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THE NATURAL HISTORY OF MOSQUITOES

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To

LEWIS WENDELL HACKETT

ABLE EXPONENT OF THE IMPORTANCE OF TRYING TO SEE THE WORLD FROM THE POINT OF VIEW OF THE MOSQUITO

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MARSTON BATES

Baltimore, Maryland
January, 1949

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CHAPTER I

INTRODUCTION

> Natural history is encumbered by multitudes of facts which are recorded only because they are easy to record.—L. C. MIALL

Mosquitoes in general, and the malaria carriers in particular, have been the subject of a tremendous amount of study, whose results have been reported in a voluminous literature. Much of this literature is an uncritical accumulation of facts that were easy to record, or of facts that were related to some momentarily fashionable subject of study, or of facts that were needed for the attainment of some immediately practical objective. As a mere accumulation of facts the literature represents, as Miall has stated, an encumbrance: but it is an encumbrance waiting to be converted into an orderly and useful structure of knowledge.

Facts form the raw material of science—the bricks from which our model of the universe must be built—and we are rightly taught to search for sound and solid facts, for strong and heavy bricks that will serve us well in building foundations, for clean and polished bricks that will fit neatly into ornamental towers. But while accumulating the bricks may be a contribution to science, we must take care that the pile does not become a hopelessly discouraging jumble. For science itself is not brickmaking—it is, at the workaday and technical level, bricklaying; and at the creative and artistic level, architecture, the designing of an edifice that will utilize all the bricks to the very best advantage.

The metaphor, of course, cannot be carried too far. The bricklayers and the architects of science are always acquiring strange, new, and beautiful bricks that make it necessary to tear their careful building down and start over. It is an unending, dreamlike game that seems to be limitless—the model of the universe will never be done, nor does any part of it seem to have a comfortable or dependable permanence. But still the bricks, as bricks, cannot be left in a jumbled pile,

and we have the task of organizing them into some sort of a pattern, however transient.

The pile of facts about mosquitoes is an impressive accumulation, one that should be useful to all biologists if only the assortment were a little better organized. Yet, as soon as one starts sorting out these facts, one finds how incomplete they are, how many more are needed if any decent theoretical structure is to be built. The bricks are not overly abundant; it is simply that they need to be sorted according to their size and shape.

That, then, is the object of this book. It certainly can do no harm, and it may serve as a convenience to the architects of biological theory. Even with all of the work that has been done on mosquitoes, the final impression of such a review is that a great deal more work is needed before we can achieve any real understanding of mosquito behavior. So the object is a double one—to indicate the needs as well as outline the resources.

Much that has been written about mosquitoes is concerned directly with methods of killing them, or of recognizing species that are dangerous as vectors of specific diseases; but the problems both of control and of identification have led to a great deal of biological work with implications that go far beyond the immediate practical objectives. Mosquito control and mosquito identification have both been the subject of many excellent and useful reviews, but in recent years no attempt has been made to summarize the knowledge that we have acquired concerning mosquitoes as living animals. The reports of original researches are scattered through a wide range of periodicals, many in medical journals not commonly available in purely biological libraries. As a result, this work has hardly been noticed by the students of biological theory, who view mosquitoes as something to be swatted, rather than as potential sources of information about the biological processes that govern the bewildering organic world that we inhabit.

The biological study of insects has in general been hampered by the tremendously complex problems of classification: before the behavior and physiology of the animals could be studied it has been necessary to name them, and this has led to what may seem to be an undue emphasis on morphology and taxonomy in entomological writing. It is only in certain groups (such as mosquitoes and butterflies) or in certain regions (such as Europe or parts of the United

States) that this primary task of classifying and describing has proceeded to a stage where the accumulation of significant observations on habit and behavior becomes practical. Much of this preliminary work remains to be done even in groups of insects of agricultural importance, because agricultural entomology has been largely developed in the temperate zone or in very restricted regions of the tropics. The importance of mosquitoes as vectors of human disease has led to their study in all parts of the world. The diseases carried by mosquitoes are particularly characteristic of tropical and relatively backward regions, which has furnished the incentive for intensive work in many such areas, while the temperate-zone fauna has been studied because of its convenient access from the great centers of learning. The result is an unusually even geographical distribution of effort. Mosquitoes are probably as completely described from the points of view of species taxonomy and life history as any group of insects; their only rivals in this respect would be certain groups that are highly prized by collectors, such as some of the families of butterflies.

It is interesting to compare mosquitoes with other groups of insects in this matter of being "well known." In general, it seems to be true that the groups of insects that have received the most attention belong to one of three classes: those that are of economic importance, those that are especially adapted to laboratory manipulation, and those that are particularly attractive to collectors. An indication of the extent to which various insects have been used in biological studies may be gained by tabulating the number of page references in the indexes of general textbooks. Wigglesworth's *Principles of Insect Physiology* (1939) furnishes a convenient guinea pig for such analysis. *Apis*, the honey bee, has considerably more page references (66) in the index than any other insect, while *Drosophila* is not far behind *Apis* in physiological studies, and in any book on genetics and evolution it is cited with overwhelmingly greater frequency than any other insect. In recent years various students in Europe and America have started making field studies of the "natural history" of *Drosophila*, and our information on this group will no doubt soon be as well rounded as it is extensive.

The history of the *Drosophila* studies is interesting, since it demonstrates what might be called the "law of the multiplication of the potential value of a subject of study": the more that is known about a given animal or group of animals, the more valuable it becomes for

further work. *Drosophila* was used at first because of the ease with which it could be manipulated in the laboratory; as studies accumulated, it became increasingly valuable for new studies merely because of the background provided by this earlier work, a process that seems to continue indefinitely, though one would expect that in the long run some limit would be met.

The order of frequency of reference to insects in Wigglesworth's index deserves further consideration. After *Apis* comes *Dytiscus*, the water beetle, with 49 references; then *Periplaneta* (cockroach) with 42; *Tenebrio* (meal worm) also with 42; *Drosophila* with 41 and *Calliphora* (blowfly) with 38. The mosquito genera come well down on the list (*Culicidae*, 11 references; *Culex*, 21; *Anopheles*, 11; *Aëdes*, 8), though the combined references make a respectable showing for the family. These various genera have been studied primarily because they are easily adapted to laboratory manipulation. Physiologists are, in fact, often criticized by other workers for using such insects for their studies instead of important pests. They use the cockroach not because it is economically an important animal, but because it is easily encountered and raised in their laboratories. Physiologists have given little attention to butterflies, because they are rather difficult to handle in the laboratory. Yet from the point of view of taxonomy and geographical distribution, butterflies are perhaps the best known large group of insects because they have been so extensively collected in all parts of the world.

The fact that diverse groups of insects have been studied from widely divergent points of view, and the resultant scattering of effort, is unavoidable in view of the nature of man and of insects. It seems, none the less, unfortunate from the point of view of the development of a unified and integrated biological science—in other words, from the point of view of the development of a general knowledge of the nature of life processes.

The scatter effect is increased by the course, perhaps largely accidental, that the biological sciences have followed in specialization. We have two widely diverging types of specialization: according to group studied and according to point of view. The first type of specialization gives us entomologists, ornithologists, herpetologists and conchologists; the second, physiologists, histologists, cytologists, and geneticists. In many cases—the taxonomists and the morphologists furnish good examples—both types of specialization may be combined,

the student being restricted both by group of organisms and by point of view.

The vastness of the biological field makes specialization imperative, though it would seem healthier to regard this as a regrettable though necessary evil, rather than as a desirable thing in itself. The daily chore of going through the contents of the endlessly multiplying biological journals, or a review of the bulging pages of the abstracting publications, sometimes gives one the impression that biology is overcome with a sterile scholasticism; that it has become hopelessly split into an enormous array of self-important little sciences, each preoccupied with the accumulation of its own peculiar kind of facts according to the forms and rituals that are prescribed by its traditions. "Biology" seems to be a completely empty concept. If you leaf through the latest editions of *American Men of Science*, you find many people who list as their fields chemistry, physics, or geology, but only here and there an instructor in some small college who dares to call his field biology. How can we have a biology if we have no biologists?

This is, of course, a passing phase. The biochemists have discovered bacteria, and the bacteriologists have discovered genetics; the geneticists have taken to describing *Drosophila* according to the rituals of the taxonomists, and the taxonomists have become remarkably familiar with translocations and polyploids. These exchanges sometimes seem sporadic and aimless, but they are bound to grow; and the end result should be a more coherent biology, cultivated by people who think of themselves as biologists, however narrow the field of their active research.

Perhaps synthesis would be facilitated by an attempt to foster research from diverging points of view on limited groups of organisms. Something of the sort is being achieved with *Drosophila*, but this work has two handicaps: the lack of economic interest and its geographical limitation. The fact that *Drosophila* studies must be primarily academic is in one sense an advantage, since there is no pressure for direct "practical" studies of insecticides or methods of control; but it is a disadvantage in securing financial support for work on a large scale and in many places. The geographical limitation of the work, for instance, is a direct result of this: natural-history studies of *Drosophila* in the field are apt to be carried out in the vicinity of the great research centers, while many of the biological processes that demand intensive study involve the tropics and areas remote from re-

search centers. Support for such work is almost unobtainable without a direct economic incentive.

Here lies the great advantage of mosquitoes as subjects of biological study. Because of their economic importance, a great deal of taxonomic and descriptive spadework has been very competently done, and a large number of investigators have worked on mosquito problems from many points of view. Perhaps the greatest gap in our general knowledge of mosquitoes lies in the field of genetics, but with the increasing interest being shown in the possible genetic make-up of mosquito populations, means will surely be found of stimulating research from this point of view. There are, of course, many other obvious gaps, but mostly of the same sort and the result of the same phenomenon—the complete lack of interest on the part of the academic biologists in mosquitoes as experimental animals. Surely if these biologists were aware of the large amount of background information that has been accumulated, and of the ease with which many species can be cultivated in the laboratory, they would appreciate the potentialities of the group for further study. Which brings us back to the *raison d'être* of this book.

The term "Natural History" has been chosen for the title in the hope of emphasizing this broad approach to a limited group of animals. Elton (1936) has pointed out that natural history—both the term and the subject—has fallen into disrepute among zoologists, and he has given an interesting analysis of the reasons for this. There seems to be no question about the disrepute, and Webster's dictionary even includes the following comment: "Now, commonly restricted to a study of these subjects in a more or less unsystematic way." This perhaps reflects the general feeling that a broad approach must necessarily be superficial; and conversely, that profoundness is an essential component of specialization.

Elton contends that "ecology" is merely a new name for "natural history," and he would define both terms as the study of animals in their natural surroundings. I should like to give natural history a more inclusive meaning, so that it would cover both the field and laboratory study of living organisms, because the two types of study are intimately related. Field observations of the behavior of single organisms or of the interrelations of communities of organisms can hardly be interpreted without laboratory analysis under conditions where experimental techniques can be applied. On the other hand, laboratory

analyses of animal or plant behavior have little meaning if they are divorced from a knowledge of the natural environment to which the animals and plants are normally subject. The understanding of the functioning of the living organism would seem to be considerably handicapped by an organization of science in which field studies are filed in the pigeonhole "ecology" and laboratory studies in the pigeonhole "physiology."

The division between ecology and physiology is essentially a division based on technique, since the methods of study of organisms in the field and in the laboratory are necessarily quite different. The difference in technique leads imperceptibly, but surely not inevitably, to a difference in vocabulary, and the vocabulary difference may create a real barrier to understanding. Karl Schmidt (1946) has remarked that ecology could be defined as "that biological science in which a spade is termed a geotome"; physiology could equally well be defined as that biological science in which objectivity is achieved by the use of Greek radicals, and clearness held to be synonymous with anthropomorphism. The vocabulary of either of these sciences is a highly specialized affair which renders their content quite unintelligible to the uninitiated.

The use of a term like "natural history" in itself symbolizes a rebellion against the endless compartmentation of the biological sciences into specialized and mutually exclusive "ologies," but it is not intended as a substitute label for the entire field of biology. Biology necessarily covers all aspects of the study of organisms and living processes without stressing one particular point of view more than another, while natural history would levy contributions from the various specialities only in so far as their material would directly help the understanding of the living, functioning, whole organism. Thus the functioning of organisms can hardly be interpreted without a knowledge of structure, and morphology as well as physiology would be drawn into this concept of natural history. The important distinction is that neither physiology nor morphology interests the naturalist as an end in itself.

Taxonomy, in so far as it involves the sorting of animals and plants into their different kinds, is the necessary first stage of any natural-history study. It is also the basic filing system on which the whole construction of observation and experiment must rest. To quote Elton again: "It was of little use making observations on an animal unless

you knew its name." Taxonomy in this sense ought to be self-limiting; its job done, once all of the species are described and adequately monographed. Unfortunately the job is so tremendously large and complex that the limit today seems even more remote than it did fifty years ago. Even among mosquitoes new species are constantly being described, and the various taxonomists have reached little real agreement as to the limits and definition of the various systematic categories—tribal, generic, specific, and subspecific. There is a very real need for encouraging both extensive and intensive work on mosquito taxonomy. The fact that so much has been done means that perhaps the basic task of naming and differentiating the separate populations can be finished.

The material reviewed in this book under the heading of "natural history" then is largely material that is usually classified as belonging to the disciplines of ecology and physiology. It is, in fact, mostly animal behavior, which is generally treated as physiology. The term "general physiology" is in very frequent use to indicate a point of view in which the student seeks for uniformities, for general basic principles, that apply to the whole animal and plant worlds. The results obtained by work carried out from this point of view form an ample demonstration of its usefulness and importance; but it has become so dominant that we tend to forget that the existence of a general physiology must necessarily imply that there is also a special or comparative physiology. In studying different populations in a limited group of animals, such as mosquitoes, one inevitably becomes impressed with the *differences* among them; and it seems to me that the isolation and description of these differences may be just as important, for biological theory as a whole, as the isolation and description of the uniformities.

One of the basic problems of biology—it is indeed a sort of unifying theme that recurs in all biological work—is the problem of evolution, of the "origin of species." It is interesting to reread Darwin's book at intervals; to me it is always surprising to find how little it is dated. This is a tribute to the cautious and critical mind of Darwin, but it is also an indictment of the progress of biology. We have accumulated a great deal of information since 1859, and there have been some very important advances in biological theory, such as that offered by modern genetics. But our progress, as compared with that of the physical and chemical sciences, seems pedestrian. It often

seems to me that biological thinking, particularly in so far as it applies to this basic problem of evolution, has got somehow confined within a groove. Whether one reads the *Origin of Species* or such recent books as those by Dobzhansky (1941), Robson and Richards (1936), Goldschmidt (1940), or any contemporary entomological textbook, one is struck by the constant emphasis on structure; and I sometimes wonder whether preoccupation with structure is not one of the confining boundaries of the biological groove.

Differences in structure are, of course, easily seen and described. The classification of insects—of most animals and plants—rests necessarily on structure, because they are studied and filed as dead organisms. It is only where structure has failed because of the size or simple organization of the organism—as in bacteria—that we have been forced to base the criteria of classification on behavior. I am convinced that it would be within the range of possibility to build up a classification of insects based on behavior, and that such a classification would be as nearly natural as the present system. Any attempt of that sort would, however, serve no useful purpose unless to make us thankful that we do not as a routine have to culture each animal before we can give it a name. In mosquitoes it is apparent that structure has, in a few cases at least, failed us—has even misled us—and there, in order to classify our animals, we may have to resort to biological procedures. These cases serve to make us aware of the somewhat arbitrary nature of our structural classification and to remind us that while structure is a convenient guide to functional divergence, it is not an infallible one.

The case of *Anopheles maculipennis*—which will come in for a deal of attention in the ensuing chapters of this book—seems to me to underline strongly the weakness of the morphological approach to problems of evolution. It even seems possible that the "species problem," so much under discussion these days, is merely an artifact resulting from this approach. Almost all attempts to define the term "species" resort to behavior concepts: a species is usually defined as a "reproductively isolated population," to use the apt phrase of Alfred Emerson, or in other terms of reproductive physiology and behavior. Yet almost all of the studies centering on this species problem are morphological: studies of structural variation, of the genetics of structural characters, of the distribution of populations defined by structural characteristics.

The economic importance of mosquitoes, and perhaps the fact that much mosquito work has been carried out by medical men unhampered by conventional entomological training, has led to the accumulation of a relatively extensive literature on their behavior. This accumulation, inadequate though it is, seems to illustrate the possibilities of studies of comparative behavior of animal populations. It may be worth while to pause here to consider behavior as a "character" in the sense of the taxonomist. The importance given to a particular morphological character in taxonomy is largely empirical; wing venation, for instance, may be a good character for defining genera in one family, while in another family no variation in venation will be found, and in another family venation will be so variable that it is considered as of no significance whatever. Within a given group of organisms, however, structural characters can generally be ranked as individual, specific, and generic. In mosquitoes, for instance, the arrangement of the bristles of the thorax of the adult seems in general to be a good generic character; coloration, genital structure, the details of setae on larvae, are specific characters. The shape of wing scales was once considered a good generic character, but it is now considered as of specific value at most.

It is similarly difficult to evaluate behavior characters. It is difficult, for instance, to define the family precisely in terms of either structure or behavior. Adult mosquitoes are in all cases flying insects with functional sucking mouth parts, and the females for the most part suck blood; the larvae are in all cases aquatic, but breathe air either at the surface or (in a few species) by attachment to aquatic plants. Mating in nature almost always occurs on the wing, the females apparently attracted to groups of sexually excited, swarming males; exceptions to this seem to be sporadic (for example, *Anopheles atroparvus*) and aberrant sexual behavior is not known to characterize any particular group within the family. Absence of the bloodsucking habit is similarly sporadic and only occasionally characteristic of groups larger than species (for example, *Megarhinus*). Moisture in some degree seems to be necessary for the initiation of the oviposition response under laboratory conditions, though there is evidence to indicate that some species may lay eggs on dry ground in nature. Most adult mosquitoes are positively phototropic to a weak light, and many to a strong light; larvae, on the other hand, are mostly negatively

phototropic except when in search of air—the act of rising to the surface for air seems to be in part a light response.

Within the family many types of behavior seem to be group (that is, generic) characters. The type of larval habitat is a group character: some genera (or subgenera) breed in tree holes, others in water at leaf bases of plants, others in temporary ground pools. Hibernation may be a group character—for instance, all *Aëdes* seem to hibernate, or pass unfavorable seasons, in the egg stage—or it may be a specific character. In *Anopheles* two similar species may have quite different methods of hibernation (for example, *claviger* in Europe as a larva, *maculipennis* as an adult). Hour of flight seems generally to be a specific character, but some groups of species show similar flight times—*Wyeomyia* all diurnal, *Anopheles* and *Culex* largely crepuscular or nocturnal.

Specific behavior characteristics seem to be much more pronounced in the adult than in the larva, and are largely connected with the primary drives of hunger, sex, and oviposition. The larva is apt to show specific physiological characteristics such as thermal death point, rate of growth, salinity tolerance, and so forth, but the behavior of larvae of related species is in general strikingly similar. In adults the conditions under which mating (swarming) occurs seem to be a specific characteristic. One would perhaps expect that differences in genitalic structure and in sexual behavior would be primary in defining species, but the differences in sexual behavior have been very inadequately studied. The stimulus necessary to initiate feeding may be in part a specific character as there is growing evidence that closely similar species may show differing host preferences. The nature of the oviposition reaction is in part a group character and in part a specific character: it is a specific character in so far as it leads to the differences that are known to characterize the breeding places of closely similar species.

Thus behavior and physiology, like structure, seem always to have both general and specific aspects. The student of structure may be interested in tracing the general characteristics of digestive, respiratory, or skeletal systems, or he may be interested in describing the differences shown by orders, families, genera, and species. In the one case we usually call him a morphologist, in the other a taxonomist. Physiologists seem almost always to have been interested in the gen-

eral aspects of function, and we have no name for the study of the differential aspects of the subject. Yet the divergence in point of view is probably as wide as that which separates morphologists from taxonomists.

In summarizing the literature on mosquito behavior, then, I have tried to stress this point of view—to stress the differences in behavior among species and groups—with the particular object of demonstrating the material of this sort that is available for studies of evolutionary phenomena. The first part of the book is organized around the life history of the mosquito, taking up in turn the characteristics of behavior, physiology, and environmental relations of the adult, egg, larval, and pupal stages. Considerable space is devoted to the relations between mosquitoes and other organisms, especially to the vector relationship with plasmodia and viruses, since this relationship has been the subject of a great deal of study. Here again I have tried to stress differences among mosquito species, since differences in susceptibility to pathogens provide valuable clues to physiological divergences. The succeeding chapters on the species problem and on mosquito classification and distribution are intended to summarize material bearing directly on the problems of evolution. Since I hope that the book will be useful for reference for people actively engaged in mosquito research, a chapter on techniques has been added.

CHAPTER II

THE ENVIRONMENT OF THE ADULT

> It is usual to speak of an animal as living in a certain physical and chemical environment, but it should always be remembered that strictly speaking we cannot say exactly where the animal ends and the environment begins.—CHARLES ELTON

THE adult and larval stages of mosquitoes are so profoundly different that it is difficult to remember that they are two aspects of the same organism. Yet an understanding of the biological characteristics of a given species must be based on a study of all of the stages: adaptations of egg, larva, pupa, or adult may be the limiting factors controlling the ecological and geographical distribution of the species population.

