageous mutations, but not the occurrence of alteration through mutation. Injurious mutations of plasmagenes can be eliminated, without interruption of reaction processes. "Neutral" mutations can be main-

tained over long periods of time and eventually acquire positive (or negative) selective value with alteration of the whole system. Mutated plasmagenes having positive selective values can be concentrated until they become effective.

These reasons suffice to account for the failure of mutation experiments on plasmagenes. These considerations also demonstrate the necessity of first investigating recombinations in cells and tissues, unless organisms containing a small number of plasmagenes are available. Only when these recombinations are well known and can be kept under control, will mutation experiments promise any success.

d. *Recombination of Plasmagenes.* Some of the theoretical premises regarding recombination of plasmon have already been mentioned. For purposes of conceptual clarification, the most important possibilities for recombination (Michaelis, 1949a, 1950a) will be reviewed here, in the order listed. They are: (1) the mixing of different cytoplasms; (2) the unequal distribution of cytoplasmic constituents during mitosis or meiosis; (3) different rates of reproduction of different plasmon constituents; (4) plasmon alteration through elimination; and (5) recombination of cytoplasmic units.

1. A union of somatic cells occurs only occasionally, in cells without solid membranes (zoological material) or after experimental grafting of cells or tissues. Experimental investigations (Küster, 1939) have shown that, even in the case of cytoplasms that are side by side in close contact, or that penetrate each other (myxomycetes) and exhibit a common cytoplasmic streaming, a true mixture—for example, the migration of plastids from one cytoplasm to another adjacent cytoplasm—is possible only under favorable conditions and when the cytoplasms are not too different.

More significant is the mixing of cytoplasms during fertilization. In isogamous organisms this is the original behavior; and even in higher plants and animals the transmission of male cytoplasmic constituents through the pollen tube or spermatozoids is more frequent than is generally assumed. However, the transmission of male cytoplasm is not identical with mixing of cytoplasms, because both cytoplasms can lie side by side in close contact and then be separated again without mixing, and because one of them can be eliminated during early embryonic cell division by degeneration, failure to multiply, or exclusion from the reproductive cells. Unfortunately, modern investigations on cytoplasmic behavior, using suitable fixative agents, have not been carried out with most organisms; nor has the variability of this behavior under experimental and natural conditions been determined. This is most regrettable, as a knowledge of cytoplasmic behavior is the primary basis for all investigations of cytoplasmic inheritance. Cytological investigations, however,

important as they may be, are not decisive even if they demonstrate no transfer of cytoplasm, because the relations of the visible amounts of cytoplasm are not so important as the transfer of single, probably invisible, units if they have the potentiality of identical reproduction.

The effect of mixture of paternal and maternal cytoplasm on the origin and maintenance of cytoplasmic differences is discussed later on (see Section IX, 4, b).

2. Some of the factors that condition unequal distribution of cytoplasmic constituents during mitosis or meiosis have already been discussed. Numerous examples could be cited, from different groups of plants and animals, in which unequal distribution of nonliving as well as identically reproducing cytoplasmic particles has been observed. In general, unequal distribution seems to occur more frequently during mitosis than during meiosis, when the chondriosomes undergo a relatively precise distribution in equal numbers by means of chondriokinesis. This precise division during meiosis might indicate that organisms in which paternal and maternal cytoplasms are not mixed perhaps show a greater constancy during reproduction than during ontogenesis. This would be a remarkable difference between cytoplasmic and nuclear inheritance.

In all cases of unequal distribution, one must distinguish between random sorting out of cytoplasm and the fixation of an unequal distribution of cytoplasm and its constituents through separation by a newly formed cell wall. Since such an unequal arrangement is frequently due to correlations between the cells and environmental factors, the unequal distribution can be directed by these factors, whereas in random distribution only an indirect influence through selective value appears probable. An example of random sorting out is the distribution of green and pale-green plastids, and probably also the formation of different types of tissues, in the irregulare alteration of the $h \times p$ hybrids in Epilobium. Contrasting examples are the behavior of cytoplasm in mosaic eggs of many animals, and, in plants, the unequal division of cells that precedes the formation of root hairs, idioplasts, stomata, and so forth.

3. The occurrence of different rates of reproduction for the different plasmon constituents can be assumed in Paramecium, under the influence of nuclear genes (Sonneborn-Preer). In Euglena, decreased multiplication of plastids results from cultivation under conditions of reduced light and appropriate nutriment. Under conditions of heterotrophic nutrition, the number of plastids per cell can decrease until colorless animals eventually result through loss of plastids (Ternitz, 1912).

4. An example of plasmon alteration through elimination is provided by the loss of potentiality for identical reproduction by some cytoplasmic units—for example, when cytoplasm is used in the formation of alloplas-

mic organelles. Degeneration of male cytoplasm has also been reported in some cases.

5. Recombination of cytoplasmic units inside the cell, similar to that encountered in chromosome mutation, is a further possibility, if these units are organized into more or less stable arrangements. No sure evidence for the occurrence of such recombination is known at present (Michaelis, 1954c).

e. *Factors Influencing Plasmon Alteration.* If we examine these various processes in relation to the material basis of plasmon, it is clear that they all can be influenced by different conditions. We may distinguish between external and internal influencing factors. Among the former, such environmental agents as temperature and cell poisons may influence the mixing and sorting out of cytoplasms by changing cytoplasmic viscosity or cytoplasmic streaming. Possibly multiplication of the plasmagenes is also affected in some way by these agents.

Much more important than the influence of these external factors is that of internal factors, such as the nuclear genes and the relations between different cells and tissues. Particularly in the closed cycle of ontogenetic development in animals will these interactions between the cells and organs of an individual limit the influence of environmental factors. Only in this way does an orderly and harmonious alteration of the whole system seem to be possible.

Presumably the modes of action of the various agents and conditions affecting alterations may be very different. In Epilobium, some of the investigated external factors were shown to exert only a *releasing* effect. It is possible that low temperature increases viscosity of cytoplasm and thus facilitates sorting out. In this case, the further process of alteration is independent of the releasing factors.

Theoretically it is possible in some cases to credit external factors with a *direct* influence, especially on the multiplication of certain cytoplasmic units. Perhaps some dauermodifications and, in Epilobium, the changed rate of alteration after treatment with radioactive isotopes and streptomycin (Table 18) originate in this way. Though some authors think it possible to assume the existence of directed adaptation on the basis of such considerations, it must be recalled that a directing influence of external factors cannot be regarded as identical with directed adaptation. An adaptation is possible only if the external factor directly favors, for example, the reproduction of such cytoplasmic constituents as simultaneously increase resistance to this same factor. Such a relationship is to be sure not impossible, but it is not probable and has in no case been proved, either in Epilobium or elsewhere.

In comparison to directed influence by external factors, such influence

on the part of internal factors seems much more likely—especially an influence of the genes on reproduction of cytoplasmic units. The influence of various *parviflorum* mutations on plasmon alteration (Table 18) can be interpreted in this sense, although the relations must be more carefully elucidated.

This kind of delayed, indirect influence of various factors, exerted through selection of plasmatypes, will be much more important than any direct influence on the multiplication of plasmon units. Such influences are no novelty, and can easily be observed in experiments with plants.

The factors that influence alterations can act on different levels of the genetic system, just as the alterations themselves may come about as a result of recombinations that take place on different levels of the system. Mutation, unequal multiplication and arrangement within the cell, and differential sorting out during cell division all cause plasmon differences *within an individual.* They can produce patterns with different plasmons and are important in connection with determination and specialization of tissue. The behavior of cytoplasm before, during, and after fertilization may be decisive for the *whole* organism.

The localization of the effect is especially important with relation to selection. A selective influence inside the cell can hardly be distinguished from a direct influence. *Selection among cells* and tissues of an individual organism takes place more easily in plants than in animals, because in the latter any imbalance on the part of some cells or tissues tends to harm the development either of these cells or of the whole organism. Such a disturbance of development, however, can be eliminated through plasmon alteration. Thus may close relations be established between gene mutation and selection within the plasmon. Ordinarily a mutation will have negative selective value, if it does not fit in with preceding reactions or if the succeeding chain of reaction does not become adapted. If, however, a muted gene is not eliminated, a selective force operates, at all succeeding steps of the reaction chain in which the mutated gene plays a part, to adapt them to the new gene. Selection during ontogeny is possible only in the plasmon. This type of selection is repeated regularly during the ontogenetic development of each individual. It is possible that regular recombinations within the plasmon are induced in this way during ontogeny. These relationships may have some explanatory value for the phenomena of determination and for Haeckel's biogenetic law.

The observations on $h \times p$ hybrids of Epilobium show that the selective value of a new plasmon combination is determined mainly by its growth rate. All the alterations encountered among the $h \times p$ hybrids are characterized by higher growth rates. Among animals, the formation of alterations with higher growth rates is limited by mutual correlation

effects. Only in the rare cases in which these are destroyed can the higher selective value of quickly growing plasmon combinations be effective. As there are certain resemblances between plasmon alterations and cancer, one might speculate that intraindividual selection of plasmon recombinations may be involved in the origin of some cancers (Michaelis, 1948e, 1949c). Lastly, it is possible that selection for plasmon types becomes effective only for the *whole individual*, in which case no significant distinction can be made between selection of genotypes and plasmotypes in populations and in successive generations.

f. *Origin and Constancy of Plasmon Alterations.* If our reflections regarding the various possibilities concerning plasmon alterations are correct, it follows that both quantitative and qualitative plasmon differences can arise, with no sharp boundary between the two. The larger the number of plasmon units involved, and the slower the sorting-out process, the more quantitative differences will prevail. In this case the alterations are gradual and transitional, with many intermediate stages. Therefore, low selective values of new plasmon combinations will favor such gradual alterations, whereas high selective values will cause rapid sorting out and sudden appearance of the alterations.

The constancy of the alterations also varies with the plasmon composition. Assuming that the plasmagenes can only manifest themselves if they are present in a certain number, and assuming furthermore that alterations originate through reduction of the number of plasmagenes, the process of alteration should be reversible if the number of plasmagenes falls below the threshold of manifestation but some plasmagenes remain in the cell. Only after complete elimination of all the plasmon units of a given type, will the alteration become irreversible, until new plasmagenes are added through fertilization. Thus the occurrence of dauermodifications can easily be explained in terms of recombination of plasmon; and also the disappearance of predetermination can be explained by elimination of plasmagenes.

If reversible alterations of plasmon arise under certain environmental conditions, and if the variation in the number of plasmagenes keeps pace with the change in the inducing factors, then plasmon alteration corresponds completely to modification. Thus one and the same change may be brought about in an organism either in the form of a modification or dauermodification or in the form of a hereditary alteration. The different characteristics of the alterations observed in Epilobium can well be interpreted in terms of a quantitative recombination of the plasmon. The fact that similar alterations are represented among widely differing hybrids is easily understandable, since the various plasmons can differ from one another only quantitatively, and may contain the same types

of plasmagenes. It would follow that they possess the same capacities for the formation of alterations.

The preceding discussion regarding plasmon recombination is by no means complete, but suffices to point out the most important principles and their bearing on some biological problems. It seemed preferable to present the various theoretical considerations, in spite of the fact that experimental proof is still lacking for many of these possibilities. Now that a case of plasmon alteration has been found, however, and since the concept of plasmon recombination is in general agreement with the behavior of cytoplasm and with the experimental facts, the new concept seems to be a useful working hypothesis for further investigations.

3. *Physiological Significance of the Plasmon*

a. *Matrocliny of the Reciprocal Differences.* In earlier work it was customary to separate reciprocal differences into two groups: matroclinous and patroclinous properties. Not infrequently the facts were distorted to fit this scheme. The matroclinous properties were explained as due to cytoplasmic inheritance. The experiments on Epilobium have demonstrated more complicated relationships. Two main groups of properties can be distinguished: matroclinous properties, and properties that are the result of changes and interference with the normal developmental pattern. It was to be expected that direct inferences about the effects of plasmagenes could best be made from investigations of matroclinous characteristics. Unfortunately, such characteristics are not frequent, occurring for the most part only in crosses without developmental disturbances. In all cases the matroclinous differences are slight; they show great variability, and can be demonstrated only by extensive measurements. Wherever it has been possible to investigate differences in a more precise manner through introduction of various genotypes, the results have shown the effect of interaction between nuclear genes and plasmagenes rather than the effect of plasmagenes per se. This behavior applies also to the investigated properties of the cytoplasm itself. Interactions between nuclear genes and plasmagenes are particularly striking among the nonmatroclinous properties and newly arising characters. In other organisms, either the influence of different genotypes on the plasmon has not been investigated, or the influence of the cytoplasm has been discovered incidentally, during analysis of nuclear genes by means of an alteration of genic action. Thus the properties of plastids, such as chlorophyll content, depend not only on the genetic nature of the plastids but also on nuclear genes and cytoplasm. So far no property has been found in which the results of such interaction can be definitely ruled out. This situation gives rise to the question: Is there such a thing as a purely

cytoplasmic effect or a purely genic effect, or must we not assume an interaction between nucleus and cytoplasm in all cases involving long reaction chains—not only in the reproduction of genetical units but also in the whole cell physiology? Under these circumstances the discovery of matroclinous properties would indicate either that the methods of analysis used detect only cytoplasmic differences, or that the influence of the cytoplasm is predominant over that of the nuclear genes (antecedence of the plasmon, in the terminology of von Wettstein, 1928).

b. *Interactions between Nuclear Genes and Plasmon* (*Michaelis, 1951c*). Interactions between nuclear genes and cytoplasm have been acknowledged in earlier works, but the plasmon has always been assigned the role of an unspecific substrate. The great number of plasmagenes, as well as the difficulty involved in distinguishing between identically reproduced plasmagenes and metabolic products, led to this assumption. On the basis of the work with Epilobium, we must conclude that the cytoplasm not only is a substrate but also contains "reaction partners" for the nuclear genes. Direct conclusions about the role of the plasmon are possible only after one has succeeded in separating the share of the plasmon from that of the nucleus in the common reaction. Since no one has succeeded yet in this attempt, only indirect conclusions can be drawn.

The interaction between the nuclear and the cytoplasmic partners must be very specific, since minute changes of one of the partners frequently upsets or disrupts the harmonious relationship. Alterations of certain nuclear genes are tolerated better, and therefore the nuclear partner is the easier to analyze. Slight alterations of the plasmon can affect the action of individual genes. Genes so affected have been designated "plasmon-sensitive" for particular plasmon-induced system alterations. The number of plasmon-sensitive genes increases with increasing plasmon differences; and with very great differences between plasmon and genome, most of the genes are plasmon-sensitive. This fact indicates that interaction with the cytoplasm is not limited to a few isolated genes, but that most—probably all—genes are directly or indirectly dependent on the relationship with the plasmon.

Little is known regarding the nature of the interaction. We can only assume that the rate and intensity of the gene reaction are changed if one of the partners undergoes alteration. Reciprocal differences can be intensified, weakened, or made to disappear entirely with changes in idiotype. Genic effects can in turn be diminished or eliminated. Intensification of gene effect seems also to be possible, if in a certain genotype the optimum of a nuclear reaction does not coincide with its possible maximum effect. Some examples of heterosis and change in gene dominance may be explained by more favorable gene-plasmon combinations. But

in all such cases it is risky to draw conclusions from the phenotype about gene reactions. The weakening of one reaction may bring the effect of another, unchanged, reaction into prominence.

c. *Specificity of the Genetic System* (*Michaelis, 1947a, 1947b*). The difficulties of defining the true action of genes become especially great when one attempts to correlate the properties of dissimilar hybrids from reciprocal crosses with certain hereditary constituents. In the experiments it has not been possible to alter the distinctive differences between reciprocal hybrids by means of nuclear genes or of different plasmons. In all cases studied, the plasmon or the nuclear genes only release a certain tendency of development, and fix the bounds within which development will proceed. The direction of the development cannot be altered, either by nuclear genes or by plasmon. As we saw earlier, the same developmental disturbances will always recur, no matter how different the genes or plasmagenes used to induce them. The remarkable fact that certain properties, which can be inherited over many generations, seem in their specific characters to be independent of the specific nature of the individual hereditary units, can be explained by the assumption that developmental tendencies are determined not only by the particular nature of the genetic constituents but also, and essentially, by the nature of their association in a network of reactions. The structure of the genetic system seems to be just as important as the nature of its components. It appears that the different components can be put together in various combinations, like the parts of a toy construction set. The constructional plan takes on a set of laws of its own, depending only indirectly at best on the structural components. The experiments with Epilobium give a first indication of how the genetical basis of such a building plan may be investigated. One uses different building materials interchangeably, as far as possible, and attempts to infer the architectural plan from the similarities and differences of reaction.

Considering first of all the developmental disturbances, we can say that many components of the genetic system, particularly lethal genes and the plasmon, are so specific that interchange leads to collapse of the whole system. But it makes little difference at what point the chain of reaction is broken; the collapse is always the same, and depends on the structure of the system.

Not all differences of reciprocal hybrids can be characterized as developmental disturbances, because under certain conditions they have positive selective value. In this case the above-outlined hypothesis will have to be reformulated. We may say, rather, that if the normal course of development is obstructed owing to the failure of one of the components of the system, alternate pathways may be open. In this case, the subse-

quent links of the reaction chain can give rise to other properties than they normally would. Although this assumption fits quite well with some of the data obtained from Epilobium experiments, it may be that in organisms of high specialization only one course of development is open. This point is of interest in connection with the distribution of hybrids showing reciprocal differences (see Section IX, 4, b), and with the question of the significance of the genetic system for evolution.

The Epilobium examples show that the gene effect depends not only on gene specificity but also, as is the case with many enzymes, on the conditions under which the genes are allowed to act. These conditions are determined by all the preceding reactions, as well as by environmental factors. The same systemic properties can be induced by quite different hereditary units, as well as by the environment. The phenomenon of phenocopies can be explained by these relations within a genetic system, and demonstrates by its occurrence the system-dependence of the involved characteristics.

In these different interrelations, the nuclear genes are the stable fixed points which make possible a permanent organization of the system. In contrast to this, the environment is the variable, primary component from which the system has developed. The plasmon, by virtue of its capacity for intraindividual alteration, occupies an intermediate position. Its participation lends flexibility and adaptability to the genetic components of the living system.

In most genetical investigations the existence and significance of the genetic system can hardly be observed. This is a consequence of the analytic method of investigating single differences that arise through exchange of a limited number of genes within a system that is kept constant. The difficulty of analyzing the plasmon has led also to the synthetic method of generalizing comparison, in which as many genes and plasmons as possible are interchanged and the differences that arise as a result are compared with one another. This method is less exact and less satisfying, to the geneticist, but its use is justified by the fact that it makes hypothesis regarding the genetic system possible.

d. *Gene Action and Gene Effect.* The significance of the new hypothesis is discussed in later sections. It must be admitted here, however, that the interpretation of the primary action of genetic units and also the analysis of gene action are rendered more difficult by it. Our experiments have shown, surprisingly, that as deviations of development arise the effects of new genes are discernible and the effects of the known genes disappear. Altering the system by means of plasmon exchange also brings about a change in gene action. For example, genes that normally cause a particular leaf shape will, when associated with a different cytoplasm, lose

this effect and cause heterosis or dwarfed growth instead. In such cases it is impossible to draw any conclusions regarding the primary action of genes. It is possible that the primary action is changed, but it seems more probable that only the end effect is changed, through alteration of the system. The experiments demonstrate only that the genes affect a chain of reactions which under certain conditions influences the shape of leaves, but which can be modified by alteration of the system in such a way that the same genes will exert more influence on the growth of shoots than on the growth of leaves. It is necessary to keep in mind that it is in principle incorrect to designate genes according to their phenotypic effects rather than their primary action. In most cases, important components of the system, which can considerably alter genic effects, are involved in the origin of dominance, epistasis, pleiotropism, and so forth.

e. *Contribution of the Plasmon to Reactions Fundamental for Life.* Direct analysis of true primary action is even more difficult for plasmagenes than for nuclear genes. The best that can be done is to draw a few indirect conclusions. A distinguishing characteristic of plasmon differences is that they induce mostly developmental disturbances or lethality, and relatively seldom morphological differences unaccompanied by harmful effects. Some authors have ascribed no significance to the plasmon because it produces "only" developmental disturbances. In my opinion, such a view is a reversal of the true state of affairs and we must, on the contrary, deduce from this disturbance-producing effect of plasmon a high degree of specificity as well as a great significance for development and for life. In a system in which one reaction follows upon another, step by step, and in which one component of the system is adapted to the other components in a specific way, even a slight alteration of the fundamental links of a reaction chain must be expected to disturb the whole course of the reaction, to a greater degree than would an alteration involving one of the final links in the chain. An exchange of components involving the last links of a reaction leaves the system unchanged, so that it cannot be detected as a constant value. Genes and plasmons that initiate fundamental reactions cannot be greatly changed without producing lethality. The plasmon, like the lethal genes, apparently participates in fundamental reactions and has great significance for the preservation of life. The large number of plasmagenes prevents a frequent appearance of mutants and facilitates this function. The more easily analyzable nuclear genes seem in general to participate in the end reactions of the chain, and are therefore more instrumental in providing the diversity and embellishment characteristic of organic life than in laying down the basic foundations of the developmental pattern. This function of the nuclear genes is facilitated by their smaller number and by their relative physiological isolation in

the nucleus. Some earlier authors were willing to credit the nuclear genes only with the production of varietal properties, and sought to discover new genetic units to account for the more important characteristics. Such a distinction between gene-dependent and plasmon-dependent reactions is certainly mistaken. But a similar trend of though is reflected, more correctly, in the hypothesis of fundamental and secondary links in a chain of reactions—whereby the whole genetic system is concerned in both types of reaction, although the plasmon participates to a higher degree in the basic reactions.

f. *First Attempts at Physiological Analysis of the Genetic System* (*Ross, 1941–1948*). According to this interpretation, the developmental disturbances found in the *Epilobium hirsutum*-Jena cytoplasm are essentially characteristic of the system structure. An analysis of this system must proceed from the phenotype and search for those physiological reactions that remain constant even when the phenotype changes greatly in response to environmental modifications. In this way one may expect to approach closer to the genetic fundamentals of the system. In the physiological investigations of Ross such a point was reached with the discovery of increased activity of oxidative enzymes in all hybrids with *h*-Jena cytoplasm, regardless of the phenotypic degree of disturbance. The activity of oxidative enzymes may also explain many properties of the disturbed hybrids. Stunting can be attributed to oxidation of growth enzymes; dwarfing, heterosis, and many other characteristics, to changes of carbohydrate balance resulting from increased respiration. It is also clear that the plasmon and many nuclear genes influence carbohydrate and auxin metabolism directly or indirectly. The environment also changes the carbohydrate balance, and thus modifies the differences between hybrids from reciprocal crosses. From the genetical investigations on Epilobium we can assume that through the interaction of plasmon and nuclear genes in each species and strain a characteristic redox equilibrium is established, and that in each of the strains the nuclear genes and plasmagenes have a different share in the development of this balance. An exchange of genes will alter the balance, and thus affect all subsequent reactions. The fact that similar relationships have been found in Phaseolus hybrids having disturbances caused by the plasmon (Geerts, 1949), and that a similar augmentation of enzyme activity and similar phenotypes can be observed in connection with other disturbances of the genetic system—for example, in virus infections—demonstrates that in the redox potential we have a system characteristic that is significant in other organisms besides Epilobium.

In a further analysis of the genetic system it would be desirable to investigate the ways in which different genes and different plasmons can

affect the redox potential. Ross has expressed the opinion that changes in the hydration of cytoplasmic colloids may be involved, but it has not been possible so far to carry out any further investigations.

g. *Additional Examples of Properties of the Genetic System.* In the experiments on Epilobium several distinct cases of *heterosis* have been observed (Michaelis, 1951b). The unusually strong heterotic effects in some alterations of the *hirsutum* × *parviflorum* hybrids can be accounted for by favorable recombinations within the plasmon, analogous to advantageous combinations of dominant nuclear genes. There are other cases in which very weak disturbances of interaction between nuclear genes and plasmagenes act in a stimulative manner. The physiological investigations indicate how such stimulative heterosis can originate. Increased respiration stimulates growth provided the reserve of carbohydrates is high or the temperature is favorable, whereas in the absence of such conditions increased respiration injures the plants. This concept of heterosis corresponds to the definition of Shull (1914). The heterosis resulting from favorable combinations, as well as the stimulative heterosis, is a property of the genetic system. In general, the same effect can be brought about through interaction between nucleus and cytoplasm as is produced by interactions within the plasmon or among the nuclear genes by themselves.

Typical examples of properties of the genetic system are *sterility* and *lethality*. As mentioned before, the interaction between nuclear genes and plasmon can be interrupted through exchange of different genes or different plasmons. Development can be halted at various points; but in most cases the same symptoms leading to death are observed. The fact that in some hybrids vegetative development is disturbed, and in others only the male gametes, is not necessarily an indication of different physiological reactions. In most hybrids the male gametes have a greater sensitivity to disturbances of the system, probably because nourishment of the pollen grains, after separation of the tetrads, is more difficult than nourishment of the female gametes and of vegetative cells. In such pollen-sterile combinations one can induce typical vegetative disturbances by increasing the number of plasmon-sensitive genes. It is clear that numerous genes and plasmons may produce sterility or lethality, either directly, by interfering with the interrelation between the genetic units, or indirectly, by increasing the sensitivity of the genetic system. The specificity of single genes is less important than the nature of their interaction and the sensitivity of the system as a whole.

A close examination of cytoplasmic inheritance shows that the plasmon participates in most reaction chains and reaction equilibria. Of the numerous possible examples, *sex determination* may be cited, even

though it has no great significance in relation to the Epilobium investigations. In Epilobium, as in other organisms, female plants originate only through high sensitivity of male gones. Anther development is inhibited as a consequence of degeneration of haplonts. In such cases it is not appropriate to speak of sex determination. But the investigations of Oehlkers (1938–1941), Hiorth (1948), and Heilbronn and Basarman (1942) demonstrate the significance of the plasmon in sex determination. Sex determination is interpreted as a reaction equilibrium within the genetic system, which can be shifted by either nuclear genes or plasmagenes, and also—during the course of further development—by the environment. This interpretation corresponds essentially to the physiological concept of Goldschmidt (1938), whose experiments on geographical strains of Lymantria (Goldschmidt, 1934) resemble the comparison of genome and plasmon in *Epilobium hirsutum* biotypes.