The larva, obviously, must grow in the situation where the adult has placed the egg; less obviously, perhaps, the adult must inhabit an area where its peculiar requirements for larval breeding can be fulfilled. It would probably be futile to attempt to determine whether a diurnal forest mosquito, for instance, occupies this habitat because the larvae are adapted to a forest breeding place such as tree holes, or because the adults are adapted to a forest climate. Again the geographical range of a given species may be controlled by, say, temperature factors acting on either the larval or adult stage, or by some peculiar requirement of egg, larva, or adult.

Thus while the aquatic environment of the larva and the terrestrial environment of the adult require totally distinct adaptations, the adaptations in the case of any given species are interdependent. The problem, in preparing a description of mosquito biology as a whole, is to place this relationship in proper perspective. The basic functions of reproduction and dispersal are properties of the adult insect, and for this reason it is perhaps most logical to consider the adult characteristics first. The primary larval function is growth. Survival through regular or irregular periods of adverse conditions—important

in determining the geographical and ecological range of a species—may depend on adaptations at any or various stages in the life history.

The present chapter is devoted to a general consideration of the environment of the adult mosquito: of field and laboratory studies of the factors governing place and time of flight and place and time of resting. The subjects of longevity, distance of flight, and seasonal fluctuation are reviewed in the next chapter under the general heading of "survival and dispersal." Sexual behavior, food habits, and egg development and oviposition are then considered in separate chapters. The oviposition habits of the adult bridge the gap to the larval environment and furnish a logical introduction to the biological characteristics of the immature stages.

THE ADULT HABITAT

It is common practice to describe the "larval habitat" of a given mosquito species, but it is difficult to think of the adult as having a characteristic habitat. The adults of each species, however, form part of some animal community, and this community and its environment form the habitat of the species. One trouble, of course, is that we usually only observe adult mosquitoes at certain modal points in their lives: often we know only where and when they bite and where they lay eggs; in some cases we know where they rest during the period that the eggs are developing, and in a few instances we have observed the circumstances of mating in nature. Even with feeding behavior our knowledge is almost entirely restricted to behavior in connection with biting warm-blooded animals: we know practically nothing about the food of males in nature, or the food of species that do not attack man or his domestic animals. A quite large number of mosquito species are known only from adults that have been bred from larvae, and in such cases our ignorance of the adult behavior and habitat is really complete.

It seems probable that the females of most mosquitoes, after taking a blood meal, spend the period during which the ovaries are developing in a stage of inactivity. A few anophelines select houses and stables for passing this period, and since the characteristics of such resting places are readily available for study, they have formed the subject of a considerable amount of investigation which is best summarized separately. The resting place, however, is certainly an important part of the adult habitat.

Perhaps in the most general sense the adult habitat can be considered to be the landscape type in which the characteristic larval breeding place of the species most often occurs. "Distance of flight" is treated at some length as a special subject in the following chapter, but we may anticipate this somewhat by making the generalization here that most mosquitoes do not wander in significant numbers far from the larval breeding place. This seems to be particularly true of forest mosquitoes, and it is probably among forest mosquitoes that both larval and adult habitats are most sharply defined.

A very large proportion of tropical forest mosquitoes are diurnal. In the tropics, at least, mosquitoes are rarely found flying in any abundance in open country during daylight hours, perhaps because no species have solved the problem of withstanding the high temperatures and low humidities of this environment (a problem that black flies and sand flies seem to have solved very successfully!). Climatic conditions in the forest are much more favorable: temperatures are lower, relative humidity higher, and light is reduced to an almost crepuscular gloom.

It is interesting that these diurnal forest mosquitoes show a definite vertical stratification, some species being found only near the ground, others only in the canopy (Davis, 1944a; Bates, 1944a; Haddow, Gillett, and Highton, 1947). Garnham, Harper, and Highton (1946) have proposed the term "acrodendrophily" for "the tendency that is shown by certain sylvan mosquitoes for haunting tree-tops." This seems a rather unnecessary complication of vocabulary.

The orientation of the adult mosquitoes is presumably governed by the vertical gradients in light, temperature, and humidity that exist within the forest, but no laboratory analysis of the possible relative importance of these different gradients has yet been made. Bates (1947a) noticed that caged mosquitoes showed a vertical orientation similar to that found in the forest, even though no environmental gradients were demonstrable in the cage. There is a correlation between adult distribution and larval breeding places, in so far as all of the ground-pool breeders fall in the class showing "preference for ground level," and the species that breed in tree holes and bromeliads show a vertical difference in larval frequency similar to that of the adults (some data in Davis, 1944b). The vertical distribution of the common forest mosquitoes of the Villavicencio area of Colombia is shown in Table I, and of two African species in Figure 1.

TABLE I VERTICAL DISTRIBUTION OF COMMON FOREST MOSQUITOES IN THE REGION OF VILLAVICENCIO, COLOMBIA

SPECIES	TOTAL SPECIMENS	PER CENT IN TREE STATIONS	COMMENT
Trichoprosopon spp.	36	0	
Aëdes serratus	299	1	Preference for
Aëdes terrens	26	4	Ground Level
Psorophora ferox	252	6	
Wyeomyia spp.	1278	11	
Sabethes cyaneus	220	28	
Aëdes leucocelaenus	143	50	
Aëdes dominici	185	56	Indifferent
Haemagogus spegazzinii	4184	77	
Anopheles boliviensis	314	79	Preference for
Sabethoides imperfectus	454	92	Upper Levels
Sabethes belisarioi	179	98	

The swarms of pest mosquitoes of the flood plains of tropical rivers are all ground-pool breeders, and Baron Humboldt, who noticed everything, has commented on their reluctance to leave the lower forest levels. "The good missionary, Bernardo Zea, who passes his life under the torments of mosquitoes, has constructed himself, near his church, upon a scaffolding of palm trunks, a little room in which he can breathe more freely. We mounted there in the evening by the help of a ladder, to dry our plants and to write up our journey. The missionary had noticed with exactness that the insects abound mostly in the lower layer of the atmosphere, that which is near the soil, up to 12 to 15 feet in height" (quoted from the translation of Howard, Dyar, and Knab, 1913, p. 10).

It is possible that nocturnal mosquitoes also show characteristic differences in level of flight, but very few data are available on this point. MacCreary (1941) made an interesting study of the altitude of flight of nocturnal mosquitoes by means of light traps run at ground level and on towers eighty and one hundred feet above ground. Mosquitoes were much more abundant at the low-level traps in all cases, but the percentage of the total captures of the different species caught in the upper traps showed differences that may well be significant. Bates and Hackett (1939) pointed out that the observed differences in the larval habitats of *Anopheles maculipennis* (*typicus*) and *A. subalpinus* could be explained if it were assumed that *maculipennis*, when ready to lay eggs, flew at a relatively low altitude and *subalpinus*

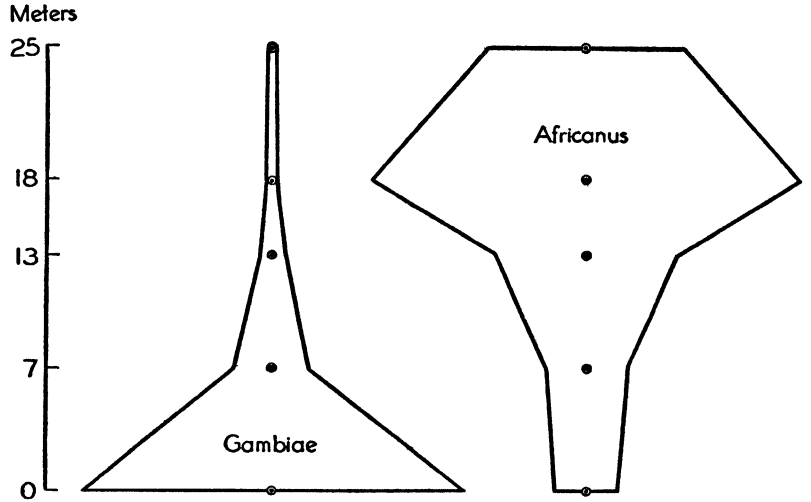


FIG. 1. RELATIVE ABUNDANCE OF *Anopheles gambiae* AND *Aëdes africanus* AT DIFFERENT HEIGHTS IN THE MAMIRIMIRI FOREST, UGANDA, DURING THE WET SEASON. (REDRAWN FROM HADDOW, GILLETT, AND HIGHTON, 1947.)

at a much higher altitude, since the chief difference between the larval habitats of the two seemed to be size of water area—*subalpinus* being rarely found in small accumulations of water.

TIME OF FLIGHT

Insect activity is generally classified as diurnal, crepuscular, or nocturnal, and all three types of behavior are well represented among mosquitoes. In general, time of flight is a group character—related species tending to show similar behavior. Thus the sabethines and certain groups of *Aëdes* are diurnal; species of *Anopheles* and *Culex* are, with very few exceptions, crepuscular or nocturnal. Diurnal mosquitoes are apt to be forest insects and, particularly in the tropics, are apt to show bright, metallic coloration.

It is, of course, easiest to measure "time of activity" of a given mosquito species by "time of biting," since this can be observed with relative facility. It seems likely, however, that biting activity shows the same general diurnal cycle as the other activities of the adult mosquito, though it may not be as precisely correlated with the daily climatic cycle as is, say, sexual activity. In general, mosquitoes that come

to bite in the day hours may also be observed to mate and lay eggs under these conditions, while mosquitoes that show crepuscular biting activity also mate and oviposit during the evening hours. That time of flight and time of biting do not necessarily correspond, however, is shown by the studies of Ribbands (1946) who found that *Anopheles funestus* and *A. melas* often did not bite until some time after they had entered houses.

Studies of the time of biting of diurnal forest mosquitoes have been made by Kumm and Novis (1938), Bates (1944a) and Haddow (1945b). From these studies, it appears that each species has characteristic flight habits, though it is not easy to define these sharply because of variations from day to day, probably related to meteorological differences.

The results obtained by Kumm and Novis with hourly captures of three diurnal species on the island of Marajó at the mouth of the Amazon are shown in Figure 2. It will be noted that these represent two distinct types of behavior: one bimodal with peaks in early morning and late afternoon, the other with a single peak at midday. In our studies of diurnal mosquitoes in the forests around Villavicencio (Bates, 1944a), we found that diurnal mosquitoes in general showed one or the other of these types of cycle, and we found it convenient to refer to a particular species as showing either the "*Psorophora ferox*" or the "*Haemagogus spegazzinii*" type of cycle. Haddow (1945b) observed the same phenomenon in the African Bwamba forests, making a primary distinction between species showing a single peak of activity in the twenty-four-hour period, and those showing two peaks of activity. Of the latter, *Aëdes simpsoni* was found to have the *ferox* type of cycle with peaks in the morning and afternoon; another group, characterized by *Mansonia fuscopennatus*, with essentially nocturnal habits, showed peaks after sunset and before dawn. Haddow proposed the term "eo-crepuscular" to describe this type of cycle, leaving the corresponding diurnal type unnamed.

The mosquitoes in the Villavicencio area that show the *Haemagogus spegazzinii* type of cycle (a single peak of activity at midday) form an interesting group of species: in general, they are apt to show also a preference for flying in the canopy zone of the forest and to have a metallic coloration. At any given hour during the day the vertical gradients in the forest are from darker, moister, and cooler in the floor zone to lighter, drier, and warmer in the canopy zone. The same

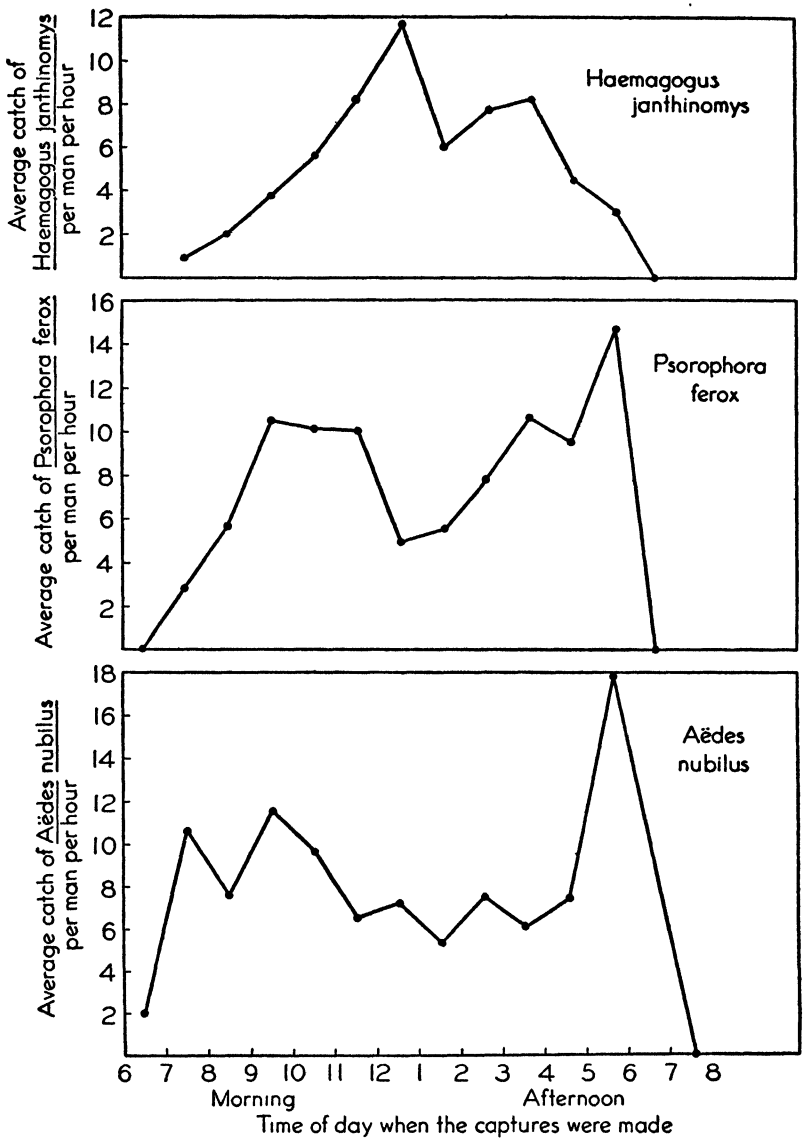


FIG. 2. BITING TIME OF THREE DIURNAL FOREST MOSQUITOES ON THE ISLAND OF MARAJÓ, BRAZIL (FROM KUMM AND NOVIS, 1938).

applies to the changes from morning to noon conditions at any given spot in the forest. It is thus perhaps not surprising that adaptations for midday activity should correspond with adaptations for life in the forest canopy. It is difficult to avoid the conclusion that the metallic coloration is also an adaptation related to this preference for lighter, drier, and warmer conditions. Haddow found that the African *Eretmapodites* showed the same type of biting cycle as the American *Haemagogus*, and his graph (reproduced here as Fig. 3) shows the relation between the daily cycles of mosquito activity, temperature, and humidity.

Species showing a single peak of activity in the night hours show an exactly reverse relation to the daily environmental cycle, as is brought out nicely in Haddow's graph of the activity cycle of *Anopheles gambiae* (Fig. 3). Relatively few data are available on the time of activity of nocturnal mosquitoes, but it is clear that the general impression that anophelines are predominantly crepuscular is not necessarily true. Probably many species show what might be called the *gambiae* type of cycle, with a peak of activity in the middle hours of the night. Thus Thomson (1941b) found that *Anopheles minimus* in India bit most commonly in the middle hours of the night; Garnham (1939) reports that *A. funestus* enters houses from 2:00 A.M. to dawn; Garnham (1945) found that *A. pharoensis* entered houses most commonly between midnight and dawn; and all night catches made in Panama by Harold Trapido (unpublished) showed that *A. punctimacula* in Panama entered stable traps most abundantly between midnight and 3:00 A.M.

Stage and Chamberlin (1945) have reported on a series of hourly catches of Alaskan mosquitoes, using a "rotary-type trap" that caught all flying insects. Unfortunately their data are not analyzed by species, so that only the general activity of all mosquitoes is shown. They found two clearly marked flight periods—at evening and dawn—and observed that the evening flight was of much greater magnitude than the dawn flight. This is also shown by the figures given by Kerr (1933) for *Aëdes africanus* and *Mansonia africana* in Nigeria. In the case of Kerr's observations, one might suppose that the majority of the hungry females would have found blood in the first flight; but this would hardly apply to the data of Stage and Chamberlin, since they were sampling mosquito flight, not females coming to bite.

Great irregularities in mosquito activity are commonly observed:

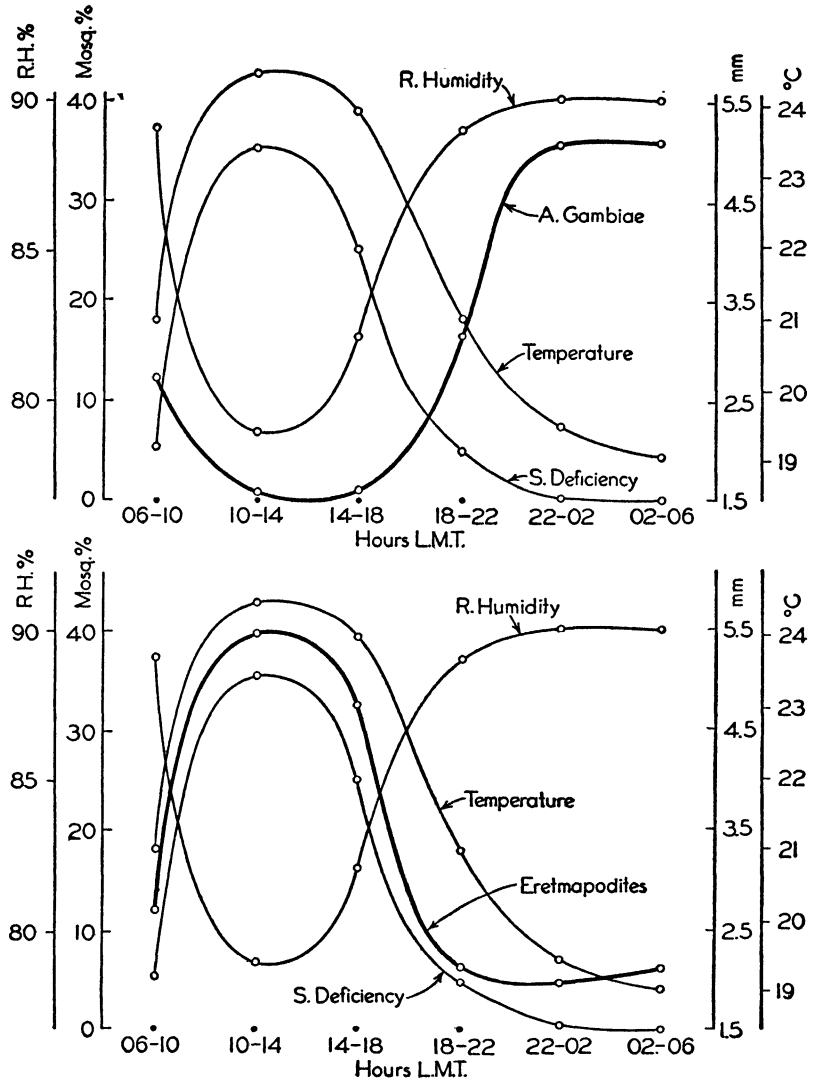


FIG. 3. RELATION OF BITING CYCLE OF THE NOCTURNAL *Anopheles gambiae* AND THE DIURNAL *Eretmapodites* TO THE DAILY CYCLES OF TEMPERATURE AND RELATIVE HUMIDITY (FROM HADDOW, 1945*b*).

a given species will bite much more abundantly on one day than on another, and in the course of a series of catches there will often be "waves" of mosquito abundance. This phenomenon has been graphically described by Shapiro, Saliternik, and Belferman (1944) in connection with nocturnal catches of *Anopheles sergenti*, and has been commented on by Haddow. Such irregularities mean that it is usually necessary to make a series of hourly captures on different days in order to get a satisfactory idea of the trend of the activity cycle of any given species, and individual captures seem often to give quite contradictory results.

ACTIVITY RHYTHMS

Evidence of physiological rhythms corresponding to the daily environmental cycle but continuing more or less independently of that cycle is fairly widespread in the animal kingdom. The literature on the subject has been reviewed by Welsh (1938). Relatively few laboratory experiments, however, have been made with mosquitoes. Exploratory experiments made in Albania with *Anopheles superpictus* (Bates, 1941b) seem to indicate that the activity cycle is to some extent independent of the environment, and thus a true physiological rhythm. This species was kept under conditions of constant, dim illumination and uniform temperature and humidity for thirty-six hours in one experiment and fifty-six hours in another, and in both experiments flight activity of the mosquitoes was renewed at twenty-four-hour intervals. This period could be reversed by subjecting the mosquitoes to light during the night hours and darkness during the day hours (this was observed also in *Anopheles messeae* by Polezhaev, 1940), so that the rhythm is relatively easily modified. A colony of *Aëdes aegypti* was kept under the same conditions as *Anopheles superpictus* in the Albanian experiments, but no evidence of activity periodicity was found: the mosquitoes were apt to fly at any time in response to a slight stimulus, as when the observer approached the cage.

In nature the physiological rhythm shown by the *Anopheles* is probably constantly controlled and modified by the environmental changes, particularly the changes in light, temperature, and humidity; but it is apparent that the possibility of an imposed rhythm must constantly be kept in mind in making laboratory experiments with behavior. The often noted refractory behavior and inconsistent results

in laboratory experiments with mosquitoes may in part be due to failure to take such rhythms into account.

RESTING PLACES

There is a rather extensive literature on the resting places of anopheline mosquitoes, in large part arising from practical malariological considerations. Where resting places are known, their study may be used as a means for determining relative abundance of different species or the seasonal or local abundance of a given species; material collected in resting places may serve for parasite indexes or for precipitin tests to determine the source of the last blood meal.

Because such studies have almost always been directly related to malaria problems, they are largely confined to anophelines, and little attention has been paid to the resting habits of other mosquitoes. The vast majority of mosquito species—including the majority of anophelines—rest in vegetation, and beating such vegetation with a net is a common method of collecting mosquitoes, as it is with many groups of insects. Field studies of the orientation of species with such habits would naturally be difficult, and our knowledge is pretty much confined to the accumulation of casual observations. Thus Barnes (1945) reports finding *Anopheles walkeri* on the dark shaded bases of mature cut-grass, *Zizaniopsis miliacea*. Wesenberg-Lund (1921, p. 38) remarks of *Aëdes cinereus* that "the mosquitoes sit deep down in the grass and do not fly up before they are disturbed."

Records of collections made by beating in different types of vegetation might reveal considerable habitat differences among various mosquito species, but I know of no such study. We have often noticed, in the forests near Villavicencio in Colombia, that the adults of *Orthopodomyia fascipes* usually rest on tree trunks, where their variegated markings make them very difficult to distinguish—presumably a case of protective coloration.

Many species of nocturnal mosquitoes, particularly of *Anopheles*, pass the day in shelters: in houses and barns, under bridges, and so forth. Studies of resting-place behavior almost always refer to such human constructions, and it is generally assumed that caves and tree holes form their natural counterpart. This shelter-seeking habit is particularly marked in the temperate zone, since it is a character of the commonest anopheline species of Europe and North America. Russell (1931) has summarized the literature on anopheline resting

places, emphasizing this difference between temperate and tropical species. The African *A. funestus,* however, shows marked house-haunting habits. De Meillon (1934) gives evidence to indicate that this species "immediately on hatching makes straight for a habitation, where it rests and feeds until its eggs are fully developed. It then flies out in order to deposit such eggs. After deposition it again flies back to a habitation."

The extensive interest in the resting places of the European *Anopheles maculipennis* and related species stems largely from the "anthropophilism-zoöphilism" theory of Roubaud (1920) and the "stabular deviation" theory of Wesenberg-Lund (1921). Wesenberg-Lund was the first to emphasize the tremendous numbers of *maculipennis* that could be found resting in stables, in contrast with their paucity in houses; and he pointed out that the disappearance of malaria in Denmark corresponded roughly with the change in agricultural practice that led to the construction of cow stables and pigsties. He postulated that this change in human habits had led to a change in mosquito habits that in turn broke the malaria transmission cycle. The work that resulted from these theories—including the study of house-haunting versus stable-haunting behavior—has been summarized in the report by Christophers and Missiroli (1934) and the books by Hackett (1937) and by Swellengrebel and de Buck (1938).

Two problems are often confused in these resting-place studies: the problem of the place where, and animal that, mosquitoes bite; and the problem of the environment selected for passing the day. Shannon (1935) presumably had this distinction in mind when he differentiated between studies of "day anophelism" and "night anophelism." Actually, in the case of the various species of the *maculipennis* group, there seems to be a high correlation between biting place and day resting place. This is shown, for instance, by the studies of Barber and Rice (1935a), who determined the source of the last blood meal for large numbers of specimens caught in houses and stables. In the case of *Anopheles sacharovi* (*elutus*), they found that 61.3 per cent of the specimens caught in houses had human blood, while 7.5 per cent of those caught in stables had human blood. In the case of *Anopheles maculipennis* (presumably a mixture of *messeae* and typical *maculipennis*), 21.2 per cent of the specimens caught in houses had human blood, in contrast with 0.5 per cent of those caught in stables. They were interested in the man versus cow preference of the two

species, but their figures also show that the mosquito does not necessarily spend the day near the source of the blood meal of the previous night.

The species of the anopheline subgenus *Nyssorhynchus* in tropical America are rather rarely found resting in shelters in the daytime; yet it was found in experiments with the insecticide DDT that spraying the inside walls of houses resulted in a great reduction of the anopheline population. Investigation of the nature of this effect led to the observation that the mosquitoes often settle on walls after biting in the house, moving away only with the change in light relations toward dawn. Something of this sort probably happens with all nocturnal mosquitoes: that is, their reactions to the night environment are not necessarily related to their reactions to the day environment. Hence the importance of Shannon's distinction between night and day anophelism.

Shannon (1935) studied the day resting places of four Greek anophelines (*superpictus, sacharovi, messeae,* and typical *maculipennis*) in two tunnels, one with the entrance inside of a stable, and the other built in a clay bank and opening directly out of doors. By means of partial partitions, he was able to get a graded series of compartments with different illumination, temperature, and humidity. He concluded that the most important factor governing orientation was light, pointing out that temperatures and humidities at dawn and until about one half hour after sunrise in all types of structures approximate those found outdoors: in short, at the time that the mosquitoes take up their diurnal resting place, light is the chief variable factor. Low light intensity is generally associated with relatively low temperatures and high humidities during the day—conditions favorable to the mosquitoes.

Shannon tried an interesting experiment with an earthenware jar which was left in a stable where it served as a resting place for *Anopheles sacharovi* and *maculipennis;* if the jar was exposed to the direct rays of the sun, the anophelines remained inside until the interior had been heated to 36°, "after which they were forced to brave the sunlight." In his tunnels, Shannon found that a light intensity of 1 to 5 foot-candles was preferred, and that no anophelines took up resting places in the absolutely dark compartments, although these were very favorable from the standpoint of longevity of caged mosquitoes. *Anopheles superpictus* tolerated higher light intensities (and

temperatures) than *A. maculipennis; Culex pipiens* selected darker (and cooler) sections than the anophelines. Eyles and Bishop (1943) investigated environmental conditions at the time of ingress and egress of *Anopheles quadrimaculatus* in shelters; they also found a direct correlation with light intensity.

The importance of light is further born out by various experiments with background color. The color preferences of resting mosquitoes were early observed; detailed studies made by Nuttall and Shipley and by Jordan and Hefferan have been quoted by Howard, Dyar, and Knab (1913, p. 216). Brighenti (1930) tried treating the walls of stables with washes of different colors; he found that mosquitoes (*Anopheles maculipennis* in the wide sense) rested most abundantly on areas of carmine red, violet, and yellow, in that order; and were least numerous in stalls painted cobalt blue or dark green.

Goodwin (1942) made studies of *Anopheles quadrimaculatus* with various types of artificial resting places; he found boxes one foot square to be the most attractive. He tried, with these boxes, the effect of height above ground, direction of opening, position in horizontal row, and different colors. He found significantly larger counts in boxes within three feet of the ground; direction of opening had no effect; in a row of boxes, more specimens tended to rest in the end boxes. He tried the following colors: white, yellow, red, blue, black, green. The largest number of mosquitoes were in the red boxes, next largest in black.

It may be noted that there is much evidence that in many insects light perception does not extend as far into the red as in man (though extending farther in the ultraviolet); in experiments with caged anophelines, we have often found that activity will cease in a red light, as in total darkness. The favorable effect of red in these experiments is thus probably due to the absence of light—from the mosquito point of view.

Various sorts of resting-place traps have been devised for catching mosquitoes, such as the box of Fletcher (1921), the earth-lined trap of Russell and Santiago (1934a), and the feather duster of Goré (1937).

LABORATORY STUDY OF ORIENTATION REACTIONS

The laboratory study of mosquito reactions is greatly handicapped by the mobility of the organisms and by the constant intervention of

escape reactions in caged animals. Yet it is only by the analysis of experiments involving single factors and simple groups of factors that we can hope to reach an understanding of the behavior of the organism in its natural environment.

Relatively little work has been done on the analysis of mosquito behavior by laboratory study: probably in part because of the difficulties, but also in part because of the unavailability of material in physiological laboratories. Most behavior work has been done on easily available animals like blowflies and fruit flies, and general physiologists seem never to have become interested in mosquito behavior. Mosquito students, on the other hand, seem rarely to have adopted the point of view of the general physiologists, and so there is a most unfortunate gap. That carefully controlled behavior work is possible on mosquitoes is shown by the studies that have been made at the London School of Tropical Medicine and Hygiene; and species such as *Culex fatigans*, *Aëdes aegypti*, *Anopheles atroparvus*, and *A. quadrimaculatus* could certainly easily be maintained as laboratory animals by the general physiologists if they became interested in the problems presented by these animals.

The orientation reactions of animals are generally referred to as "tropisms," and form the subject of a very voluminous literature. Many classifications of animal tropisms have been proposed, and the whole subject has been reviewed in a recent book by Fraenkel and Gunn (1940). These authors consider that the term "tropism" should be abandoned entirely as far as animal reactions are concerned, and they propose instead what they consider to be a more objective classification of "kineses, taxes and compass reactions." Mast (1941) in a review of the book has pointed out that the mere shift in terminology does not necessarily mean a gain in objectivity—does not necessarily result in the elimination of anthropomorphic implications.

A classification like that of Fraenkel and Gunn, however objective, certainly results in the introduction of a special jargon that may readily confuse the nonspecialist and make it difficult for him to follow developments in that particular field. The term "tropism" seems to be firmly entrenched, and any attempt, however laudable, to eliminate it from the zoological vocabulary is probably foredoomed to failure. In the course of general studies it may be impossible to make the behavior analysis necessary to determine the type of response governing a particular reaction, and it perhaps does not matter in such a

case whether the response is called "phototropism" or "orientation to light"—though the latter type of phraseology would seem to me preferable.

The principal factors that govern the environmental orientation of a mosquito seem to be *light, temperature,* and *humidity*. Reactions to *chemical factors* come into play chiefly in the search for food and for oviposition site and will be considered in relation to those activities; it is possible that reaction to *sound* is important in sexual behavior.

Light. In some ways, light is the easiest of the physical environmental factors to study in the laboratory, and it seems to be the predominant factor in guiding many mosquito orientations, yet comparatively little experimental work seems to have been done on it. The study is complicated because the light reaction of a mosquito may depend both on the kind of mosquito and on the activity rhythm of the mosquito. Two different species may react quite differently to a given experimental situation. It is also possible to set up identical experiments with mosquitoes of the same colony and at one time get one result and at another time another result, apparently depending on the physiological state of the mosquito. This is quite reasonable in that at one time light must serve to guide the mosquito to a resting place, and at another time serve to stimulate it to activity or to lead it to, or keep it in, its flying environment.

Almost all mosquitoes show a "tropo-tactic" reaction to light, that is, a reaction directly to or away from a source of illumination (contrasting with "kinetic" reactions which are carried out by random movements). Careful laboratory studies seem to be lacking, and most observations are based on the use of light traps in the field (Headlee, 1937; Johnson, 1938; Carnahan, 1939; MacCreary, 1939). The attraction of mosquitoes to light is perhaps such a common laboratory observation that no one has thought to make comparative studies of the differences among species. The attempts of mosquitoes to escape from cages must be largely light reactions; and many species, when they escape, head directly for the windows. There is surely, however, a measurable difference in the intensity of the reaction in different species. In Colombia, for instance, we have the impression that *Haemagogus spegazzinii* shows the strongest positive light response of any species that we handle; by utilizing this response the mosquito can easily be manipulated, as it will invariably go to the end of a glass tube directed toward the light, or to the side of a cage toward the light—

a reaction that is by no means so invariably true of other species, even from the same habitat.

Certain anophelines, and perhaps other nocturnal mosquitoes, show an avoidance of strong light, and if offered a choice of resting places will choose the darkest location, other factors being equal. Once a resting position has been taken, however, the mosquitoes tend not to move, even though light conditions are changed (de Meillon, 1937). Hundertmark (1938a) showed that in an apparatus with a series of compartments lined with paper shaded from white through grays to black, *Anopheles atroparvus* tended to select the darkest compartment during the day hours, and the lightest during the evening hours, showing the shift in reaction with activity state that was noted above. Anophelines do not, however, universally show this light avoidance during the day hours: it is, perhaps, a characteristic of the species that are normally found resting in the dark corners of buildings during the daytime (for example, members of the *maculipennis* group). Species that are never or rarely found in buildings (the Egyptian *A. pharoensis*, members of the American *Nyssorhynchus* group) show a strong positive reaction to a dominant light source during the day hours.

Experiments with the relative attractiveness of light of different wave lengths have been made with light traps in the field. Headlee (1937) found that blue light was most attractive, white light least; the one being 21.5 times as attractive as the other, calculating the number of mosquitoes caught per microwatt of radiant energy at a distance of 10 inches. Red and green-yellow light were also more attractive than white. Johnson (1938) found that the attractiveness of traps was increased as colors were tried through the visible spectrum from red to violet.

Some studies have been made on the influence of light (especially of different colors) on mosquitoes searching for food and oviposition places; such experiments can, however, most conveniently be considered in connection with the discussion of food and oviposition.

Temperature. The effect of temperature on mosquitoes has to be considered from two more or less distinct points of view: the effect on the mosquito of a gradient in temperature, where the mosquito can show a preference; and the effect on the mosquito of a constant environmental temperature, where no choice is possible.

If temperature plays a part in the habitat orientation of mosquitoes,

its effect should be demonstrable by experiments with temperature gradients. Such experiments are difficult because of the problem of producing a temperature gradient while maintaining a constant relative humidity, and the only detailed experiments that seem to have been made with mosquitoes are by Martini and Teubner (1933) and Thomson (1938). Martini and Teubner used an apparatus with a series of ten chambers, in each of which the temperature and humidity could be controlled; for temperature experiments, they maintained uniform humidities by using different desiccating solutions. Thomson studied the humidity reactions of the mosquito first, and found a range of humidity in which the animals seemed insensitive to differences; he then regulated the absolute humidity in the temperature gradient so that the extremes of humidity eventually set up were well within the inactive zone.

Martini and Teubner experimented with *Aëdes aegypti* and overwintering *Anopheles maculipennis* (in the wide sense); with a temperature gradient between 32° C. and 15° C. (the humidity varying between 68 per cent and 72 per cent), 50 per cent of the *aegypti* went to the coolest compartment (observations over a three-day period), *A. maculipennis* showed a more random distribution, with a definite predominance in the compartments at 19° and 20°. In a second experiment with *maculipennis*, essentially the same results were obtained.

Thomson's experiments are hardly comparable with those of Martini and Teubner, as he used a quite different technique, exposing his mosquitoes (*Culex fatigans*) to five-degree temperature changes in different parts of the temperature scale. As he found considerable behavior differences in mosquitoes in different stages of development, he made separate experiments with newly emerged females, hungry females, blood-fed females, and females with mature ovaries. He found that "the most striking feature of behavior at all stages was the strong avoidance of high temperatures. This was strongest in hungry females, less strong in the blood-fed females and those with mature ovaries, and least strong in the newly emerged females." Newly emerged females avoided the warm side of the experimental container when the extreme temperatures were 25° C. and 30° C., but with choices below 25° C. they either were indifferent to the temperature gradient or showed a slight preference for the warm side. It is interesting that when the temperature extremes were narrowed to 29° C. and 30° C., there was still a clear avoidance of the warmer side of

the container. Since the temperature gradient was set up across a container 20 cm. in diameter, this meant that the mosquitoes were reacting to an average temperature difference of 0.05° C. per centimeter across the dish. At lower temperatures, the mosquitoes were much less sensitive to slight differences.

Humidity. Martini and Teubner, using their ten-chamber apparatus at constant temperatures and with varying humidities, found that *Theobaldia annulata* clearly preferred the highest humidities, the reaction being sharper at high temperatures than at low. *Aëdes aegypti* showed a less pronounced preference for the highest humidities, but the experiments were made at 20°, which the authors consider may have been too low for this tropical species. A series of experiments with *Anopheles maculipennis* showed a general preference for high humidities, though the reaction was by no means always sharp.

In the humidity experiments of Thomson, the mosquitoes (*Culex fatigans*) were kept in the dark at a constant temperature of 25° C. in a container 20 cm. in diameter in which a humidity gradient was maintained by means of different humidifying solutions on opposite sides of the container. The mosquitoes were experimented with separately according to their stage of development, as in the temperature experiments. In all cases, the strongest reaction was an avoidance of the higher humidity when the alternatives were both above 95 per cent relative humidity. This reaction was most pronounced in the fed and mature females, less pronounced in the newly emerged females, and weakest in the hungry females. Blood-fed females were found to be sensitive to a difference of 1 per cent relative humidity across the 20 cm. diameter of the dish when the alternatives were both near saturation: which would mean that the mosquito was reacting to a humidity difference of 0.05 per cent per centimeter across the dish.

Mosquitoes in all stages of development showed a slight but regular avoidance of low humidities, provided a sufficiently large humidity range was present (at least 40 per cent difference between the two sides of the dish). Within the range between 30 and 85 per cent relative humidity, the mosquitoes were completely unaffected by humidity differences when the difference was 40 per cent or less.

CHAPTER III

SURVIVAL AND DISPERSAL

> "Well, in *our* country," said Alice, still panting a little, "you'd generally get to somewhere else—if you ran very fast for a long time as we've been doing."
>
> "A slow sort of country!" said the Queen. "Now, *here,* you see, it takes all the running *you* can do, to keep in the same place. If you want to get somewhere else, you must run at least twice as fast as that!"—LEWIS CARROLL

THE three topics of longevity, flight range, and seasonal distribution are grouped together under this heading of "survival and dispersal." The survival of the species, of course, depends on adaptations of all stages, so that the factors governing adult longevity are but one aspect of the general problem. Dispersal, on the other hand, is purely an adult function, since the eggs are stationary and the range of movement of the larvae very limited. An exception would be the domestic *Aëdes aegypti,* which has spread through much of the tropical zone of the world because of its habit of laying eggs in domestic water containers; the eggs may remain dormant for long periods of time, and thus be transported by man along with the containers. This, however, is surely a happy accident from the point of view of *Aëdes aegypti,* and not a mechanism of dispersal designed as such by natural factors. Seasonal distribution—the response of the species population to the annual climatic cycle—is of direct interest in relation both to species survival and species dispersal. It may provide clues both to optimal climatic conditions and to limiting climatic conditions which govern survival and affect dispersal.

LONGEVITY OF THE ADULT

The study of mosquito longevity is rendered difficult by the impossibility under most circumstances of making direct observations. Data on the average length of life of adult mosquitoes of different species under different climatic conditions would be of great value from the standpoint of ecological theory, especially for the study of what has

aptly been called population dynamics. It is also of obvious and immediate medical interest in the study of disease transmission. Longevity studies under controlled laboratory conditions can be made quite readily, but numerous questions arise when an attempt is made to interpret these results in terms of natural conditions. Studies of survival in nature by the use of marked mosquitoes are subject to the same limitations as flight-range studies by this method, and, indeed, low percentage of recoveries renders the results quite meaningless.

Sinton and Shute (1938) in a review of the literature on mosquito longevity point out that this may be influenced by four types of factors which may be paraphased thus:

1. Characteristics of the mosquito species.
2. Activities of the individual mosquito.
3. Climate.
4. Incidence of parasites and predators.

Characteristics of mosquito species. It seems quite clear that under given environmental conditions each mosquito species has characteristic average survival in the adult stage and that various mosquito species show considerable differences in survival under identical conditions. It is also likely that there would be considerable differences among species in survival under natural conditions. Perhaps one of the factors that determine whether a given anopheline species will be a vector of malaria is its longevity in nature. It seemed to us, for instance, that the complete absence of quartan malaria and the comparative rarity of other types in parts of Egypt where *Anopheles pharoensis* was very abundant might be explained if this species had a comparatively short life as an adult. Malaria was intense in the parts of Egypt where *A. sergenti* occurred, and under laboratory conditions we found that *sergenti* lived much longer than *pharoensis.* Mayne (1930) found that under given laboratory conditions *A. annularis* (*fuliginosus*) survived much longer than *A. culicifacies* or *A. subpictus.* Sinton and Shute (1938) found *A. atroparvus* to be hardier than *A. maculipennis* or *A. messeae.* Treillard (1938) found that *Anopheles minimus,* a malaria vector, lived five to ten times as long as *A. vagus,* which is not a vector.