These few examples may suffice to demonstrate the general principle of the new hypothesis.

4. *Frequency of Occurrence of Cytoplasmic Inheritance and Plasmon Differences (Michaelis, 1951d)*

Since the first demonstration of cytoplasmic inheritance in mosses by von Wettstein (1926) and the first proof in higher plants (Michaelis, 1929, etc.), many other examples of cytoplasmic inheritance have been discovered. But in spite of the relatively large number of such examples it cannot be denied than in an even larger number of organisms no cytoplasmic influence is demonstrable. This brings up the question of whether or not cytoplasmic inheritance is widely distributed, and why in many organisms no cytoplasmic differences can be observed.

a. *The General Occurrence of Plasmagenes.* The opinion, held by some, that cytoplasmic inheritance is limited to a few organisms cannot be correct, since the capacity for identical reproduction constitutes one of the most basic properties not only of heredity but also of life. It cannot be assumed that the essential "life substance," the cytoplasm—which can never be replaced or formed anew—possesses the capacity for reproduction only in certain organisms, or that this reproduction takes place in an identical fashion only in some organisms. Nuclear genes and plasmagenes are both reproduced identically by the system of the cell; and the system of nuclear and cytoplasmic hereditary constituents, demonstrated in Epilobium, must be present in an analogous form in all organisms. Consonant with this opinion is the fact that there is no evidence that the nucleoproteins (which have been correlated with identical reproduction) occur only in the cytoplasm of certain organisms.

The examples that have demonstrated cytoplasmic inheritance in a more or less precise manner (Caspari, 1948; Michaelis, 1951d) are distributed among all the groups of the plant kingdom. Cytoplasmic inheritance is known among the cryptogams—in fungi and mosses—and among the monocotyledons and many families of choripetals and sympetals. Among animals, also, there are several different classes in which cytoplasmic inheritance seems probable. The list of organisms exhibiting cytoplasmic inheritance will undoubtedly grow more impressive when all genetically investigated hybrids have been subjected to reciprocal crosses, and when adequate attention has been given to slight reciprocal differences and to differences between reciprocal crosses as to segregations of recessive, plasmon-sensitive genes. Cases of different crossibility in reciprocal hybrids must also be taken into consideration. It is clear that the simple observation of differences among reciprocal hybrids does not constitute proof of cytoplasmic inheritance; but these instances cannot be overlooked in any consideration of the general distribution of plasmons, as long as the concept of cytoplasmic participation in inheritance is not refuted by investigations of possible sources of error. Further examples may be concealed by gradually decreasing maternal predetermination.

b. *The Plasmon in Similar Hybrids from Reciprocal Crosses.* With all these possibilities taken into account, the fact remains that most hybrids in animals and many hybrids in plants show no differences in reciprocal crosses. Since examples of maternal inheritance are relatively rare among animals, the hypothesis has been advanced that maternal inheritance in plants results through the plastids alone. Although it is more difficult than is generally supposed to distinguish between plastid inheritance and cytoplasmic inheritance, the existence of cytoplasmic inheritance dependent on several components of the cytoplasm other than the plastids has been demonstrated in Epilobium. It is very probable that, in other cases as well as in Epilobium, all cytoplasmic components having the capacity for identical reproduction participate in inheritance. There are plenty of reasons to account for the absence of differences in many reciprocal hybrids.

The observations made on Epilobium agree with the hypothesis that the cytoplasm takes part in all vital reactions and that nucleus and cytoplasm are very delicately balanced in relation to each other. Each disturbance of this balance produced by crosses results in lethality, sterility, or other developmental disorders. This fact makes the detection and investigation of plasmon differences very difficult. In all cases showing really large differences, incompatibility prevents comparison by means of crossing. Even the growth (and also the germination?) of pollen tubes can be inhibited by the presence of unsuitable cytoplasm in the style tissue. In

other cases, the hybrid from one of the reciprocal crosses is lethal, and becomes viable only after an exchange of the disturbing cytoplasm. Von Wettstein discovered in mosses a close correlation between taxonomic relationship and plasmon differences, which prevents crossing between groups that are too dissimilar. Therefore the conclusion seems justified that exchange of components among widely differing genetic systems is unsuccessful in proportion to the extent to which these components participate in vital reactions.

All the cytoplasmic differences investigated in Epilobium are so slight that differences between plasmagenes themselves cannot be analyzed. These become recognizable only in disturbances between cytoplasm and plasmafremde genes. To find and analyze cytoplasmic differences we need test genes or test genomes that react on these differences without inducing lethality. If the differences are to be manifested in the F_1, the plasmon-sensitive genes must be dominant. Exact comparison of reciprocal F_2 segregations has seldom been carried out. In Epilobium, the investigations of cytoplasmic inheritance have yielded results only because a whole series of test genomes of different strengths were available and could be used according to requirement. Even in Epilobium, however, such test genomes are not frequent (see Table 7), and can be found only by systematic search, using large assortments and numerous crosses. Such a procedure is very important in any investigation into cytoplasmic inheritance.

But crossibility and the use of test genomes are not sufficient for the detection of plasmon differences. If these differences are recognizable only through disturbances of gene-cytoplasm balance, the organism must be able to tolerate these disturbances, and the degree of disturbance induced by the test genomes must fall within the limits of this tolerance. Organisms undoubtedly differ with respect to limits of tolerance, and some highly specialized reaction systems probably have so low a tolerance that the smallest deviation in any basic component of a reaction chain is lethal. The degree of tolerance probably constitutes one of the evolutionary potentials of the organism (Section IX, 6, b, viii). Apparently in Epilobium the range of tolerance is relatively wide. It can be divided into a broad scale, reaching from normal development, through stimulative heterosis, quantitative weaknesses, and qualitative differences, all the way to sterility and lethality.

Of course, maternal inheritance as an expression of cytoplasmic inheritance is to be expected only in those organisms in which the cytoplasm is derived exclusively from the female parent. Among the many isogamous organisms, the method of reciprocal crosses is not applicable in the detection of cytoplasmic differences, and less impressive methods must be

used. It is not surprising, therefore, that cytoplasmic inheritance has been found so seldom in isogamous organisms.

But in oögamous organisms also, transmission of cytoplasm by both parents is not rare. The spermatozoids contain cytoplasmic elements, and the pollen tube can transmit cytoplasm. Unfortunately, there have been few attempts to discover in what species and under what conditions the transmission of male cytoplasm occurs, or to determine the behavior of the male cytoplasm after fertilization. As long as such data are lacking, any discussion concerning the distribution and importance of cytoplasmic differences must remain problematic. Some conclusions can be derived from the behavior of the male plastids and chondriosomes. It is known that *status paralbomaculatus* (Correns) is not infrequent in plants, and we must assume that the plastids enter the egg cell with the aid of the cytoplasm and along with the cytoplasm. The frequent occurrence of *status paralbomaculatus* contradicts the textbook opinion that in the fertilization of higher plants the cytoplasm is derived exclusively from the maternal parent. In animals, one can identify male mitochondria in the ova after fertilization. Therefore the transmission of male cytoplasmic constituents can be expected to occur even more frequently in animals than in plants.

Among all organisms in which transmission of male cytoplasm takes place, constant maternal inheritance can be expected only in those few cases where the new combination of the maternal cytoplasm with the hybrid genome has a positive selective value and the male cytoplasm has been either eliminated or diluted to the point of ineffectiveness. In all other cases the maternal influence must disappear sooner or later. Heretofore such cases have been excluded from heredity without further investigation, under the name of predetermination and maternal after effects. Obviously, the more frequent the transmission of male cytoplasm is, the less frequent must be the examples of cytoplasmic inheritance.

Of course the existence of cytoplasmic inheritance is independent not only of the methods available for its investigation but also of the occurrence of plasmon differences. The various possibilities regarding the origin of cytoplasmic differences have already been taken up (see Section IX, 2), but I will discuss here briefly the influence of mixing of cytoplasm during fertilization on the occurrence of cytoplasmic differences. In isogamous organisms the frequent union of approximately equal amounts of cytoplasm tends to equalize all cytoplasmic differences. In organisms living in a closed reproductive community under identical environmental conditions, cytoplasmic differences will usually be absent. Differences arise only after geographic, physiological, or genetic isolation, and they increase with the duration of this isolation. In my opinion this

explains why cytoplasmic differences increase with decreasing taxonomic relationship, rather than the assumption that plasmagenes are the factors responsible for the properties characterizing the higher taxonomic groups. The hypothesis that plasmon differences are brought about by different environmental conditions can provide additional explanation of the fact that cytoplasmic differences of similar extent are found among geographical strains of a given species grown under different ecological conditions. An increase in difference in the amount of cytoplasm contributed by the maternal and paternal gametes has an effect similar to that of decreasing frequency of the mixing of plasmons by isolation. Oögamy favors the establishment of cytoplasmic differences. It is interesting in this connection that a higher level of organization is always correlated with oögamy. The importance of cytoplasmic inheritance for evolution may explain such relationships, since oögamy permits the occurrence of greater cytoplasmic differences without preventing recombinations between different cytoplasms. An exclusively maternal transfer of cytoplasm appears to be unfavorable for diversity of cytoplasms, because the casual loss of some sorts of plasmagenes cannot then be compensated for by mixture with male cytoplasms, and will result in definite progressive loss of phylogenetic potentialities.

After occasional union of different kinds of cytoplasm a large number of widely different combinations will at first arise. Under the selective influence of nucleus and environment, the offspring in succeeding generations will sooner or later tend to reach a certain combination of genome and plasmon. But complete uniformity will be attained only in populations with a very limited ecological variability.

These considerations can provide only a provisional model, which will vary for each material. It may be demonstrated that the behavior of cytoplasmic constituents during fertilization is one of the most basic factors relating to cytoplasmic inheritance and to the distribution and manifestation of plasmon differences. It is very necessary to take all these considerations into account, and to investigate them with the aid of modern methods.

5. *Significance of Cytoplasmic Hereditary Factors in Development and Determination*

The experiments on Epilobium have led to the hypothesis of a genetic system in which the multiplication, constancy, and effectiveness of the various components depend on the structure of this system and on the properties of all its components. In particular, the action of nuclear genes can be altered by changes in the plasmon. In this system the nuclear genes are relatively constant within the individual, whereas the plasma-

genes can be recombined much more easily during ontogeny, in a reversible or irreversible manner, and this in turn may alter the effect of nuclear genes. Such alterations of the genetic system by plasmon recombination have heretofore been found in only a few experiments; but theoretical considerations make it seem likely that such alterations do occur more frequently, especially in all cases where the cells divide unequally or where an unequal distribution of cytoplasm and its constituents becomes fixed as a result of cell division. In plants, and particularly in animals, the developmental potencies often become limited through unequal distribution of cytoplasm. It is not difficult to suppose that the alteration of developmental potencies can be caused by cytoplasmic alteration of the genetic system. This supposition is made more probable by the facts that nucleoproteins play a significant role in neural induction in amphibians, and that animal eggs show organization and stratification not only of nonliving substances such as yolk and enzymes but also possibly of self-reproducing particles such as basophilic granules or mitochondria. It is probable either that the plasmagenes can act as inductors or that the composition of the plasmon can be affected by inductors, and therefore that plasmon alterations are concerned in many determination processes.

A detailed discussion of the problem would exceed the limits of this article. We shall mention only a few direct conclusions from the experiments, and several parallels between the experiments and characteristic processes of development.

a. *Reversibility of Plasmon Alterations.* First we should discuss the apparent conflict between cytoplasmic inheritance and the fact that determinations are not transmitted from one generation to the next. It will be recalled, however, that in Epilobium a series of similar alterations of reciprocal differences has been found, of which some are inherited over many generations, whereas others disappear with fertilization but remain constant during vegetative reproduction, and still others show very slight constancy even in vegetative reproduction. I can only suppose that this series is related to similar cytoplasmic alterations. Experimental demonstration is impossible, when the alteration disappears with fertilization. The parallels between phenotypically similar hereditary alterations, after-effects, and modifications have been discussed earlier. Theoretically the absence of constant inheritance can be explained in many different ways through recombination (see Section IX, 2, d–f).

In many determination processes, the totipotency and the capacity to form gametes are lost, and any proof of cytoplasmic inheritance is thus impossible—just as in many Epilobium alterations—because no genetic experiments can be made. Constancy in vegetative transmission (for example, in tissue cultures or cancer metastases) is an imperfect

substitute; yet under certain conditions it does supply important indications regarding the participation of self-reproducing particles, even though conclusions about the localization of these particles in genome or plasmon can be arrived at only indirectly, at best.

Transmission of cytoplasmic alterations from one generation to another cannot be expected, either, in cases where the alteration comes about through elimination of one type of plasmagenes and the second parent contributes this type of plasmagenes in fertilization. Transmission is lacking also when the alteration arises through a loose combination of certain types of plasmagenes into reactive groups and when this association is broken up at fertilization. Aside from the observation that many cytoplasmic properties undergo sudden changes at the time of fertilization, we have no definite proof of the existence of such a process; but it may prove helpful to keep this possibility in mind.

Reversible determinations may also arise, through intraindividual plasmon alterations, if the relative amount of a particular type of plasmagenes is altered quantitatively and if the effectiveness of this type is limited by a threshold in such a way that it is not completely eliminated.

It is obvious that these considerations are highly speculative, and that it will be difficult to back them up with data arrived at by genetical methods. But it is an essential fact that the alterations of cytoplasmic inheritance in Epilobium show some parallels with the processes of determination, even though at present these parallels appear to be superficial. In the following sections some of these parallels may be recalled.

b. *Pattern Formation in Epilobium Alterations.* It was mentioned earlier that the irregulare alteration of the *hirsutum* × *parviflorum* hybrid is composed of several plasmon types, which are separated from one another during leaf development and form irregular patterns on the leaves. The fact that despite extensive multiplication by cuttings no altered shoots arise during the first year under normal conditions shows that the various cytoplasmic constituents remain in the same combination in the initial cells of the growth point. Only in the resting buds of hibernating organs and during seed formation does the process of separation of cytoplasmic constituents also extend to a considerable number of the initial cells of shoots. This, however, constitutes the essential point of difference between the formation of patterns in the irregulare alteration and the formation of patterns in other organisms, and makes it possible to demonstrate that hereditary elements participate in pattern formation. The irregularity of the patterns can be explained by random separation of cytoplasmic constituents. Regular patterns can be expected if the direction of this separation is influenced by environmental condi-

tions, or by correlation between different cells, or when specific plasmon alteration takes place only in certain layers of tissue. This type of behavior is perhaps represented in the origin of the diversivirescens alteration, which forms regular periclinal chimeras. Demonstration of the cytoplasmic nature of most diversivirescens alterations is possible only because of the constancy they exhibit during vegetative multiplication, for the diversivirescens plants are sterile.

c. *Relation between Cytoplasmic Inheritance and Age* (*Michaelis, 1951d*). Experiments described earlier demonstrated the influence of age on the origin of cytoplasmic alterations. So far, it has not been possible to carry these experiments far enough to determine whether the increased frequency of alterations with increasing age is simply a function of time (*e.g.*, through concentration of alterations and mutations), or whether processes peculiar to aging (*e.g.*, through aging of the colloids) are involved. Both possibilities seem reasonable.

Whereas in plants the accumulation of cytoplasmic alterations simply provides new material for selection, most cytoplasmic alterations in the closed system of animals limit developmental potencies or harm the organism, especially if they are induced by extreme environmental conditions rather than by the system itself and therefore do not fit into the normal, harmonious process of development. It seems probable that some plasmon alterations, originating in the course of time and as a consequence of special conditions, have a significant share in the processes of aging.

d. *Relation between Cytoplasmic Inheritance and the Biogenetic Law.* In the open system of plants, a single plasmon alteration tends to change only the selective value of some cell descendants, and within the individual plant the new plasmotype can displace the old. If the alteration survives fertilization, a new strain will arise.

In the Epilobium hybrids, similar alterations arise during the course of time in all individuals of the same combination. It is quite possible that other organisms can give rise to only one specific type of alteration, and that therefore each individual changes in the same fashion. This requires, however, that there be a definite and sufficiently high level of plasmon lability, and that the conditions of selection be identical in all individuals of the species. Such a possibility should have particular importance in cases where cytoplasmic alteration disappears with fertilization. It is surprising that the emergence of the adult form from the juvenile stages in Acacia, Hedera, Chamaecyparis, and others shows a high degree of resemblance to the origin of cytoplasmic alterations, occurring either suddenly or by means of transitional stages after a certain period of development. The high stability of these forms during

vegetative propagation, and their disappearance after fertilization, also correspond to some cytoplasmic alterations (Michaelis, 1951a).

The assumption that in certain idiotypes the same plasmon alterations recur regularly may contribute to an understanding of the law of recapitulation. Let us assume a genetic system in which the different members of the system are adapted to one another, and also in which the nucleus can induce alterations of the plasmon directly or indirectly and the limits of these alterations are determined by the genotype. Then it seems quite possible that in the course of evolution regular sequences of plasmon alterations arise, and that such recombinations of the system, originating during phylogenetic development, are repeated during the ontogenetic development of each individual (see Section IX, 2, e).

e. *Cytoplasmic Inheritance and Cancer* (*Michaelis, 1948e, 1949c*). According to the described theory, it can be expected that plasmon recombinations occur with relative frequency in animals. Presumably not all the alterations will fit into normal ontogenetic development. In a constant genotype most of the alterations will never manifest themselves, since they will be selected against. Occasionally, however, alterations with positive selective value will appear. As was noted in plants, tissues with higher growth rates may arise, and will be able to displace the normal tissues if at the same time the correlations and regulations among tissues, which normally prevent separate growth, are disrupted. Such autonomously growing tissues derived from plasmon alterations would necessarily show all the characteristics of cancer. Actually, in the origin of cancers several distinctive features can be observed which are also characteristic of cytoplasmic alterations. The high constancy of cancer cells during vegetative reproduction has led to the theory of the mutational origin of cancer. The fact that the carcinogen benzpyrene is stored exclusively in cytoplasmic constituents and at the sites of ribonucleoprotein production; the long latent period between the occurrence of a carcinogenic injury and the appearance of cancer; the increase in frequency of cancer with increasing age; the occurrence of additive series of cancer inductions, leading through several stages of predisposed cells to a directed, parallel origin of cancerous cells at several places; the reversibility of some preneoplasms and warts: these and other phenomena are hardly comprehensible if cancer originates through mutation of nuclear genes. But in the event of the origin of cancer through cytoplasmic alterations such phenomena are to be anticipated. Thus it is a feasible hypothesis that a large proportion of cancers originate through plasmon alterations.

In connection with this hypothesis, however, one limitation must be noted. The autonomous, unchecked growth of cancer is the expression of a system-determined characteristic, and therefore it may be brought

about not only through alteration of the cytoplasmic component of the system but also through a virus or through mutation of the karyotic component. The concept of origin of cancer by gene mutation does not contradict the new hypothesis. This is all the more true because gene mutation can follow plasmon alteration, and vice versa. It will not be easy to determine how often gene mutation, and how often cytoplasmic alteration, is the primary cause of cancer.

6. *The Role of Cytoplasmic Inheritance in Evolution* (*Michaelis, 1949a, 1950b*)

The importance of cytoplasmic inheritance for evolution has heretofore been underestimated. Dobzhansky (1937), Darlington (1939), and Wright (1945), for example, assign to the plasmagenes only a secondary role in evolution. Lamprecht alone (1944) considers the plasmon as a species barrier, because in species hybrids the normal interactions between nuclear genes and cytoplasm can be so disturbed that sterility or lethality results. The low evaluation of the plasmon with relation to evolutionary processes is undoubtedly based on the infrequent occurrence of differences between reciprocal hybrids among plants and animals. It was pointed out earlier in this discussion (see Section IX, 4) that the occurrence of reciprocal differences is not a valid basis for conclusions about the significance of the plasmon. The new experiments on Epilobium provide a basis for inferences that lead to new possibilities and require a revision of opinions regarding the evolutionary importance of the plasmon. At the present stage, however, definitive conclusions cannot be drawn, and the ideas presented below can constitute only a preliminary sketch.

The most important new fact is that the concept of the genetic system, which according to earlier opinion consisted only of nuclear genes, can be rounded out through recognition of extranuclear hereditary elements, which, in their primary alterability by mutation, are probably subject to laws similar to those governing the nuclear genes, but which, on account of their large numbers and their behavior during cell division and fertilization, can be recombined subsequently in a quite different manner. The introduction of such elements into our picture of the genetic system gives rise to several new possibilities regarding evolution. All the existing theories of evolution, of course, remain valid; but they can be expanded, probably in such a way as to remove the last difficulties in the interpretation of evolution.

a. *The Significance of the Plasmon.* In view of the large number of plasmagenes of the same type present within the cell, we can surmise that n most cases the mutation of one of numerous plasmagenes has no physio-

logical or morphological effect. A mutation becomes recognizable only after accumulation of the mutated plasmagenes. Similarly, it is not likely that selection exerts its influence on the single, individual plasmagene. Probably it affects only the multiplication of the mutated plasmagene, indirectly, by way of its action on the whole system. Thus a large number of different "alleles" can accumulate in the cytoplasm, creating a reservoir of diversity from which the best plasmagenes can be withdrawn and concentrated during ontogeny and phylogeny. The multitude of differently mutated plasmagenes, and their various combinations into cytoplasmic constituents, may be one basis for the enormous variability among protoplasmic proteins, which can be demonstrated, for example, by serological methods.

A second basis seems to be the alterability of the sum of the plasmagenes within an individual under the influence of the whole system and of the environment. This alterability provides potentialities for ontogenetic and phylogenetic development that would seem to be impossible to such an extent unless one takes the plasmon into consideration. For these we refer the reader to previous sections, and mention specifically here only the phenomena of directed alterations,[3] transitional alterations, and the parallels between hereditary alterations, dauermodifications, and determinations. Every unbiased person will recognize that such behavior of plasmon gives rise to new evolutionary possibilities. But it should be emphasized that, at the present stage of investigation, we can speak only of possibilities. Their true significance must be elucidated by further investigations on the frequency of these plasmon alterations; and in the end the paleontologists have the last decision as to whether the experimental findings can explain their observations. At present it would be just as erroneous to place too great hopes in the new possibilities as to underrate them.

The underestimation of the plasmon in relation to evolution has a methodical and epistomological basis. As was pointed out in Section IX, 4, the demonstration of plasmon differences is very difficult from the standpoint of method, and is possible only in certain materials and under certain conditions. Plasmon differences can very easily be overlooked in the usual crossing experiments. Furthermore, from the very beginning many examples of plasmon alterations have been excluded from genetics under the headings "dauermodifications," "delayed effects," "environmental modifications," and "determinations." The practical difficulties

[3] A useful example of directed alteration as it relates to evolutionary significance is the retardation of plastid multiplication that occurs in flagellates under poor conditions of lighting—a retardation that can cause the elimination of plastids, and that results in the production of animal species parallel to the green flagellates.

of drawing a line of demarcation between plasmon alterations and true modifications should not cause us to overlook the plasmon alterations in theoretical discussion.

But on the other hand the importance of the plasmon in evolution must not be overemphasized, for the plasmon does not act autonomously. It is incorporated in the genetical system as a whole, and the possibilities for cytoplasmic alteration are limited by the more stable genome. This interrelationship was discussed in Section IX, 2, e.

b. *The Significance of the Integration of Nuclear Genes and Plasmagenes in a Single System.* The stability and lability of genome or plasmon of course constitute the basis of evolution. But just as essential is the fact that two hereditary elements possessing different, nearly contrasting potentialities for alteration are united in a harmoniously functioning system. The genome, on the one hand, whose mutants appear in an alternative fashion and are quickly accessible for selection, has a higher vegetative constancy and can undergo recombination only during the alternation of generations. On the other hand, the plasmon, whose mutants are slow in appearing, can be altered within one individual and remains constant during alternation of generations if the cytoplasm is not transmitted by the father. From the combination of two such components arises a genetical system that is at the same time stable and capable of adaptation. In this system the relative importance of the combined components can be regulated to some extent by the number of units and by the degree of oögamy. It is not surprising that the highest organization of this system is associated with a definite degree of oögamy. Though some paleontologists and taxonomists consider it impossible to explain in terms of mutation and selection the origin of complicated organs, in which the function and selective value of the individual parts depend on the presence of all the other numerous parts, this difficulty scarcely seems to arise in a system of nuclear genes and plasmon. The following sketch, by no means complete, outlines several essential points which may clarify the significance of such a system for evolution.

(i) *Mutual influence on stability.* After an exchange of genomes, stable plasmons can undergo alterations with a higher frequency; and in altered plasmons, genes can exhibit a somewhat higher lability. The causes of such reciprocal influence are unknown. If physiological relations between cytoplasm and nucleus play a role in the reproduction of nuclear genes and cytoplasm, it is possible that disturbance of these relations brings about mutations. The harmonious balance between the different components of the system can be restored more quickly by increase of mutations and alterations than by spontaneous mutation and alteration.

(ii) *Changes in manifestation.* In a divergent or altered cytoplasm,

the manifestation and dominance of genes can be changed (Michaelis, 1951c). Numerous types that differ greatly in morphology and physiology can arise, and provide new material for selection (Fig. 6). Some types displaying heterosis, giant growth, or pulviniform growth may acquire positive selective value, and under certain conditions (*e.g.*, low temperatures) become superior to the normal type. The stunted Epilobium dwarfs that can arise in the cytoplasm of *E. hirsutum* Jena have a positive selective value in winter temperatures and furnish a parallel to the pulviniform plants of the Arctic and of the high mountains. The introduction of nuclei into a "foreign" cytoplasm can lead to the morphological expression of latent genotypes that have a quite definite geographical distribution. When nuclei from Western European *hirsutum* strains are introduced into the cytoplasm of *hirsutum* Jena, there arises a group with clearly distinguishable morphological characteristics, which without this cytoplasm would not be recognizable (see Section V, 3, f). Likewise, latent mutants may become visible in a "foreign" cytoplasm.

The introduction of various genes into certain cytoplasms reveals different possibilities for development in the cytoplasm also. If one developmental pathway in the cytoplasm becomes blocked, by the failure of a link in the reaction chain, another pathway is opened up, giving rise to completely new types and physiological relationships.