These laboratory experiments must, of course, always be interpreted with caution, since species that would be found in diverse environments in nature may show abnormal behavior when kept in

a standard laboratory environment. Thus in Colombia (Bates, 1947b) we found that *Aëdes serratus* and *Psorophora ferox* would live for very long periods when kept in small cages in a cellar where the temperature was quite constant at 25° and the relative humidity at 90 per cent. *Haemagogus spegazzinii* under these conditions survived for only four or five days at most, though we had good reason to believe that its average life in nature was as long or longer than the *Aëdes* and *Psorophora.* When the mosquitoes were kept in individual shell vials at higher temperatures the *Haemagogus* survived much longer than either the *Aëdes* or *Psorophora.*

Activities of the individual mosquito. The influence of the activities of the individual mosquito on survival are shown by the difference between hibernating and active specimens of the species that hibernate as adults. The evidence with regard to *A. atroparvus* has been summarized in some detail by Sinton and Shute under the heading "the metabolic and biological activities of the female mosquito in relation to its span of life." Hibernating individuals, in a condition of "gonotrophic dissociation," may live for months, and *atroparvus,* at least, transmits malaria while in this condition. (See summaries in the books by Hackett, 1937, and Swellengrebel and de Buck, 1938.)

Climate. Many laboratory studies have been made showing the effect of temperature and relative humidity on mosquito survival, and there is no question but that longevity in nature varies with seasonal and geographical changes in climate. The season of malaria transmission, for instance, may be determined not only by conditions favoring anopheline abundance, but also by conditions favoring greater individual length of life. The effect of climate on the survival of *Anopheles culicifacies* is nicely shown by the experiments of Russell and Rao (1942b) with survival in a large outdoor cage. A chart summarizing the results of their experiments is reproduced in Figure 4.

It is very difficult to evaluate temperature and humidity factors separately in relation to mosquito longevity. Most insects are unable to survive at very low humidities, probably because of inability to control water loss (see Wigglesworth, 1939, pp. 354–359). In general with mosquitoes the higher the humidity the longer the survival (for example, the experiments of Hundertmark, 1938b; Leeson, 1939), but in many cases, at least, this does not apply with very high relative humidity. Thomson (1938) found that in selection experiments *Culex fatigans* avoided very high humidities. Mehta (1934a) found that hu-

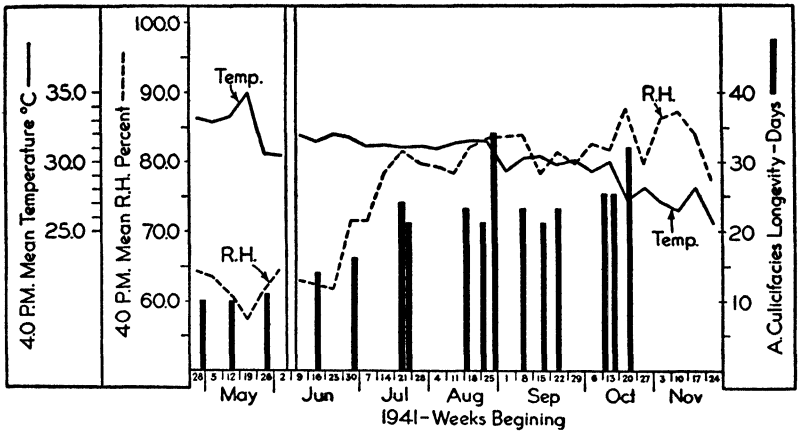


FIG. 4. LONGEVITY OF *Anopheles culicifacies* IN AN OUTDOOR CAGE IN RELATION TO SEASONAL CHANGES IN TEMPERATURE AND HUMIDITY (FROM RUSSELL AND RAO, 1942*b*).

midities above 90 per cent were injurious to *Anopheles subpictus* in the laboratory. Rajindar Pal (1943) in a study of the longevity of *Anopheles culicifacies* under controlled temperature and humidity conditions, found that at low temperatures (12.7° C.) a saturated humidity was favorable, but that at higher temperatures (25° C. and 30° C.) survival at a relative humidity of 60 per cent was appreciably longer than at either higher or lower humidities (20, 40, 80, and 100 per cent). Russell and Rao (1942b) have summarized various papers indicating that lower humidities may be favorable at relatively low temperatures.

Under constant humidity conditions, the lower the temperature the greater the longevity of the mosquitoes. The increased longevity may have no biological advantage, however, since all metabolic processes (for example, egg development) are similarly retarded (Bates, 1947b).

Incidence of parasites and predators. Sinton and Shute (1938) have reviewed the evidence of many experiments on the effect of plasmodium infection on mosquito longevity. It can be said that there is no evidence that such infection has any unfavorable effect on mosquito survival. This seems also to be true in the case of virus infections. On the other hand nematodes, particularly filaria and mermithids, may cause a very heavy mortality (see Chap. XIII). It would be a prac-

tically impossible task to gather statistics on the effect of predators on adult mosquito populations in nature, but it is my impression that predators are more important as enemies or larvae than of adults—that the chief limiting factors in adult survival are probably climatic.

This leaves unanswered the basic question of what is the mean length of life of a given species in a particular natural environment. Russell and Rao (1942b) from observations on *Anopheles culicifacies* in a large outdoor cage came to the conclusion that the death rate was 50 per cent every two days; the "probable duration of life" (that is, the age at which half of the population dies) was thus 2.0 days, and the "average life" was 4.0 days; the maximum longevity of females varied from 8 to 34 days. Most students of the subject have concluded that mortality in an adult anopheline population is by geometric progression, as shown by Russell and Rao, but few have arrived at as short an "average life" as these authors found.

For a species to be an efficient vector of malaria, a considerable number of individuals would have to live for more than 10 days to transmit *Plasmodium vivax* or *P. falciparum* or for more than 20 days to transmit *P. malariae*. Boyd (1930) suggested approaching the problem by a comparison of the percentage of individuals of an anopheline population showing oöcysts with the percentage showing sporozoites, since an interval of 7 to 10 days would elapse on an average between the two stages. From a series of *Anopheles albitarsis* dissections in Brazil, he concluded that the mortality was somewhat more than 50 per cent in this interval (6.2 per cent of the mosquitoes with oöcysts and 2.8 per cent with sporozoites). This is similar to the surmise of Hackett (1937, p. 67), based on rate of adult disappearance after suppression of breeding, that adult mortality is somewhat more than 50 per cent a week. Under special circumstances, such as hibernation, adult life may be very prolonged, and it is probable that many individuals in such cases live for six or seven months (for example, observations of Cambournac and Hill, 1939, on *Anopheles atroparvus*). All authors are in agreement that the life span of the males of a given species is much shorter than that of the females.

DISTANCE OF FLIGHT

The question of the extent of dispersal of anopheline mosquitoes is of direct importance in malaria-control operations, and consequently has received a great deal of attention from medical entomologists.

Studies of rate and extent of dispersal of organisms are also of very considerable interest from the point of view of general biology: they may indicate the extent to which a species becomes broken up into more or less genetically independent local populations (the raw material of evolutionary change) and the extent and rapidity with which a species population can take advantage of, or be adversely affected by, new or changed environmental situations. Wolfenbarger (1946) has written a general summary of the literature on the dispersion of insects and other small organisms.

Two methods have been commonly used in mosquito dispersal studies: the release and recapture of marked individuals, and observation of dispersal from known breeding places. Neither method is apt to give very satisfactory results. Experiments involving the release of marked mosquitoes require great labor and a rather complex organization for the collection and identification of the released specimens, and even under optimum conditions only a very insignificant proportion of the marked mosquitoes is ever recaptured. The method of direct observation is also of limited usefulness because it is rare that dispersion from a single breeding place can be studied.

As might be expected, the studies that have been made show that range of flight is a characteristic of the species of mosquito and of local topography, so that it is not possible to make a rule of thumb for malaria-control officers. In general, anopheline mosquitoes do not spread in great numbers far from their breeding places, and larval control measures for towns and villages are generally restricted to the immediate periphery of the area to be protected. Hackett (1937, p. 206) has remarked that in the tropics control work over an area with a radius of a kilometer will protect a center, but in Europe it has generally been found that control work must be carried out to a radius of 4 or 5 km., probably because the species of the *Anopheles maculipennis* group show an unusually wide range of dispersal. Malariologists also recognize a rather obvious general rule that the distance of flight of anophelines in significant numbers depends on the size of the breeding place.

The method of studying mosquito activity by the recapture of marked specimens was first proposed by Zetek (1913), who tested a number of dye solutions. Earlier experiments with this method have been reviewed by Russell and Santiago (1934b and 1934c). The method of marking mosquitoes with the metallic gold, silver, and

bronze powders used by printers was first introduced by Majid (1937), and this technique has since come into wide use because of the ease with which the metallic particles can be detected on recaptured mosquitoes by examining them with a dissecting microscope. Weathersbee and Hassell (1938) were able to identify adult mosquitoes that had bred from larvae fed with vital stains and this method offers the possibility of studying dispersion from a particular breeding place, though it has not yet been adequately tested under field conditions.

Perhaps the most thorough study of the flight range of marked mosquitoes is that made by Russell, Knipe, Rao, and Putnam (1944) with *Anopheles culicifacies* in the Madras Presidency. They used the dusting method of Majid and found that this resulted in no damage to the mosquitoes, in contrast with a 30 per cent or more mortality in experiments made with liquid stains. All of their marked mosquitoes were released at a central point and attempts at recapture were made in 80 calf-baited traps arranged in concentric circles at distances of one-quarter, one-half, three-quarters, and one mile from the central point of release. The statistics of their experiment are interesting, in that they show the large amount of labor necessary for the application of this type of study. A total of 54,950 marked specimens were released; 207,800 mosquitoes were captured and examined for markings, 601 marked specimens being found—a recovery of 1.1 per cent.

The results of the trap captures are summarized in Table II. Captures were also made in near-by villages and "the total observed flight range was between 1.5 and 1.75 miles. Nine specimens made this flight in a single night and seven others made it in two nights. Three of the flights were made against the wind." Males, as can be seen from the table, did not disperse as widely or as rapidly as females.

TABLE II RECAPTURES OF MARKED *Anopheles culicifacies* IN FLIGHT-RANGE EXPERIMENTS OF RUSSELL, KNIPE, RAO, AND PUTNAM

TRAP DISTANCE	MALES			FEMALES			PER CENT IN TRAPS	
IN MILES	1st day	2nd day	Total	1st day	2nd day	Total	Males	Females
0.25	56	15	71	157	23	180	67.6	39.7
0.50	13	3	16	99	14	113	15.2	24.9
0.75	5	5	10	75	18	93	9.5	20.5
1.00	6	2	8	54	13	67	7.6	14.8
Totals	80	25	105	385	68	453	99.9	99.9

In the quarter-mile traps more specimens were recovered in the "with-wind" quadrant than in the "against-wind" quadrant (251 to 69), but in the one-mile traps the captures were the same (18). "These data indicate that *culicifacies* can and do fly against the wind in good numbers. In longer flights, the numerical difference between those going with the wind and those going against it is not marked, but in shorter flights considerably more specimens flew with the wind than against it."

The percentage of recovery in staining experiments is generally less than 1 per cent, and it is very difficult to form much of a picture of the flight habits of a given mosquito species from such meager returns. Recoveries are apt to be made at widely dispersed points, and about the most that can be proven is that occasional specimens of the particular species tested do fly for one, two, or three kilometers. Shapiro, Saliternik, and Belfermann (1944), for instance, released over 10,000 marked *Anopheles sergenti* in Palestine, and recovered only one specimen; this, however, was found on the second night after release at a distance of over 4 kilometers. The problem is to judge whether such recoveries represent exceptional individuals or a general trend. The frequency with which marked mosquitoes have been recovered at considerable distances from the point of release, however, would seem to indicate that relatively long flights are at least not uncommon among a wide variety of anophelines.

Russell and others (1944) included some speculation as to what happens to the 99 per cent of the marked mosquitoes that are never seen again. They believe that *Anopheles culicifacies* predominantly selects shelters for resting places, and that the failure to recover a higher proportion of mosquitoes in their experiments reflects the high mortality rate in nature. This brings in the problem of where most anophelines do rest during the day. It is a common experience of entomologists to find larvae of some mosquito species abundantly, and yet rarely encounter the adults; again the sexes are produced in about equal numbers in nature, but even in the case of species with pronounced shelter resting habits, such as the members of the *Anopheles maculipennis* group, only an insignificant number of males turn up in the shelters. It seems likely that with many species, at least, our methods of collection yield a quite inadequate sample of the population, which in turn makes the interpretation of flight experiments very difficult.

One of the most interesting studies of the flight habits of anophelines by direct observation was carried out by Swellengrebel and Nykamp (1934) in connection with the land-reclamation project of the Zuider Zee in the Netherlands; summaries of this study have been made by Hackett (1937, p. 206), and Swellengrebel and de Buck (1938, p. 110). The new reclaimed land (polders) was too salty for anopheline breeding during the first year, so that any adults found there must have come from older land. Swellengrebel built two pigsties at 3 and 5 kilometers from the nearest old land and collected on an average 13 females and 2 males daily at the 3-kilometer pigsty, and 7 females and 1 male daily at the 5-kilometer pigsty. Larval breeding had not yet begun in the new polder in the following year, and at this time an additional pigsty was built at 10 kilometers. The mosquito catch increased considerably in all of the pigsties because of the abandonment of larval control in adjacent old land, and the average daily catch was 69 anophelines at the 10-kilometer pigsty. The species concerned in these observations was *Anopheles atroparvus*, and they show very clearly that both the males and the females may disperse in appreciable numbers for considerable distances from their breeding places.

Hopkins (1941) reported finding *Anopheles funestus* in considerable numbers at a point 2 miles from the nearest breeding place; he considered that this was an unusual occurrence, explained by the size of the breeding area and the favorable prevailing wind. De Meillon (1934) was able to make a study of the dispersal of *A. funestus* from a breeding place in the Transvaal, finding that 80 per cent of the adults were found within a half a mile (0.8 km.).

Data on the flight range of mosquitoes other than anophelines are rather meager, though there is evidence that some of the "pest mosquitoes" of the genera *Aëdes* and *Psorophora* may make long flights in considerable numbers. Studies of such species have been made by Horsfall (1942), Clarke (1943), and MacCreary and Stearns (1937), all reporting dispersal in significant numbers over several miles. MacCreary and Stearns, collecting *Aëdes vexans* and *A. sollicitans* at light traps in Delaware Bay, found a higher proportion of males at a trap 8.4 miles from land than at nearer traps, which contrasts with the usual results in studies of anophelines. Matheson (1944, p. 43) quotes a report by Curry of collecting numbers of *Aëdes sollicitans* on shipboard 110 miles east of Cape Henry.

Mosquitoes that stray into the upper atmosphere may get carried for very great distances, and it is interesting that Glick (1939) in airplane trapping experiments in Louisiana caught 11 specimens of *Anopheles quadrimaculatus* at altitudes up to 1,000 feet, and found a specimen of *Aëdes vexans* at 5,000 feet.

SEASONAL DISTRIBUTION

There is a considerable accumulation of observations on variation in seasonal abundance of mosquitoes, particularly anophelines, since the importance of the subject in relation to malaria studies is obvious. Such studies also, however, are of general biological interest. Students of the statistical aspects of natural selection and of the distribution of genetic changes in populations have pointed out that the spread of variants through a population is bound to depend in great part on the nature of the fluctuations in population density; and the subject of seasonal and annual cycles has thus become of basic interest in relation to the processes of evolution. Such studies also form an integral part of any ecological work, since the environmental characteristics change in time as well as in space. Indeed, the adaptations of a particular species to factors in the physical environment (climate) may be as clearly shown by the seasonal distribution of the species as by the habitat or geographical distribution—and it may be as difficult to isolate the particular limiting factors that control abundance in time as it is to isolate those that control distribution in space.

Any datum on mosquito abundance is bound to be an index, since there is no way of making a census of the actual number of individuals found in a particular area. For the most part the index serves only as a guide to population fluctuations in a given place, or to variations from place to place: the rise and fall of the index is assumed to correspond directly to fluctuations in the absolute populations, but we have no idea of the real size of the population at any time or place.

As far as I know, only one attempt has been made to determine the actual *density* of population of adult mosquitoes in a particular area: this is the study carried out by Eyles and Cox (1943) in the Reelfoot Lake area of Tennessee. They used the so-called "Lincoln index" as developed by C. H. N. Jackson for population studies of *Glossina*. By this method, a random sample of individuals is marked and released in a given area; at some later date a random sample from the area is caught and examined for markings: the proportion of marked

individuals to total sample in the second capture should be the same as the proportion initially marked to the total population of the area. There are, of course, many variables and possible sources of error in applying such an equation to mosquito studies—these are discussed in the paper by Eyles and Cox—but the method nevertheless has very interesting possibilities. In the Reelfoot Lake experiment, Eyles and Cox arrived at a population estimate of 8,450 to 14,750 females of *Anopheles quadrimaculatus* per acre in July and August.

A variety of sampling methods has been used for studying seasonal fluctuations in populations of adult mosquitoes, and the subject perhaps warrants some discussion.

Analysis of miscellaneous collections. A good idea of mosquito prevalence can often be obtained by the accumulation of what might be called "miscellaneous observations." This is particularly true in areas where the fauna has been intensively studied from other points of view. A particularly good illustration of the method is the "Life-History Calendar of the British Mosquitoes" given by Marshall (1938). Such studies are very useful in connection with general ecological work, but they do not permit statistical handling.

Captures at bait. Perhaps the simplest form of standardized capture is the routine collection of mosquitoes from human or animal bait. The method is chiefly useful for diurnal mosquitoes, as the captures depend on being able to see the mosquitoes. The papers by Kumm and Novis (1938) and Bates (1945) may be cited as examples of the type of results that can be obtained by this method.

Captures in resting places. This is one of the most extensively used methods of checking on mosquito abundance. It is sharply limited, however, to mosquitoes with known resting habits, such as the species of the European *Anopheles maculipennis* group. The results may be expressed by either of two methods: total mosquitoes caught in a particular situation or mosquitoes caught per man-hour of collecting. The former method is feasible where stables, for instance, are well constructed so that it is possible to be reasonably sure that all mosquitoes resting in the enclosure have been counted. The expression of results per man-hour perhaps puts a greater premium on the skill of the particular collector, but the method seems to give a working idea of relative abundance, at least of certain species of anophelines, and it has been extensively used in checking on malaria control operations.

Captures in traps. Three main types of traps have been used in studies of mosquito prevalence: light traps (Headlee, 1932), resting-place traps (Russell and Santiago, 1934a), and stable traps (Magoon, 1935; Shannon, 1939; Bates, 1944b). Any trapping method seems to have a selective action—that is, certain species may enter the traps more readily than others—so that traps must be used with caution for determining the relative abundance of different species. Trapping methods are, however, more easily standardized than other types of capture and for that reason may give a more accurate idea of fluctuations of abundance of a particular species than other methods. Factors that may cause variations in light-trap captures have been discussed by Huffaker and Back (1943), and the limitations of the stable-trap method have been discussed by Gabaldon and others (1940) and Bates (1944b). The ideal study of seasonal variation in mosquito populations would involve several methods of sampling of both the larval and adult stages, as was done, for instance, in a study of the anophelines of southeastern Madras by Russell and Rao (1941).

Types of seasonal cycles. In the temperate zone seasonal changes are primarily related to the cold period (winter), in the tropics to periods of dry weather (dry season, or seasons). In both cases the type of seasonal distribution shown by a given species of mosquito seems to depend on the type of adaptation shown by the species for surviving the unfavorable season, that is, on method of hibernation or aestivation. In the temperate zone, mosquitoes may show hibernation adaptations in the egg, larval, or adult stage; or in the warmer parts of the temperate zone, they may show no special hibernation mechanism, but simply continue development at a slower rate directly dependent on the decreased temperature.

The temperate-zone situation, being the best known, may be considered first. The accompanying table, copied from Wesenberg-Lund (1921) shows the types of seasonal distribution found in Denmark. This table is, of course, an oversimplification, as Wesenberg-Lund points out in his discussion, but it is for that very reason a very convenient guide. The species marked with an asterisk are also found in England, which makes it possible to check the Danish observations with English material summarized by Marshall (1938) and thus gain an idea of the effect of the milder English climate in modifying the species behavior. The life histories of the North American mosquitoes have not been so well summarized, but the situation clearly parallels

that found in Europe and generalizations from the one fauna apply equally well to the other. It may be convenient to name the different types of seasonal cycles after well known species that show each particular type of cycle, as is done below.

I. The *Aëdes cinereus* type of cycle. This species, both in Denmark and England, has only one generation a year, and its life history in the United States seems to be very similar (Matheson, 1929, p. 107). Hibernation is in the egg stage. This type of cycle is typical of the majority of the aëdine mosquitoes of the colder parts of the temperate zone.

II. The *Aëdes caspius* type of cycle. This is essentially only a modification of the *cinereus* type of cycle: hibernation is in the egg stage, but there may be an indefinite number of generations a year. *Aëdes caspius* has a very wide range in the Old World and in warmer areas (that is, North Africa) the species may breed continuously. This modification of the *cinereus* cycle is perhaps typical of aëdine mosquitoes in the warmer parts of the temperate zone. Many species which show a *caspius* cycle in the southern part of their range are single-brooded (*cinereus* cycle) in the north.

III. The *Anopheles claviger* type of cycle, illustrated in Wesenberg-Lund's table by *Aëdes rusticus*, *Theobaldia morsitans*, and *Mansonia richiardii*. Hibernation is in the larval stage and there is apt to be only one generation a year in northern countries such as England and Denmark. *Anopheles claviger* presents perhaps the best studied example of larval hibernation—a phenomenon that will be discussed along with other aspects of larval behavior in a later chapter.

IV. The *Culex pipiens* type of cycle: hibernation in the adult stage. The various mosquitoes of the *Anopheles maculipennis* group also show this type of hibernation.

The physiological adaptations that determine whether suspension of development (diapause, hibernation) occurs in the egg, larval, or adult stages are profoundly different, and studies of these various mechanisms will be discussed in later chapters. The different types of cycles result in quite different curves of adult abundance. Species with the *cinereus* and *caspius* types of cycles tend to appear in great numbers, quite abruptly: with the *cinereus* type of cycle there will be but one annual wave of adults, while with the *caspius* type there may be several such waves, depending largely on the distribution of rains and floods. It is interesting that *Aëdes communis*, which is clas-

TABLE III LIFE-HISTORY CALENDAR OF DANISH MOSQUITOES (AFTER WESENBERG-LUND, 1921)

SPECIES	MONTH I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
I												
Aëdes cinereus *	•	•	•	•	•–	–+	+	+	+	•	•	•
Aëdes maculatus	•	•	•	•–	–	+	+	+	+	•	•	•
Aëdes vexans *	•	•	•	–	–	+	+	+	•	•	•	•
Aëdes annulipes	•	•	•	–	–	+	+	+	+	•	•	•
Aëdes excrucians	•	•	•	–	–	+	+	+	+	•	•	•
Aëdes flavescens	•	•	•	–	–	+	+	+	+	•	•	•
Aëdes detritus *	•	•	•	•	•	•	–	+	+	•	•	•
Aëdes punctor *	•	•	•	•	–+	+	+	•	•	•	•	•
Aëdes cataphylla	•	•	•	–	–+	+	•	•	•	•	•	•
Aëdes diantaeus	•	•	•	•–	–+	+	+	+	+•	•	•	•
Aëdes geniculatus	•	•	•	–	+	+	+	+	+•	•	•	•
	•	•	•	–	–	+	+	•	•	•	•	•
Aëdes communis *	–	–	–	–	–	+	+	•	•	•	•	•
	+	+	+	+	+	•	•	•	•	–	–+	+
II												
Aëdes caspius *	•	•	•	•–	–•	+	•	•–	+	+•	•	•
Aëdes dorsalis	•	•	•	•–	–•	+	•	•–	+	+•	•	•
III												
Aëdes rusticus *	–	–	–	–	–+	+	•	•	•	–	–	–
Theobaldia morsitans *	–	–	–	–	–+	+	+	+•	–	–	–	–
Mansonia richiardii *	–	–	–	–	–	+	+	+•	–	–	–	–
IV												
Theobaldia annulata *	+	+	+	+	+•	+•	+•	•+–	+–	–+	+	+
Culex pipiens *	+	+	+	+	+•–	+•–	+•–	+•–	+•–	–+	+	+

Key— • = egg; – = larva; + = adult; * = found in England (Marshall, 1938).

sified in the table as having the *cinereus* type of cycle because there is apparently only one generation a year in Denmark, can apparently grow at very low temperatures, so that all stages may be found in the midwinter months. Species with the *claviger* type of cycle may be either single- or many-brooded, and in more southern latitudes they tend to show an early spring peak of adults. Species with the *Culex pipiens* type of cycle are probably always many-brooded, and the species population is at a minimum level during the winter months, building up by successive generations during the period of favorable temperatures.

Within the tropics suspension of mosquito development is never necessary because of unfavorable temperatures, but it may be necessary because of periodic or irregular absence of water from breeding places. There are many types of rainfall cycles in the tropics, ranging from climates with no rainfall, to climates with constant heavy rains, though perhaps the commonest type of cycle involves one or two dry seasons alternating with rainy seasons. In such cases adaptations for passing the unfavorable dry period are generally classed as aestivation phenomena. The only well known type of developmental interruption in tropical mosquitoes involves the egg stage, though it is possible that other mechanisms may exist.

Because developmental interruption is primarily related to water fluctuations in breeding places, the most logical classification of types of seasonal distribution would seem to be by type of breeding place, as was done by the author (Bates, 1945) in a study of the annual cycles of mosquitoes in the region of Villavicencio, Colombia. From this point of view, tropical mosquitoes fall into four broad classes.

I. Mosquitoes that breed in permanent or semipermanent pools. Most anophelines belong in this class, and the chief variation is apt to be in extent of area available for breeding. Pools, ponds, and marshes may become greatly reduced in the dry season, but it is rare for this type of habitat to disappear altogether from an entire region. Where ponds dry up completely, some stream habitats may take on pond-like characters and become inhabited by mosquito species that are normally found in ponds. The pond- and marsh-breeding species may thus become very scarce during a severe dry season, the population building up with succeeding generations with the onset of the rains.

II. Mosquitoes that breed in stream associations. These are numer-

ous in the tropics, including many species of anophelines, and they are interesting because the seasonal distribution is the reverse of that of the pond breeders: maximum density is reached during the dry season, and the problem of the species is how to survive the hazards of the rains. Tropical streams in periods of heavy rainfall are subject to sudden and intense floods, and larval breeding seems only possible during the stable dry seasons. The contrast between seasonal distribution of a stream-breeding species (*Anopheles pseudopunctipennis*) and a pond-breeding species (*Anopheles albimanus*) is nicely shown in the study of Kumm and Zúniga (1944) in El Salvador. On the completely arid coast of Pacific Peru, where mosquito breeding is limited to stream habitats, the abundance of *Anopheles pseudopunctipennis* is governed by periodic floodings due to the melting snows.

III. Mosquitoes that breed in temporary pools. The fauna of rain pools consists mostly of species in which development may be indefinitely suspended in the egg stage—a characteristic of the genera *Aëdes* and *Psorophora*. The larvae of some of these species grow with incredible speed, and an isolated heavy rain may be followed in a week or so by the abrupt appearance of great numbers of adult mosquitoes of this group. The characteristic of rain-pool breeders in the tropics is abrupt and large fluctuation in the adult population.

IV. Mosquitoes that breed in container habitats. The water content of container habitats (a term proposed by Shannon, 1931, to cover tree holes, water-containing plants, water in fallen leaves, and so forth) is also subject to great variation. In open containers, such as fallen leaves or coconut husks, the temporary nature of the container precludes the usual egg type of developmental interruption. I suspect that species breeding in such habitats survive unfavorable periods mostly as adults. We have observed in Villavicencio that adults of *Trichoprosopon digitatum*, a species that breeds in such transient containers, are extraordinarily hardy under laboratory conditions, surviving in open cages for months. In the case of tree holes, a given breeding place may have a long history, and the aëdine mosquitoes that occupy such habitats all show the phenomenon of suspension of egg development. Water fluctuations in protected tree holes are not as abrupt as in open ground pools, and changes in adult populations are correspondingly more gradual. The inhabitants of water-containing plants in tropical America are predominantly

sabethine mosquitoes, and little is known about their biology. It seems likely that in many cases, at least, the eggs can withstand a certain amount of desiccation.

The majority of studies of the seasonal distribution of mosquitoes that have been made in various parts of the world have been concerned with anophelines known or suspected to be vectors of malaria, with the object of studying the relation between anopheline abundance and human malaria. Numerous papers of this type have been published in the malariological journals, such as the *Journal of the Malaria Institute of India* and the *Rivista di Malariologia.*

CHAPTER IV

THE SEXUAL BEHAVIOR OF MOSQUITOES

> These factors—natural and artificial selection, the manner of breeding characteristic for the particular organism, its relation to the secular environment and to other organisms coexisting in the same medium—are ultimately physiological, physical, and chemical, and yet their interactions obey rules *sui generis*, rules of the physiology of populations, not those of the physiology of individuals.—TH. DOBZHANSKY

THE study of the sexual behavior of animals is surely a basic problem in any investigation of the mechanism of evolution, since it is by differences in sexual behavior that the genetic discontinuity of species populations is maintained in nature, yet naturalists in general seem hardly to have recognized the importance or significance of the subject. The mechanism of the transference of hereditary characters has received a vast amount of study, has become a separate and special science in itself (genetics), but the behavior of animals incident to the bringing together of the genetic materials has received scant attention. As R. A. Fisher (1930, p. 130) has remarked: "The grossest blunder in sexual preference, which we can conceive of an animal making, would be to mate with a species different from its own and with which the hybrids are either infertile or, through the mixture of instincts and other attributes appropriate to different courses of life, at so serious a disadvantage as to leave no descendants." Whether two species can cross, whether the hybrids are fertile or sterile, is rather an academic question if in fact the two species do not mate with each other. And all that we know of animal populations in nature indicates that in the vast majority of cases each mates with its own kind. From this point of view it becomes important to find out what behavior mechanisms maintain this reproductive isolation; under what circumstances these mechanisms break down, permitting hybridization; and what circumstances lead to the formation of distinct be-

havior mechanisms which result in the sexual fission of previously interbreeding populations.

Our knowledge of mosquito behavior is inadequate for the formulation of any very clear or definitive answers to these questions: but a beginning has been made, at least in the accumulation of observations. The stimulus for much of the study of sexual behavior in mosquitoes has been the immediate practical need for finding methods of inducing mating in captivity, so that different species can be maintained as laboratory colonies. This work has shown the tremendous diversity of sexual behavior among similar mosquito species, and indicates that the subject can be analyzed, to some extent at least, by experimental laboratory methods.

Copulation in mosquitoes seems almost invariably to be associated with the "swarming" of the males: a phenomenon that has often been observed, but that is difficult to describe or study because of the conditions under which it usually takes place (dim light, inaccessible locations). Insects (and many other animals) commonly show complicated behavior patterns in connection with mating. The word "courtship" is usually applied to such behavior in birds and mammals, and it has sometimes been used for certain types of insect-mating behavior, though its anthropomorphic implications would seem to make it an inept word, at least for insects. The morphologists commonly distinguish between "primary sexual characters" and "secondary sexual characters"; the first term referring to the morphology of the sexual organs themselves, and the second term to accessory structures limited to one sex, such as scent glands, special types of ornamentation, and so forth. A similar distinction would seem useful for behavior. In this sense behavior patterns associated with mating would be considered secondary sexual characters, except for the "primary" act of coitus itself. The term "epigamic behavior," which has come into rather wide usage for such activities, seems more appropriate than "courtship."

In the case of mosquitoes, the swarming of the males may serve to bring the sexes of a particular species together by placing the mating act in a special environment. Such a restriction, however, might also conceivably serve as a handicap if mating failed to occur when the special conditions were not present: it is certainly a handicap to raising many species in the laboratory. It has been suggested that epigamic behavior may serve as a sexual stimulus to the female, or that

it may be a necessary preliminary stimulation for the males. But this fails to explain why such stimulation should be needed in some species and not in others. It seems to be generally true that secondary sexual characters, whether structural or behavioral, show sharp specific distinctions. Systematists thus put great stress on differences in genital armature, and on various secondary sexual characters of the male such as scent glands or highly modified palpi in species that have morphologically identical females.

Many insect species seem to get along very well without obvious special epigamic behavior patterns, or without obvious secondary sexual morphological characters; yet if such structures and behavior have no "survival value," it is difficult to understand why they occur so commonly and so widely through the animal kingdom. Perhaps their chief function is as "recognition characters" for ensuring the correct association of species in mating; and it may well be that in groups where secondary sexual characters are apparently absent, it is merely that they are not obvious to the human observer.

FACTORS INFLUENCING SWARM FORMATION

There is a fairly extensive literature on the mating habits of mosquitoes, which has been partially summarized by Howard, Dyar, and Knab (1913), Banks (1919), Corradetti (1937a), Marshall (1938), and Bates (1941b). Part of the interest in this subject stems from the discovery of the diverse habits shown by the closely similar species of the *Anopheles maculipennis* group in Europe. Roubaud, who was one of the first to study this question, proposed that species that would mate readily in small cages be termed "stenogamous" and those that seemed to require outdoor conditions "eurygamous." This division is based on the assumption that the prime difference in mating habits among mosquito species is space requirement. It seems probable, however, that many other factors are involved, and that any classification of mating habits would have to take such other factors into account.

Anopheles atroparvus, which formed the basis of Roubaud's concept of stenogamous mating behavior, seems to be exceptional among species that have been carefully studied in that swarming of the males is facultative, as has been clearly shown by the observations of Cambournac and Hill (1940). *Atroparvus* males will mate in cages (and in nature) with resting females; yet under certain conditions they also form swarms. In the case of other species, mating seems always

to be preceded by swarming of the males, though the swarms may be formed in small cages, and there seems to be every gradation among species that have been studied in the amount of space required for mating.

Important factors governing swarm formation and orientation would seem to be: activity state of the mosquito, spatial relations, light, wind, sound, association of species, temperature and humidity.

Activity state of the mosquito. The influence of a diurnal physiological rhythm on the behavior of the mosquito was discussed in a section of the preceding chapter. The effect of this rhythm on sexual activity in certain species is quite clear. In experiments with *Anopheles superpictus* kept in a uniform environment (Bates, 1941b), it was found that swarms were formed at approximately twenty-four-hour intervals, apparently in close association with the general activity rhythm of the colony. There is also considerable negative evidence on this point: mosquitoes that are not in an active state cannot be induced to swarm even when placed in apparently ideal environmental conditions. The experiments of Cambournac and Hill (1940) with *Anopheles atroparvus* show this. They observed this mosquito swarming regularly in a stable every evening, but when they attempted, during the day, to reproduce evening conditions in the stable, full of resting mosquitoes, they were unable to elicit any response. Mating is not universally conditioned by such a rhythm, however, because in the case of *Aëdes aegypti*, for instance, mating will invariably follow any disturbance of the caged mosquitoes which causes them to start flying.

Spatial relations. The spatial orientation of the swarm is obviously one of the most important factors influencing epigamic behavior, but it is an extraordinarily difficult factor to study. In our big outdoor cage in Albania (Hackett and Bates, 1939) there were four points over which swarms of *superpictus* regularly formed, but we were unable to find anything in common among these four points that was not also common to many other parts of the cage where swarms did not form. By modifying these locations we could prevent the formation of the swarm or cause it to disperse after it had formed, but we were unable to introduce new spatial relations and predict how these would affect the swarm.

Factors governing the orientation of *superpictus* swarms are made even more obscure by a field observation by Dr. J. S. Kennedy while

he was working in Albania. He found a swarm of several hundred *superpictus* males inside a stable near Tirana, 30 to 60 cm. above the floor in the lightest part of the room. The swarm was complex, that is, with several distinct foci. The position of the foci was apparently determined by patches of fresh dung. On the second evening the swarm was in a different position in the stable and again over fresh dung. The interesting thing is that he noticed another swarm of *superpictus*, much smaller (ten or twenty individuals), outside the stable about one and one-half meters above the middle of the roof. This swarm started later than the indoor one, but broke up at about the same time, apparently on account of an increase in wind strength.

We thought that color of background might be an important factor, but we were never able to demonstrate this with any of the Albanian mosquitoes, except that occasionally by changing the color of the background where the swarm had formed, we were able to cause it to disperse. In the case of *Anopheles multicolor* in Egypt, however, we once observed that a small swarm had formed over a piece of white paper in a cage, and we discovered that if this paper were moved slowly around in the cage the swarm would follow. In this case it was quite clear that the orientation was to light reflected from the paper, since the same result could be secured with a mirror, but not with dark colored paper. We had tried similar experiments with *superpictus* and *labranchiae* in Albania, but without results. It is interesting that Cambournac and Hill (1940) found that *atroparvus* swarms would orient to white paper. Recently in Colombia, we found that a white surface under a dim light would serve for the orientation of swarms of *Anopheles pseudopunctipennis* under cage conditions.

The orientation of mosquito swarms over contrasting and sharply defined points has often been observed in nature. The repeated observation of *Culex pipiens* swarming over church steeples and chimneys is an example: cases in which such swarms have caused an alarm of fire are quoted by Howard, Dyar, and Knab. Staley, quoted by Marshall (1938), reports seeing this species swarming "over low bushes," "on the lee side of a hedge," "near the gable of a building," "above a chimney-pot," and in a variety of other situations. He points out that, as a rule, swarming takes place either over, or to leeward of, some prominent object (such as a hedge) according as the air is calm or disturbed. A number of mosquito species have been observed to

form swarms over the head of a man, and may follow him as he walks slowly about (Matheson, 1929; Bates, 1941b).

The observations of Dyar (1920) on the swarming habits of species of *Aëdes* in the Canadian Yukon Valley show how similar species may have distinct swarm orientations.

> The swarming habits of the common males at White Horse were constant and interesting. The town is in the sandy level river-flat with a high bluff behind, formerly the river margin. On walking toward the bluff any still evening, males were encountered, first the *callithotrys* in the tops of small pines; next *prodotes* over open spaces between pines and willows; then, on reaching the high spruce trees, *lazarensis*, high up opposite the ends of projecting branches; and lastly in openings between tall spruce, over willow bushes, *punctor*, and an occasional *excrucians*, high up and flying wildly. At Dawson, *pullatus* appeared over willow bushes on the hillside any time after 4 P.M. that the sun went behind a cloud.

Light. Most of the descriptions of mosquito swarming involve crepuscular species under twilight conditions. It is probable that the strictly diurnal mosquitoes of the tropics would have quite different mating habits, but little is known about these. In the case of species with crepuscular swarming habits, it is clear that light would play an important role in influencing the formation of the swarms. We first discovered that we could control the swarming of mosquitoes in the laboratory when we went into the insectary in Albania one night to see what was going on. We switched on the light, which happened to have a dim bulb, and a minute or so later we were surprised to find that a large swarm of *superpictus* had formed in the middle of the room. We at once concluded that the control of light was the answer to the problem of mating under laboratory conditions, and thereafter most of our attention was given to this factor. Since most of the experiments were made with *superpictus*, the following discussion will be limited to this species.

We soon found that when the mosquitoes were in a suitable activity state, the formation of a swarm was a response to a change in light intensity; a change from bright light to dim light, or from darkness to dim light was equally effective. The optimum light for *superpictus* appeared to be on the order of one foot-candle. Swarms seemed never to occur in complete darkness or in light or more than about 10 foot-candles in a closed room, though under outside conditions swarms were observed when the light was between 10 and 15 foot-candles.

Once a swarm had formed it was relatively stable, and the light intensity could slowly be increased up to about 50 foot-candles before the swarm would disperse. After dispersal, however, the swarm would not reform until the light had again been reduced to about 3 foot-candles. Swarms dispersed at once if all lights were turned off, and would reform in 30 seconds to a minute when a light of about one foot-candle was again turned on. We made a series of experiments with lights of different color, but we were unable to reach any positive conclusions with regard to the effectiveness of different wave lengths because of the difficulty of distinguishing between the effect of color and intensity, especially since we had no idea of the relative sensitivity of the mosquitoes to light of different wave lengths. We got the impression, however, that blue-green light was the most effective and red light the least effective.

It seems probable that the light intensity most favorable for swarming differs in different species. In our big cage in Albania, we found that the swarms of *Anopheles superpictus* formed a few minutes earlier than *Anopheles maculipennis*. Shute (1930) found that after swarms of *Culex* and *Chironomus* had broken up, *Mansonia richiardii* formed at the same spot. He found that the reverse succession occurred in the early morning. The *Mansonia* started to swarm at three o'clock, being replaced by *Culex pipiens* at about four o'clock. This looks like a clear case of difference in optimum light intensity for swarming.

We have found control of light to be important in inducing mating under laboratory conditions with many species of mosquitoes in experiments in Albania, Egypt, and Colombia. Similar experience has been reported by Davies (1944) with *Anopheles claviger* and Thomson (1947) with *A. gambiae* and *melas*.

Wind. It has frequently been observed that mosquito swarms fail to form, or are soon dispersed, on windy evenings. We found that swarms in a large cage could be caused to disperse by turning on an electric fan (Bates, 1941b).

Sound. A swarm of male mosquitoes produces a very characteristic humming sound and it seems perfectly possible that this sound serves to attract the females, though there is very little experimental evidence to support this. The swarming males themselves are readily influenced by sound, and observations on this point are fairly numerous. (See Howard, Dyar, and Knab, 1913, pp. 114–117.) *Superpictus* swarms in

the big cage in Albania responded immediately to a low hum, middle C being about the most effective pitch. The swarm would lose its form, many of the males coming and buzzing around the observer's head, the abnormal behavior persisting as long as the hum was maintained. When humming was continued intermittently for some time, the response became weaker, and after fifteen minutes of trials, there was no response at all, the swarm remaining undisturbed. Whistling had no effect on the swarms. Curiously, the hum response did not appear with swarms in a cage of room size or smaller, which made experiments with the phenomenon difficult. We tried using tuning forks in a room-sized cage, but this was also ineffective. In the big cage we could detect no difference between *superpictus* and *maculipennis* in the response to humming.

It has been frequently observed that the humming of electric wires is attractive to mosquitoes, and it has been suggested that traps be built on this principle of construction. It is notable that all observations of this sort involve the attraction of males, and the assumption is that the attracting sound must resemble that produced by a female mosquito. It is very difficult, however, to understand the function of swarming in the mosquito life history, unless the females are attracted to the sound produced by the swarming males. Kahn, Celestin, and Offenhauser (1945) made electric recordings of the sounds of *Anopheles quadrimaculatus, Aëdes aegypti, A. albopictus,* and *Culex pipiens.* They found that "despite the great variety of sounds, each genus and species have tonal emanations which are so distinctive in character that an experienced observer can not only readily distinguish one genus from another, but . . . can also distinguish males from females of the same species. . . . The noise of a single female will cause the males of the same species to burst into an answering chorus."