(iii) *Plasmon alteration as a compensatory factor.* When most properties are determined by the system as a whole, the same property can be changed as well by the genes as by the plasmon. The plasmon can be altered intraindividually, and in the direction of harmonious development. Gene-determined developmental disturbances can be eliminated more rapidly by plasmon alterations (Michaelis, 1949g) than by spontaneous mutations. Hence the plasmon seems to be adapted to the function of restoring a harmonious equilibrium within the system after major gene or genome mutations (polyploidy!).

(iv) *Selective value as a system-dependent property.* Naturally, selective value, just as many other properties, is determined by the genetic system as a whole. The negative selective value of most gene mutations therefore constitutes no valid objection to the significance of gene mutation in phylogeny. In a more or less specialized system any major alteration of one component is bound to disturb the functioning of the system. But negative selective value can be eliminated during ontogeny or phylogeny by changes of the system, either through plasmon alteration or through exchange—for example, introduction of the mutated gene into another cytoplasm.

(v) *Inter- and intra-individual selection.* In considering selection, we must distinguish between the selection of systems (individuals) by the

environment and selection within the system. In the latter case, the selective influence of the system as a whole and of its components will in general be greater than the influence of the environment. The importance of selection becomes significantly enlarged by the introduction of the concept of intraindividual selection. Just as selection within a population can alter the population in a directed manner, so intrasystemic selection, theoretically, can alter the system in a directed manner. The difference between directed alteration of populations and of individuals is not so great as was visualized by the old theories of evolution, from the exclusive standpoint of the individual.

In most cases, intraindividual selection in its physiological aspects can be examined only with difficulty. It cannot be ascertained in paleontological material. Thus the finding of the paleontologists—that in most cases quality and quantity of new formations are determined not by environment and selection but autogenically by the organism—is understandable. Intraindividual selection can bring about preadaptation.

(vi) *Macro- and micro-evolution.* Many paleontologists make a distinction between microevolution and macroevolution. As most gene mutations scarcely change the fundamental structure of the main branches of the phylogenetic tree, some paleontologists assume that there is a special mechanism for macroevolution. In a genetical system which represents not only the simple sum of hereditary elements but an organized association of these elements governed by its own set of laws, the mutation of single hereditary elements can never obliterate the difference between the main evolutionary branches. The different mutants are the absolutely essential material of evolution. Addition of mutants produces microevolution; their harmonious combination in an organized system seems to be the basis of macroevolution. This organization is facilitated by the alliance of the more inflexible nuclear genes with the more easily alterable plasmon. It would not be correct, however, to attribute microevolution to nuclear genes and macroevolution to the plasmon. The essential factor is not the material localization of the hereditary elements, but the manner in which they are combined into a system. It might be more correct to interpret microevolution in terms of the alteration of secondary and peripheral reaction chains, and macroevolution in terms of the reconstruction of primary links in the reaction chain during the embryonic stage. Microevolution might also be explained by mutation, and macroevolution by the complicated combination of important mutants of the different carriers of heredity.

(vii) *Rate and direction of evolution.* If the hypothesis that has been discussed is correct, then the rate of evolution is independent of the time of origin of mutation. A number of mutants, with their preservation, is a

prerequisite of evolution. But their favorable combination has no relation to the time of occurrence of the mutations. The evolutionary possibilities provided by mutation can be realized suddenly and explosively. The plasmon alterations in Epilobium may serve as a model of this. After a suitable genotype has resulted from crossing, under certain environmental conditions numerous alterations suddenly arise on each plant, within an ordinarily constant cytoplasm. Thus a number of new types originate, from which various courses of alteration can branch out (Fig. 5). It cannot be decided whether these alterations are only a model for the origin of new types, or actually an example of evolution; but it seems possible that in rare cases similar processes lead to the sudden formation of new types.

If such processes occur during ontogeny, it seems possible that they are repeated during further phylogenetic development (see Section IX, 5, d). On the supposition that alterations of plasmon, in particular, can be recapitulated, perhaps the share of the plasmon in evolution may be roughly estimated from the frequency of ontogenetic recapitulation.

The hypothesis also adds to the understanding of orthogenetic development. Plasmon alteration can be directed by selection, especially by intrasystemic selection. Furthermore, the possibilities of alteration are limited by the structure of the system and the comparative stability of the nuclear genes. These two factors alone serve to explain orthogenesis. Each system, moreover, possesses only certain restricted possibilities for progressive development. This can be demonstrated in the technical development of any constructional plan.

The irreversibility of evolution is especially easy to understand in a system in which all the parts are mutually dependent upon one another, can be recombined in different ways and at different times, and are subject to very different requirements for selection. It is impossible to reverse all these different conditions in the correct order.

(viii) *The different stages of evolution.* The paleontologists distinguish different stages of evolution—for example, according to the terminology of Schindewolf (1950), the stages of typogenesis, typostasis, and typolysis. Typogenesis seems to be possible only in systems in which the specialization of the system and the adaptation of its members to one another are so slight that mutation and combination are facilitated by a wide range of tolerance (see Sections IX, 3, c and IX, 4, b). We can assume that at this stage the mutation rate is much higher than the rate of visible mutants, because many mutations are not manifested. The demonstration of mutations and genotypes that do not manifest themselves at origin, but only after the range of tolerance has been narrowed down through alteration of the genotype or plasmotype, makes it seem probable

that the morphologically discernible stage of typogenesis is preceded by a latent phase, during which the initial pattern of species formation is not yet morphologically recognizable. Only after the limits of tolerance have been narrowed down, can the developmental patterns established in the preparatory phase become suddenly visible. This is a further possibility which can be used to explain the explosive origin of species in the stage of typogenesis.

With regard to the plasmon, reciprocal differences may be relatively frequent during typogenesis in cases of extreme oögamy, since several courses of development are possible. Perhaps several potentialities for development lie dormant in the species of Epilobium that have been investigated. Evidence of typogenesis as a historical process, however, is not obtainable by genetical methods.

During the stage of typolysis the specialization of the genetic system is probably too great and the range of tolerance too narrow. All those mutations that are essential for evolution and all the important alterations brought about by exchange or recombination are at this stage lethal. With increasing specialization of the genetic system, plasmon and genome differences act as barriers between species. Since in the stage of typostasis the mutation rate may be approximately equal to the rate of appearance of mutants, probably during the stage of typolysis the rate of mutants is again smaller—not as a consequence of a failure of mutations to be manifested, but as a consequence of loss through lethality.

Naturally, in this fragmentary, general survey, many of the ideas presented are very hypothetical, many of the conclusions may have to be corrected, and many disturbing gaps still remain to be filled in. This is not surprising, in a field in which for the most part only occasional observations have been made and hardly any systematic investigations have been carried out. It appeared useful, however, not only to discuss the experimental facts but also to demonstrate the theoretical frame of reference, with a view to stimulating new work on cytoplasmic inheritance and the pursuit of investigations that are no longer possible for the author alone.

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The Genetics of *Colias* (Lepidoptera)

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I. Introduction

The genus *Colias* is present in most of the nontropical continental areas of the world, and even near the Equator *Colias* species occur in western South America where high mountains provide a temperate climate (see Table 13, *c*). There is a numerical predominance of species which live in mountains of temperate regions and at high latitudes. This suggests that the living *Colias* are derived from one or a few types with a genom profoundly selected for adaptation to a climate like that now characteristic of boreo-alpine zones.

Talbot (1935), in a recent catalogue, recognized 57 species in the subgenus *Colias* (he considered *Zerene* a second subgenus). One of these (*C. ponteni* Wallgr.) is probably based on mislabeled material. Ignoring the

many other changes in the Talbot list which will be required, we are left with 56 species. These are distributed in the following regions:

Palearctica	— 37
Nearctica	— 11
[Holarctica	— 4]
South America	— 8

One Palearctic species (*C. electo* L.) extends to South Africa, but no species is solely or principally African. The South American species are well set off from those of the Northern Hemisphere and appear to have descended from a common ancestor from the North not shared with any living northern species. Aside from this single development in the Southern Hemisphere, the most notable characteristics of *Colias* distribution are: (1) the impressive number of species (23) found solely in central Asia; (2) the absence of *Colias* from the Australian region and oceanic islands; and (3) the close genic interrelationships which appear to exist among all the *Colias* of the Northern Hemisphere, as deduced from their wing characters and biology and from the frequent natural interspecific hybridization. The relative lack of morphological differentiation throughout these species makes it quite impossible to master their taxonomy without biological information. Some representative species are shown in Figs. 2 and 3.

Colias fits into the very distinct family Pieriididae and the subfamily Coliadinae (Ford, 1941). Its nearest relatives seem to be the group of "Large Sulphur Butterflies" (*Phoebis*, *Anteos*, *Catopsilia*, etc.) (Klots, 1933). Some of their genic similarities to *Colias* will be reviewed below.

II. Sexuality of Lepidoptera

It is generally stated that in the Lepidoptera the female is the heterogametic sex. Seiler (1914, 1919), Doncaster (1914), and others have shown that the female may have a pair of dissimilar sex chromosomes (ZW type) or an unpaired sex chromosome (ZO type) homologous to the similar pair regularly present in the male (ZZ). Actually the chromosomes of the two sexes have been compared in relatively few species of Lepidoptera, and there is a possibility, at least in the butterfly family Lycaenidae, that the male may sometimes be heterogametic.

The secondary sexual characters, unlike many of those in the Mammalia and Aves, seem to be independent of gonadal control. In *Lymantria dispar* L. and perhaps all Lepidoptera, gonadectomy and gonadal exchange have little or no effect on the striking sexual differences in color,

body and wing shape, antennal form, and behavior (Meisenheimer, 1909; Makino and Saito, 1953). Goldschmidt (1923) has analyzed in detail the nature of sexuality and the control of secondary sexual characters in the Lepidoptera. Sexual color characters, such as female dimorphism for the "alba" gene of *Colias* and for the "valezina" gene of *Argynnis paphia* L. and male and female dimorphism in *Lymantria* races (Goldschmidt, 1920) are not controlled by hormones of the classical type distributed in the blood. The distribution of color in half—and—half and one-fourth—three-fourths gynandromorphs of these and many other species indicates that the chromosome complement of each sex in each pigment-producing cell expresses its "andrases" or "gynases" directly in the pigment of that cell. Morgan's (1914) explanation of the action of a sex-limited gene in males of a bug, *Euschistus*, fits the "alba" and "valezina" genes nicely: "The chemical interaction between two X's and the rest of the cell is of such kind that it produces a female, and the female complex, as such, is inimical to the development of a spot [male sex-limited character in *Euschistus*] and favorable for the development of the accessory organs of reproduction and of all secondary sexual characters of the female, while XY and the rest of the cell is inimical to the development of the accessory organs and of the secondary sexual characters of the female, and favorable for the development of the accessory sexual organs and of the secondary sexual [characters] of the male."

Figure 1 shows a previously unreported gynandromorph of *Colias philodice* Latr., in which the right wings have the female pattern and the left wings have the male characters. It was taken at South Meriden, Connecticut, by Harry L. Johnson. The specimen is of special interest because the female side is "alba." If the expression of the "alba" gene were normally inhibited in males by a sexual hormone originating in the gonads or elsewhere and circulating in the blood, the gene would be completely expressed in the absence of the male hormone (or presence of a facilitating hormone in females) or completely suppressed in the presence of the male hormone. With such a hormonal mechanism, the same uniform presence or absence of "alba" coloration would also be expected in this gynandromorph, but such is not the case. Thus, as Morgan presumed, each male cell must produce its own "alba" suppressor, or each female cell must produce its own "alba" facilitator. The sexual characters for wing size and pattern in *Colias* appear to be under the same intracellular control. No supportable explanation is apparent for the small patches of "alba" color on the male side. Possibly the individual was orginally a male (ZZ chromosomes), but one of the first two blastomeres lost a Z and thus became female (ZO), with each blastomere developing into one side of the imago. The femaleness in small patches on the male side could

then be caused by further losses of Z much later, in development of wing imaginal discs. Since this gynander is almost certainly the result of an intrarace pairing, there is no need to invoke hypotheses of differential strength in the sex chromosomes from the two parents, as is done for some *Lymantria* intersexes. A rather different gynandromorph of *C. eurytheme* Bdv., with wings on both sides largely male, has been figured and discussed by Gerould (1925). Gynandromorphs carrying the "alba" gene have also been reported for *Colias lesbia* Fab. (Turner, 1927) and *Eurema hecabe simulata* (Talbot, 1939).

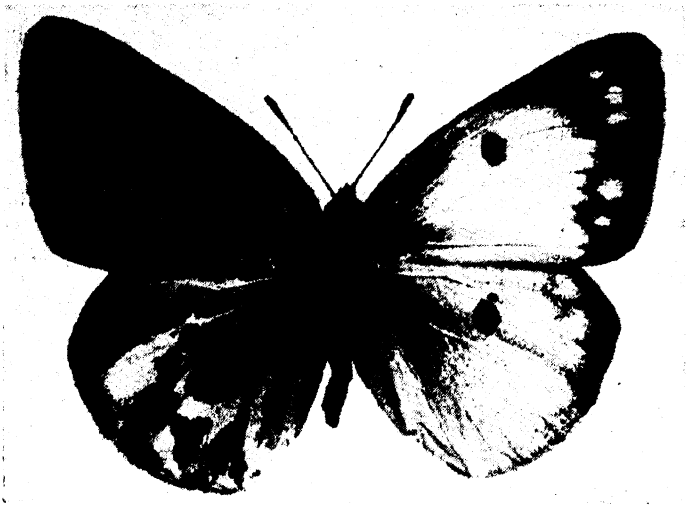


FIG. 1. Gynandromorph of *Colias philodice*, taken flying at South Meriden, Connecticut. The right side is female and "alba," the left side is male and yellow, except for the "alba" patches in the hindwing. The black margin outside the largest "alba" patch is also femalelike, indicating that these "alba" islands are physiologically female. Photographed with a blue filter (Wrattan No. 47) to increase the contrast between yellow and white.

Cockayne (1932) explained the sex-limited expression of autosomal genes in Lepidoptera in terms of an "activator" gene located on the W chromosome of the female. Males of *Colias*, lacking the W chromosome, would also lack the "activator" gene and therefore be unable to express the "alba" gene present on an autosome. Cockayne recognized the difficulties posed by the bilateral gynandromorphs of *Colias* in which the female side is "alba," the male side colored. He accounted for these by assuming that such bilateral gynandromorphs are produced by binucleate ova, one nucleus fertilized by a Z sperm, one by a W sperm. He stated that these gynandromorphs "cannot arise by the loss of a Z chromosome, as some gynandromorphs arise, because there would be no activating gene to aid the autosomal dominant." This is circular reasoning and does not make the Cockayne hypothesis more supportable than

that of Morgan. It may well be that the chemistry of maleness of Lepidoptera, produced by the double strength of Z relative to the autosomes, is the inhibitor of expression of sex-limited genes, rather than there being a specific activator gene on the W chromosome producing expression of the gene in females. Cockayne does not account for one-fourth and three-fourths gynandromorphs of *Colias*, in which the female parts are phenotypically "alba," nor does he mention the "alba"-colored patches in the male side of bilateral gynandromorphs. These forms are more easily explained in terms of loss of a Z chromosome than in terms of his "activator" on the W chromosome.

III. Chromosomes of *Colias*

Federley (1938, 1942) has described briefly the chromosomes of five species of *Colias;* additional counts of the chromosomes of two of these and of two more species have been reported by Kernewitz (1915), Lorković (1941), and Maeki and Makino (1953). The numbers are shown in Table 1.

Federley found that the six metaphase oöcytes of *C. palaeno* L. all had one chromosome conspicuously larger than the others, but that in *C. hecla* Lef., *C. croceus* Fabr., *C. nastes* Bdv., and *C. hyale* L. no single distinctively large chromosome was present. The female *C. hyale* from Esbo was noteworthy in having in each of the five oöcytes examined 32 chromosomes, of which 4 were always bound together and the other 28 were singletons. Although the data are insufficient for certainty, it appears that *C. palaeno* has the ZO type of sex chromosomes, the males having diploid = 60A + $Z_1Z_1Z_2Z_2$ and the females having diploid = 60A + Z_1Z_2. However, the expectation would be that half of the oöcytes have 32 chromosomes (30A + Z_1Z_2) and half, 30 chromosomes (30A + 0). Instead, Federley found that all six metaphase oöcytes had 31 chromosomes, of which one (Z_1Z_2?) was large.

With a basic haploid number of 31, *Colias* is typical of the Lepidoptera. Most of the species of the Order known cytologically have numbers in the narrow 29 to 31 range, the most important exception being the family Lycaenidae, whose members generally have haploid numbers of 23 or 24.

In *Bombyx mori* L., where the female is certainly the heterogametic sex, crossing over takes place only in the male (Naville, 1937). It will be of great interest to learn enough about linked genes in *Colias* and other Lepidoptera to see whether crossing over is generally confined to the homogametic sex in the Lepidoptera. Among the Diptera, crossing over likewise occurs only in the homogametic sex (female) in *Drosophila, Phormia,* and *Calliphora.*

TABLE 1
Haploid Chromosome Numbers of *Colias*

Species	*Origin*	*Number studied*	*Spermatocyte* I	*Spermatocyte* II	*Oöcyte I*	*Reference*
C. myrmidone	Germany?	1 pupa	—	30–31	—	Kernewitz, 1915
C. palaeno	Finland	2 ♂♂, 2 ♀♀	31 (4 cells), 32 (11 cells)	31 (1 cell), 32 (13 cells), 33 (2 cells), 34 (2 cells)	31 (6 cells)	Federley, 1938
C. hecla	Finland	1 ♀ (9 cells)	—	—	31	Federley, 1938
C. croceus	Yugoslavia	5 ♂♂	31	31	—	Lorković, 1941
(= *edusa*)	Azores	2 ovaries	—	—	31	Federley, 1942
C. hyale	Yugoslavia, S.E. France	2 ♂♂	31	31	—	Lorković, 1941
	Åland, Finland	1 ♀ (6 oöcytes)	—	—	31	Federley, 1942
	Esbo, Finland	1 ♀ (5 oöcytes)	—	—	32	Federley, 1942
	Esbo and Borgå	3 ♂♂ (25 cells)	31	—	—	Federley, 1942
C. erate	Sapporo, Japan	?	31	—	—	Maeki and Makino, 1953
C. nastes	Finland	?	—	—	31	Federley, 1942

IV. Larval Genes

The higher insects (Holometabola) are characterized by a remarkable developmental sequence. The larval body serves as an *in vivo* culture medium in which small groups of cells (imaginal discs) grow slowly, and later very rapidly, to produce the tissues of the future adult insect. Most of the larval tissues undergo histolysis during the brief period of metamorphosis when the definitive imaginal form replaces the larval form. This process seems to be a peculiarity of all of the orders of Holometabola and to have no counterpart in the Hemimetabola, the Paleoptera, or the primitively wingless insects; however, the histology of postembryonic development must be investigated for a wide variety of insects before one can generalize with confidence on the phylogenetic scope of the phenomenon.

Of special interest for geneticists is the fact that a single individual can have two very different bodies in contact with the environment during different periods of its postembryonic development. The two bodies, one succeeding the other rather suddenly, may differ not only in form but also in environment. Consider, for example, the great dissimilarity in habitat and habits between a larval *Drosophila* living in a slime-flux and the imago flying freely and probably doomed if one small drip from the fluxing tree falls on it. In many animals with marked metamorphosis (*e.g.*, Amphibia), the single genom carries the genic controllers of the structure and functioning of two environmentally dissimilar forms. But in the Holometabola, probably far more than in other metamorphosing animals, the complex of genes controlling larval adaptive characters can cease functioning after metamorphosis, and an almost completely new complex of imaginal adaptive genes can begin functioning after having been unexpressed in the larva.

With Lepidoptera most larval variants appear to be unrecognizable in the imaginal stage, and recognition of imaginal variations cannot be anticipated by the biologist in the larval stage. This applies to genes controlling colors, pattern, and special adaptive structures of either the larva or adult and not, of course, to factors affecting general viability, fertility, and size. A few of the species with conspicuous larval polymorphism are *Vanessa atalanta* L., *Papilio glaucus* L., *Celerio lineata* Fab. and many other Sphingidae, and *Lycaenopsis pseudargiolus* Bdv. and Lec. Some and perhaps all of the variations are hereditary. Thus far no correlation has been found between these larval variants and imaginal forms. Similarly, special imaginal genes such as "alba," "pocahontas," and "glaucus" (see Section V, 6, below), are not recognizable in the larvae. See the review by Tanaka (1953) of the present knowledge of

mutant genes which affect, respectively, the larval, pupal, and imaginal characters of *Bombyx mori.*

In *Colias* the only genetic analyses of larval characters are those by Gerould. He (1921) found in an inbred stock of *C. philodice* Latr. an allele, recessive to the wild type, which caused the larvae to be blue-green in color, rather than the usual grass green. He believed that the gene acted to modify or remove the usual yellow pigment of the hemolymph, carotin. This substance is presumably derived from the leaves eaten by the larvae. In its absence the hemolymph, larval and pupal cuticle, imaginal eye, and egg shell lacked the normal yellow color. Professor Gerould also found that when braconid parasites developed in "blue-green" larvae and emerged to pupate, the cocoons they spun were white, lacking the yellow color of cocoons from the same parasite developing in normal *Colias* larvae. The "blue-green" gene did not affect the general viability, but homozygous "blue-green" males usually would not mate.

The next year, after outcrossing and subsequent inbreeding of the "blue-green" stock, Gerould (1926) found by chance a similar new allele, "olive-green." This was also recessive to the wild type. In individuals homozygous for the recessive allele the older larvae and the imaginal eyes were olive-green in color, and scales on the underside of the hindwings had a distinct orange cast. The haemolymph in "olive-green" larvae showed no recognizable difference from that in normal larvae. The "olive-green" adults mated and oviposited readily, but the eggs usually did not develop; Gerould attributed this to sterility factors brought out by close inbreeding and independent of the "olive-green" expression of the gene. The "olive-green" allele in the larva and in the imaginal eyes was masked by the "blue-green" allele when both were present. Gerould obtained an apparent 9:4:3 ratio when individuals heterozygous for both genes were intermated; in three such broods there were 166 wild type: 82 "blue-green": 65 "olive-green" larvae, and these produced 128 wild type: 61 "blue-green": 52 "olive-green" adults.

Neither the "blue-green" nor "olive-green" mutants have been recognized among the F_1 larvae of over fifty wild *Colias* females from Connecticut from which progeny have been reared, nor have the mutants been noticed in about twenty subsequent F_2 and F_3 broods reared. By chance Gerould (1921) obtained evidence of the rigorous selection brought to bear against the "blue-green" larvae by predators and presumably helping to keep the gene extremely rare. One of his New Hampshire broods was left on clover plants outdoors, without protective cages (it was expected that this was not a brood of special genetic interest). Wild House Sparrows (*Passer domesticus* L.) discovered sooner than Professor

Gerould that the brood included some homozygous recessive "blue-green" larvae. Apparently able to see the mutant larvae more easily than normal grass-green larvae, the sparrows consumed nearly all the "blue-green" larvae while failing to find many of the grass-green ones.

Larval populations of *Colias eurytheme* and *C. philodice* are polymorphic, the variability being greater in some regions than in others. From Louisiana larvae of *C. eurytheme*, Floyd (1940) described six different color patterns, one of which may have been homologous to the "blue-green" mutant discovered by Gerould. Gerould and others have referred to pattern variations in *Colias* larvae, but the heredity is not yet understood. The variants with increased spotting or striping occur in Connecticut stocks at all seasons, but they are proportionately most numerous in the fall.

V. The Sex-Limited "Alba" Gene

1. *Inheritance*

Special female dimorphism occurs in many, and perhaps all, species of *Colias*. One form has the ground-color of the upperside white, and one has the color of the same area a shade of yellow or orange. Gerould (1911) first showed for *Colias philodice* that the "alba" allele is dominant, always expressing itself phenotypically in the heterozygous female. His experiments also showed that it is autosomal rather than sex-linked.

Gerould's notation (*W* for the "alba," or white, allele and *w* for the colored allele) is here replaced by *A*, for "alba," and *a*. The white female form of many species of *Colias*, *Eurema*, *Phoebis*, and other Pieriididae has been known for a very long time by the latin name *alba* or a similar variant like *albula*. A move is under way to introduce into the *Règles Internationales de la Nomenclature Zoologique* a provision whereby the same form in a series of related species will carry the same name in each species. If this highly desirable action is taken, as seems likely, *alba* will presumably be the name fixed for the white female form of *Colias* and related genera. *A* and *a* seem to be far better international notations than *W* and *w* in this case, and the change is here put forward while the literature on the genetics of the "alba" gene is still small.

Gerould first believed the "alba" gene to be dominant in the female, recessive in the male. No white male was produced in his many breeding experiments, although his broods 1910g and 1910h resulted from pairings in which both parents were believed to be heterozygous (Table 4). The ratio of "alba" to yellow females in the two 1910 broods is near 2:1($\chi^2 = 0.500$; $P = < 0.50, > 0.30$) and very far from the expected 3:1($\chi^2 = 6.259$; $P = < 0.02$). Gerould reasonably accounted for the

shortage of "alba" females in terms of lethality for those homozygous for the dominant allele (AA). Such lethality might also have explained the absence of phenotypically "alba" males, since the sex-ratio indicated no higher mortality for females than for males. A few wild white males of *C. philodice* were known to butterfly collectors, and Gerould supposed them to be AA males which survive very rarely.

As he was able to prove (1923), these suppositions about inheritance of male coloration were incorrect. The males with all three genotypic combinations, aa, Aa, or AA, are phenotypically always yellow. No characters have been found for distinguishing phenotypically females of the two "alba" genotypes or males of the three genic combinations, although this was attempted with scanty material by Hovanitz (1944a). The genotypes and phenotypes of the alleles of the "alba" gene in *C. philodice* are as follows:

Genotype	♀ Wing Color	♂ Wing Color
AA	White	Yellow
Aa	White	Yellow
aa	Yellow	Yellow

Thus, the gene for "alba" is transmitted to the daughters exactly alike by both males and females, but its wing-color expression is completely suppressed in males. Such autosomal genes are appropriately termed *sex-limited genes*. The inheritance of white wing color in males will be reviewed in Section VI, 1, below.