Association of species. Swarms including the males of more than one species of mosquito have occasionally been observed in nature. The following cases are quoted by Marshall (1938): *Anopheles algeriensis* males along with "clouds of *Aëdes cinereus* males" (observed by Edwards); *Aëdes detritus* "swarming over reeds or low bushes, in many cases accompanied by males of *Aëdes caspius*"; *Theobaldia subochrea* participating in swarming of *T. annulata.*

In a few laboratory trials in Albania, we found that the swarming of one species seemed to have absolutely no effect on the other species that might be in the cage at the same time. At one time we had *super-*

pictus, sacharovi and *maculipennis* in the same room. *Superpictus* and *sacharovi* swarms would be formed at the same time, but with completely distinct orientation (*superpictus* over the middle of the floor, *sacharovi* over the observer's head), while *maculipennis* males would continue to fly about the room without being in the least affected by either of the swarms. We tried also mixing other species with *superpictus* in small cages, but without any effect.

Temperature and humidity. At twilight, when mosquito swarms are usually formed, correlated changes occur in three factors of the environment: light, temperature, and humidity. Changes in temperature and humidity seem to have some effect in influencing the general activity of mosquitoes, but the effect of these factors is generally completely overshadowed by concurrent changes in light. In swarming behavior, as distinguished from general activity, we were never able to find that change in temperature had any effect.

SEXUAL SELECTION AND DIFFERENCES IN FEMALE BEHAVIOR

That there would be differences in female behavior corresponding to the differences in male behavior might be assumed on theoretical grounds, but it has so far been demonstrated experimentally only in the work of de Buck, Schoute, and Swellengrebel (1934). These authors had no difficulty in inducing *atroparvus* to mate in the laboratory but they were unable to get laboratory fertilization in *messeae*. The *messeae* females were, however, fertilized by *atroparvus* males, and when the reciprocal cross was tried, a few *atroparvus* females were fertilized by *messeae* males. Only 4 per cent of the *atroparvus* females were so fertilized, in contrast to 32 per cent of the *messeae* females when put with *atroparvus* males, but this 4 per cent is a very significant figure, since there is no case known of the *messeae* male fertilizing its own female under laboratory conditions. The Dutch authors also found that *atroparvus* males did not fertilize the *messeae* females nearly as readily as they did their own females—32 per cent in comparison with 95 per cent. We also observed a relatively low fertilization rate in cross-mating when we repeated these hybridization experiments (Bates, 1939a), but the conditions under which our experiments were made were not sufficiently standard to warrant comparisons among species.

While Dr. I. M. Puri was visiting the Tirana laboratory, he showed us how we could mark mosquitoes by dusting them with gold and

silver powder, using the technique developed by Majid (1937). By this method we could mark females of different species of the *maculipennis* group, otherwise indistinguishable, and thus determine the relative fertilization rates with males of a single species. Dr. Puri helped us to make three experiments, the results of which are summarized in Table IV. From this it appears that *atroparvus* males fertilize their own females more readily than they do those of *maculipennis*, but that there is little difference in this regard between the females of *atroparvus* and of *labranchiae*. This is not surprising, since *labranchiae* and *atroparvus* are found to be quite similar by almost any test.

TABLE IV RESULTS OF EXPERIMENTS IN WHICH MALES OF *Anopheles atroparvus* WERE LEFT WITH FEMALES OF VARIOUS SPECIES

EXPERIMENT	NO. ♂♂	♀ SPECIES	MARKING	NO. ♀♀	NO. NIGHTS	PER CENT FERTILE
A	40	atroparvus	Gold	20	3	95
		maculipennis	Silver	20	3	65
B	40	atroparvus	Gold	20	2	95
		labranchiae	Silver	20	2	100
C	45	atroparvus	Gold	15	2	85
		labranchiae	Unmarked	15	2	85
		maculipennis	Silver	15	2	55

The exact nature of the behavior differences between females is obscure, but a few observations that we have made on laboratory behavior may be of interest in this connection. We first came to realize the importance of female behavior from our experience with *Anopheles claviger*. We found that the males of this species could readily be induced to swarm in small cages, and grappling pairs were frequently observed falling out of such swarms; yet very few of the females in these cages were found to be fertilized. Apparently the difficulty was that intromission had not yet occurred when the grappling pairs hit the bottom or sides of the cage, thus interrupting the behavior sequence. We subsequently observed the same phenomenon with *Anopheles multicolor* in Egypt: the males would swarm readily in a cage 50 cm. high, but the fertilization rate would be much lower in such a cage than in one a meter high. From these and similar observations it would seem that in some species intromission can occur while

the female is in a resting position (for example, *atroparvus*), but that in other cases the mosquitoes must be in flight. Of species with this second type of behavior, probably some require a much longer flight distance than others. The cage requirements of a particular species may thus depend on two different factors: the spatial relations of the male swarm and the mating behavior of the females. Copulation in *maculipennis* (*typicus*) in the Albanian big cage seemed always to occur in flight; and in all of the cases that we could observe the act was completed on the wing, the pairs flying horizontally across the cage.

All observers have commented on the relative scarcity of mating pairs in swarms of mosquitoes found in nature, and Cambournac and Hill (1940) even suggest that in the case of *atroparvus* swarming has largely lost its functional character, being a sort of vestigial ritual. Certainly swarming behavior is incomprehensible unless the females are attracted to the swarm, yet the evidence of such an attraction is slight. Under laboratory conditions the females of all of the species that we worked with seemed to be completely indifferent to swarms of males, even when these were only a few centimeters away. We came to the conclusion that under these circumstances mating was entirely a matter of chance, depending upon whether a female happened to fly within the orbit of the swarm. In small cages we occasionally observed cases in which the same female mated several times: she would fall out of the swarm with one male and, flying up again, would cross the swarm once more, be grabbed by another male, and so forth. We could always induce mating by releasing a female within the swarm.

This negative laboratory evidence cannot, of course, be used as an argument that the swarms do not exert an attraction for females in nature. The attractive stimulus would presumably be sound, and sound stimuli in cages may be sufficiently distorted to be inoperative, as is shown by the failure of males to react to humming under such circumstances. An interesting experiment would be to release virgin females in the field in the vicinity of swarms and observe their direction of flight.

INHERITANCE OF EPIGAMIC BEHAVIOR PATTERNS

Roubaud has studied the inheritance of sexual behavior (Roubaud, Colas-Belcour, and Gaschen, 1933), but it is difficult to interpret his

results. As Tate and Vincent (1936, p. 142) have pointed out, much further work on the genetics of behavior will be necessary before the inheritance of such characters can be discussed with confidence. One observation that we made in Albania in connection with the hybridization experiments with the *Anopheles maculipennis* group (Bates, 1939a) is of considerable interest in this connection. We found that the F_1 males of the *atroparvus* x *maculipennis* cross, which were completely sterile (gonads atrophied), formed into a swarm and attempted to mate if placed in a small cage (50 cm. on a side) which was illuminated from above with a dim blue light. This behavior seemed identical with the behavior of *labranchiae* under the same circumstances, but differed sharply from the behavior of either parent. From this observation it is probable that the sexual behavior pattern was not dependent on the functioning of the primary sexual organs, and that it may have a complex genetic basis, since the characters of the F_1 seem to differ from the parental characters and may represent a blend.

It is, of course, well known that sexual behavior in insects is not governed by gonadal hormones, since the copulatory instinct is not modified by castration. Wigglesworth (1939, p. 383) gives references to various experiments of this sort.

In the case of *Anopheles quadrimaculatus*, Boyd, Cain, and Mulrennan (1935) found that there was a gradual adaptation in their colony to life in an artificial environment. They found that after thirty-six generations the mosquitoes would mate in a small cage (30 inches square by 36 inches high), which they presumably would not do before. The swarming behavior apparently was not lost, since they remarked that copulation occurred in flight. If the sexual behavior pattern has a fixed genetic basis, as seems likely, such an adaptation could only be explained on the assumption of variability in the original population, subject to subsequent selection. Since mosquito populations are presumable genetically diverse, such variability is not improbable. We hoped that we might be able to secure a strain of typical *maculipennis* that would mate regularly in cages by selection from the eggs laid in the Albanian big cage and from occasional egg groups laid in room-sized cages, but all such attempts failed. We also failed to observe any increase in adaptation to cage life in *Anopheles superpictus* over a period of two years, judging by regular checks on the percentage of fertilized females in the colony.

SPECIAL TYPES OF BEHAVIOR

The vast majority of descriptions of epigamic behavior in mosquitoes involve swarms of dancing males. The conspicuousness of these swarms facilitates observation; epigamic behavior patterns involving single males or single pairs, if they exist, would be much less apt to be observed, and I have come across only a few cases of field observation of other types of behavior.

The males of *Aëdes aegypti* may often be observed on solitary patrol flights, though small groups of males (thus constituting a "swarm") is perhaps the more common behavior. The males of this species are also reported to take up a position on "the dark colored clothing of a person sitting quietly, watching their chance to pounce upon a female coming to feed" (Howard, Dyar, and Knab, 1913, p. 275). This is similar to the behavior of the males of *Aëdes dominici*, a diurnal forest mosquito. These males can often be observed hovering over men or animals in the forest, either singly or in groups (but never with any group-swarming orientation) dashing at females that come to feed. They will attack almost any female mosquito, though, of course, copulation is only successful with their own species.

Haddow (1946) has remarked that the male genitalia of species of the African genus *Eretmapodites* present an extraordinarily complex structure. He suggests that this may be necessitated by their "peculiar and violent copulatory habits. The male having established contact with the female, the latter almost immediately settles. The male now hangs head-downward, suspended solely by his terminalia, and may retain this position for over an hour, frequently quivering rapidly and violently throughout."

Perhaps the most curious mating behavior of any mosquito is that of the New Zealand *Opifex fuscus*. This has been described by Kirk (1923) (quoted by Edwards, 1932, p. 128) as follows:

O fuscus breeds in brackish or salt-water pools on rocky coasts a little above high-water mark, generally those containing an abundant growth of *Enteromorpha*. Males on emergence skim or skate over the surface of the water, the tips of the tarsi being raised. "They adopt a very alert attitude, peering down into the water and often thrusting the head below the surface to get a clearer view. Their object is to capture the female pupae that are about ready to emerge. If the surface of the water is broken by a rising pupa a few inches away, the male darts at the spot with wonderful

quickness. The capture of the pupa is made by means of the anal forceps, the great ungues of the male not being used in these circumstances. The grip is usually secured on the frontal ridge along which the puparium would split in a case of unaided emergence; but often the grip is to one side of the ridge. In either case the puparium is ruptured a little to one side of the ridge, and the anal forceps of the male rest upon the thorax of the pupa, working backwards and extending the slit. In a few cases, the slit is greatly extended, but generally the male ceases active efforts in this direction as soon as the head and part of the thorax are clear. Emergence from this point is generally affected by the gradual straightening of the legs of the young imago, which rises slowly and steadily, without jerky movement until nearly the whole of the abdomen is clear. The male has kept the forceps in close contact with the body of the imago, two or three segments of his abdomen being inside the puparium. If, as is usually the case, the imago is a female, connection is affected before her abdomen is quite clear, and when emergence is complete copula has already begun" (Kirk).

The large front claws of the male, and also the proboscis, are used for fighting away rivals, though they do not appear to effect any damage.

HYBRIDIZATION EXPERIMENTS

Relatively few attempts at hybridization have been made with different mosquito species, though such experiments would seem to be of great interest in connection with the problem of defining the limits of specific populations. The most extensive study of hybridization among a group of related species involves the European populations of the *Anopheles maculipennis* group. De Buck, Schoute, and Swellengrebel (1934) first found that hybrids of these species were partially or completely sterile; further studies were carried out by de Buck and Swellengrebel (1935, 1937), Corradetti (1934, 1937a), and Bates (1939a). The results obtained in all of these studies are in close agreement. The Albanian experiments were summarized thus (Bates, 1939a):

atroparvus ♂

x *messeae* ♀. F_1 eggs fail to hatch, or larvae die in first stage.

x *sacharovi* ♀. F_1 consisting of males only, and these with atrophied gonads.

x *maculipennis* (*typicus*) ♀. Healthy and vigorous F_1 adults, but both sexes sterile.

x *subalpinus* ♀. Healthy and vigorous F_1 adults, but males sterile; females normal [at least, many ovipositions obtained from every experiment; no dissections were made]. These females back-crossed with *atroparvus* males: F_2 females normal, males sterile (25 dissections). These females back-crossed with *atroparvus* males; F_3 females normal, 18 per cent of the males normal (11 dissections). In other words, no normal males are found until the third cross, when the composition of the strain is ⅛ *subalpinus*, ⅞ *atroparvus*.

x *melanoon* ♀. Healthy and vigorous F_1 adults; females normal, males sterile (30 dissections). These females back-crossed with *atroparvus* males; F_2 females normal, 8.3 per cent of the males normal (12 dissections).

x *labranchiae* ♀. Healthy and vigorous F_1 adults; females normal, males sterile (31 dissections). These females back-crossed with *atroparvus* males; F_2 females normal, 20 per cent of the males normal (33 dissections).

Sweet and Rao (1937) distinguished two types of *Anopheles stephensi* from various parts of India on the basis of measurements of the ova; the typical form was readily colonized in small cages in the laboratory, but the form which they named *mysorensis* proved to be extremely difficult to maintain in captivity. In a subsequent article (Sweet, Rao, and Rao, 1938) hybridization experiments between these forms were described. They found that "there would seem to be a quite definite natural barrier to successful cross-breeding, since with crosses in both directions only a small minority of the females laid eggs and a still smaller minority laid viable eggs." In such experiments, the F_1 eggs show, of course, the characters of the female parent, since the egg shape and size is a property of the parental ovary and not of the hybrid embryo contained within the egg. In the few cases in which it was possible to get an F_2 or F_3 generation, egg characters were found to be intermediate.

The common domestic mosquito of the tropics of the world (*Culex fatigans*) has long been recognized as distinct from the corresponding mosquito of the temperate zone (*Culex pipiens*), the two being distinguishable by many characters, including the structure of the male genitalia. Weyer (1936) found that these two species were readily crossed, and that the hybrids were completely fertile. The male genitalia of the F_1 generation showed characters intermediate between the two parents, but in the F_2 generation the genitalia were found to be very variable, including in some cases characters that were distinct from those of either of the ancestral species, which suggests that the genetic control of the genital characteristics has a complex basis.

Weyer also tried crossing the two European "races" of *Culex pipiens* (treated by Marshall, 1938, and others as distinct species, *Culex pipiens* proper and *Culex molestus*). He found these also to be easily hybridized; but since the two forms are separated largely by biological characters, the analysis of the hybrid characteristics proved difficult. These experiments are discussed in more detail in Chapter XVI.

Toumanoff (1937, 1938, 1939) has reported on experiments with crossing *Aëdes aegypti* and *A. albopictus*. The F_1 of the male *aegypti* x female *albopictus* cross all resembled *albopictus;* conversely, the *albopictus* x *aegypti* cross resembled *aegypti;* he also found that the crosses with material from some localities were successful, and with material from other localities not, suggesting geographical diversity. De Buck (1942) failed in attempts to cross laboratory strains of these two species in Holland: the females were fertilized, but no embryonic development occurred except for one larva that hatched from eggs laid by an *aegypti* female, but that died in a few hours.

GYNANDROMORPHS

The term "gynandromorph" is applied to insects that show a sort of hermaphroditism in which certain parts of the body reveal male characters, others female characters. This phenomenon is possible because sex determination in insects is directly dependent on the genetic composition of the individual cells of different parts of the body and not, as in other animal groups, indirectly through circulating hormones. The genetic basis of sex determination and of the type of genetic accident that produces gynandromorphs has been studied in *Drosophila* and a few other insects, and the results have been briefly summarized by Wigglesworth (1939). A very large number of gynandromorphs have been described among the Lepidoptera, perhaps because they are especially common in that order, or perhaps because the sexual dimorphism of many butterflies makes a mosaic gynandromorph, with patches of the pattern of both sexes, particularly striking.

Among mosquitoes only a very few gynandromorphs have been described, and such forms are probably very rare, since immense numbers of mosquito specimens are examined in detail daily in many parts of the world in connection with study and control projects. Marshall (1938) has discussed the seven British records of gynandromorphs: four of *Aëdes punctor*, one of *Aëdes detritus*, one of

Culex pipiens, and one of *Culex molestus*. In addition, three mosquito gynandromorphs from Germany have been described: one of *Aëdes punctor* by v. d. Brelje (1923); one of *Aëdes aegypti* by Martini (1930, p. 248); and one of *Culex pipiens* by Weyer (1938).

In the New World, Felt (1904) described *Culex abserratus* (= *Aëdes implacabilis*) on the basis of a gynandromorph specimen from New York, and Theobald (1907, p. 4) has described a gynandromorph of *Aëdes pullatus*. In the course of studies of mosquitoes at the Villavicencio laboratory in Colombia we have come across two gynandromorphs of *Haemagogus spegazzinii* among bred specimens, and one of *Culex coronator*, caught in a stable trap.

CHAPTER V

FOOD HABITS OF THE ADULT

"And what does it live on?"
"Weak tea with cream in it."
A new difficulty came into Alice's head. "Supposing it couldn't find any?" she suggested.
"Then it would die, of course."
"But that must happen very often," Alice remarked thoughtfully.
"It always happens," said the Gnat.—LEWIS CARROLL

THE blood-sucking habit is almost universal among mosquitoes, and it is probable that in most cases blood as a food is obligatory—that is, the females cannot develop eggs on other foods. Since the blood-sucking habit (and consequent parasitism) is the direct basis of the economic interest of mosquitoes to man, it has been the subject of a considerable amount of study. Yet our knowledge is far from adequate, especially with regard to the differences in behavior that characterize particular species, and with regard to the specific stimuli that govern attraction and the biting reaction under either field or laboratory conditions.

As is well known, it is only the females that bite. A few species and groups are not known to bite warm-blooded animals, and in these cases it is usually assumed either that they feed on cold-blooded animals or on plants. Relatively little is known about the food habits of such species, or of the food of male mosquitoes under natural conditions. The present chapter is thus concerned chiefly with the behavior of mosquitoes in relation to warm-blooded vertebrate hosts.

FACTORS GOVERNING THE SEARCH FOR FOOD

Each mosquito species characteristically bites at a certain time of day, and this time of biting is presumably governed by the physiological rhythm of the species and by reactions to the daily climatic cycle of light, temperature, and humidity. Since "time of biting" is commonly taken as an index of "time of activity," it was discussed in

the second chapter along with other general environmental reactions.

In addition to the general factors which control hunger in the mosquito, there must be specific stimuli which lead the mosquito to its host. These stimuli may be similar in all mosquitoes that bite warm-blooded animals, though the relative importance of different classes of stimuli may vary with the species of mosquito, and the threshold of stimulus necessary to elicit a response may also vary, since it is well known that different species of mosquitoes show characteristic host preferences which could only be explained on some such basis. Classes of stimuli that may act to elicit and control the biting response will be discussed in the present section, and observations on differences in host preference among different mosquito species in the ensuing section of this chapter.

The amount of study that has been devoted to the biting response of mosquitoes seems remarkably small when one considers the obvious practical and theoretical interest of the subject. Almost every malariologist is convinced that anopheline mosquitoes are attracted to potential hosts from considerable distances, yet no attracting stimulus has yet been found that exerts a demonstrable influence more than a few centimeters away from the host. The studies that have been made are mostly concerned with smell, temperature, moisture, carbon dioxide, color, and movement.

Smell. One would expect smell to be the primary stimulus in guiding a mosquito in its search for food. Insects are known to have, in very many cases, an extraordinarily keen sense of smell, and because of the location of the chemoreceptor organs in the paired antennae they are able to locate the direction from which a smell comes and thus orient themselves toward the source (for references, see Wigglesworth, 1939). This ability is shared by so many different insects that it can safely be assumed to exist in mosquitoes even though in this particular case direct experimental evidence is lacking. In insects that show sharply characterized host limitation (selection of food plant by Lepidoptera, for instance), it can often be demonstrated that the guiding factor in determining the host is a particular odor, and it is possible to induce a butterfly to lay eggs on almost any plant if this plant is first sprayed with a distillate of the normal host plant. It is surprising in view of this to find an almost complete lack of experimental evidence to support the possible role of smell in governing the host-seeking of female mosquitoes.

Perhaps the most complete set of experiments with the chemotropic reactions of mosquitoes was made by Rudolfs (1922) in New Jersey. He tested the reaction of freshly caught *Aëdes sollicitans* and *Aëdes cantator* to a large number of substances—both natural secretions and pure chemicals. He found, to quote his summary, that

> perspiration, blood, urine and sebaceous secretion (human and cow) proved inattractive. Some of their constituents and intermediate decomposition products were decidedly attractive (phenylalanine, hemoglobin). Several amino acids influenced the activity of mosquitoes or induced them to suck. Carbon dioxide and ammonia, ultimate decomposition products of the human body, proved to be strongly activating, inducing the insects to stab and to exhibit "satisfaction" or "pleasure." A combination of CO_2 and NH_3 with a particular temperature and degree of moisture (such as to reproduce the conditions of the breath) was highly activating. It seems that these stimuli, produced in quantity by the human body, play an important role in the attraction of mosquitoes. This explains why a motionless sleeper and animals of great variety, amphibians, reptiles, birds and mammals, are all subject to attack.

Negative results with possible skin odors, similar to those obtained by Rudolfs, have been obtained by other workers using animal secretions or blood products. The experiments of van Thiel (1937, 1939) may be cited in this connection. He found that the sweat and blood of man or pig exerted no attraction for mosquitoes. Van Thiel was not able to find that the more or less volatile amino acids, decomposition products of sweat, exerted any attraction, which contradicts some of the results of Rudolfs. The experiments of van Thiel and Rudolfs are in agreement on the strong attractiveness exerted by carbon dioxide.

We made a few experiments in Albania, testing the attraction of different substances that were put in small traps which were left in rooms containing large numbers of anophelines. We were never able to trap any mosquitoes when we used absorbent cotton that had been rubbed over a sweaty animal as bait, although it was easy to trap the mosquitoes with vegetable baits, such as slices of apple or sugar solutions.

Temperature and moisture. Reuter (quoted by van Thiel, 1939) made a very interesting set of experiments testing the attraction of *Anopheles atroparvus* to an "imitation arm." The arm consisted of a glass cylinder, 18 inches long and 2 inches in diameter, through which

water, mixed to the desired temperature from cold and hot pipes, was allowed to flow. Two strips of filter paper about 2½ inches wide were hung on the arm; one strip was moistened by distilled water and the other by the solution to be tested. The outer surface of the paper was kept at 30° to 35° C., approximating the temperature of the human arm. These experiments indicated that the stimulus of temperature alone was sufficient to make the mosquitoes try to bite the glass tube or the paper covering it. If the stimulus of temperature was absent, no response was observed, even in cases where there was a substance on the tube that exerted additional attraction. The critical temperature below which attraction did not occur seemed to be 28° C. Van Thiel concluded from this that temperature was the "basic" stimulus for the biting reaction.

If wet and dry papers were placed on the warm glass cylinder, more mosquitoes were attracted to the wet paper than to the dry. This was taken to indicate that moisture may be a secondary attractive stimulus. Studies were made using this apparatus with various substances that might presumably attract mosquitoes, with the results mentioned above: no attraction was found from sweat or the volatile acids contained therein, although a slight attraction was found with defibrinated blood and pig faeces. The attraction to blood, however, was not always clear, and frequently mosquitoes tried to bite the warm glass tube without being attracted to near-by blood. In one experiment distilled water was found just as attractive as blood, and in another red colored water was equally attractive.

Crumb (1922) early noted the attractive effect of a moderately high temperature on mosquitoes. He found that a joint of stove pipe placed in the woods and heated by an alcohol lamp attracted about as many mosquitoes as were attracted by persons in the vicinity. In laboratory experiments with *Culex pipiens*, the maximum response occurred at temperatures between 32° and 43° C., these being 8° to 16° higher than the surrounding air. Temperatures above 49° and below 30° proved less attractive, and at 60° C. the mosquitoes were entirely dispersed.

Carbon-dioxide tension. Rudolfs observed that when breath was introduced into a tube containing mosquitoes, they were induced to stab the glass walls vigorously. Similar reactions have been observed by almost everyone who has handled mosquitoes in the laboratory: caged mosquitoes will almost always bite more readily if they are

first "activated" by breathing into the cage. We had great difficulty in Albania in inducing *Anopheles maculipennis* to bite when we put an arm in the cage, but we found that they would bite fairly readily if the head was inserted. It is a common observation that in nature many species of mosquitoes attempt to bite around the head more frequently than on other parts of the body. Haddow (1945b) has shown this nicely with *Aëdes simpsoni* by analyzing a series of captures according to the part of the body attacked.

The attractive stimulus of breath would presumably be caused either by temperature, moisture, or carbon dioxide. Rudolfs found that under the conditions of his experiments, temperature and moisture were not in themselves attractive and came to the conclusion that carbon dioxide, especially in combination with favorable temperature and moist air, was the attractive agent. Van Thiel came to the same conclusion with regard to carbon dioxide, and his experiments are perhaps clearer. He used a funnel which was pressed against the center of the screened side of the cage from the outside and connected with glass tubing to a tank containing carbon dioxide or compressed air. The gas could be bubbled through a flask of warm water to produce favorable temperature and moisture conditions. To gauge attractiveness, he counted the number of mosquitoes that approached the mouth of the funnel in five-minute intervals. With this apparatus, van Thiel found that moisture and temperature by themselves exerted some attractiveness, but that this attractiveness was appreciably increased by the presence of carbon dioxide. He came to the conclusion that the attractiveness of animal skin is due not only to moisture and warmth, but also to transpired carbon dioxide. He inferred, which seems logical, that such attractive agents probably operate only over short distances, perhaps not more than 25 centimeters. This leaves the problem of attractiveness over greater distances still unsolved.

Color and movement. MacGregor (1915) observed that *Aëdes aegypti* will bite a black guinea pig much more readily than a white one, and Brett (1938), also working with *Aëdes aegypti*, found that if he placed different colored gloves over his hand, the colors with the lowest reflection factor were most attractive, black being the best. These experiments support a phenomenon that is very commonly observed by both field and laboratory workers: when animals are used as mosquito bait, darker colored individuals of a particular species seem always to be more attractive than lighter colored ones.

In the case of diurnal mosquitoes particularly, it seems likely that visual stimuli play an important part in the biting reaction. It may be because of this that conventional stable traps give rather unsatisfactory results with such species. With tropical forest mosquitoes, one gets the impression that movement is important. Thus Haddow, Gillett, and Highton (1947) remark of the African *Eretmapodites* that they "are best captured by moving from point to point, with brief halts in favourable spots, and in the present series the stationary baits attracted small numbers only."

Other factors. While the biting behavior of a mosquito seems to depend, in part, upon the activity state of the animal, the dependence is not as sharp as appears to be the case with sexual behavior. During an experiment with diurnal rhythm carried out in Albania with *Anopheles superpictus*, the mosquitoes were given an opportunity to bite for five minutes at two-hour intervals over a forty-eight-hour period, and some specimens attempted to bite on every such occasion. In this species and under the conditions of this experiment then, there appeared to be a clear periodicity in sexual behavior and no periodicity in feeding behavior. The experiment was, however, carried out under highly artificial conditions, and in nature it is generally true that mosquitoes bite only during their period of general activity. Studies of the general environmental conditions most favorable for biting have been made by several people: the study of Rudolfs (1923) may be cited as an example.

In the laboratory, nocturnal mosquitoes will often bite readily only in complete darkness, and diurnal mosquitoes will sometimes bite more readily in darkness than in light. In this latter case, the explanation is probably in the elimination of disturbing escape reactions on the part of the mosquitoes. Mosquitoes can also be induced to bite more readily under laboratory conditions if they are subjected to a sudden shift in temperature, particularly from cold to warm conditions. The explanation of this may be in the heightened general activity of the mosquitoes.

It is well known that mosquitoes do not generally take a blood meal during the first day of their adult life. This is generally explained as due to the slow hardening of the mouth parts which are, at first, not sufficiently strong to penetrate skin. Seaton and Lumsden (1941) carried out some experiments with *Aëdes aegypti*, in which virgin females, separated in groups according to age from one to six days old,

were given equal opportunity to feed. They found that the largest number of mosquitoes fed in the three- to four-day-old groups. The feeding reaction in this case was, perhaps, somewhat delayed as most mosquitoes seem ready to bite when they are over one day old.

Seaton and Lumsden also found there was no difference in the feeding rate with *Aëdes aegypti* between virgin and fertilized females. This is possibly a characteristic of the species that they worked with, as we found in Albania that with *Anopheles atroparvus*, the biting rate was distinctly higher in fertilized than in unfertilized females. This has also been reported for *Anopheles quadrimaculatus* by Burgess and Young (1944).

STUDIES OF HOST PREFERENCE

Roubaud, Wesenberg-Lund, and Grassi, in 1920 and 1921, independently reached the conclusion that the explanation of "anophelism without malaria" in many parts of Europe lay in the food habits of *Anopheles maculipennis*. Roubaud thought that there were two "physiological races," one which he called "anthropophilous" (adapted to feeding on man) and the other "zoöphilous" (adapted to feeding on large domestic animals). Wesenberg-Lund thought that in Denmark *Anopheles maculipennis* had gradually changed its feeding habits with the change in agricultural practice that led to the housing of large numbers of cattle in stables: the mosquitoes had become primarily associated with cattle instead of with man. Grassi, like Roubaud, thought that there was a biological race that did not bite man.

These theories, especially that of Roubaud, attracted a great deal of attention and started a series of investigations that are still in progress. The story of these investigations has been interestingly summarized in the book on malaria in Europe by Hackett (1937). The old "species," *Anopheles maculipennis*, was found to consist of at least six or seven independent and distinct populations, each with characteristic habits including different types of host preference. The differences were found not to be absolute, in that one population would bite man exclusively and another not at all, but rather to be in the degree to which man was subject to attack. The situation has been summarized thus by Bates and Hackett (1939):

> Growing knowledge of these forms suggests that the present use of the terms "anthropophilous" and "zoöphilous" is unfortunate since the former implies a special preference for man over all other animals, and the

latter a preference for any other animal to man. It has been our experience, in common with that of others, that *atroparvus, labranchiae* and *sacharovi* will bite several different animals quite readily, including man and rabbits. *Maculipennis* (*typicus*), *messeae* and *subalpinus*, however, have a narrower range of hosts. They are reluctant to feed on man and rabbits in the laboratory, but they will bite a calf when it is put in a room where they are kept. A term like "zoöphilous," then, which includes calf and rabbit in the same category but excludes man, is misleading. All of these mosquitoes seem to prefer to bite cattle when man is the alternative, and the difference between the types seems to be that some are more general in their feeding habits than others, or perhaps that some respond to weaker stimuli than others. It appears that even a "non-anthropophilous" form like *messeae* may in some instances be a malaria carrier, as in certain purely *messeae* regions in Hungary, so that such terms have only a relative significance at best.

A very large proportion of the studies of the host preferences of various anopheline species have been carried out by the precipitin-test method of identifying the source of the mosquito blood meal. This method of identifying a particular blood specimen by reactions with specific antisera was first devised by Uhlenhuth and applied to mosquitoes by Bull and King (1923). Rice and Barber (1935) devised a technique whereby large numbers of individual mosquitoes could be tested for source of blood without undue difficulty, and their method has come into rather wide use. The precipitin-test technique, however, has a number of limitations that seem not always to be kept in mind. It can only be applied to species that have the habit of resting indoors after taking a blood meal, since otherwise it is impossible to find sufficient numbers of individuals with fresh blood. There is, then, always a bias, depending on where the mosquitoes are caught, since mosquitoes found in houses are most apt to have human blood, in stables cow blood, and so forth. The precipitin test offers a valuable method of checking on resting habits, since specimens found in houses with cow blood, for instance, have obviously wandered from the place where the blood was ingested; this is, however, a distinct problem from that of host preference.

The method perhaps has its most useful application in host preference studies as a method of determining the animal species attacked when mosquitoes are released in an enclosure containing various possible hosts. Thus Hu and Yu (1936) used this method with *Anopheles hyrcanus sinensis*, liberating hungry mosquitoes in a room containing

several possible hosts, and subsequently identifying the source of the blood meal. They obtained the following result with this species:

Animal species	Cow	Goat	Pig	Man	Dog	Cat	Fowl
Per cent biting	47.6	25.0	13.8	4.8	3.6	3.4	1.8

The possibilities of the precipitin method in large-scale field studies are shown by the study made by Barber and Rice (1935a) in Greek Macedonia. They tested the source of the last blood meal of over 18,000 anophelines; among these were 54 sporozoite positive specimens of *Anopheles sacharovi* (*elutus*) of which only 27 per cent had taken the last blood meal on man. The comparative frequency with which this species bites man, however, was shown by the percentages caught in stables positive for human blood: for *sacharovi* this was 7.5 per cent; for *maculipennis*, 0.5 per cent; for *superpictus*, 1.6 per cent; and for *algeriensis*, 0.2 per cent. This might be interpreted as showing a greater tendency on the part of *sacharovi* to wander, except that captures in houses showed the reverse tendency: 61.3 per cent of the *sacharovi* from houses were positive for man, as compared with 21.2 per cent of *maculipennis*, 29.7 per cent of *superpictus*, and 15.8 per cent of *algeriensis*.

Various experimental methods of approach to the problem of host preference have been tried. Corradetti (1937b) exposed a single batch of mosquitoes of a given species to a particular host on successive days, recording the number biting each day through five days. He tested by this means *labranchiae*, *sacharovi*, *atroparvus*, *maculipennis* (*typicus*), and *melanoon* on man, ox, pig, donkey, rabbit, and chicken. Around 40 per cent of the *labranchiae*, *sacharovi*, and *atroparvus* bit on the first day, and the difference in behavior with different hosts was very slight. The *maculipennis* and *melanoon* showed a much lower percentage biting on the first day, and only about half bit in the course of the five days of the experiment; in both cases, a significantly higher percentage bit the ox.

Rice and Barber (1937) tested man versus cow by placing the mosquitoes in a cage of metal screening 20 cm., in diameter and 30 cm. long, arranged so that one open end was in immediate contact with the bare abdomen of a man and the other with the clipped or shaved side of an animal. After an interval of one half to one hour of exposure, the mosquitoes were removed and the source of blood of engorged specimens determined by precipitin tests. They tried *sacharovi*,

maculipennis, and *superpictus*, finding that 45.2, 15.7, and 27.3 per cent respectively fed on man. They thus concluded that under these conditions *sacharovi* was indifferent, while *maculipennis* and *superpictus* showed a definite preference for cow over man.

Van Thiel (1939) made an extensive series of experiments using man and pig as alternative hosts. In experiments with *atroparvus* in the Netherlands, using a "choice apparatus" in which the two hosts were one meter apart, he found that pig was preferred to man, but that the difference was due primarily to temperature, since an electric heater with wet towels would attract more mosquitoes than either man or pig. When the same apparatus was tested in Corsica with *labranchiae* and *sacharovi*, no difference in attractiveness was found between man and pig. Van Thiel concluded that experiments in which alternative hosts were separated by a small distance were unreliable, in so far as the factors governing the choice were probably quite different from those operating in nature. In particular he was impressed by the apparent importance of temperature and the unimportance of smell, considering that at greater distances, as in nature, smell must be operative. He made a series of tests with another apparatus in which man and pig were placed in separate traps within a large caged area. With this he found that *atroparvus* definitely preferred the pig, and that the arrangement of electric heater and wet towels showed no attractiveness. When this apparatus was tested in Italy with *labranchiae* and *sacharovi*, he found that man was preferred to pig, concluding that these species showed a true "anthropophilism."

From these various experiments it is clear that different species of mosquitoes will show different host behavior under given experimental conditions, but that the experimental conditions are as important as the hosts. Generalization from laboratory experiments to nature is obviously hazardous, as van Thiel has pointed out, but the subject is of such considerable interest from the point of view of malaria epidemiology that it would seem to warrant more detailed investigation. It would, in particular, be valuable to have an experimental method whereby species in different parts of the world could be compared with regard to host-seeking habits.

It may be that the flight habits of a particular mosquito species are an important factor in determining what host it is apt to bite under natural conditions. This is suggested by some experiments reported by Causey, Deane, and Deane (1943) with *Anopheles gambiae*, a

species notorious as a malaria vector because of its close association with man. These authors found that in cages *A. gambiae* would bite a wide range of animals, showing no particular preferences. In nature, they bit man freely within houses, but when "both human and animal bait were planted just outside houses that had been shown to be heavily infested with *A. gambiae* . . . no specimen was captured . . . outside the house," although *Nyssorhynchus* species came commonly to bite. They found adults of *gambiae* in inhabited houses only, with one exception—and in the exceptional case it was found that a herd of goats was stabled in the house at night. From this it seems possible that *Anopheles gambiae* is not so much "man-loving" as "house-loving," and that it just happens that man is the primary house-inhabiting animal in the tropical areas where *gambiae* lives.

A curious instance of host preference has been reported by Galliard (1936) and confirmed by Wanson and Nicolay (1937). These authors found that *Culex fatigans*, when first brought into the laboratory, would attack man and guinea pig indiscriminately, but that after five or six laboratory generations females of certain strains would feed only on man. In general, attempts to show that an individual mosquito that has taken a blood meal from a particular kind of animal is more apt to bite that same type of animal a second time have failed, and a more detailed analysis of the phenomenon reported by Galliard would be very interesting. It seems to be a general experience of people working with *Culex* and bird malaria that certain strains bite more readily than others; and particular laboratory strains may be highly prized for this reason. It may be that strains with particular biting habits can be established by selection from a mixed stock.

MECHANISM OF BITING

Gordon and Lumsden (1939) have reported some very interesting observations on the mechanism of biting in *Aëdes aegypti*. By means of a very ingenious apparatus, they were able to observe with a microscope the penetration of the mosquito's proboscis in the transparent tissue in the webs of the feet of frogs. It had generally been assumed that the proboscis of the mosquito in penetrating the tissue remained rigid, acting like a hypodermic needle, but Gordon and Lumsden were able to observe repeatedly a very marked active flexibility at the labral tip, the organ being turned this way and that within the tissue until a capillary was finally encountered. They ob-

served two types of feeding; one which they call "pool feeding" and the other "capillary feeding." In the first case a capillary is ruptured by the tip of the fascicle and blood is sucked from the small pool formed by the ensuing haemorrhage. More frequently,

blood was taken up as a result of the fascicle penetrating into the lumen of a capillary. The usual representation of the fascicle penetrating a large

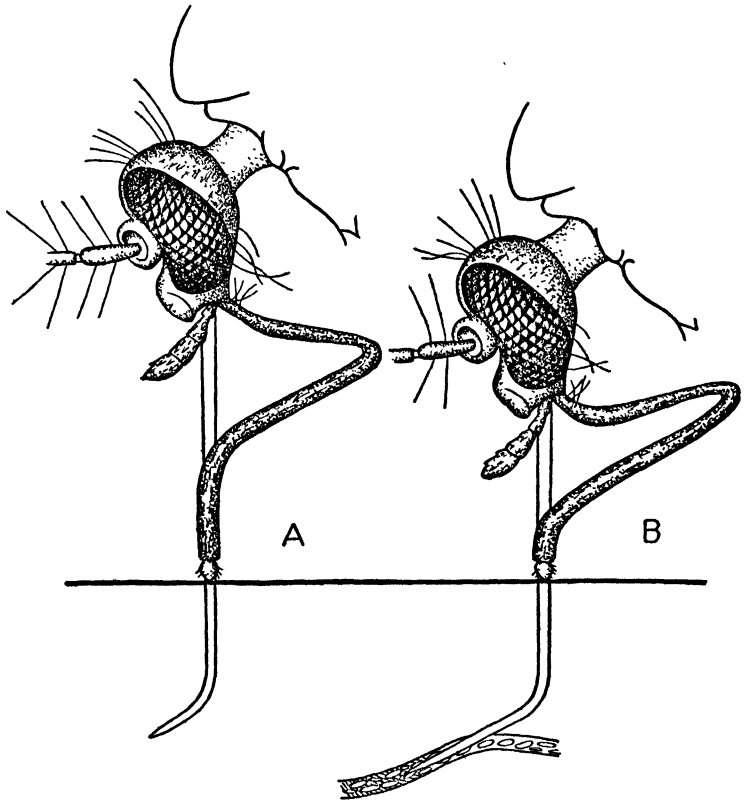


FIG. 5. STAGES IN THE INTRODUCTION OF THE FASCICLE BY *Aëdes aegypti* IN BITING, AS OBSERVED IN THE WEB OF THE FROG'S FOOT. (*A*) THE ACTIVELY FLEXIBLE TIP OF THE FASCICLE, HAVING ENTERED THE WEB AT RIGHT ANGLES TO THE SKIN SURFACE, HAS CUT A CURVED COURSE TENDING TO BECOME PARALLEL TO THE CUTICLE. (*B*) THE PASSIVELY FLEXIBLE PORTION OF THE FASCICLE HAS FOLLOWED THE CURVE PREVIOUSLY TUNNELED OUT BY THE CUTTING TIP, WHICH HAS PENETRATED A SMALL CAPILLARY (FROM GORDON AND LUMSDEN, 1939).

capillary and coming to rest at right angles to it with its tip in the lumen, is a similar misconception to that of considering the fascicle as a more or less rigid structure. It is true that this attitude was occasionally observed, but the normal method of capillary feeding appeared to be that the fascicle, penetrating a capillary at any angle, continued to pass along its lumen. The extent to which the fascicle traversed the capillary varied considerably: sometimes it would pass up the lumen for almost a quarter of its length; more often, however, it only penetrated a short distance, the tip being usually bent just at its entrance.

Gordon and Lumsden were able to make a few observations on the ejection of the salivary fluid from the fascicle. This ejection seemed to occur at various stages of penetration, so that it is probable that "saliva gains access to the tissues, to any haemorrhage that may have been produced by the mosquito, and directly to the capillary circulation." The mode and place of salivary ejection is, of course, of great interest in connection with the problems of disease transmission by mosquitoes.