Gerould's results in the F_1 and F_2 of his brood 1909a demonstrated conclusively that the "alba" allele is dominant in the female. A wild "alba" female, already fecundated by an unknown wild male, was brought into the laboratory. Of her daughters, 7 were "alba" and 16 yellow. One of these yellow daughters mated with a male sib and produced 15 daughters, all of which were yellow. The F_1 brood (7 "alba":16 yellow) could be explained by having the "alba" allele either dominant or recessive. If it were dominant, the unknown wild father must have been homozygous for the yellow allele; if it were recessive, the father must have been heterozygous. However, in the F_2 brood (all 15 females yellow) the yellow mother must have been homozygous.

Letting Y and y stand for the two alleles, *with yellow dominant*, the crosses would appear thus:

1. Wild ♀ "alba" (yy) × wild ♂ (Yy) →
   7 ♀♀ "alba" (yy) + 16 ♀♀ yellow (Yy);
2. F_1 ♀ yellow (Yy) × F_1 ♂ sib (Yy) →
   0 ♀ "alba" + 15 ♀♀ yellow (YY, Yy) (expect 3 yellow:1 "alba").

Since this is far from the expected 3:1 ratio ($\chi^2 = 5.000$; $P = < 0.05$), the dominance of the yellow allele is not confirmed.

Letting *A* and *a* stand for the two alleles, *with "alba" dominant*, the results of the actual crosses fit the expectations; thus:

1. Wild ♀ "alba" (*Aa*) × wild ♂ (*aa*) →
   7 ♀ ♀ "alba" (*Aa*) + 16 ♀ ♀ yellow (*aa*);
2. F_1 ♀ yellow (*aa*) × F_1 ♂ sib (*aa*) →
   0 ♀ "alba" + 15 ♀ ♀ yellow (*aa*).

A crucial test showing that the "alba" gene is autosomal, rather than sex-linked with the male heterogametic, is found in crosses in which the F_1 of a yellow female include both "alba" and yellow daughters. If there had been male-sex-linkage, only one female phenotype would have appeared in the F_1:

♀ *aa* × ♂ *a*Y → all ♀ ♀ *aa* (yellow) + ♂ ♂ *a*Y, or
♀ *aa* × ♂ *A*Y → all ♀ ♀ *Aa* ("alba") + ♂ ♂ *a*Y.

Edwards, Gerould, and the writer have proved that the "alba" gene of *C. philodice* is not male-sex-linked, by rearing both "alba" and yellow daughters from each of several yellow females (Table 3, *a*). The cross is diagrammed thus:

♀ *aa* × ♂ *Aa* → ♀ ♀ *Aa* ("alba"): ♀ ♀ *aa* (yellow)
+ ♂ ♂ *Aa*: ♂ ♂ *aa*.

Similar confirmation has been obtained for four other species (Table 3, *b*, *d*, *e*, and *f*).

Proof that the gene is autosomal, not sex-linked with the female heterogametic (the usual sex-chromosome situation in Lepidoptera), is more difficult to obtain because the genic constitution of the male is not expressed phenotypically. The determining test requires that both female forms appear among the daughters of a cross between an "alba" female and a male known to be homozygous for the recessive allele. Such a cross is Gerould's 1914d, in which an "alba" female *C. eurytheme* was mated to a male *C. philodice* whose mother and 41 female sibs were all yellow. The F_1 of the brood 1914d (see Table 4, *c*) contained 24 "alba" females and 10 colored females. Had the "alba" gene been female-sex-linked, the cross could have produced only colored daughters, diagrammed as follows:

♀ *A*W × ♂ *aa* → ♀ ♀ all *a*W + ♂ ♂ all *Aa*.

Brood 44–61 of Komai and Aé (1953) for *Colias erate poliographus* Motsch. is probably another example of this cross, but the published data are inadequate for certainty.

TABLE 2

F_1 of *Colias* Crosses of Homozygous Recessive Parents

[♀ yellow (*aa*) × ♂ *aa*]

No "alba" daughters produced

Brood	*Origin*	*aa* ♀♀	♂♂	*Reference*
	a. *Colias philodice*			
—	Colorado	15	20	Edwards, 1887
—	Colorado	13	3	Edwards, 1887
1909 2y	New Hampshire	15	20	Gerould, 1911
1920S	New Hampshire	118	127	Gerould, 1923
1920θ	New Hampshire	13	21	Gerould, 1923
1921mc	New Hampshire	20	2	Gerould, 1923
58	Connecticut	34	24	Remington, unpubl.
154	Connecticut	30	50	Remington, unpubl.
	b. *Colias eurytheme*			
1913c	Ariz. or Colo.	206	214	Gerould, 1923
1914x	Ariz. or Colo.	136	149	Gerould, 1923
Table 12-E	Illinois	20	?	Hovanitz, 1944a
61A-A	Connecticut	14	8	Remington, unpubl.
	c. *Colias philodice–eurytheme* hybrids			
1914a	N. Hamp. & Ariz.-Colo. (♀ *philo.* × ♂ *eury.*)	40	122	Gerould, 1923
1914h	N. Hamp. & Ariz.-Colo. (♀ F_1 hybrid × ♂ *philo.*)	36	24	Gerould, 1923
Table 3	California (wild ♀ F_1 hybrid)	13	8	Hovanitz, 1944a
51	Connecticut (♀ *eury.* × ? ♂)	14	35	Remington, unpubl.
57	Connecticut (♀ *eury.* × ? ♂)	39	43	Remington, unpubl.
	d. *Colias christina* Led.			
Bean 12	Alberta	5	?	Gerould, 1923
Bean 14	Alberta	5	?	Gerould, 1923
Bean 15	Alberta	4	?	Gerould, 1923
Bean 16	Alberta	5	?	Gerould, 1923
	e. *Colias myrmidone*			
IX	Graz, Austria	23	15	Pieszczek, 1917
XIV	Graz, Austria	14	22	Pieszczek, 1917
XV	Graz, Austria	40	46	Pieszczek, 1917
	f. *Colias erate poliographus*			
C1	Honshu, Japan	51	57	Komai and Aé, 1953
H19	Honshu, Japan	16	13	Komai and Aé, 1953
H47	Honshu, Japan	36	30	Komai and Aé, 1953
H48	Honshu, Japan	13	11	Komai and Aé, 1953
H52	Honshu, Japan	14	22	Komai and Aé, 1953
H60	Honshu, Japan	22	37	Komai and Aé, 1953
H74	Honshu, Japan	14	23	Komai and Aé, 1953
H76-5	Honshu, Japan	13	9	Komai and Aé, 1953

TABLE 3

F_1 of the Cross [♀ yellow (*aa*) × ♂ *Aa*] in *Colias*

Brood	*Origin*	*"alba"* ♀♀	*aa* ♀♀	♂♂	*Reference*
		a. *Colias philodice*			
—	Colorado	3	1	11	Edwards, 1887
1909b	New Hampshire	5	10	19	Gerould, 1911
1910e	New Hampshire	13	14	59	Gerould, 1911
1910f	New Hampshire	7	5	10	Gerould, 1911
1910i	New Hampshire	30	14	27	Gerould, 1911
1910k	New Hampshire	19	19	46	Gerould, 1911
1911χ	New Hampshire	1	2	13	Gerould, 1923
1920λ	New Hampshire	28	28	77	Gerould, 1923
1920ο	New Hampshire	59	76	146	Gerould, 1923
60	Connecticut	6	6	20	Remington, unpubl.
70B-A	Connecticut	9	13	16	Remington, unpubl.
	♀ *Totals*	180	188	(expected 1:1; $\chi^2 = 0.174$, $P > 0.50$)	
		b. *Colias eurytheme*			
*Table 8-D6	California	5	21	57	Hovanitz, 1944a
Table 12-A	California	21	18	?	Hovanitz, 1944a
		c. *Colias philodice–eurytheme* hybrids			
—	Colorado	3	1	11	Edwards, 1887
6	Connecticut (♀ *eury.* × ♂ ?)	3	1	2	Remington, unpubl.
50	Connecticut (♀ F_1 hybrid × ♂ *eury.*)	1	7	20	Remington, unpubl.
	♀ *Totals*	7	9	(1:1; $\chi^2 = 0.250$, $P > 0.50$)	
		d. *Colias christina*			
Bean 1	Alberta	7	4	?	Gerould, 1923
Bean 7	Alberta	2	2	?	Gerould, 1923
Bean 9	Alberta	2	7	?	Gerould, 1923
Bean 10	Alberta	2	6	?	Gerould, 1923
Bean 11	Alberta	7	5	?	Gerould, 1923
	♀ *Totals*	20	24	(1:1; $\chi^2 = 0.364$, $P > 0.50$)	
		e. *Colias myrmidone*			
*XVI	Zeltweg, Austria	10	42	67	Pieszczek, 1917
*XVIII	Graz, Austria	2	4	10	Pieszczek, 1917
		f. *Colias erate poliographus*			
H27	Honshu, Japan	6	17	55	Komai and Aé, 1953
H43	Honshu, Japan	8	17	10	Komai and Aé, 1953
H54	Honshu, Japan	42	32	64	Komai and Aé, 1953
H68	Honshu, Japan	22	17	30	Komai and Aé, 1953
	♀ *Totals*	78	83	(1:1; $\chi^2 = 0.155$, $P > 0.50$)	

* More than one *aa* mother may have contributed ova to these broods.

TABLE 4

F_1 of the Cross [♀ *Aa* × ♂ *aa*] in *Colias*

Brood	*Origin*	*"alba"* ♀♀	*aa* ♀♀	♂♂	*Reference*
		a. *Colias philodice*			
—	West Virginia?	0	4	1	Edwards, 1878
—	West Virginia?	1	3	?	Edwards, 1878
1909a	New Hampshire	7	16	39	Gerould, 1911
1910b	New Hampshire	22	28	79	Gerould, 1911
1910d	New Hampshire	15	13	30	Gerould, 1911
1910g	New Hampshire	38	22	63	Gerould, 1911
1910h	New Hampshire	13	8	16	Gerould, 1911
1911α	New Hampshire	8	17	28	Gerould, 1923
1911β	New Hampshire	13	11	25	Gerould, 1923
1911γ	New Hampshire	28	21	42	Gerould, 1923
1919B	New Hampshire	28	38	64	Gerould, 1923
1919C	New Hampshire	67	36	77	Gerould, 1923
1920μ	New Hampshire	14	10	20	Gerould, 1923
1921c	New Hampshire	14	9	45	Gerould, 1923
1921f	New Hampshire	20	13	40	Gerould, 1923
1921h	New Hampshire	13	12	26	Gerould, 1923
1921p	New Hampshire	65	69	217	Gerould, 1923
1921q	New Hampshire	21	17	36	Gerould, 1923
59	Connecticut	14	9	15	Remington, unpubl.
62	Connecticut	8	9	16	Remington, unpubl.
155	Connecticut	5	9	18	Remington, unpubl.
	♀ *Totals*	414	374	(expected 1:1; $\chi^2 = 2.030$, $P > 0.10, < 0.20$)	
		b. *Colias eurytheme*			
1913a	Colo. or Ariz.	29	24	57	Gerould, 1923
1913b	Colo. or Ariz.	27	20	49	Gerould, 1923
1913m	Colo. or Ariz.	16	11	38	Gerould, 1923
1914y	Arizona	13	15	29	Gerould, 1923
1916 I	Colo. or Ariz.	62	53	102	Gerould, 1923
1916 II	Colo. or Ariz.	28	29	39	Gerould, 1923
Table 12-G	Illinois	18	21	?	Hovanitz, 1944a
53	Connecticut	3	9	11	Remington, unpubl.
72	Connecticut	8	9	12	Remington, unpubl.
	♀ *Totals*	204	191	(1:1; $\chi^2 = 0.428$, $P > 0.50$)	

TABLE 4 (*continued*)

Brood	*Origin*	*"alba"* ♀♀	*aa* ♀♀	♂♂	*Reference*
	c. *Colias philodice–eurytheme* hybrids				
1913o	Colo.-Ariz. & N. Hamp. (*eury.* ♀ × *philo.* ♂)	36	35	72	Gerould, 1923
1913s	Colo.-Ariz. & N. Hamp. (*eury.* ♀ × F_1 hybrid ♂)	35	38	108	Gerould, 1923
1913u	Colo.-Ariz. & N. Hamp. (*eury.* ♀ × F_1 hybrid ♂)	15	13	36	Gerould, 1923
*1914d	Colo.-Ariz. & N. Hamp. (*eury.* ♀ × *philo.* ♂)	24	10	20	Gerould, 1923
	♀ *Totals*	110	96	(1:1; $\chi^2 = 0.971$, $P > 0.30$)	
	d. *Colias christina*				
Bean 3	Alberta	10	10	?	Gerould, 1923
	e. *Colias croceus* (= *edusa*)				
(4 "alba" ♀♀)	England	110	125	302	Frohawk, 1901
(sev. "alba" ♀♀)	England	115	100	175	Burkhardt, 1948
1 "alba" ♀	England	13	12	32	Warrier, 1951
	f. *Colias myrmidone*				
VII	Graz, Austria	1	4	6	Pieszczek, 1917
XIII	Graz, Austria	4†	5	5	Pieszczek, 1917
XVII	Zeltweg, Austria	18	17	28	Pieszczek, 1917
	g. *Colias erate poliographus*				
A1	Honshu, Japan	35	35	69	Komai and Aé, 1953
C8	Honshu, Japan	40	36	83	Komai and Aé, 1953
C9	Honshu, Japan	48	35	61	Komai and Aé, 1953
H63	Honshu, Japan	30	22	49	Komai and Aé, 1953
*H44-61	Honshu, Japan	8	21	29	Komai and Aé, 1953
	♀ *Totals*	161	149	(1:1; $\chi^2 = 0.465$, $P > 0.40$)	

* Parental genotypes known certainly before F_1 obtained.

† Two of these ♀♀ were form "agnes," a half-orange, half-"alba" intermediate.

TABLE 5

F_1 of the Cross of Heterozygous Parents (♀ "alba" (*Aa*) × ♂ *Aa*) in *Colias*

Brood	*Origin*	*"alba"* ♀♀	*aa* ♀♀	♂♂	*Reference*
		a. *Colias philodice*			
1920t	New Hampshire	95	28	130	Gerould, 1923
1921d	New Hampshire (sib × sib *ex* 1:1 brood)	28	0	30	Gerould, 1923
1921o	New Hampshire	49	25	80	Gerould, 1923
1921t	New Hampshire	10	2	19	Gerould, 1923
	♀ *Totals*	182	55	(expected 3:1; $\chi^2 = 0.4064$, $P > 0.50$)	
		b. *Colias eurytheme*			
*Table 7-A	California	37	20	?	Hovanitz, 1944a
*Table 7-E	California	34	15	?	Hovanitz, 1944a
*Table 7-F	California	14	4	?	Hovanitz, 1944a
*Table 8-D5	California	15	1	33	Hovanitz, 1944a
	♀ *Totals*	100	40	(3:1; $\chi^2 = 0.952$, $P > 0.30$)	
		c. *Colias philodice* × *eurytheme* hybrids			
Table 3	California	13	43	68	Hovanitz, 1944a
61H	Connecticut	19	5	9	Remington, unpubl.
		d. *Colias christina*			
Bean 5	Alberta	14	7	?	Gerould, 1923
		e. *Colias croceus* (= *edusa*)			
Chapman	S. France	52	19	79	Main & Harrison (Anon., 1905)
Willian ♀	England	26	7	35	Bowden (Anon., 1946?)
	♀ *Totals*	78	26	(exactly 3:1)	
		f. *Colias myrmidone*			
X	Graz, Austria	9	5	14	Pieszczek, 1917
XII	Graz, Austria	7	1	5	Pieszczek, 1917
		g. *Colias erate poliographus*			
H26	Honshu, Japan	35	5	57	Komai and Aé, 1953
H75	Honshu, Japan	18	1	14	Komai and Aé, 1953
*H55-13	Honshu, Japan	7	0	8	Komai and Aé, 1953
*H55-58	Honshu, Japan	4	1	5	Komai and Aé, 1953
*H55-59	Honshu, Japan	6	2	18	Komai and Aé, 1953
*H55-62	Honshu, Japan	9	0	4	Komai and Aé, 1953
*H55-103	Honshu, Japan	8	3	8	Komai and Aé, 1953
*H44-48	Honshu, Japan	11	1	3	Komai and Aé, 1953
*K2-16	Honshu, Japan	16	0	7	Komai and Aé, 1953
	♀ *Totals*	114	13	(3:1; $\chi^2 = 14.760$, $P < 0.01$)	

* Parental genotypes known certainly before F_1 obtained.

Seven of the nine possible mating combinations are known to have produced offspring in the laboratory. Tables 2–8 show for all species of *Colias* the known broods whose genotypes are reasonably certain. Small broods have been omitted from groups with numerous examples.

TABLE 6
F1 of the Cross [♀ *aa* × ♂ *AA*] in *Colias*

Brood	*Origin*	*"alba"* ♀♀	*aa* ♀♀	♂♂	*Reference*
		a. *Colias eurytheme*			
Table 8-C	California	11	0	16	Hovanitz, 1944a
61	Connecticut	32	0	17	Remington, unpubl.
		b. *Colias erate poliographus*			
H21	Honshu, Japan	14	0	12	Komai and Aé, 1953
H44	Honshu, Japan	26	0	16	Komai and Aé, 1953
H55	Honshu, Japan	54	0	47	Komai and Aé, 1953
H76-4	Honshu, Japan	23	0	23	Komai and Aé, 1953
K2	Honshu, Japan	44	0	62	Komai and Aé, 1953

TABLE 7
F1 of the Cross [♀ *AA* × ♂ *Aa*] in *Colias*

Brood	*Origin*	*"alba"* ♀♀	*aa* ♀♀	♂♂	*Reference*
		Colias eurytheme			
Table 7-C	California	15	0	?	Hovanitz, 1944a
Table 7-G	California	25	0	?	Hovanitz, 1944a
Table 8-D7	California	29	0	?	Hovanitz, 1944a

2. *Lethal Modifiers*

The question of lethality in the homozygotes of the "alba" alleles has been the object of a series of studies. The apparent 2:1 broods obtained from Gerould's early *Aa* × *Aa* crosses were offset by all-white female broods obtained from supposed *Aa* × *Aa* crosses (see Table 5, Brood 1921d, and Table 8, Broods 1921a, 1913t) and by 3:1 broods in some cases (Table 5, Broods 1920ι and 1921t). Gerould (1923) therefore set aside his earlier view that the *AA* genotype is lethal and substituted an hypothesis involving a recessive lethal gene closely linked with the factor for "alba" and yellow female coloration. The lethal combinations would be

AlAl, *Alal*, *alAl*, and *alal*.

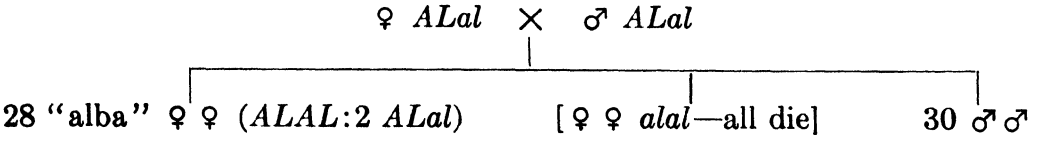
For example, the parents of Brood 1921d were sibs from Brood 1921p, in which there were 65 "alba" ♀♀ : 69 *aa* ♀♀. Thus, the genotype of each

TABLE 8

F_1 of Crosses in *Colias* with ♀ "Alba" and One Parent Homozygous *AA*

Brood	*Origin*	*"alba"* ♀♀	*aa* ♀♀	♂♂	*Reference*
	a. *Colias philodice*				
1920β	New Hampshire (sib × sib *ex* 3:1 brood)	24	0	17	Gerould, 1923
1920ε	New Hampshire (sib × sib *ex* 1:0 brood; F_1 include *Aa*)	15	0	2	Gerould, 1923
1920π	New Hampshire (sib × sib *ex* 1:0 brood)	21	0	29	Gerould, 1923
1920ρ	New Hampshire (sib × sib *ex* 1:0 brood)	10	0	14	Gerould, 1923
1920υ	New Hampshire (sib × sib *ex* 3:1 brood)	10	0	7	Gerould, 1923
1921a	New Hampshire (sib × sib *ex* 2:1 brood)	19	0	10	Gerould, 1923
	b. *Colias eurytheme*				
Table 12-B	California	28	0	?	Hovanitz, 1944a
	c. *Colias eurytheme* ♀ × *philodice* ♂				
1913t	Ariz.-Colo. & N. Hamp. (♀ *AA*? × ♂ *Aa* or *aa*)	39	0	94	Gerould, 1923
	d. *Colias myrmidone*				
VI (2 ♀♀)	Zeltweg, Austria	11	0	7	Pieszczek, 1917
XI	Zeltweg, Austria	19	0	19	Pieszczek, 1917
	e. *Colias erate poliographus*				
B2	Honshu, Japan	55	0	63	Komai and Aé, 1953
B2-12	Honshu, Japan	14	0	18	Komai and Aé, 1953
B4	Honshu, Japan	28	0	19	Komai and Aé, 1953
B44	Honshu, Japan	35	0	51	Komai and Aé, 1953
B83	Honshu, Japan	37	0	43	Komai and Aé, 1953
B84	Honshu, Japan	60	0	63	Komai and Aé, 1953
K1	Honshu, Japan	16	0	13	Komai and Aé, 1953
K3	Honshu, Japan	16	0	19	Komai and Aé, 1953

parent would be expected to be *Aa* or *aa*. However, Brood 1921d contained 28 "alba" ♀♀ and no *aa* ♀♀. With the linked lethal, Gerould diagrammed the brood as follows.



No other simple explanation is apparent for this one difficult brood. The others can all be explained without invoking such a lethal gene. Gerould's pedigrees show that one or both parents of Brood 1921a could have been homozygous *AA*, since their parents (♀ p 43 and ♂ p 2) could both have been *Aa*. Likewise the mother of Brood 1913t (♀ d 5) could have been homozygous *AA*. He apparently overlooked these possibilities in his preoccupation with the hypothetical linked lethal. Although Hovanitz (1944a) concluded that "the homozygous dominants seem to be lethal or semi-lethal with certain modifier environments"—an hypothesis which "supersedes that of Gerould"—it must be noted that his evidence is slight and that this view differs very little from the earliest hypothesis of Gerould. But it is in fact an obvious supposition to be derived from data on the geographic distribution of "alba" frequencies in natural populations (see Section V, 5, d). Perhaps an equally reasonable and simpler explanation is that even without special modifier genes each of the three possible genotypes is highly vigorous in certain environments and relatively less viable and fertile in others; in other words, it is the environments which differ rather than (or in addition to) the genic modifiers. It is difficult to account for the maintenance of such prominent female polymorphism unless the heterozygote is heterotic, *i.e.*, adaptively superior to either homozygote, wherever the populations are polymorphic. The level of the "alba" allele in each population may be controlled by the viability of the two homozygotes relative to each other and to the heterozygote. Dobzhansky and his collaborators have reported such a situation with inversion types and other genic complexes in an extensive series of papers on natural populations of various *Drosophila*, especially *D. pseudoobscura*. Only detailed genetic and physiological testing of *Colias* stocks with various geographic origins can give satisfactory conclusions.

The Japanese race of *Colias "hyale"* (*recte C. erate*) studied by Komai and Aé (1953) appears to show the reverse of Gerould's early New Hampshire lethality with the dominant homozygotes of *C. philodice*. They have omitted details one would like to see in their published data, but supposed *Aa* × *Aa* crosses gave in total the remarkably high F_1 ratio of 65 "alba" to 8 *aa* daughters and 75 sons. For an unaltered 3:1 ratio, the probability is less than 0.01 that such deviation could be attributed to chance. The *aa* homozygotes of both sexes of *C. erate poliographus* seem to have low viability in Japan.

3. *Chemistry of Orange, Yellow, and White Pigments in* Colias!

The names by which members of the family Pieriididae are known colloquially, such as "the Sulphurs," "the Whites," "les Saufrés," "die

Weisslinge," and probably even "butterflies" and "die Schmetterlinge," refer to the principal coloration of the adult insects. These white, yellow, and orange pigments (Fig. 2) belong to the class of Pteridines known as the *pterins* (Gates, 1947). The white pigment is *leucopterin*, the yellow pigment is *xanthopterin*, and some of the red pigment is *erythropterin*. All three are present in *Colias croceus* and presumably *C. eurytheme*, the color of the orange males and females being due to a mixture of xanthopterin and erythropterin (Schöpf and Becker, 1936), whereas the "alba" females are pigmented largely by leucopterin (Ford, 1947).

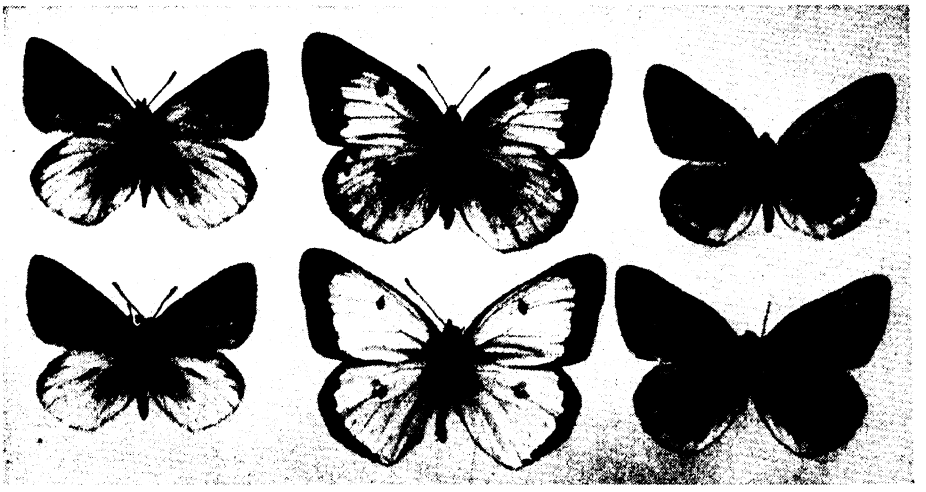


FIG. 2. Three species of *Colias* from separate continents, each showing a different condition of yellow-orange pigments. *C. chrysotheme* (right), from Moravia, has the ground-color orange on both pairs of wings. *C. philodice* (center), from Connecticut, has the ground-color yellow on both pairs of wings. *C. dimera* (left), from Baños, Ecuador, has the ground-color of the forewings orange and of the hindwings yellow, with the discal spot streaked instead of rounded. Females are above, males below. Photographed with a green filter (Wrattan No. 11) to increase the contrast between yellow and orange.

The investigation of these pigments has an interesting history. Hopkins (1889) extracted from the European pierid butterfly, *Gonepteryx rhamni*, a yellow pigment which he considered closely related to a known derivative of uric acid and which gave the murexide test. He named it "Lepidotic acid" (1891). In his major treatise on pierid pigments, Hopkins (1895) also reported two similar substances extracted from wings of various Pieridídae. He identified the white wing-color of three species of *Pieris* as actual uric acid. Hopkins found that orange coloration was usually produced by an increase in "Lepidotic acid," but he noted indications of a red pigment, closely related to the yellow, in *Delias* and other genera. The yellow pigment ("Lepidotic acid") was obtained from *Colias croceus, C. fieldi* Mén., *C. hyale, Phoebis argante* Fab., *Eurema lisa* Bdv.

and Lec., *Anthocaris cardamines* L., *Delias eucharis* Drury, and *Dismorphia praxinoë* as well as *G. rhamni*. The same pigment was believed to be identified in the "material voided from the rectum of a Pierid, showing that in the coloured insects as well as the white, a normal excretory product subserves the purpose of ornament."

After thirty years the study of these pigments was resumed by Wieland and Schöpf (1925), who succeeded in crystallizing the yellow pigment from *G. rhamni* and named it "Xanthopterin." The next year these authors (Schöpf and Wieland, 1926) crystallized the white pigment from an extract from the wings of about 25,000 *Pieris napi*. They named it "Leukopterin" and found that it differs fundamentally from uric acid, thus discarding Hopkins's early view. They also showed (Wieland *et al.*, 1933) that no pterins are present in the excreta of adults of species having pterins in the wings. Becker (1937) proved further that pterins are absent from the Malpighian tubules of *G. rhamni*, *Pieris*, and *Vespa*, all known to have pterins in the wing-scales or body cuticle. Ford (1947) was able to confirm Hopkins's observation that pterins are present in most or all Pieridídae and absent from the other families of butterflies. Ford's supposition of the widespread presence of pterins in moths (1937) has not yet been confirmed. In addition to Lepidoptera, xanthopterin has been isolated from the cuticle of the Ascalaphid Neuroptera, of one of the homopterous Cicadidae, and of eight families of Hymenoptera (Schöpf and Becker, 1933; Becker and Schöpf, 1936), and from the eyes and ova of Orthoptera (Goodwin, 1952).

Hopkins's third pigment is probably the pterin which Schöpf and Becker (1936) found with xanthopterin in *Colias edusa* (= *C. croceus*) males and four other Pieridídae and named "Erythropterin." They reported the presence of four additional pterins in Pieridids other than *Colias*, namely "Chrysopterin," "Mesopterin," "Anhydroleukopterin," and "Guanopterin." Little is known of the first three, but Purrmann (1940) found that "Guanopterin" is actually isoguanine.

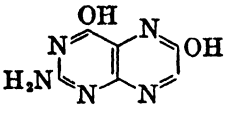
The first successful synthesis of pterins was by Purrmann, who prepared leucopterin (1940) and xanthopterin (1941).

The numbers of specimens required for these chemical analyses were substantial. In Hopkins's early work he used 500 *G. rhamni*, 200 *Eurema lisa*, and many others. With the help of Bavarian school children, Wieland *et al.* (1933) were able to gather 215,000 *Pieris*. Tartter (1940) extracted 170 g. of leucopterin from wings of 850,000 mixed *P. napi* L., *P. rapae* L., and *P. brassicae* L.

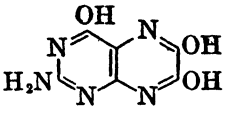
The wings of males have been used in most of these studies. However, Makino, Satoh, *et al.* (1952) demonstrated for *Pieris rapae* that the pterin content of wings of males and females is very different. Both sexes of *P.*

rapae have predominantly white wings, with some black markings. Extracts from the wings of males proved to contain abundant leucopterin and small quantities of uric acid, xanthopterin, and "xanthopterin *a*." Extracts from the wings of females yielded only a small quantity of precipitate, identified as "xanthopterin *b*" and "a trace of xanthopterin." We have no knowledge of how widespread these sexual differences may be among the Pieriididae.

The structural formulas for leucopterin and xanthopterin are given below. It can be seen that xanthopterin is converted to leucopterin with the addition of one oxygen atom— a difference controlled genetically by the "alba" allele of *Colias*.



Xanthopterin



Leucopterin

4. *Distribution among Species of* Colias *and Other Pieriididae*

Enough is known of the inheritance of the "alba" allele in 6 species of *Colias* to show that it is inherited consistently as a sex-limited dominant gene expressed only in the female (Tables 2–6). A rapid search of the literature was made to survey the occurrence of the "alba" gene in the 56 species of *Colias* recognized by Talbot (1935).

1. "Alba" the wild-type female form:
   a. Colored (*aa*) ♀ ♀ unknown—5 spp.
   b. Colored ♀ ♀ known—3 spp.
2. Colored (*aa*) ♀ ♀ the wild type; "alba" distinctly scarcer:
   a. "Alba" abundant—16 spp.
   b. "Alba" rare—11 spp.
   c. "Alba" unknown—13 spp.
3. Both sexes whitish—1 sp.
4. Coloration masks gene if present—1 sp.
5. Insufficient information—6 spp.

Of the last 6 species, 5 are South American. Of the 48 species for which good records were found, in only 13 is the "alba" form apparently unknown. On the other hand, 5 of the 48 better known species seem to lack the colored (*aa*) form completely, *i.e.*, *C. chlorocoma* Christ., *C. sieversi* Grum., *C. sifanica* Grum., *C. montium* Ober., and *C. phicomone* Esp. The first 4 are from central and western Asia, from which collecting records are few. It seems probable that the "alba" and colored (*aa*)

female forms will be discovered in every species of *Colias*, if in fact specimens are not already present in collections.

The "alba" genotype of the generotype of *Colias*, *C. hyale* L., is difficult to determine. In Europe the females are consistently whitish yellow. These females may all be genically equivalent to the "alba" form of *C. croceus*, *C. philodice*, etc. Whether this is so or not, clear-cut female dimorphism due to the "alba" gene seems not to have been recognized in *C. hyale* by European lepidopterists. The Asiatic *Colias* populations long known as races of *C. hyale* have been transferred to *C. erate* Esper, on genitalic as well as wing-pattern characters (Talbot, 1939). Thus the presence of female dimorphism in *C. erate poliographus*, investigated genetically by Komai and Aé (1953), throws no light on the genotype of the true *C. hyale* in Europe.

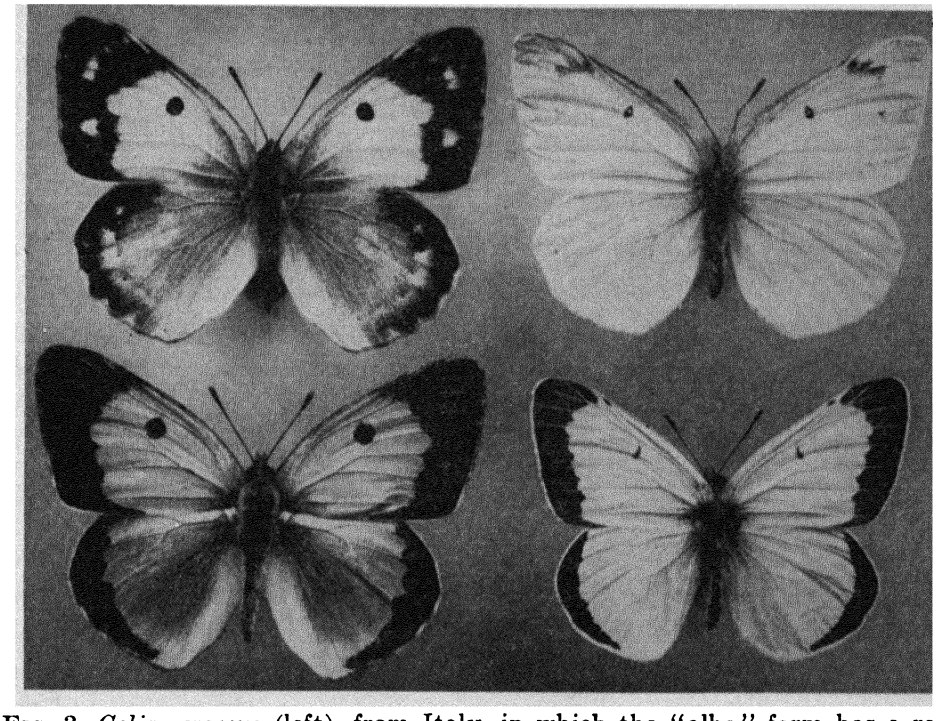


FIG. 3. *Colias croceus* (left), from Italy, in which the "alba" form has a rather high frequency. *C. alexandra* (right), from Colorado, in which the frequency of the "alba" form is very low. "Alba" females are above, colored males below. Photographed with a green filter (Wrattan No. 11).

The eight species with "alba" as the principal female form are from mountains or from the far North, but no simple generalization is possible coupling "alba" with high latitudes and altitudes. In Boulder County, Colorado, for example, *Colias scudderi* Reak. is most abundant at elevations between 9500 and 11,000 feet. There at least 80% of the

females are "alba." From 9500 down to 7000 feet *C. alexandra* Edw. (Fig. 3) flies in great numbers, and above 11,000 feet *C. meadii* Edw. is common. Both of the higher and lower altitudinal flankers of *C. scudderi* have colored females outnumbering "alba" at least 20 to 1. Similarly in the Himalayan region at least three species with most or all females "alba" (*C. cocandica* Ersch., *C. alpherakyi* Stgr., *C. montium* Ober.) and at least five with all known females colored (*C. marcopolo* Grum., *C. ladakensis* Feld., *C. berylla* Fawc., *C. nina* Fawc., and *C. dubia* Elwes) fly at altitudes above 12,000 feet. In the Himalayas *C. erate* lacks the "alba" form completely, although "alba" females of *C. erate* are numerous at sea level much farther to the east.

C. tyche Boeb. has both sexes white, and Professor J. Herrera Gonzalez, of Santiago, has informed me that a similar situation characterizes a Chilean *Colias* now being investigated. The chemistry and genetics of this case will be of great interest.

It is reasonable to suppose that this gene in *C. philodice* is homologous to that producing the "alba" form in the other species of *Colias*. It is inherited in interspecific crosses between *C. philodice* and *C. eurytheme* in the same manner as in either supposedly pure species (Gerould, 1923; Hovanitz, 1944a; Remington, Tables 3 and 5, above).

Female dimorphism involving color differences similar to those produced by alleles of the *Colias* "alba" gene are well known in Pieriididae of several other genera. Although homology may be less probable here, it is reasonable to expect that the white female form in most of these species will also prove to be controlled by an autosomal dominant sex-limited gene. Table 9 shows representative species of Pieriididae in which white and yellow or orange female forms are known.

The chemical nature of these pigments in many of the forms has not yet been investigated, but the few determinations which have been made indicate that their chemistry is the same as that of *Colias*. It is known that in *Gonepteryx rhamni* the white is leucopterin and the yellow is xanthopterin. The white of *Euchloe* spp. is leucopterin. It will be seen that in *Colotis*, *Phoebis*, and *Eurema*, as in *Colias*, the males and most females are colored, with white females rare, whereas in *Appias*, *Euchloe*, *Anthocaris*, *Kricogonia*, and others the usual color for both sexes is white (leucopterin), and the second female form is yellow or orange. This is a notable reversal of effect from the sexual suppressors, the carrier males being able to express only the oxidized pterin in one group and in the other group only the reduced pterin. It will be of great interest to learn (1) whether the allele producing malelike (white) females in the *Euchloe* group is dominant and (2) whether the allele producing malelike (colored) females in *Phoebis* and *Eurema* is recessive, as in *Colias*.

TABLE 9
Some Pieriididae with Two ♀ Forms: White and Colored

Comparative forms with monomorphic ♀ ♀ are in parentheses ( ).

Species	*Principal ♀ form*	*♂ color*	*Locality*
Appias albina dorada C. and R. Feld.	white	white	Ceylon, India
Appias paulina galathea Feld.	white	white	Nicobar Is.
Pieris sisymbrii Bdv.	white	white	W. No. America
Euchloe ausonides Bdv.	white	white	W. No. America
(*Euchloe charlonia doveri* Evans	white	yellow	N., W. Baluchistan)
(*Euchloe charlonia lucilla* Butl.	yellow	yellow	N.W. India)
Anthocaris sara Bdv.	white	white	W. No. America
(*Anthocaris pima* Edw.	yellow	yellow	Arizona)
Nathalis iole Bdv.	yellow	yellow	No. America
Kricogonia lyside Godt.	yellow	whitish	West Indies
Kricogonia castalia Fabr.	whitish	whitish	West Indies
Ixias pyrene cingalensis Moore	yellow	yellow	Ceylon
(*Ixias pyrene sesia* Fabr.	yellow	yellow	India)
Colotis calais modestus Butl.	salmon-pink	salmon-pink	Ceylon, India
Colotis fausta faustina C. and R. Feld.	salmon-buff	salmon-buff	No. India
(*Colotis fausta fausta* Oliv.	salmon-buff	salmon-buff	N.W. India)
(*Colotis fausta fulvia* Wall.	white	orange-yellow	Ceylon, S. India)
Catopsilia florella Fabr.	yellow	white	Africa, Asia
Phoebis sennae eubule L.	yellow	yellow	No. America
Dercas verhuelli doubledayi Moore	yellow	yellow	No. India, Burma
(*Dercas verhuelli parva* Evans	white	yellow	Burma)
Anteos maerula Fabr.	yellow	whitish	Neotropics
Gonepteryx rhamni L.	white	yellow	Eurasia
(*Gonepteryx cleobule* Hbn.	orange-yellow	orange-yellow	Canary Is.)
(*Gonepteryx farinosa* Zell.	white	yellow	Near East)
Eurema hecabe simulata Moore	yellow	yellow	Ceylon, India
Eurema hecabe contubernalis Moore	yellow	yellow	India, Burma
Eurema l. laeta Bdv.	yellow	yellow	Ceylon, India
Eurema lisa Bdv. and Lec.	yellow	yellow	No. America
Eurema jucunda Bdv.	yellow	yellow	S. No. America
Eurema gundlachia Poey	orange	orange	So. and No. America
Eurema xanthochlora pomponia Hopf.	yellow	yellow	Peru
(*Eurema candida papuana* Butl.	white	yellow	Australia)
(*Eurema messalina* Fabr.	white	white	W. Indies)
(*Eurema boisduvaliana* Feld.	yellow	yellow	Mexico)

5. *"Alba" Frequencies in Natural Populations*

a. *Long-Term Changes.* There is little evidence for or against the occurrence of a change in frequency of the "alba" allele over a period of years. Gerould (1923, 1941, 1946) has taken samples of *C. philodice* at Hanover, New Hampshire, during a period of 35 years (Table 10). Because the 1911 sample was taken early in the season, it probably is not reliable for a year-to-year comparison with the four late-summer samples, but from 1923 to 1945 there may have been a real increase in the

TABLE 10
"Alba" Frequencies in *C. philodice* at Hanover, N.H.

Year	*Months*	*"alba"* ♀♀	*yellow* ♀♀	*Frequency* (%)
1911	May–June	10	52	16.13 ± 4.68
1923	July–Aug.	4	37	9.76 ± 4.63
1940	Sept.–Oct.	42	202	17.21 ± 2.41
1943	Sept.–Oct.	161	528	23.37 ± 1.61
1945	Sept.–Oct.	26	98	20.97 ± 3.66

frequency of "alba" females. The difference in the 1923 and 1943 frequencies is barely statistically significant.

b. *Seasonal Changes.* Data on seasonal variation in the frequency of "alba" females are much better. Hovanitz (1944b) took a series of large samples of *C. eurytheme* in each of several localities in California. The most relevant serial samples are assembled in Table 11, a. In the San Fernando Valley the 25 September chrono-population has an "alba" frequency significantly higher than the populations of 22 June and 29 September–9 October. At Rosedale the late June population has a significantly lower "alba" frequency than do the August and September populations. No other pairs of chrono-populations in each locality show statistically significant differences in frequency, but there is a general suggestion in most localities that the "alba" frequency rises as the season advances. Since all of these samples were taken in California during the same year (1942), it is possible that unusual climatic events of that year produced natural selection favoring "alba" females over *aa* females, but that some or most other years would show the reverse trend. A similar increase in "alba" frequency in Japan is indicated by data in Table 11, b. Scudder (1889) long ago stated that the "alba" form of *C. philodice* is rare in the first generation and "is often very common late in the season." In spite of disagreement by Edwards (1878) and Gerould (1923), there is reason to suppose that Scudder was correct but probably overstated his case.

TABLE 11
Seasonal "Alba" Frequencies in *Colias* ♀♀

Locality	*Time*	% *"alba"* ♀♀	*Total* ♀♀
a. *C. eurytheme*, 1942, in California (from Hovanitz, 1944b)			
San Gabriel Valley	"Spring"	33.33 ± 7.27	42
San Gabriel Valley	May–June	28.15 ± 1.55	906
San Gabriel Valley	Late summer	33.37 ± 1.65	812
San Gabriel Valley	Autumn	28.75 ± 1.82	612
San Fernando Valley	25 May	30.57 ± 3.04	229
San Fernando Valley	22 June	28.43 ± 2.01	503
San Fernando Valley	25 July	35.97 ± 4.06	139
San Fernando Valley	1 Aug.	35.96 ± 4.50	114
San Fernando Valley	25 Sept.	37.00 ± 1.34	1292
San Fernando Valley	27 Sept.	32.82 ± 1.38	1161
San Fernando Valley	29 Sept.	29.00 ± 1.26	1307
San Fernando Valley	1 Oct.	30.04 ± 1.11	1708
San Fernando Valley	3 Oct.	28.09 ± 1.38	1068
San Fernando Valley	5 Oct.	26.01 ± 1.18	1380
San Fernando Valley	7 Oct.	30.00 ± 1.26	1330
San Fernando Valley	9 Oct.	27.77 ± 1.47	929
Rosedale	25 June	53.33 ± 1.31	1440
Rosedale	21 Aug.	64.16 ± 1.68	812
Rosedale	22 Sept.	67.65 ± 2.88	272
Base	25 June	52.68 ± 1.28	1528
Base	21 Aug.	53.73 ± 2.51	389
Base	25 Sept.	55.56 ± 4.86	188
Tracy	7 June	64.67 ± 2.37	300
Tracy	14–16 Aug.	67.88 ± 1.36	1180
b. *C. erate poliographus* (from Komai and Aé, 1953)			
Gotemba, Japan	June–Aug. 1949	68.57 ± 5.55	70
Gotemba, Japan	Sept.–Oct. 1949	95.65 ± 4.25	23
Gotemba, Japan	June–Aug. 1951	61.25 ± 5.45	80
Gotemba, Japan	Sept.–Aug. 1951	74.07 ± 8.43	27

c. *Changes through the Day.* The frequency of "alba" females of *C. eurytheme* in flight has been shown to change with some regularity during the course of each day (Hovanitz, 1948). In Table 12, a, the data are given for the three days on which Dr. Hovanitz took large samples through most of the flight period. These show that the "alba" frequency was higher early and late in the day than toward the middle of the day. They also show that the total number of females flying declined toward the middle of the day but that the orange females decreased propor-

tionately less than did the "alba" females. There was a subjective element in the collection of the data; the observer made the counts while walking rapidly through the infested field, and the specimens were not actually captured. It is not known whether the same frequency change

TABLE 12
Changes in Frequency of Flying "Alba" during the Day

Time	*Total* ♀♀	*"Alba"* ♀♀	*Frequency* (%)
a. *Colias eurytheme* in California (Hovanitz, 1948)			
Bakersfield, June 6			
8:50 A.M.–9:55 A.M.	431	220	51.04 ± 2.41
10:00 A.M.–11:10 A.M.	441	245	55.56 ± 2.37
11:30 A.M.–11:55 A.M.	177	82	46.33 ± 3.75
12:00 Noon–1:05 P.M.	298	145	48.66 ± 2.89
1:30 P.M.–2:30 P.M.	189	83	43.92 ± 3.61
3:00 P.M.–4:35 P.M.	420	203	48.33 ± 2.44
5:05 P.M.–6:15 P.M.	348	183	52.59 ± 2.68
Bakersfield, June 7			
6:55 A.M.–7:45 A.M.	203	121	59.61 ± 3.34
7:50 A.M.–8:30 A.M.	161	87	54.04 ± 3.83
10:00 A.M.–11:10 A.M.	527	272	51.61 ± 2.17
12:30 P.M.–1:30 P.M.	263	110	41.83 ± 3.04
1:40 P.M.–2:25 P.M.	222	85	38.29 ± 3.26
3:55 P.M.–4:55 P.M.	325	172	52.92 ± 2.77
5:00 P.M.–5:55 P.M.	237	128	54.01 ± 3.14
Brawley, June 10			
5:55 A.M.–6:50 A.M.	1113	264	23.72 ± 1.27
8:05 A.M.–9:00 A.M.	1158	226	19.52 ± 1.16
10:05 A.M.–11:00 A.M.	1043	175	16.78 ± 1.15
12:05 P.M.–1:15 P.M.	967	167	17.27 ± 1.19
2:30 P.M.–3:40 P.M.	1036	178	17.18 ± 1.14
5:20 P.M.–6:25 P.M.	1310	267	20.38 ± 1.01
b. *Colias croceus* in England (Ford and Dowdeswell, 1948)			
Penzance, August			
Up to 11 A.M.	34	13	38.24 ± 8.33
Noon to 3:30 P.M.	24	4	16.67 ± 7.61
After 5:30 P.M.	10	3	30.00 ± 14.91

characterizes *C. eurytheme* in other parts of its range; possibly in cooler and more northern localities the midday dip in "alba" frequency is slight. The Connecticut populations have proved to be too small to provide satisfactory data for comparison with those of Dr. Hovanitz. Ford and Dowdeswell (1948) took similar records for *C. croceus* in England. Although their numbers were too small to demonstrate the "alba" fre-

quency difference statistically, the figures indicate the same change found by Hovanitz. Their data are copied into Table 12, a.

The "alba" phenotype appears to be less able than the *aa* phenotype to tolerate the high temperature and strong sunlight of midday. Tables 12, a, and 13, a, show that at Brawley and other localities where the temperature and solar radiation are much higher than in the Bakersfield area, the general "alba" frequency is relatively much lower. Extrapolation of the trend suggests that no region is likely to have such high temperatures and solar radiation that the "alba" form will disappear from the population of *C. eurytheme*. Extrapolation in the other direction further suggests that no part of the range in which *C. eurytheme* can survive is so cool that the "alba" gene becomes fixed in the population.

d. *Geographic Differences.* There is abundant evidence to show geographic differences in "alba" frequency of several species of *Colias*. Gerould (1923) summarized records of *C. palaeno* in Europe, where the southernmost populations have the lowest "alba" frequency. Hovanitz (1944b) demonstrated conclusively that in the large portion of California where *C. eurytheme* occurs exclusively of *C. philodice* there is a steep and rather regular gradient between high "alba" frequencies to the north and low frequencies to the south. Table 13, a, shows a number of these frequencies arranged by the longitude of the localities.

The high northward frequency of "alba" females is not always found in other species. In New England *C. philodice* seems to show the reverse, with a southward series of frequencies of 0.10, 0.15, and 0.37, although some of the samples are small (Hovanitz, 1944c). Extension farther southward of this series is hindered by a large admixture there of *C. philodice* with *C. eurytheme;* many "alba" females of these species are difficult to place in the correct species.

In the Andes Mountains of Colombia and Ecuador Hovanitz (1945a) obtained substantial samples of *Colias dimera* Dbldy. and Hew. in eight localities. Frequency data for these samples are given in Table 13, c. An even latitudinal trend is not apparent, but the lowest "alba" frequency was found at the lowest altitude, and the highest frequency was found at the highest altitude. The reason for the great change in "alba" frequency from that at 2600 meters to that at 2700 meters is not clear.

In Japan, where "alba" females outnumber *aa* females of *C. poliographus*, northern Honshu populations have a significantly higher "alba" frequency than do southern Honshu and Kyushu populations (Komai and Aé, 1953). Frequencies in seven localities are shown in Table 13, b.

Hovanitz (1950) has recently prepared a comprehensive survey of his records of the frequency of the "alba" allele in numerous North American populations of fifteen species of *Colias*. He has illustrated geo-

TABLE 13
Geographical Changes in Frequency of "Alba" ♀♀

Longitude	*Frequency* (%)	*Locality*
a. *Colias eurytheme* in California, all 1942 (Hovanitz, 1944b)		
38°0′ ±	66.04 ± 3.70	San Francisco Bay
37°44′	67.50 ± 1.22	Tracy
37°23′	72.60 ± 0.96	Westley
36°57′	61.72 ± 1.69	Madera
36°20′	59.48 ± 1.88	Hanford
35°50′	65.37 ± 1.59	Allensworth
35°35′	63.08 ± 0.89	Wasco
35°25′	58.36 ± 1.09	Rosedale
35°14′	53.11 ± 1.09	Base
34°43′	38.83 ± 4.81	Lancaster
34°36′	34.41 ± 2.28	Palmdale
34°33′	39.34 ± 6.25	Bouquet Canyon
34°16′ ±	30.30 ± 0.42	San Fernando Valley
34°04′ ±	30.19 ± 0.94	San Gabriel Valley
33°47′	29.41 ± 2.02	Santa Ana
33°47′ ±	22.65 ± 1.82	San Jacinto Valley
33°36′	12.95 ± 2.85	Thermal
33°08′	18.47 ± 2.19	San Pasqual-Escondido
33°08′	17.80 ± 1.49	Kane Springs
32°58′	14.48 ± 2.06	Brawley
32°40′	12.99 ± 1.57	Calexico
b. *Colias erate poliographus* in Japan (Komai and Aé, 1953)		
38°16′	75.44 ± 5.70	Sendai (1951)
36° ±	74.25 ± 1.79	Oiwake (1950)
36° ±	77.27 ± 4.47	Tokiwa (1951)
35°25′ ±	73.53 ± 7.57	Arakawa (1940)
35°19′	70.97 ± 3.08	Gotemba (1949, 1951)
35°0′	55.96 ± 4.75	Kyoto (1951)
33°34′	53.42 ± 5.82	Fukuoka (1949)
c. *Colias dimera* in the northern Andes Mts. (Hovanitz, 1945a)		
6°50′ N.	34.17 ± 3.06	Cerrito, Colombia (2700 m.)
5°40′ N.	14.85 ± 1.83	Tunja-Paipa, Colombia (2600 m.)
4°36′ N.	12.67 ± 1.75	Bogotá, Colombia (2600 m.)
2°25′ N.	56.19 ± 4.84	Puracé, Colombia (2800 m.)
1°14′ N.	51.01 ± 4.10	Pasto, Colombia (2700 m.)
0°50′ N.	75.00 ± 3.00	Ipiales, Colombia (2950 m.)
0°14′ S.	48.54 ± 2.65	Quito, Ecuador (2800 m.)
1°40′ S.	49.32 ± 3.36	Riobamba, Ecuador (2800 m.)

graphic differences in frequency with several maps showing an exact figure calculated for each locality. His summary of the geographic correlations is worth quoting *verbatim:*

> With but few exceptions, the frequency of the white females in all *Colias* described has been highest in the northwestern part of North America. For those species that extend that far, the area of highest concentration of the responsible dominant allele is in central Alaska. For most species, the area of lowest concentration of the white allele is the prairie region of southern Alberta and Saskatchewan. Notable exceptions to this generalization are to be found in the *C. christina–alexandra* complex. However, as will be shown in a later detailed study of species habitats, these discrepancies are accounted for by the nature of the specific habitat occupied by this species. Other significant occurrences of a low frequency of the white female in an area expected to be high (such as *C. philodice* in New England) are as yet unaccounted for.

Of the thousands of specimens included in the 1950 survey, perhaps the majority are from field samples taken by Dr. Hovanitz. However, he included many hundreds of specimens examined in most of the North American museums (see also Hovanitz, 1944c). Without any doubt, these museum specimens usually represent biased samples, especially for populations in which the real "alba" frequency was high or low. One example to be found in earlier papers (Hovanitz, 1944b, 1944c) illustrates this point. Museum material of *Colias eurytheme* showed "alba" frequencies of 0.47 in northern California and 0.43 in southern California, but very large random samples of the populations gave "alba" frequencies of 0.64 in northern California (11,616 ♀ ♀) and 0.26 in southern California (8,412 ♀ ♀). Generalizations drawn from such biased samples cannot be accepted without reservation. No distinction is made in the tables to show which are the Hovanitz samples and which are museum samples. A further question comes from the failure to include temporal data. As discussed above, it seems likely that in any one population the frequency of the "alba" allele varies from year to year and for multibrooded populations fluctuates from generation to generation each year. Furthermore, there are indications in some regions that the frequency has continued to decrease or increase, essentially in one direction, for long periods. Many of the museum specimens used by Hovanitz were presumably very old and not necessarily comparable with samples of recent populations. And finally, my large reared series show that the correct species of a substantial proportion of the "alba" females of *C. philodice* and *C. eurytheme* cannot be positively determined when nothing is known of the brothers, *aa* sisters, or parents of a given "alba" female. Therefore, in most U.S.A. localities other than California, the confusion of species may be expected to weaken gene-frequency generalizations for these two species and certain other difficult species-pairs. It is quite possible that

most of the stated frequencies are correct and that new information will simply strengthen the correlations drawn, but no fully satisfactory basis exists in the 1950 paper for weighing the conclusions.

6. *Similar Genes in Some Other Animals*

Sex-limited genes in Lepidoptera are probably of very wide occurrence. In the Papilionidae, as in the Pieriididae, these seem to be expressed only in females. The inheritance of the dark form of the North American *Papilio glaucus* has not been definitely worked out, but it appears only in the female and is probably controlled by a dominant allele. Most of the females east of the Great Plains and south of 40° N. latitude are of this "glaucus" form, with the usual yellow ground-color of the wings completely suffused with blackish brown. North of this region and in peninsular Florida the dark female is absent except in a rather steep cline, with the dark form disappearing. To the north all females are of the "turnus" form, with the familiar "Tiger Swallowtail" colors. By analogy from Old World mimicking complexes, it has been claimed that the dark female mimics a distasteful model, presumably *Battus philenor* L. (Mead, in Edwards, 1877). The evidence for mimicry in this case is nonexistent at present. That there is sex-limited inheritance with the dark allele dominant is suggested by one case reported by Edwards (1877), as follows. John Akhurst reared from a single wild yellow female from Brooklyn, New York, "a large number of butterflies." Two F_1 females were dark; the others (number not stated) were yellow. Assuming that these two female forms are controlled by a single pair of alleles, the question arises whether they are sex-linked or autosomal, and whether the dark allele is dominant or recessive. The crosses in which a yellow mother could have produced both yellow ("turnus") and dark ("glaucus") daughters are diagrammed below. *G* and *g* are used to represent the two alleles with the allele for the dark female dominant; *T* and *t* represent these alleles where the one for the yellow female is considered dominant.

1. Autosomal, "glaucus" dominant:

   ♀ *gg* × [♂ *Gg*] → ♀ *Gg* (dark): ♀ *gg* (yellow) + ♂♂.

2. Autosomal, "turnus" dominant:

   a. ♀ *Tt* × [♂ *Tt*] → 1 ♀ *TT* (yellow): 2 ♀♀ *Tt* (yellow): 1 ♀ *tt* (dark) + ♂♂.

   b. ♀ *Tt* × [♂ *tt*] → ♀ *tt* (dark): ♀ *Tt* (yellow) + ♂♂.

3. Sex-linked, "glaucus" dominant:

   ♀ *g*W × [♂ *Gg*] → ♀ *G*W (dark): ♀ *g*W (yellow) + ♂♂.

4. Sex-linked, "turnus" dominant:

$$\text{♀ } TW \times [\text{♂ } Tt] \rightarrow \text{♀ } tW \text{ (dark)} : \text{♀ } TW \text{ (yellow)} + \text{♂♂}.$$

In view of the extreme rarity of the "glaucus" females in Brooklyn, the probability is very low that both parents could have carried the "glaucus" allele. Possibilities 2a and 2b are thus the least likely. All three other alternatives require the mother to have been pure for the "turnus" allele and the father heterozygous. Sex-linked genes expressed solely in

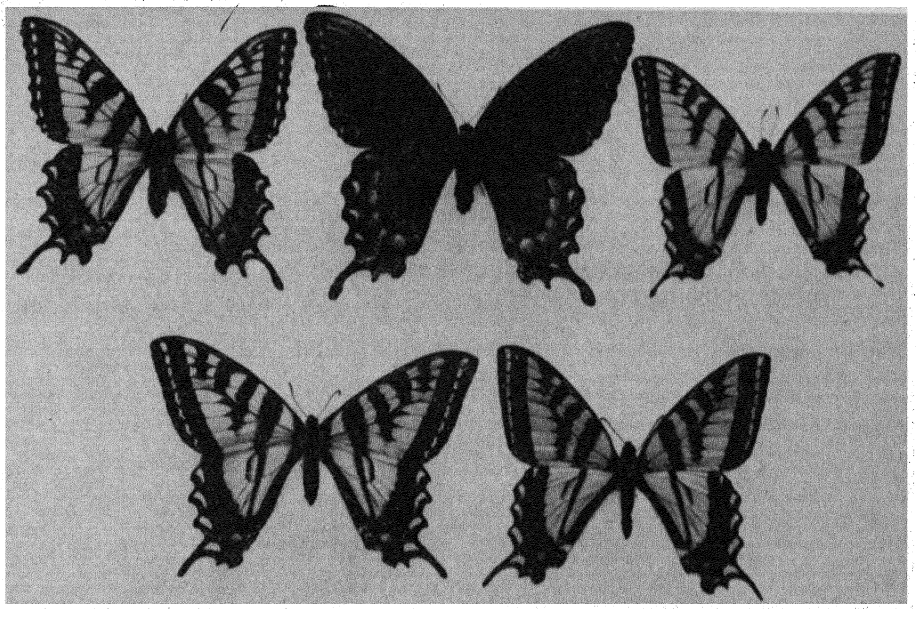


FIG. 4. *Papilio glaucus* (above) and *P. rutulus* (below), allopatric sibling species from North America. *P. glaucus* has female polymorphism, with a malelike form (left, above) and a black form (center, above). *P. rutulus* has only the malelike female. The right specimen, above and below, is the male.

one sex are very unusual; on the other hand, in butterflies autosomal sex-limited genes expressed only in the female are well known (*vide Colias* "alba," above, and *Hypolimnas* and *Papilio* female polymorphics, below). Consequently it is to be expected that the "glaucus" allele will prove to be an autosomal dominant. The physiological expression of the "glaucus" gene is probably intracellular, like that of the "alba" gene in *Colias*, not limited sexually by a hormone distributed in the blood. Bilateral gynandromorphs of *P. glaucus*, with the female wings dark, have been found from time to time. It is interesting to note that the allopatric sibling species, *Papilio rutulus* Lucas, shows no female dimorphism, although the latitudinal range is essentially the same as for *P. glaucus;* these two species are shown in Fig. 4.

Several mimicking species of *Papilio* exhibit extreme female polymorphism, with the males probably carrying the genes but never expressing them. Each of several discontinuous female forms mimics a different distasteful species. Genetical studies of three of these species have shown that the strikingly different female forms are controlled by simple autosomal Mendelian factors. In *P. memnon* a single dominant allele converts the tailless female form "isarcha" into a female form "achates," which has the familiar tails found on many Swallow-tail butterflies. The other sex-limited genes known for *P. memnon*, and for *P. polytes* and *P. dardanus*, affect the colors and wing-patterns and are thus similar in action to the "alba" gene of *Colias*. Species of the nymphalid genus *Hypolimnas*

TABLE 14
Genotypes of Mimicking ♀ *Papilio* and *Hypolimnas*

	♀ *Form A*	♀ *Form B*	♀ *Form C*
P. memnon L. (Indomalayan)	*LLAA, LLAa, LlAA, LlAa* ("achates" ♀)	*LLaa, Llaa* ("isarcha" ♀)	*llAA, llAa, llaa* ("laomedon" ♀)
P. polytes L. (Indomalayan)	*CCRR, CCRr, CcRR, CcRr* ("romulus" ♀)	*CCrr, Ccrr* ("polytes" ♀)	*ccRR, ccRr, ccrr* ("cyrus" ♀)
P. dardanus Brown (African)	*CCHH, CCHh, CChh, CcHH, CcHh, Cchh* ("cenea" ♀)	*ccHH, ccHh* ("hippocoon" ♀)	*cchh* ("trophonius" ♀)
H. misippus L. (African)	*MM, Mm* ("misippus" ♀)	*mm* ("inaria" ♀)	

may have nonmimicking males, with the females polymorphic and mimicking distasteful butterflies of the family Danaidae. In *H. misippus* L. the genic control of the two female forms is known to be by a single pair of sex-limited alleles (Anonymous, 1911, 1912b). *H. dubius* Beauv., however, has both sexes mimetic and dimorphic, with the form "dubius" controlled by a presumed autosomal dominant allele and the form "anthedon" by the recessive allele (Anonymous, 1912a). The known genotypes of female forms of *Hypolimnas misippus* and the three species of *Papilio* are shown in Table 14. It is assumed that these are autosomal, not sex-linked genes, but for *H. misippus* and probably the *Papilio* species the data are inadequate to exclude sex-linkage.

Gerould (1923) has suggested that in flight the "alba" females of *Colias* closely resemble various species of *Pieris*, but he noted that no

protective qualities are known for *Pieris*, and he therefore referred to this as pseudo-mimicry.

One of the Hesperiidae, *Poanes hobomok* Harris, has two female forms commonly co-occurring in many populations in eastern North America. These forms are comparable to the "glaucus" and "turnus" forms of *Papilio glaucus* females. The distribution of the dark female known as

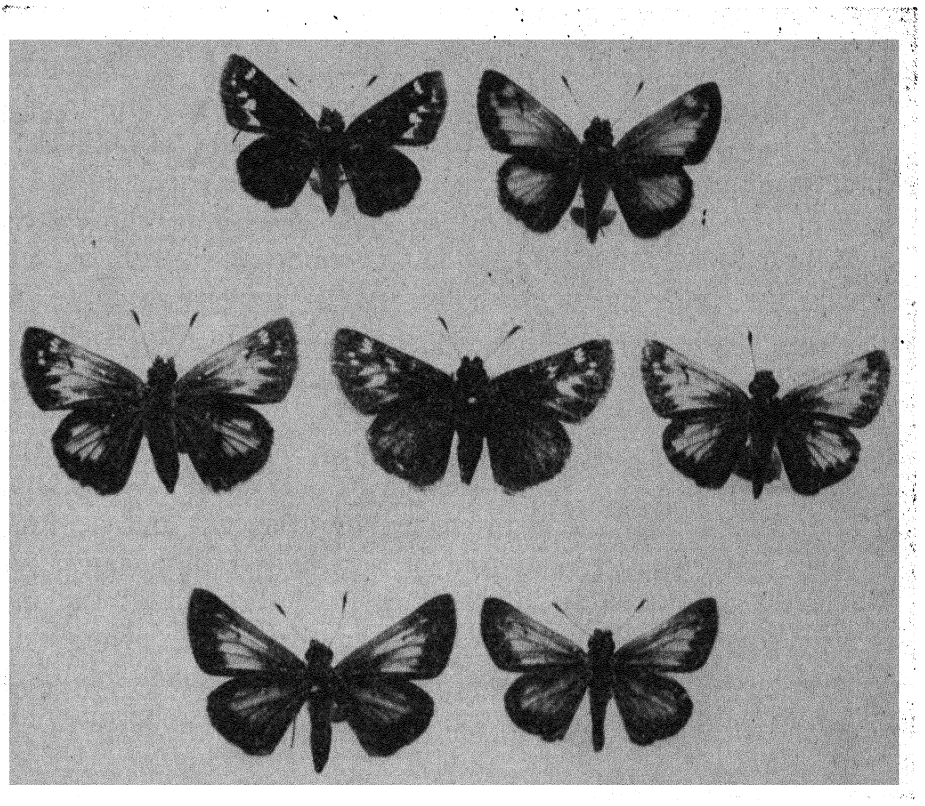


FIG. 5. Three species of *Poanes* (Hesperiidae), showing notable differences in the color of the female. *P. zabulon* (above) has the female always dark, unlike the male. *P. aaroni* (below) has the female always malelike. *P. hobomok* (center row) has the female dimorphic, with one form malelike (left) and the other dark like the female of *P. zabulon*. The pair of *P. aaroni* is from New Jersey; the specimens of *P. zabulon* and *P. hobomok* are from Connecticut. The right specimen in each row is the male.

"pocahontas" is the reverse of that of the dark female of *P. glaucus*, in that the lowest frequency of the dark form is in the southern part of the range. Nothing is known of the inheritance of these two sex-limited forms of *P. hobomok*. Other species of *Poanes* are monomorphic in both sexes, but the females may be orange-yellow and malelike, as in the "hobomok" form (*e.g.*, *P. aaroni* Skin.), or blackish and conspicuously different from the males, as in the "pocahontas" form (*e.g.*, *P. zabulon* Bdv. and Lec.). Thus, one or the other allele is fixed in most species of *Poanes*, but in *P.*

hobomok both occur, suggesting adaptive superiority of the heterozygote. The three species of *Poanes* noted are shown in Fig. 5.

In a moth, *Spilosoma lutea* Hufn., Lempke (1948) has shown that the sex-limited dark female form is probably controlled by a recessive autosomal allele.

Few sex-limited forms with male polymorphism are known to lepidopterists. One is the yellow-or-white dimorphism of *Parasemia plantaginis* L. This case has been discussed by many authors, and the dominance of the simple white allele was finally demonstrated by Suomalainen (1938). One of his broods, resulting from the cross of a white male with its sib, contained 41 white males, 10 yellow males, and 24 females. This must have been a cross of heterozygous sibs with an expected 3:1 ratio ($\chi^2 = 0.791$; $P = > 0.30$). The "basipuncta" gene, controlling a yellow spot at the base of the forewing in male *Lasiocampa quercus* L. of Europe, is a recessive sex-limited gene (Lempke, 1951). The only example known to me of conspicuous male sex-limited dimorphism in a butterfly is the "nig" form in *Lycaenopsis pseudargiolus* in the southeastern United States. Its genic control has not been studied.

Genes expressed only in one sex are almost unknown outside the insects, but it is possible that many rare genes now considered sex-linked, in man and other animals, will prove to be autosomal and sex-limited. Perhaps the first recorded example of sex-limited inheritance is the horned-hornless gene of sheep. This differs from the "alba," "glaucus," and other genes of Lepidoptera, because sexuality merely reverses dominance; females and males are phenotypically alike for each homozygous genotype, but the heterozygotes are horned in one sex, hornless in the other.

VI. Other Genes Affecting Wing-Markings

1. "*White Males*"

The occurrence in *Colias* of males with the ground-color white, as in "alba" females, is rare but has been known for many years. The earliest published record I have found is by Edwards (1892), for *Colias philodice*, but there are probably earlier European references. Gerould (1911) mentioned two other white males of *C. philodice*. He was misled by these instances into thinking that they represented the homozygous *aa* males which rarely survive, but we now know that this whiteness is inherited independently of the "alba" gene. White males are also known for *C. phicomone* (Verity, 1911); *C. hyale* (Metschl, 1922); *C. eurytheme* (Hovanitz, 1944a); *C. aurorina* Herr.-Sch. (Reichel, 1950); *C. croceus* (Warrier, 1951); *C. erate poliographus* (Komai and Aé, 1953); and probably other species.

Detailed comparisons of various white males of *C. philodice* and *C. eurytheme* (Hovanitz, 1944a) and *C. poliographus* (Komai and Aé, 1953) show that phenotypically these specimens are a diverse lot. It is likely that there are several different genes which can produce the whitish ground-color.

The whitish pigment is supposedly due to leucopterin, as in the "alba" females. The "alba" substance is leucopterin, which is oxidized xanthopterin, which is doubtless the ground-color pigment of normal males of *C. philodice* and *C. poliographus*. It would not be surprising, therefore, to find that leucopterin is formed with several quite different oxidizers, each controlled by a separate mutant allele. Some blanching agents may simply produce leucopterin in the usual sites of xanthopterin, but some may decolorize other pigments as well, producing varying degrees of extreme albinism. Certain generalizations are now possible from the known cases of these "white male" types.

1. These genes, unlike the "alba ♀" gene, are recessive to the alleles for yellow or orange pigment.

2. The genes are not sex-limited, males and females occurring with expected frequency in laboratory broods; most of these recessive alleles produce in females phenocopies so similar to the familiar "alba" females that only the males are noticed in the field by collectors.

3. Viability is usually greatly reduced in recessive whites, with many dying before development has been completed.

4. The alleles are probably rather frequent in heterozygotes in some wild populations, perhaps maintained by heterosis, but the low viability of homozygous recessives keeps recognizable individuals very rare in nature.

These "white male" forms have been reared from at least six different wild female stocks of four species of *Colias*. In three instances offspring were obtained from the recessive whites.

1. The most "alba"-like form was discovered among the F_2 of a wild *C. philodice* (♀ 58) from Connecticut (Remington, 1954a). A normal yellow female produced 24 males and 34 females, all with pure yellow ground-color. From two brother-sister crosses 6 pure yellow F_2 of ♀ 58 were reared, but from the third sib cross a white male and 3 females and 6 males, all normal yellow, were reared. This white male had the same ground-color as many "alba" females (sex-limited dominant gene) and showed normal viability. It was mated with a normal sister, but the few ova she laid failed to hatch. The white male again copulated, this time with a nonsib, ♀ 62C, which had 8 "alba" sisters, 8 yellow sisters, and 14 yellow brothers. This cross produced 9 females and 14 males, all wild-type yellow, as expected. Four brother-sister matings, obtained with

great difficulty owing to the lateness of the season, yielded only 9 F_2 of the white male; 3 females and 5 males had the wild-type yellow coloration, but the sixth male had extensive white areas on otherwise yellow wings. Assuming that this semiwhite male is homozygous for the white allele, the expected ratio of 3 yellow to 1 white was not significantly missed ($\chi^2 = 0.926$, $P = > 0.30$). It has been established that this white form is probably controlled by a single autosomal recessive allele.

2. The same summer a second wild yellow female *C. philodice*, from the same clover field in Connecticut, produced 6 F_1, of which 4 females and the only male are whitish. The male and 1 female are pale creamy yellow ("blonde") with the hindwing cell spot completely decolorized. The other 3 white females look phenotypically normal for the dominant "alba" allele. None of the "blonde" sibs copulated, but an "alba" sister (♀ 75A) was outcrossed to a male probably homozygous for the recessive allele of the "alba" gene. From this cross 14 "alba" females, 20 yellow females, and 27 yellow males were reared; as expected, none appears to be "blonde." Ninety-four F_2 of ♀ 75A were reared from 8 brother-sister crosses: 1 "blonde" female, 11 "alba" females, 33 yellow females, and 49 yellow males. The "blonde" female had two sibs, a yellow male and a yellow female. Since one-half of the F_1 of ♀ 75A were expected to be homozygous for the wild-type allele and one-half heterozygous for the "blonde" allele, it was expected that in one-fourth of the brother-sister matings the male and the female would both be heterozygous. These would be expected to produce 25% "blonde" offspring. Thus, about 6 of the 94 F_2 of ♀ 75A would be expected to have been "blonde." The one actually observed gives a low probability that such a deviation is due to chance alone (P = between 0.02 and 0.05). Low viability for the "blonde" homozygotes may explain these findings. This "blonde" form seems also to be controlled by a simple recessive autosomal allele, quite likely at a different locus from that in the 58H brood.

3 and 4. Komai and Aé (1953) reared a white male in the F_2 of each of two broods of *Colias erate poliographus*. One seemed to have normal viability and was "perfectly white." The other male was slightly yellowish, and its veins failed to harden—a lethal condition in the reproductive sense. All sisters of both white males were said to be "alba." No further rearings are mentioned for either brood. Several important details were omitted from the paper.

5. Hovanitz (1944a) reared 2 white males and a white female among the 28 F_2 of a wild *C. eurytheme* female from Rio Hondo, California. The original parent, all of her 33 F_1 females, and 34 F_2 females in two broods included only pure orange adults. The 6 female sibs of the white female and males were likewise orange. This white form was clearly controlled

by an autosomal recessive allele. Phenotypically, it was at best semi-lethal; there were various abnormalities in expansion and hardening of the wings, pigmentation of the blood, and functioning of the proboscis. One-fourth of this brood would be expected to have been white; the lower observed frequency may have been due to some pre-imaginal lethality. None of these white *C. eurytheme* mated, and all their sibs were accidentally killed.

6. Warrier (1951) reared a substantial brood of *C. croceus* from England showing an extremely albinic form ("cinerascens"). Unlike the other recessive whites considered above, Warrier's *C. croceus* had the normal black markings almost completely blanched. This was a particularly interesting brood because the dominant sex-limited "alba" allele was also present. From a wild "alba" female, Warrier reared 22 normal orange and 10 "cinerascens" males, 10 normal orange females, 9 normal "alba" females, 2 orange-type "cinerascens" females, and 4 "alba"-type "cinerascens" females. The 12:13 ratio of orange-type to "alba"-type females indicates that for the "alba" gene the wild female was heterozygous (*Aa*) and her unseen mate homozygous (*aa*) (see Table 4, above). The ratio of 41 normal to 16 "cinerascens" sibs is near a 3:1 ratio ($\chi^2 = 0.2865$, $P = > 0.05 < 70$), indicating that the "cinerascens" allele is recessive and that the parents were both heterozygous for it. It is unexpected that two wild *C. croceus* were carrying an allele so rare phenotypically as this one undoubtedly is. Warrier found the mating vigor of all the "cinerascens" sibs very low. None of the males would mate, but after several attempts, a "cinerascens" female copulated with a normal male sib and then did not oviposit. Later this female paired with a male from an unrelated brood. It laid 90 eggs, from which 72 F_1 were reared. Of these, 41 males and 18 females had normal orange wings, 12 females were "alba," and 1 male was "cinerascens." Three brother-sister pairings from these produced 31 plus "a considerable number of offspring," but because of winter food-deficiencies they had to be ill-fed. No imagines which eclosed were of the "cinerascens" form. Cockayne (1951) reasonably suggested that there was selective mortality of "cinerascens" in the last two generations, which were reared under unfavorable conditions.

2. *Melanistics*

Extreme melanism characterizes the wild type of a few scarce species of *Colias*. In most species, individuals with a strong suffusion of dark pigment are very rare. However, their repeated though infrequent occurrence, known for several species, suggests that these forms express a regular part of the normal genic variability of most *Colias* populations

and that these forms remain rare because they are adaptively inferior in present-day environments. Individuals fully suffused with black or dark gray have been recorded for *C. philodice* from Palmyra, New York; Montreal, Quebec; and Plainfield and Concord, Massachusetts (Scudder; 1889; Edwards, 1877–1878; Gerould, 1911), for *C. eurytheme* from Webster Groves, Missouri (O'Byrne, 1932), and for *C. euxanthe* Felder from the Andes Mts. (Fassl, 1915). Unrecorded melanic specimens of *C. electo* from Africa and *C. alexandra* from Wyoming are in the collections, respectively, of the American Museum of Natural History and Yale University.

Other blackish forms of normally light-colored Pieriididae are known. A melanic male of the almost pure white *Pieris napi pseudonapi* B. and McD. has been recorded from Colorado (Remington and Remington, 1943). There is a less extreme melanic form (known as "phileta") of female *Ascia monuste* L. in the southeastern United States. The dark female form appears not to be induced genetically, but rather environmentally, with crowding during larval growth. The dark females thus produced become migratory and perhaps gregarious (Nielsen and Nielsen, 1950) and are therefore biologically comparable to the migratory phase of grasshoppers in various parts of the world.

Nothing seems to be known of the genetics of the melanic forms of *Colias*. The genetically known melanic forms of various moths in industrial areas have usually proved to be controlled by unifactorial dominant or semidominant alleles (see the recent review by Ford, 1953). However, it would be unwarranted to generalize for the Pieriididae from results with these families of moths. Gerould (1923) noted in passing that his O^{77} ♀ of *C. philodice* was "a melanic mutant" and that the F_1 brood "mc" was melanic. Female O^{77} was mated with a male from the field (and presumably homozygous nonmelanic). It is therefore likely that this melanic allele is dominant over the wild-type allele. Gerould gave no description of the O^{77} ♀ or her offspring, but he has informed me (*in litt.*) that the female progeny were "more than commonly swarthy beneath," so this brood presumably is not of the heavily and generally suffused type described above.

3. *Discal Spot*

All species of *Colias* have a prominent discal spot in the hindwing. The spot is typically of various shades of yellow or orange in yellow *Colias*, like *C. philodice* or *C. hyale*, and reddish in orange forms, like *C. eurytheme* and *C. croceus*. The variability in the color of the spot in yellow forms is much greater in some species (*e.g.*, *C. philodice*) than in others (*e.g.*, *C. alexandra*). Komai and Aé (1953) found that in male *C.*

poliographus the allele for orange spot is probably dominant over the allele for yellow spot.

In Connecticut *C. philodice* it appears that in the male the yellow and orange spots are under unifactorial control, with the allele for yellow recessive to the allele for orange (Remington, 1954b). The homozygous recessive female shows a pale orange spot rather than the yellow of the homozygous recessive male. All populations of *C. philodice* probably exhibit marked genetic polymorphism in this spot.

4. *Inheritance of Orange and Yellow*

Several of the species of *Colias* are reported to have both yellow and orange forms, although the record is confused. *C. chrysotheme* Esper, *C. myrmidone* Esper, *C. aurora* Esper, and *C. diva* Grum. are usually orange but are said to have yellow females occasionally. *C. erate* is usually yellow, but an orange form is known for both sexes. *C. aurorina* seems to be generally orange, but race *libanotica* Led. is yellow. In *C. wiskotti* Stgr. males are usually orange-yellow and females usually orange but occasionally yellow. The somewhat related genus *Eurema* includes similar orange-yellow dimorphism, notably in the orange *E. nicippe* Cramer, with its rare yellow form. The inheritance of the two forms is unknown in all these examples. The two genetically best known *Colias* are the North American *C. eurytheme* (orange) and *C. philodice* (yellow). There is free hybridization between these close relatives, and some evidence has been found concerning inheritance of the orange and yellow parental types. Gerould (1943) obtained progeny from several crosses between orange and yellow *Colias*. Some F_1 hybrids were then intermated, and others were crossed with both parental types. Climatic conditions have a marked effect on the intensity of orange color (see below), individuals genetically pure orange showing a great increase in yellow color during the early spring and late fall (Gerould, 1943; Hovanitz, 1945b). Unfortunately, Gerould's F_2 and backcross broods emerged in late fall and are not as instructive in considering inheritance of orange and yellow color as broods emerging in summer would be. Nevertheless, it is clear that the F_2 may include both the pure orange and the pure yellow parental types. The phenotypic alteration of the orange pigment may account for the spread in the F_1 and the apparently evenly graded series in F_1, F_2, and backcross broods found by Gerould and Hovanitz (see Hovanitz, 1944a, Table 1) and confirmed in my unpublished results from Connecticut crosses. When F_1 hybrids are crossed with pure yellow *C. philodice*, about one-half of the progeny are phenotypically pure yellow, and one-half are like the F_1 hybrid parent. The sum of the published evidence and my newer data shows that it is possible that the orange and yellow wing-colors are con-

trolled by one or at most two pairs of genes having no dominance. Hovanitz (1944a), without adequate evidence, has stated: "The segregations show that the factors controlling the orange coloration are multiple factor ones." He mentioned rare yellow "sports" in pure orange populations, but apparently no genic analysis was attempted. These may have been either immigrants or mutants carrying exactly the same yellow allele as *C. philodice.*

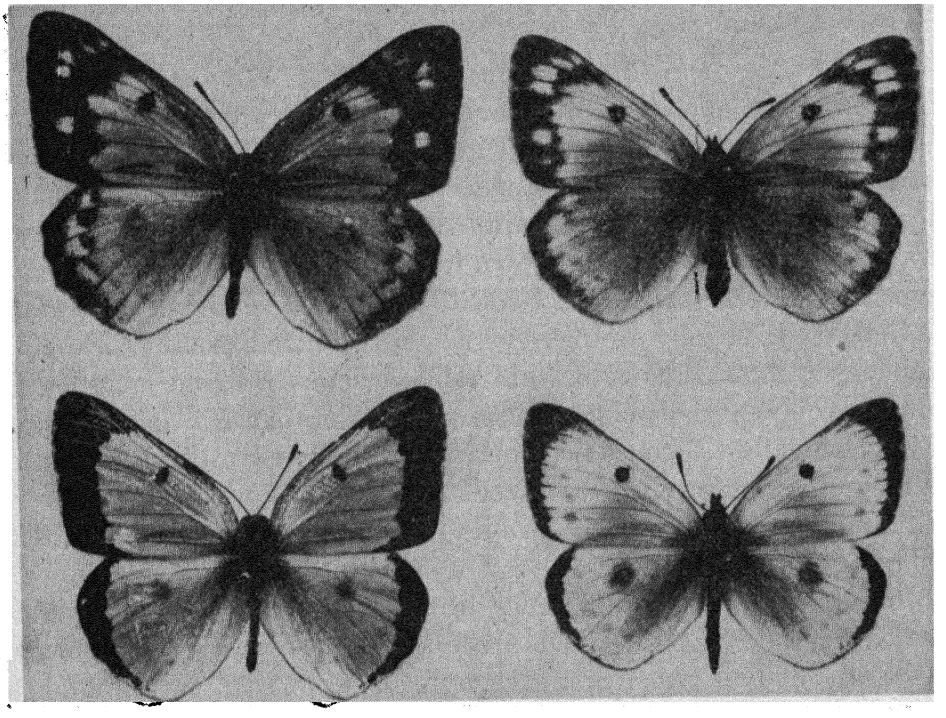


FIG. 6. Seasonal (nongenetic) variation in *Colias eurytheme.* The two pure orange summer sibs (left) are F_1 of a wild Connecticut female (No. 61). The two winter sibs (right), reared in a greenhouse, are F_3 of female No. 61. In the photograph orange color is made to appear dusky and yellow color whitish. The winter sibs show the usual orange ground-color replaced by yellow in the areas farthest from the body; the black margins are also greatly reduced, and the size is somewhat smaller. Photographed with a K-2 filter to increase the contrast between orange and yellow.

The climatic factors causing the marked decrease in orange pigment on the outer half of the wings in early spring and late fall have not been certainly determined. Pure *C. eurytheme* broods reared in midwinter in Connecticut in my greenhouse are fair duplicates of genically F_1 hybrids reared in midsummer (see Fig. 6). F_1 hybrids, on the other hand, show winter coloration much less orange than their midsummer genic counterparts and have narrower black margins. Temperature cannot be the only factor, and it is not likely to be the primary one. Day-length and perhaps the average total hours of unclouded sunlight per day appear to be prin-

cipal causes of the yellow shift in winter. They may act directly on the butterflies or nutritionally by changing the foodplants. An opposite effect in winter is seen on the underside of the wing; this becomes noticeably darkened, by an increase in black scales scattered over the surface. In wild populations it is well known that all spring individuals of *C. eurytheme* are small, yellowed above, and darkened below, and that such individuals may sometimes be found in early winter; this form is known as "ariadne." *C. philodice* also shows the reduction in size and the darkening below, but the yellow pterin pigment seems to be unaltered.

In Lepidoptera environmentally induced variations of striking types seem to be commoner than conspicuous genetic variations. These have been reasonably well described for most butterflies, although in the past there were many instances in which two seasonal forms were regarded as separate species. The published analyses of the control of variants such as "dry" and "wet" forms and green and brown chrysalids are often flatly contradictory, and much carefully controlled experimentation, associated with chemical analyses, is needed. The role of genes in permitting these environmental variations and the adaptive significance of such nonhereditary polymorphism are challenging questions for every naturalist.

I am grateful to B. W. McFarland, my colleague on the staff of the Connecticut Agricultural Experiment Station in New Haven, who prepared the illustrations with me. All are new for this paper.

VII. Summary

1. The genus *Colias*, with about 60 known species, is represented in most parts of the world. There appear to be close genic interrelations among the species, and natural hybridization occurs between many pairs of species, particularly in North America.

2. The Lepidoptera known cytologically are characterized by the ZO and ZW type of sex chromosomes, with the female heterogametic (ZO or ZW) and the male homogametic (ZZ). Secondary sexual characters are independent of gonadal control or of hormones circulating in the hemolymph. A new bilateral gynandromorph of *Colias philodice* is figured. The right side is female and "alba," and the left side is male and normal yellow except for "alba" patches in the distal part of the hindwing. It is suggested that these patches are produced over cells which have become femalelike by losing one Z chromosome late in development, with the entire insect presumed to have a uniform *Aa* or *AA* genotype for the "alba" genes. It is not known whether *Colias* females are ZO or ZW. The chromosome number for the five species studied is

typically 31 or 32. As in Diptera, it appears that crossing over in Lepidoptera may be confined to the homogametic sex (male).

3. The inheritance is known for two genes, "blue-green" and "olive-green," expressed in *Colias* larvae. Each is recessive to the wild-type allele. With one the larva and the imaginal eyes are bluish. With the other the larva and the imaginal eyes are yellower than usual. When a larva is homozygous for both "blue-green" and "olive-green," the "blue-green" seems to mask the "olive-green"; the F_1 of heterozygous parents give a 9:4:3 ratio of forms. Predator-pressure probably is important in keeping the frequency of these genes very low in natural populations.

4. Most species of *Colias* have populations in which the females are dimorphic for expression of two alleles at the "alba" locus. "Alba" is a dominant autosomal gene and is sex-limited, being expressed only in females. Its suppression in males is attributed to the "andrases," or chemical maleness of each cell.

5. The viability of the "alba" phenotype differs in various environments. This difference may be regarded in terms of the presence or absence of lethal and semilethal modifiers or in terms of viability modifiers being expressed or unexpressed in different physicochemical *milieux*. In general, for each species of *Colias* the frequency of the "alba" phenotype is highest: (a) in the north; (b) at the highest elevations; (c) in the earliest seasonal broods; and (d) at the earliest (coolest) part of the day.

6. The wing-colors of the Pieriididae may be primarily orange, yellow, or white. The pigments belong to the class known as pterins; the red pigment is erythropterin, the yellow is xanthopterin, and the white is leucopterin. It has been shown that leucopterin is oxidized xanthopterin. "Alba" females of *Colias* differ from yellow females in this apparently simple oxidation.

7. Many genera of Pieriididae other than *Colias* have dimorphic species, with one form white and the other yellow or orange. In some this is female sex-limited; in others both sexes are dimorphic.

8. Outside the family Pieriididae there are several more or less well-known species with balanced sex-limited female polymorphism. The inheritance of these forms is known in part for *Papilio memnon*, *P. polytes*, *P. dardanus*, and *Hypolimnas misippus*. Little is known of the heredity of the balanced dimorphism in females of *Papilio glaucus* and *Poanes hobomok*, but in each it is suspected that the allele controlling the blackish form is dominant over the allele controlling the malelike form. Male polymorphism due to genetic factors is very unusual among Lepidoptera.

9. Recessive genes controlling whitish wing-colors similar to those controlled by the dominant "alba" gene are rare. They are quite independent of "alba" genetically and are expressed in both sexes.

10. Balanced polymorphism for the hindwing discal spot probably characterizes natural populations of *Colias philodice*. The allele for pale spot is recessive to that for redder spot.

11. *C. philodice* and *C. eurytheme* are most easily differentiated in museum specimens by the yellow and orange wing-colors, respectively. Laboratory crosses show that these colors may be controlled by one or two pairs of genes, with no dominance. Both "pure" parental types are recovered in the F_2 and in backcrosses. Phenocopies of the hybrid types are produced in genetically pure *C. eurytheme* by spring and autumn environments.

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Artificial Insemination and Livestock Improvement

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The first experimental demonstration of the feasibility of artificial insemination was by Spallanzani in dogs in 1780. In farm animals, it was first used successfully in horses in the 1890's. Much research was devoted to the techniques of A.I. in Russia prior to the World War I, mostly by Ivanov. After the war, it was used on a very large scale not only in horses but also in cattle and sheep, and many of the advances in technique are in fact due to Russian workers (Goode and Rudduck, 1948). Its value has not been confined to domestic animals, and it has been of great value to geneticists working with laboratory animals, apart from the great increase in our knowledge of the physiology of reproduction that has been gained from it. In the laboratory, it has been used mostly in the rabbit, as for instance in the work on production of polyploids by the treatment of sperm by Häggqvist and Bane (1950).

Outside Russia, the first commercial use of A.I. was in Denmark in 1936, and an Artificial Breeding Association was set up in New Jersey, U.S.A., in 1938. Although the first impetus in the spread of A.I. was undoubtedly the possibility of the wider use of proved sires and, in some cases, the eradication of venereal disease (Anderson, 1948), it became obvious that it was actually for many farmers a cheaper way of getting a cow in calf than was natural service. During and immediately after World War II, the movement gained ground slowly, but in the last five years there has been great expansion and the A.I. center has become an accepted part of the dairy industry in many countries. The historical development of A.I. has been described in Perry *et al.* (1949).

This review will be mostly concerned with the genetic implications of A.I. in livestock improvement. Before discussing this in detail, however, it will probably be worth-while presenting briefly the biological and agricultural limitations upon, and conditions for, its use.

I. Biological Limitations

Of the biological factors limiting the use of artificial insemination, the most important are:

1. The extent to which the semen can be diluted and retain its fertilizing power.
2. The length of time the semen can be stored without losing fertilizing power.
3. The ease of detection of heat in the female.

The following table, taken from Perry *et al.* (1949), summarizes the relevant information on the first two points on animals that are of practical interest.

	Females inseminated per ejaculate	Survival time (days)
Bull	100–600*	4–6
Ram	40–100	5–7
Horse	8–12	1–2
Boar	4–8	¼
Cock	6–10	Small

* Current practice in U.S.A. is 1000.

Thus communal, as distinct from private, use of A.I. is only possible in the cow, the sheep, and the horse. The preservation of the semen by deep-freezing, which is discussed later in this review, will alter the position, but this development is so new that its possibilities have not yet been fully exploited. The detection of heat is not a problem in temperate countries, but in tropical breeds of cattle, the heat period is very short (~2 hours) and may be almost undetectable.

II. Agricultural Limitations

We may distinguish broadly two situations in which artificial insemination is used in farm animals:

1. Where its use is temporary only, as for the following reasons:

   a. In order to effect a rapid genetic change to an already existing desirable genotype. This may involve only a few females, as, for instance, the introduction of the polled gene into a cattle population (as has been done in Herefords in Great Britain using semen flown from the United States), or may continue over

three or four generations in the grading-up to a known breed. In this respect, the existence of a commercial A.I. system facilitates the change of breed type within a country, as in the switch to Friesians from Dairy Shorthorns in Great Britain.

b. In order to control venereal disease, such as trichomoniasis. A.I. has been used in East Africa for this reason.

c. To make possible the wide use of individual famous sires.

2. Where it forms a permanent part of the agricultural system. Its use will then depend mainly on the relative cost to the farmer of artificial and natural service.

The discussion of agricultural limitations will refer only to the factors governing the economic efficiency of such a permanent system and in particular to the use of A.I. in dairy cattle, as most of the information available comes from that animal. The communal use of A.I. is then most economical in the following situations.

1. The density of the cattle population should be high. The wages and transport of inseminators form more than half the usual operating costs and the more dense the population, the less time and money is wasted in going from farm to farm.

2. It will be more attractive to the owner of the small herd.

From the farmer's point of view, the value of A.I. will depend on the relative cost of getting his cow in calf by artificial and by natural mating. The main cost of the latter is the feeding, maintenance, and depreciation of the bull, which in general amounts each year to the same as the insemination of about 40 cows by A.I. If his herd is small, it will pay him to use A.I. rather than to keep a bull. For this reason, too, the herds using A.I. are not a random sample of the dairy herds in the area but contain a higher proportion of small herds. A.I. has its greatest use in England and Denmark, where the average herd size is less than 10, and has been used little in New Zealand, where the average herd size is over 40.

3. A consideration of minor importance is that calving should not be concentrated in one season of the year.

The A.I. center has to be maintained over the whole year and the number of bulls and vehicles required is determined by the peak demand for inseminations. This is another reason why the spread of A.I. has been slow in New Zealand, where 70% of the cows calve in July and August because of the reliance on grass for food.

It was noted above that from biological considerations A.I. is of general value only in the horse, the cow, and the sheep. The horse has declined so much in agricultural value in the past decades that the dis-

cussion of A.I. in horse breeding is of academic interest in many countries. It appears still to be used in Russia. In sheep, A.I. has the drawback that sheep flocks are usually large and that mating is very seasonal. For that reason, it appears to have no permanent place as it has in dairy cattle. However, it is being used extensively in grading-up in Russia and in some South American countries, *e.g.*, Brazil and Uruguay. In farms of the Ministry of Agriculture of the U.S.S.R., in 1949, there were 15,253 A.I. stations on collective farms and 12.5 million ewes were inseminated (Sozial Zivotn, 1950).

Although A.I. has no commercial use in poultry because of the large flock size and rapid decline of fertilizing power with time, it nevertheless has a place in breeding flocks. The recent popularity of the battery system and the deep litter system has meant that, if hens are to be selected on the basis of performance under the system, A.I. is by far the most convenient way of mating them. In addition, the recent emphasis on family selection on part records only has meant breeding from cockerels, which are often not very successful in natural mating. For these reasons, all matings are made by A.I. in some research stations.

III. The Operation of an A.I. Center for Dairy Cattle

The operation of an A.I. center will vary in detail in different countries, but in Great Britain a typical center holds 20–30 bulls by each of which 1000–1500 cows are got in calf each year. Higher figures than this are common in the United States. The bulls will probably be kept at a central station to which there will be attached about three subcenters which serve as semen storage depots and as communications centers for their local areas. For his fee, the farmer is entitled to an unlimited number of services for any cow which requires more than one. The usual criterion of efficiency of the method is the "3-month non-returns" (often referred to briefly as the conception rate)—the proportion of cows for which a second service has not been requested within three clear calendar months of the first. This will depend on other factors besides the actual pregnancy of the cow but is a reasonable practical standard. It is normally in the region of 65–70% and is one of the main factors controlling the economic efficiency of the station, as Edwards (1949) has pointed out. Each cow that needs a free service means a visit by an inseminator for no financial return.

One of the advantages of A.I. is the ease with which some venereal diseases of cattle, for instance trichomoniasis, can be controlled, as the bulls at a center are under much closer veterinary supervision than those kept by individual farmers for natural service. Disease control has been the main reason for the introduction of A.I. in some areas. Although

it is of great value in many cases, all the entries are not on the credit side. The recent increase in *Vibrio foetus* in Great Britain, for instance, has shown that a disease which the veterinary authorities do not have under control may be spread by A.I. A center is also at a great disadvantage in an outbreak of an infectious disease such as foot-and-mouth disease, as the passage from farm to farm of inseminators is an obvious possible source of infection. The necessary ban on their movement means that a farmer who might have kept a bull himself before the spread of A.I. may not be able to get his cows in calf for several months, depending on the severity of the outbreak.

In spite of these minor difficulties, the spread of the method in dairy cattle has been very rapid in countries where the agricultural conditions are suitable. The following table, taken in the main from Bonadonna (1952), shows the proportion of dairy cattle artificially inseminated in various countries.

	Year	Proportion of cows artificially inseminated
U.S.A.	1951	16
Canada	1952	9
England and Wales	1952	33
France	1951	15
Belgium	1951	7
Holland	1951	24
Germany (West Zone)	1950	4
Norway	1952	5
Denmark	1951	58
Sweden	1951	16
Finland	1951	7

Unfortunately, no adequate statistics are available for the whole of the Soviet Union, but A.I. is undoubtedly being used there on a large scale.

IV. The Present Effect of Artificial Insemination on Production

It was commented above that A.I. is used in dairy cattle primarily because it is cheaper than natural service. It is important to know whether A.I. has effected a genetic improvement in the herds where it was used. It was hoped in many countries that the widespread use of bulls bred in more fashionable herds than those from which the farmer would usually buy his bulls for natural service would itself cause an increase in yield in the first generations of A.I. In most countries, the A.I. system has been genetically only an appendage to the pedigree breeding system in that the breeding operations in the herds using A.I. had no effect on the genetic constitution of the pedigree herds. The herds using A.I. are

generally smaller than average and are in general nonpedigree. Denmark is an exception because in that country A.I. merely replaced the already flourishing system of bull societies. It has been normal practice there that a fashionable bull may be used in a great many herds in distinction to the prevailing custom in Great Britain and the United States that a famous bull should be rigidly reserved by his owner so that his influence spreads mainly through the sale of his sons. In Denmark, many of the A.I. bulls are sons of bulls that have themselves been used in A.I., although they may well have been bred by natural service. The distinction between the pedigree herds, on the one hand, and the herds using A.I., on the other, does not have the rigidity that it does in other countries. For that reason, although much information could be obtained on the early results of A.I. in Denmark, it would not be of great interest because A.I. was merely a continuation of the bull societies and therefore would not be expected to have any immediate genetic effect.

Unfortunately detailed analyses of the production records of animals got by A.I. are very rare. Much rather valueless data on this point has been published from time to time. The results given have often been selected so that they are illustrative of particular instances rather than a critical analysis of the whole situation. Distinction is not always drawn between the records of the daughters of A.I. bulls got by natural mating and on the basis of which the bulls may have been bought, and those of the daughters actually got by A.I.

A survey of some 1500 first lactation records of heifers got by A.I. in England has been made, covering three breeds (Robertson and Rendel, 1954). In the majority of the herds in the survey, only a proportion of the heifers were by A.I. bulls, the remainder being either bought in or got by natural service on the farm. These "non-A.I." heifers can be looked upon as representatives of the stock that the farmers would have in the absence of A.I. and serve therefore as a natural standard of comparison for the performance of the A.I. heifers. One is asking in effect: "Are the A.I. bulls genetically superior to the bulls that the farmer would otherwise have used?" An estimate of the average effect of A.I. was got by weighting the comparison in each herd-year according to the number of heifers in the two groups. The daughters of the A.I. bulls had an average superiority of 1 gallon of milk, which is the same as saying that they were genetically equivalent in yield to the bulls used for natural service. They were however significantly superior in fat content (0.06%). When a survey was made of the bulls used for natural service, it was found that the great majority of them were pedigree, some from well-known herds. The lack of superiority of the A.I. bulls reflected rather on the acumen of their purchasers and on the information available to them. When a

classification of herds was made into high- and low-yielding groups (based on some measure not entering into the comparison, such as the yield of mature cows), it was found that the difference between A.I. and non-A.I. animals was the same in herds at all levels of yield. This must indicate that the great differences in average milk yields between the herds in the sample were due, as far as could be ascertained, almost entirely to differences in management rather than to genetic differences. On the other hand, evidence was found that as much as 50% of the variation between herds in fat percentage might be due to genetic differences.

The majority of these A.I. bulls were bred in fashionable herds and were bought mostly on pedigree evidence, very few being proved at the time. They proved themselves not genetically superior in yield to the less fashionable bulls and at the same time, they could neither improve the bad herds nor the good. There would seem therefore to be little genetic difference between herds within a given breed type. This is not unexpected when one considers the continual interchange of genes there is between herds. In Great Britain, only about 10% of all pedigree dairy heifers have homebred sires. There is a continual flow of genes from the dominant group of herds to the rest of the pedigree herds and from them to the nonpedigree herds that must continually level out any genetic differences that may exist at any time (Robertson and Asker, 1951). Within each herd there remains plenty of variation waiting to be utilized, and there were in fact significant differences between A.I. bulls in their progeny averages both for yield and for fat content.

From the United States the data on the production of A.I. bred animals are rather scanty. Perry (1949) has given a survey of the fat yield of cows got by A.I. in New Jersey and New York. Unfortunately, the evaluation of the success of A.I. is by a comparison of daughters with dams. As, on the average, the dams will be milked about 5 years before their daughters, many of their lactations must have fallen during the war years when production averages were lower than before or after the war. In New York, 362 heifers bred by A.I. had an average fat yield of 5 pounds or 1% more than their dams. In New Jersey, the 430 A.I. heifers had an average fat production of 14 pounds or 3% more than their dams.

A further analysis of records from New York State (C. R. Henderson, private communication) has shown that, as in English records, the difference in fat yield between A.I. and non-A.I. heifers in the same herds is small and is unrelated to level of production. There is also some evidence that the ranking of bulls in breeding value is not the same in all farms.

More detailed information is available from New Zealand, where A.I. has been used so far only on an experimental scale so that a high proportion of good progeny-tested bulls could be used (New Zealand Dairy

Board Repts., 1951, 1952). These two reports summarize the fat production records of 1127 Jersey heifers bred by A.I. bulls. The heifers were judged on a comparison with mature cows in the same herd after age correction. The results are summarized below.

	No. of daughters	Superiority over mature cows in same herd
Merit sires used in 1948	685	+29 lb fat
Merit sires used prior to 1948	253	+3 lb fat
Other sires	189	+7 lb fat
Total	1127	+19 lb fat

It must be noted that 549 of the heifers in the first group were by only two sires and showed an average superiority of 30 pounds. Some data are also given for results in different herds but not in sufficient detail for an analysis to be made. The general suggestion is that rather more of the variation between herds is genetic than in the British results. There seems to be more variation between herds in fat content than in the latter, possibly because many of the herds are grading up to Jersey.

The value of A.I. as a method for the improvement of production does not lie only on the genetic side. The farmer who is losing money with cows producing an average lactation of 500 gallons, no longer has the excuse that in any case his neighbor's cattle are genetically better than his and that he cannot afford to buy any better. Certainly the use in A.I. of the same bulls in herds at different production levels has shown unequivocally the reason for the low production of the poor herds. It seems probable then that the immediate emphasis in the improvement of such herds should be on management.

The possibility that deleterious recessives might be spread by the use of A.I. has often been brought up as one of its disadvantages. This is a peculiar point of view, as such genes cannot be passed on unless they are already present in pedigree cattle of the same breed. A rather similar view is that A.I. will lead to inbreeding because of the restriction in the number of bulls used. But, after the use of A.I. for several generations, the genetic make-up of the commercial cattle will be identical with that of the pedigree animals and their degree of inbreeding will depend only on the breeding operations in the important pedigree herds. Indeed, the use of A.I. does in a sense lessen the risk that the individual farmer will suffer financial loss by having a high proportion of calves born homozygous for a deleterious recessive. Although an individual bull heterozygous for such a gene will pass it on to many offspring, the average gene frequency in the A.I. bulls will not be greater than that in the pedigree breed as a whole. An individual farmer using A.I. will normally have the

progeny of many bulls in his herd at the same time, and so is much less likely to have a recessive at high frequency than if he used only one bull in natural service. Indeed, the chance of such a bull, heterozygous for a gene already present at an appreciable frequency (say 5%) in a breed, being detected is much greater in A.I. than in natural service. The A.I. bull leaves many more offspring than a bull in natural service and occurrences of homozygous recessives can readily be noted and his heterozygosity discovered. In A.I. the farmers are using bulls which are not their own property and about whose shortcomings they are therefore prepared to complain loudly. In natural service, the sporadic occurrences of recessive homozygotes can easily be overlooked or, if necessary, concealed. The most vigorous efforts to purify a breed from such a gene have been made in the case of paralysis of the hindquarters in Red Danish cattle (Nielsen, 1951). In herds using A.I. bulls, a special effort is made to trace all homozygotes born, involving a payment to the veterinary surgeon reporting it and to the owner of the herd in which it was born. It is then possible to publish "blacklists" of known heterozygotes, even though these contain some of the most fashionable sires in the breed, and also a list of the bulls that can be presumed to be free of the gene, on the basis of considerable A.I. use without the occurrence of a recessive homozygote in their progeny (Pedersen and Andersen, 1950).

Only in the New Zealand records has a marked improvement been brought about by the bulls used at the A.I. stations, and in this case, they were selected with extreme care. In the American and English results, in which A.I. was on a commercial, rather than an experimental, level and where it was not possible to obtain a high proportion of proved sires, the improvement in production has been small indeed. It must be remembered that comparisons can only be made for recorded herds, which in all countries have a higher average production than that of all herds. However, in the English data, there was no significant improvement even in the poorest of the recorded herds, and it is a reasonable presumption that these are not much different from many nonrecorded herds in cattle and management.

The genetic situation may vary in different countries, but the conclusion from these results must be that there is not a great genetic difference between the A.I. bulls and the cattle in the herds in which A.I. is used. We must therefore act upon the assumption that the continued use of such bulls will be on the average genetically neutral and that the continuation of the present system, in which a succession of bulls are brought in to the centers, can be of little interest to the geneticist. The value of the A.I. system to the geneticist lies in the possibilities of the improvement of the cattle in the unit from within. The results from the

use of superior progeny-tested bulls in A.I. in New Zealand are encouraging evidence that the material for such improvement is available.

V. A.I. Stations as Centers of Dairy Cattle Improvement

The use of the existing artificial insemination stations in the genetical improvement of dairy cattle does not present any new theoretical problems—rather some interesting variations on an old theme. It has become a commonplace that the rational method of improving dairy cattle is by progeny testing. But many of the proponents of progeny testing do not seem to have realized that in breeding units of conventional size, its efficient use is by no means simple and may in some situations be almost impossible. The general problem has been considered theoretically by Dickerson and Hazel (1944) and with more specific reference to A.I. in dairy cattle by Robertson and Rendel (1950).

There are three essentials in any progeny-testing scheme:

1. The efficient use of superior bulls.
2. Accurate progeny information on which the bulls can be judged.
3. A choice between bulls for which this information is available.

The most important point regarding the use of superior sires is that they must pass on their genes through their sons as well as through their daughters, *i.e.*, the unit must be to some extent genetically isolated from the rest of the breed. The selection program depends for its success on an increase of the frequency of the desirable genes, and the continual introduction of unknown genes from outside can only retard progress. Such a progeny-testing program at an A.I. station would mean a radical change in policy. At the present moment, the flow of genes is from the pedigree herds into the commercial herds using A.I.; and, however good an A.I. bull is, if he is merely used to breed rather more cows in commercial herds, his genetic effect on the unit can be temporary only, because when the next group of bulls is bought in, that effect is diluted by the new inflow of genes. It follows then that the use of bought-in progeny-tested bulls, whether they have been tested in the unit or in outside herds, is not an intelligent long-term program. The next lot of proved bulls will be as good as the last, no better and no worse, and unless the breeding herds from which the bulls are brought are themselves improving, the cattle in the A.I. unit will remain at a steady genetic level. If a good bull is found, his genes must be passed on through his sons by testing them as potential successors to their father.

In herds of conventional size, in which perhaps less than 10 heifers come into milk each year, it is virtually impossible to make effective use of progeny-testing. The breeder cannot at the same time have an adequate number of daughters on which to judge each bull and a choice between

bulls. He may have some choice in the sense that he could retain bulls that he considers have bred well and discard the poor ones, without ever having used more than one sire at a time. If he is fortunate enough to find such a superior sire, he must breed sons from him. If his herd is of the size that he will usually not use more than one sire at a time, he will then find himself faced with inbreeding problems such as are discussed later in this review.

From these rather qualitative arguments, it seems obvious that the effective use of progeny testing as a method of dairy cattle improvement requires larger breeding units than are usually available to the individual breeder. The A.I. center is the ideal solution to this problem in that the genetic control of a large cow population is in the hands of the few people who are responsible for the bull-purchasing policy. The main theoretical discussion of a progeny-testing scheme in the framework of an A.I. station is in Robertson and Rendel (1950) and Rendel and Robertson (1953). The general structure of such a scheme is that a proportion of the inseminations are by superior proved bulls, chosen on their A.I. daughters' performance, and the remainder are by young bulls, the sons of the best proved bulls available out of the best cows in the unit. When their daughters' records become available, the worst of the bulls are discarded and the better taken in as proved bulls. The paper was devoted to a consideration of the optimum structure and probable annual genetic improvement for breeding units of different numbers of recorded cows.

The two main variables to be considered in computing the optimum structure are:

1. The proportion of inseminations by young bulls.

The supply of future proved bulls depends on an adequate number of matings being made by young bulls but the more matings that are made by the existing proved bulls, the more can their genes be passed on to future generations. We must therefore balance the utilization of the present proved sires against the need to provide new proved sires in the future. If a very high proportion of the matings are to young sires, the scheme gradually becomes one of family selection rather than of progeny testing. After proving, the superior sires would then only be used to breed sons to be tested and not to breed cows in the commercial herds in the unit.

2. The number of heifers on which each young bull should be tested.

If a certain number of matings are to be made by young bulls each year, how many bulls shall they be divided between? If, say, two superior sires are required each generation, the problem is to maximize the superiority of these chosen animals—to balance the accuracy of the judgment against the width of the choice. If many sires are tested and each has

only a few daughters, there is plenty of choice but little information on which to base it. At the other extreme, if we test a few bulls on many daughters, there is plenty of information but little choice.

On the basis of assumptions of various agricultural parameters involved, *e.g.* the proportion of young bulls that will die before their daughters come into milk, and so on, and taking a heritability of 0.25 for milk yield, it is possible to predict the probable annual genetic improvement for units of a given structure. The genetic improvement arises in four ways.

1. In the use of the best sires available to breed young bulls for testing, improving the genes passing from male to male.

2. In the use of proved bulls on a proportion of the cows, improving the genes passing from male to female.

3. In breeding young bulls for testing from the best cows in the unit, improving the genes passing from female to male.

4. In the culling of cows early in their life for low production, improving the genes passing from female to female. In an A.I. station, this is a matter for the individual herd owner.

The expected annual genetic improvement is found by dividing the average genetic improvement along the four paths by the generation length. In a progeny-testing scheme, the latter may be expected to be longer than in usual breeding practice because of the greater age of the sires. It might be expected to be 5½ to 6 years instead of the usual 4½ in dairy cattle. The balancing of the two important variables cannot be done algebraically and must be done by calculations of annual genetic improvement for a series of selected values. These have led to the following conclusions:

1. As was obvious on qualitative reasoning—that the rate of genetic improvement under progeny testing increases as the number of cows in the breeding unit increases.

2. That the two main variables can vary widely without greatly affecting the rate of improvement. In general, at least a quarter of the cows should be mated to young bulls which should be judged on at least 20 daughters each.

3. That the greater part of the improvement comes from breeding new young bulls from the old proved sires.

In a unit of 2000 recorded cows, the probable rate of improvement in milk yield, assuming a phenotypic coefficient of variation of 20% and a heritability of 0.25, is about 1.5% a year, which is over twice as rapid as the maximum expected in herds of conventional size in the absence of progeny testing. It would therefore seem desirable to take advantage of the opportunity which the A.I. centers present as large breeding units,

rather than have them continue as mere appendages of the existing pedigree breeding system.

Since these calculations were made, some of the assumptions have been found to have been somewhat optimistic. In particular, it was assumed that for every 3 cows presumed in calf in recorded herds to a young bull, one heifer lactation would be obtained on which he could be judged. In practice, the figure turns out to be 6, the difference being partly due to the sale of cows in calf so that their offspring cannot be traced. The problems of interpretation of the progeny test under A.I. conditions will be discussed in the following paragraph, but it appears that because of variations in management between herds and the small size of herds using A.I., about 50% more daughters will be required for a test of the same accuracy than under a uniform level of management with the same intraherd heritability. These factors will modify the optimum structure in the direction of testing rather fewer young bulls by mating them each to more cows. Because of (2) the rate of genetic improvement will not be materially altered.

The daughters of bulls used in artificial insemination will usually be scattered through many herds at different management levels. The evaluation of the breeding value of the sire from these records has been discussed in detail by Robertson and Rendel (1954). The choice of the most accurate simple method will depend on the circumstances at the individual station. The problem is somewhat complicated by the fact that in some stations the farmer may choose the semen he requires (possibly at a slightly higher fee). Some bulls, which are thought to be superior, will have a larger proportion of such "nominated matings" than others and will probably have daughters in rather better herds. If this constitutes a serious bias, it appears that comparison with heifer contemporaries in the same herd is the most accurate simple method for milk yield. Even if bulls are used at random and the farmer has no choice, the contemporary comparison will still be better than other simple methods provided the variation between herds is more than one quarter of the total. As mentioned above, because of the low herd size, the effective heritability of an individual record for a progeny test by the contemporary comparison under English conditions is only two-thirds the intraherd heritability, and therefore 50% more daughters are needed for the same accuracy. Fat content, less affected by environmental changes, does not present such difficulties and a simple average of the daughters' records would seem to be quite adequate. These methods give only an estimate of the relative production of the actual daughter groups—the prediction of the breeding value of the sire (as measured by his future daughters) requires a regression of these averages to the population mean by a

factor depending on the effective heritability of the character concerned and the number of daughters available.

Two theoretical points should be dealt with in considering the efficiency of such a breeding unit. The first is the degree of inbreeding in each generation that it is proper to allow. Inbreeding in this respect has two effects. In the first place, it has a direct and depressing effect on yield and on other desirable characteristics of dairy cattle such as viability and fertility. In a progeny-testing program, the inevitable concentration of blood lines will mean that a proportion of the expected gains will be lost in inbreeding depression. This concentration will arise mostly from the breeding of young sires from a small number of proved sires. In this case, if the effective number of sires from which sires are bred is N_{mm} (in Wright's sense) and that from which females are bred is N_{mf}, then the expected inbreeding per generation is given by

$$\frac{1}{8}\left(\frac{1}{4N_{mm}} + \frac{3}{4N_{mf}}\right)$$

If we take as probable figures for our proposed scheme, $N_{mm} = 2, N_{mf} = 12$, this gives a value of 2.4% per generation. Unfortunately, we know far too little about the expected effect of inbreeding on yield. Several analyses have been made bearing on this point but they are by no means unanimous in the answer. If we take the most pessimistic view, that 1% increase in the inbreeding coefficient means 1% decrease in yield, then the expected decline in yield each year due to inbreeding is about 0.5%, or one-third of the expected gain from selection. The dilemma is that selection can only be made more intense by decreasing N_{mm} and this means an increase in inbreeding. Exactly where the optimum lies is uncertain, but it is probable that to take N_{mm} equal to two will be not too far out. The magnitude of the effect of inbreeding on other characteristics such as fertility is not known with any certainty, but what meager information we do possess suggests that the inbreeding rate mentioned above can readily be tolerated in the early generations of such a scheme.

This general problem, like most of the others we are discussing, is not new to the animal breeder, but it seems for some reason not to have been encountered in dairy cattle breeding. The question is: "What I am to do to avoid getting too inbred even though I think my cattle are better than anybody else's?" Apart from the theoretical discussion of the procedure in a unit that is genetically closed, there remains the practical solution of continually introducing a little outside blood but not being finally committed to its inclusion in the unit until it has been proved. This has been solved by the leading beef breeders in Great Britain by occasionally buying in a young bull from another herd by a sire of their own breeding

out of a good cow. Only heifers by him are taken into the herd until he is thoroughly progeny-tested and then, if he proves to be good, his sons are used. A somewhat more violent method would be to introduce a small proportion of sires from outside the unit and not to use sons of them unless their daughters milk well. In a pilot scheme, this would have the advantage of providing a control for the success of the selection program.

The second and longer term effect of inbreeding is the exhaustion of genetic variability within a unit due to homozygosis. This has been discussed by Rabasa (1950) and by Rendel and Robertson (1951). It is a second-order problem in the sense that it will only arise after the inbreeding has risen to a high level, which will take a long time in cattle breeding. In the above scheme, for instance, it will take twenty generations or 100 years to halve the genetic variation.

A question of wider relevance concerns the integration of such a scheme with other units in which similar schemes might be operated. The problem is whether it is desirable to use the whole A.I. population of a region of, say, 500,000 cows as one breeding unit or whether it should be split up into independent units. Undoubtedly there would be some genetic advantages in the latter system. The operation of several independent units would lead to some diversification of the cattle in the separate units by the chance fixation of desirable gene combinations, as has been suggested by Wright (1939). There could then be some selection between units. In some units, the inbreeding, low though it is, might lead to a decline in desirable characters. This could be easily remedied if the unit was merely one of several. There is also the possibility that different genetic combinations are required for different parts of the country. This could be easily secured with local units, genetically semi-isolated from each other. However, no matter how great the theoretical advantages of such an arrangement, it would no doubt be rather difficult to operate in practice. The appearance of an outstanding bull at one center would immediately lead to a demand for his semen or that of his sons at other centers. But these problems, though they need to be discussed before they arise, would not have practical importance for many years.

Though it is comparatively simple to discuss the optimum theoretical structure for a breeding unit of, say, 2000 cows using A.I., it is by no means cheap to put the results into practice. Normally only a proportion of the animals in the unit are recorded—in Great Britain about 20%—and only these are effective in the progeny-testing program. A total population of some 10,000 cows, of which 2000 were recorded, in normal operation might require up to 10 bulls at any time. A progeny-testing scheme, testing 4 young bulls each year, will eventually require the maintenance on the station at any time of some 25 bulls. Each year, in

the early stages, 2 young bulls will have to be bought above the usual requirements at, say, £300 a year (and they may still have to be bought in from members, when the scheme is operating fully) and 15 extra bulls will have to be maintained at the cost of £100 a year. The total extra cost would then be of the order of £2000 a year, representing an increase of 20% over the present cost of insemination. However, though these annual costs may appear large, they should quickly be offset by the increased income due to the improvement of the cows in commercial herds.

VI. The Application of Progeny-Testing Schemes at A.I. Centers

In several countries, the first practical steps have been taken to make efficient use of progeny testing in A.I. units. In most centers, however, bulls are still bought in from outside the unit, which means that the rate of genetic improvement is tied to that in the herds from which the bulls were bought. In Great Britain, the first start was made by the Milk Marketing Board (1949) who have now used several teams of about four young bulls at the same time at one center. However, what is to be done with the bulls that are found to be good when their daughters come into milk, apart from using them more extensively in A.I., seems to be not yet clear. In this scheme, the bulls in the testing teams are used in herds at a level of production rather above average, which, although simplifying in some measure the interpretation of the tests, has the disadvantage of not using a proportion of the herds and therefore not utilizing to the full the advantages that A.I. units offer. However, in this preliminary work, many of the practical problems that are likely to arise in the course of such a program will be solved (Brown, 1954).

More recently, with the financial help of the Agricultural Research Council, a more rigid scheme modelled on the theoretical discussion above has been started at the A.I. station at Cambridge on the Friesian breed. It is hoped to test 4 young bulls each year on some 20–25 daughters, and after the initial period of some 5 years, to breed new bulls by the best two proved sires available out of the best cows in the unit. Each young sire will be mated to at least 20 of his daughters for their first calf so that some selection can be made against deleterious recessives in the early stages of the scheme. Selection will be mostly for yield, but some attention will be paid to fat percentage and a watchful eye will be kept on other characters such as calf mortality, fertility, and longevity. This is, of course, a long-term program, and even a preliminary assessment of the success or failure of the scheme cannot be expected for at least ten years.

In the New York Animal Breeders Cooperative, considerable differences were found in the breeding values of the bulls used, and from the best of these sons are now being brought into service in the A.I. unit (New York Artificial Breeders Cooperator, March, 1953).

In Denmark, as was pointed out earlier, the relationship of the A.I. system to the pedigree breeding system has been quite different from other countries and this has led to interesting developments in the field of progeny testing. Since the beginning of the century, there has been considerable interest in progeny testing in Denmark, and progeny investigations (Afkomsundersøgelse) of the bulls used in the leading herds have been published regularly since that time. They are based primarily on daughter-dam comparisons. The decline in the level of feeding during the World War II made such comparisons of little value. However the spread of A.I. and the considerable use of individual bulls that it led to, made possible the setting up of special stations for the progeny testing of A.I. bulls (Afkomsprøve). There are now 18 stations throughout the country, testing each year about 50 bulls on 20 daughters each. The heifers are taken in on September 1st each year and must satisfy certain conditions of age and time of calving. Every effort is made to keep the level of management the same at all stations and, judging by the results to date, this has been successful. Very great differences in the average yield of daughter groups have been found—in the 160 bulls of the Red Danish breed so far tested, the average 304-day yield ranges from 1120 gallons to 710 gallons and the fat content from 4.71% to 3.85% (Afkomsprøver med Tyre, 1953). By this means, possibly one half of all the young bulls used at A.I. centers can be tested *after* they have been used considerably. As a result of the tests, it has been found that the best bulls are used more in the year after the test and the worst bulls are used very much less. However, at the moment this system, excellent though it is, is mainly a method of getting accurate progeny tests on a section of the A.I. bulls. If, and only if, the published results of these bulls have an effect on the breeding of the future bulls used in A.I. stations, will they make any permanent contribution to the improvement of the cattle. There are at the moment very few signs that this is so but, as the results continue to come in, we may possibly see them having more influence.

To a geneticist, the special stations are exceedingly valuable as a source of data from a controlled environment and as such the stations are without parallel. But the animal breeder must ask whether equivalent information on the same bulls is not already available from the many other daughters that they have had in commercial herds. The progeny tests are not cheap to run, and it does in fact cost almost as much to test a bull in these stations as it does to keep him alive till he is 5 years old.

Of course, the stations have a demonstration value over and above their immediate purpose in judging the bulls. In addition, the test information is simple to interpret and available for all to see. The problem of genotype-environment interaction must be considered in this context. The bulls that appear best under the high management test stations may not be the best under commercial conditions. Investigations into these points are in progress at the moment. The next ten years will show whether the special stations will revolutionize Danish cattle breeding as the publication of the progeny investigations did in the past.

The possibilities of setting up similar stations in Great Britain has been considered, but it is doubtful whether conditions are suitable at the moment. In Denmark, some bulls cannot be tested because sufficient daughters cannot be found to satisfy the conditions, and this would probably be even more difficult in other countries. The possibility of the Danish scheme rests on several factors:

1. The uniformity of agricultural conditions over the whole country and the long tradition of cooperation amongst the farmers.

2. The high proportion (50%) of cows in recorded herds in which pedigree records are kept by which heifers can be identified.

3. The high level of freedom from bovine tuberculosis and other infectious diseases.

It will be realized from the different schemes that have been discussed in this section that a good beginning has been made in the integration of of progeny testing methods into the A.I. organization. In the course of the next few years, these pioneering efforts will give valuable practical experience in the organization of such schemes. The Milk Marketing Board scheme and the special Danish stations are, at the moment, only methods of finding out the bulls with a high breeding value. However, the effort devoted to their discovery may be largely a waste of time if intelligent use is not made of them. The other two schemes have to some extent attempted a solution to this problem. However, we are in this matter in a period of trial and error but with dairy cattle it is unfortunately a long time before the verdict is given.

VII. The Effect of Recent Developments in A.I. Technique

In the past few years, research into the development and extension of A.I. methods has led to interesting possibilities in livestock improvement.

The work which promises to have the most immediate effect on the technique and organization of A.I. is on the deep-freezing of bull semen by Polge *et al.* (1949) and by Polge and Rowson (1952a, b). It was commented above that the short effective life (6 days) of bull semen leads to a high proportion of semen being wasted. By the addition of 10%

glycerol to the buffered semen, it is possible to freeze the mixture slowly to solid CO_2-alcohol temperatures (−79°C) without affecting its fertilizing ability on thawing. At this temperature, the semen can be preserved for long periods. At the Cambridge A.I. center, calves have been born from the deep-frozen semen that has been preserved for 12 months and cows are pregnant by semen that has been frozen 19 months. Even after this period, the conception rate is still as high as for fresh semen. The success of the method, even thus far, will have far-reaching effects on the operation of A.I. centers and will require the modification of some of the comments made above. No semen need be wasted, and the normal demands of the insemination service could be met with a third to a quarter of the bulls at present used. Transport problems from the center to the subcenter will become much simpler, as the time between freezing and thawing is irrelevant. One large central bull station could then supply semen to a very large area. The long-distance transport of semen, especially to tropical countries, is simplified, and after the semen reaches its destination many more cows can be inseminated with it, because it does not have to be used immediately.

Although the method has obvious advantages in the grading-up A.I. scheme, its value in a progeny-testing scheme is not great. In fact, the possibility (as yet not realized) that semen from a fashionable sire may be used many years after his death raises up horrifying possibilities of inbreeding. In the scheme outlined earlier, which might be carried out at A.I. stations, a limit has already to be placed on the least number of proved sires in each generation that will be used as sires of young bulls, and this could already be overstepped without the use of frozen semen. It would however be valuable in removing the chance that a young bull might die before his daughters come into milk and he be therefore lost to the scheme. It might indeed be possible to progeny-test a bull without his material presence at the center being required.

Much work has recently been devoted to superovulation and egg transplantation in cattle. It is possible by suitable hormone injections to obtain a large number (up to 50) of eggs from the same cow, although the number falls off after repeated attempts. Unfortunately it has so far been impossible to transplant these eggs successfully into other females, although this has been done in laboratory animals such as mice and rabbits. In the cow, the susceptibility of the uterus to infection depends on the stage of the oestrous cycle. It is possible to inseminate a cow because at oestrus the resistance to infection is high. Five days later, the suitable time to put in a fertilized egg, the resistance is much lower and all such attempts have led to uterine infection (Brock and Rowson, 1952). If such a technique were developed to the state of simplicity of the present

A.I. service, it would allow an improvement in the selection on the female side. This would not mean a great increase in the rate of improvement in a unit that is already operating an efficient progeny-testing scheme. Selection is already possible in females on their own performance, although the proportion of cows in the herd from which heifers must be reared is high. The technique would have most of its effect in increasing the intensity of selection of cows from which heifer replacements are reared. In an efficient progeny-testing scheme, the effect of this would not be great. In the absence of progeny testing, it would have more effect and might almost double the rate of improvement in small herds. It would seem to have more application in experimental breeding work and greatly facilitate, say, the formation of inbred lines of dairy cattle by the production of large contemporary full-sib groups.

VIII. Summary and Discussion

Artificial insemination has now an accepted role in the dairy industry of many countries. It has attained this position as a cheap and efficient way of getting cows in calf. What information we have on its initial genetic effects does not suggest that there have been great improvements in cattle in commercial herds from the bulls that have so far been used except in New Zealand, where its use was experimental with consequent greater choice of sires. In any case, any grading-up that there was to be done (and that remains doubtful) would have been as good as completed in two generations or ten years. Under existing conditions we may therefore expect the use of A.I. to be genetically neutral in the future even though it has not been in the past. We must therefore look further ahead than the mere continuation of the process of importation into the A.I. system of fresh bulls generation after generation. The solution to the problem is that the A.I. units must themselves play a constructive part in dairy cattle breeding instead of tagging along at the heels of the existing bull breeding herds.

The existence, in the A.I. centers, of large breeding groups is a magnificent opportunity for the rational use of progeny testing, in which, after finding good bulls, their successors should be bred from within the unit and progeny-tested in their turn. The exact organization of such a breeding unit still remains to be worked out, and tentative efforts are now being made in several countries. The problems that such schemes involve are often sociological and psychological, and possible solutions will have to be judged on these as well as on genetic grounds. For instance, is the Danish method of special progeny-testing stations with high progeny averages and no complications in interpretation better than progeny testing in the commercial herds, where the average produc-

tion will certainly be lower and the averages need more adjustment? Can sufficient pedigree breeders be made interested in the scheme so that their herds can be used for bull-breeding? If the method is to have wide use, will it be possible to organize a series of units almost isolated genetically or must the whole breed be the breeding unit? Is the existing migration of cows from herd to herd sufficiently great to be a block to further improvement in the long run? These and many other questions like them will have to be answered, and some of the answers can only be got from practical experience and not in the office or the laboratory. From these lines of attack could come a revolution in the breeding of dairy cattle which would not be without its effects in the improvement of other farm animals.

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