A few studies have been made of the properties of the salivary secretion of mosquitoes. The general subject is reviewed in a paper by de Buck (1937) and in the paper by Gordon and Lumsden, quoted above. De Buck found that the salivary secretion of *Anopheles maculipennis* contained both a haemagglutinin and anticoagulin. He did not find any agglutinating property in the salivary secretion of *Anopheles plumbeus* or *Culex pipiens*, and this property in the secretion of *Theobaldia annulata* was weak. Gordon and Lumsden failed to find any agglutinin in the secretion of *Aëdes aegypti*. It thus seems very probable that the chemical composition of the saliva varies greatly from one species of a mosquito to another.

Various authors have become interested in the question of the amount of blood taken up by a mosquito, largely because of the relation of this to the number of gametocytes ingested in malaria-infection experiments. Mosquito species differ greatly in this regard. Thus Boyd, Carr, and Rozeboom (1938) found that *Anopheles albimanus* took 1.3 mg. of blood, while *A. quadrimaculatus* took 3.2 mg. Hovanitz (1947) investigated the variation in the amount of blood ingested by *Aëdes aegypti*. He found that this amount varied from 0.7 to 3.9 mg. Some individuals took up to 1.5 or 1.7 times their original weight in blood.

FOOD OF MALES

There are a few records of male mosquitoes biting man—several instances are quoted by Howard, Dyar, and Knab, for example (1913, p. 109). Such behavior is certainly abnormal and may always involve morphologically abnormal individuals (gynandromorphs). Such a case has been reported by Edwards (1917):

> While watching one day a number of *Aëdes punctor* feeding on my hand and ankle, I suddenly noticed that one of them was apparently a male. I at once secured it, and kept a look out for others. In about ten minutes I had taken three in the act of sucking, while a fourth had escaped. Probably more could have been secured if time had allowed, but unfortunately I had no other opportunity of visiting the locality until the mosquito season was over. A close examination later of the three specimens taken showed that none of them were normal males, but all three had one or more female characters on one or both sides of the body. It seems not improbable, therefore, that other male specimens of various mosquitoes which have occasionally been recorded as biting were really partly hermaphrodite.

Male mosquitoes are occasionally caught in stable traps. The distribution of such captures by species in two years of trapping at Villavicencio, Colombia, was as follows:

	FEMALES	MALES
Anopheles spp. (mostly rangeli)	24,992	2
Culex spp.	4,452	9
Mansonia fasciolata	5,160	1
Psorophora cingulata	8,943	76
Psorophora ferox	105	3
Aëdes serratus	2,012	1

In this case one specimen (*Culex coronator*) proved to be a gynandromorph. The males were presumably attracted to the trap by the odor of sweat or faeces, and the relative abundance of the *Psorophora* males probably reflects some distinctive food habit.

It is customary, in maintaining laboratory colonies of mosquitoes, to provide either sugar solutions or fruit as food for the males (and as supplementary food for females). It is assumed that in nature plant juices, particularly flowers, form the principal food of males, and of

females in the case of species not known to suck blood. Early records of mosquitoes as flower visitors have been summarized by Knab (1907) and quoted in the first volume of the monograph by Howard, Dyar, and Knab (1913, p. 110). Subsequent records (for example, Britten, 1937; Philip, 1943) add little to the general fact that mosquitoes of both sexes are fairly frequently found at flowers.

SPECIAL FOOD HABITS

A rather large number of mosquito species are not known to suck blood. In many cases it may be that the mosquitoes have specialized but unknown host habits, since observations are largely limited to man and domestic animals. It has been assumed that certain mosquitoes may be specialized for feeding on cold-blooded vertebrates —amphibia and reptiles—but there are few direct observations. Certain mosquitoes have been noted biting other insects, and a few cases are cited by Howard, Dyar, and Knab (1913). In a few mosquito groups, the blood-sucking habit is apparently entirely absent. Thus the large, brightly colored tropical mosquitoes of the genus *Megarhinus* appear to be exclusively flower feeders, and the proboscis is so modified that it could not be used for piercing skin. The genera *Uranotaenia* and *Deinocerites* are sometimes thought to have lost the blood-sucking habit, but Remington (1945) found that *Uranotaenia lowii* would bite amphibians, though refusing to bite reptiles or invertebrates. Dr. R. B. Hill tells me that he has seen *Deinocerties* bite horses in Jamaica.

Perhaps the most extraordinary food habits of mosquitoes are those of the Oriental and African genus *Harpagomyia*. Edwards (1932, p. 92) has summarized accounts of their habits thus:

> The habits of the adults are of exceptional interest and were first observed in detail by Jacobson in Java, having been confirmed subsequently by James in Ceylon and Farquharson and others in tropical Africa. The flies haunt tree-trunks where ants of the genus *Cremastogaster* are found, and obtain their food from the ants; the proboscis of the mosquito is evidently highly specialized for this purpose, and they probably do not feed in any other way. The *Harpagomyia* places itself directly in front of an advancing ant, sometimes even nipping the ant between its front legs, and not releasing it until it stops and opens its jaws, when the mosquito thrusts the swollen tip of its proboscis into the ant's mouth and rapidly absorbs the food offered. While the interchange is taking place the ant strokes the

tip of the mosquito's proboscis with its palpi. When not in use, the proboscis of the mosquito is folded backwards under the body, a most unusual position in this family. While feeding the wings are vibrated and the hind legs held high so that the tarsi curve forward over the head, as in *Wyeomyia* and related genera.

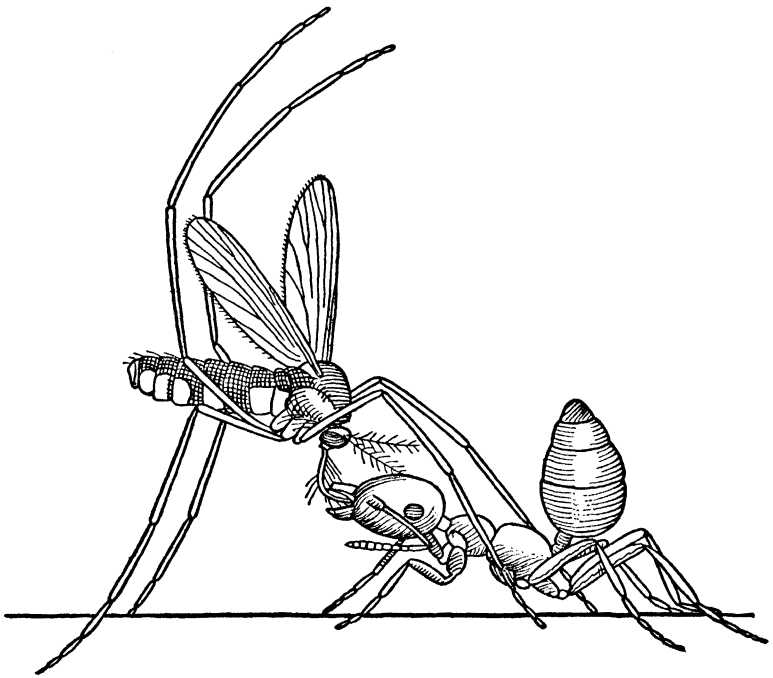


FIG. 6. *Harpagomyia* MOSQUITO TAKING FOOD FROM THE MOUTH OF A *Cremastogaster* ANT (FROM *The Standard Natural History* BY PERMISSION OF F. WARNE & CO.).

CHAPTER VI

EGG DEVELOPMENT AND OVIPOSITION

> It is becoming quite a common thing for zoölogists to study living animals, often, it is true, under artificial conditions in the laboratory, but still living.—CHARLES ELTON

JUST as our knowledge of the food habits of adult mosquitoes is largely confined to behavior in relation to warm-blooded vertebrates, so also our knowledge of egg development is largely based on studies of the digestion of blood meals. The behavior and physiology of mosquito groups such as *Megarhinus* and *Uranotaenia* that are unimportant from the point of view of human economy are totally unknown. As always, studies center on the all important genus *Anopheles*, with supplementary work on the easily handled species of *Culex* and *Aëdes*. In *Anopheles*, egg development is dependent on a blood meal, and various studies have been made of the effect of different types of blood or of blood fractions on egg development and on the number of eggs laid. The effect of environmental factors, especially temperature, on egg development has also attracted some attention. For the ecologist, however, the greatest interest centers in the behavior of the female mosquito once the eggs are developed. The study of the behavior of the ovipositing female would seem to be basic to an understanding of the environmental relationships of a particular species, and it is surprising to find that relatively little attention has been given to this important subject.

NECESSITY FOR BLOOD

Since egg development in mosquitoes is so generally contingent on a blood meal, it may be well to consider first the isolated cases of autogeny (egg development without blood) that have been described. The term "autogeny" was proposed by Roubaud (1929) in connection with a study of "biological races" in the French *Culex pipiens*. He found that there were two types of *pipiens* in France: one characterized by cyclical hibernation (for which he proposed the elegant

word "asthenobiosis"), requiring blood for egg development ("anautogeny") and mating only in large cages ("eurygamy"); the other with the opposite characteristics.

Of the numerous words coined by Roubaud for phenomena of mosquito biology, "autogeny" has perhaps gained the widest currency, and the concept has stimulated a considerable amount of research. The whole problem of racial differentiation in *Culex pipiens* has been reviewed by Tate and Vincent (1936) and by Marshall (1938). It is now generally recognized that two biologically distinct mosquitoes were formerly included under the name *pipiens*, and Marshall and Staley (1937) have proposed that these should be considered as distinct species. They would restrict the name *pipiens* to the mosquito requiring blood for egg development and a large space for mating, and apply the name *molestus* Forskal to the "autogenous" and "stenogamous" form. These authors have pointed out various morphological differences between the two mosquitoes, and their action in treating them as nomenclatorially distinct species seems logical.

It is clear that both types of *Culex* occur in North America (Richards, 1941), but the subject has received scant attention there. The phenomenon of autogeny was observed in New Jersey by J. B. Smith many years ago. He stated (1904, p. 26): "It is certainly proved by my own experience that *Culex pipiens* may oviposit without food other than that which could be found under the net covering a common wooden pail in which the parent developed."

The difference between *pipiens* and *molestus* with regard to autogeny is clear and unequivocal. The one never develops eggs without a previous blood meal, while the other can be maintained for an indefinite number of generations with no adult food other than some fruit such as raisins. De Boissezon, in a series of papers (for example, in 1933), has maintained that the ability of adult mosquitoes to lay eggs without blood meals depends on the larval food, and that autogeny results from a larval food rich in iron. Other workers have failed to confirm his results, in so far as they might be used to explain the behavior difference between *pipiens* and *molestus*. Tate and Vincent (1936), for instance, were unable to get eggs from *pipiens* raised in De Boissezon's lentil medium. Both Tate and Vincent, and Marshall have maintained thriving colonies of *molestus* over periods of years with only vegetable food for the adults. Roubaud (1933) thought that when colonies were kept for a long time without blood meals

they tended to die out—a phenomenon for which he proposed the term "spanogyny."

Culex molestus, if given the opportunity, will bite readily; it bites man, in fact, more readily than does *pipiens*, which prefers birds. Egg development in *molestus* after a blood meal is rapid, and from the data given by Tate and Vincent, it seems that more eggs are laid with a blood meal than without. "Bloodthirstiness" is not lost even after many completely autogenous generations. The possible physiological difference between *molestus* and *pipiens* that enables the one to develop eggs without blood seems not to have been investigated; perhaps it is related to a greater efficiency on the part of *molestus* in carrying over nutrient materials from the larval stage. The two species may be crossed readily in the laboratory, and Tate and Vincent report a series of such hybridization experiments. Autogeny characterized at least a small percentage of the hybrid offspring, though sometimes it did not appear until the F_2 generation.

Oviposition without a blood meal by species that normally bite has also been reported for *Theobaldia subochrea* in England (Marshall and Staley, 1936) and for *Aëdes concolor* in Australia (Woodhill, 1936). No *Anopheles* has as yet been reported to lay eggs without a previous blood meal, though Cambournac and Hill (1939) in one instance obtained a few eggs from an *Anopheles atroparvus* that had never taken a blood meal; in this case, the eggs failed to hatch.

EFFECT OF TYPE OF BLOOD ON EGG DEVELOPMENT

In the case of anautogenous mosquitoes—a category that includes the vast majority of species that have been studied in the laboratory —interest naturally centers on the property or component of blood that makes it necessary for egg development. Woke (1937a; 1937b) made a rather detailed study of the effect of various blood components and of blood from different species of animals on egg production in *Aëdes aegypti*. Previous workers (particularly Gordon, 1922) had found that whole blood was necessary for egg development in this species and Marchoux and Simond (1906) even reached the conclusion that "living blood" was necessary. Woke, using rabbit blood, found that the production of viable eggs in normal numbers followed the ingestion of "drawn, untreated whole blood, whole blood diluted with an equivalent volume of water, defibrinated, decalcified, and heparinized blood; and that the treatments neither destroy nor lower

the nutritive qualities of whole blood for egg production." His data are summarized in Table V.

TABLE V THE EFFECT OF VARIOUS BLOOD COMPONENTS ON EGG PRODUCTION IN *Aëdes aegypti* (WOKE, 1937)

LOT NO.	BLOOD COMPONENT	NO. OF MOSQUITOES	AVERAGE NO. OF EGGS PER MOSQUITO
1A	Erythrocytes of rabbit's blood suspended in Ringer's solution	64	50.7
1B	Erythrocytes of rabbit's blood suspended in Ringer's solution. The volume of packed red cells constituted 36 per cent of the suspension	69	67.6
2	Erythrocytes of chicken's blood suspended in Ringer's solution	60	86.8
3	Plasma of rabbit's blood	60	37.9
4	Serum of rabbit's blood	63	62.4
5	Haemoglobin from red cells of rabbit's blood	61	65.1

The discrepancy between the results obtained by Woke and those of other authors may be related to differences in technique. Woke fed his mosquitoes by inducing them to pierce a rat skin stretched over a small paraffin cell containing the nutrient material to be tested, thus closely approximating natural conditions of feeding. It is noteworthy that all of the blood fractions—erythrocytes, plasma, serum, and haemoglobin—from which Woke secured eggs are rich in protein, and it seems possible that the necessary food for egg development is highly concentrated protein rather than some particular substance, such as haemoglobin. Thus Fielding (1919) has reported obtaining a few fertile eggs from *Aëdes aegypti* fed on peptone and sugar.

In another series of experiments, Woke found that there was a significant difference in the number of eggs laid by *Aëdes aegypti*, depending on the source of the blood. Mosquitoes that had fed on man and rhesus monkeys laid significantly less eggs than mosquitoes that had fed on rabbit, guinea pig, canary, turtle, or frog. His data are summarized in Table VI. This same phenomenon has been observed by Roubaud and Mezger (1934) for *Culex pipiens*, and by Tate and Vincent (1936) with the same species. It is interesting that Tate and Vincent failed completely to obtain eggs from *pipiens* fed on citrated human blood, though some eggs were obtained from females fed on citrated canary blood (not as many as from mosquitoes that had en-

gorged directly on canaries, however). Their experiments were designed primarily to test the possibility of reactivating hibernating mosquitoes, and so their results are difficult to interpret in terms of normal food requirements for the species. Human blood may, in general, be rather poor food for mosquitoes, since Roy (1931) found that *Anopheles stephensi* laid only about half as many eggs after feeding on man as after feeding on rabbit, guinea pig, or rat.

Various authors have given figures on the number of eggs that may be laid by one female mosquito. Such figures, based on laboratory observations, have little meaning because it is difficult to estimate how much the laboratory environment may influence egg development and oviposition. Certainly most mosquitoes develop from 100 to 300 eggs on a single blood meal. Marshall (1938) has summarized the records for various British species for maximum number of eggs in one batch: these vary from 104 for *Aëdes geniculatus* to 433 for *Culex pipiens*. He mentions the case of a female of *Theobaldia subochrea* observed by Staley to lay 1,036 eggs in five egg rafts over a period of 33 days.

FAT PRODUCTION DURING HIBERNATION

In the case of mosquito species that hibernate in the adult stage, prehibernation blood meals may serve for the formation of a large reserve of fat instead of for the development of eggs. This condition was termed "gonotrophic dissociation" by Swellengrebel (1929), and has been the subject of a considerable amount of investigation by various authors, summarized in the books by Hackett (1937) and Swellengrebel and de Buck (1938). Swellengrebel originally intended the term "gonotrophic dissociation" to apply to *Anopheles atroparvus*, which takes occasional blood meals all through the hibernating period: the condition found in *Anopheles messeae*, in which a large fat body is formed, capable of sustaining the mosquito throughout the winter, was termed "gonotrophic concordance." The first term, however, may serve a more useful purpose if it is applied broadly to all cases in which a blood meal is utilized for maintenance (fat production) instead of reproduction (egg development). Malariologists are interested in the phenomenon because it accounts for the curious seasonal distribution of malaria transmission in the Netherlands and similar countries. To the general biologist, however, the chief interest is perhaps in the possible mechanism of this functional change.

TABLE VI EGG PRODUCTION OF *Aëdes aegypti* FOLLOWING INGESTION OF BLOOD FROM DIFFERENT SPECIES OF VERTEBRATES (WOKE, 1937b)

LOT NO.	ANIMAL	NO. OF FEMALES	FIRST BLOOD MEAL			SECOND BLOOD MEAL			AVERAGE NO. OF EGGS PER MG. OF NUTRIENT FOR BOTH MEALS
			Mg. of Nutrient Ingested	Total No. of Eggs Produced	Eggs per Mg. of Nutrient	Mg. of Nutrient Ingested	Total No. of Eggs Produced	Eggs per Mg. of Nutrient	
1	Man	28	72.8	2473	34.0	64.8	1558	24.0	29.2
2	Monkey	26	54.4	1711	31.5	52.7	1310	24.8	28.2
3	Rabbit	23	50.7	2696	53.2	44.4	1958	44.1	48.9
4	Guinea pig	28	53.4	2955	55.3	49.2	2419	49.2	52.4
5	Canary	26	54.3	2649	48.8	50.5	1798	35.6	42.4
6	Turtle	25	47.0	2362	50.2	41.4	1735	41.9	46.3
7	Frog	26	45.1	2625	58.2	44.7	2100	47.0	52.6

Roubaud seems to consider that most hibernation phenomena are the result of some inner rhythm in the species population, more or less independent of environmental control, though coinciding with environmental rhythms. He calls this "asthenobiosis" or "spontaneous biological inertia" (Roubaud and Colas-Belcour, 1926), and regards gonotrophic dissociation as a particular case. It is likely that gonotrophic dissociation is not the result of any single environmental factor, such as temperature. Fat production sometimes sets in in adults in the fall when the mean temperature is still above the level at which mosquitoes are active in the spring; and various workers have found that hibernation is not necessarily broken if the mosquitoes are simply brought into a warm laboratory. Yet a regular physiological cycle of the type described by Roubaud, superimposed on a population with a varying and irregular number of annual generations, seems impossible.

In Albania we kept laboratory material of many species of *Anopheles* active all through the winter months in an insectary where light, temperature, and humidity were kept as far as possible at midsummer conditions. We came to the conclusion, from various observations, that fat production (gonotrophic dissociation) was a result of any combination of factors that tended to restrict the activity of the mosquito, and that the release from hibernating conditions was caused by factors that tended to stimulate activity. Thus fat production in wild mosquitoes in the fall was associated not only with a drop in temperature, but with the shortest period of evening twilight, with an appreciable shortening of the length of day, and with the onset of the fall rains, all of which would tend to keep the mosquitoes quiescent.

OTHER FACTORS INFLUENCING EGG DEVELOPMENT

The speed of egg development, like other physiological processes in insects, is dependent on environmental temperature. Shlenova (1938) studied the effect of temperature on *Anopheles messeae*, and found that blood digestion was accelerated by a rise in temperature up to 30° but was retarded at 35°. At temperatures above 15° it was accelerated by high humidity, perhaps because evaporation at low humidities reduced the body temperature of the mosquito. He found that in small mosquitoes blood was digested more rapidly than in large ones.

We found with *Haemagogus spegazzinii* in Colombia that the per-

centage of mosquitoes laying eggs under laboratory conditions varied with the environmental temperature (Bates, 1947b). Significantly less specimens laid eggs at a constant temperature of 30° than at 25° or with alternating temperatures of 25° and 35°, and egg production was almost completely inhibited at a constant temperature of 35°. Eggs were laid a day sooner at 30° than at 25°, but 35° had no further accelerating effect on time of laying. In current experiments with *Anopheles pessôai* we find that, on an average, eggs are laid 6.7 days after the blood meal at 20°, 6.0 days at 25° and 4.0 days at 30°. Such temperature relations probably vary greatly with different species of mosquitoes.

Egg development after a blood meal seems in part to be dependent on the presence of spermatozoa in the spermatheca of the mosquito. Roy (1940) has proposed the term "spermathecal stimulation" for this phenomenon. He found that a blood meal led to egg formation in virgin females of *Anopheles stephensi* and *A. annularis*, but failed to do so in *A. subpictus*. We found similar specific differences among the anophelines in Albania: unfertilized females of *A. superpictus* and the species of the *maculipennis* group would rarely develop eggs after a blood meal, while unfertilized *A. algeriensis* would lay sterile eggs quite freely. Our most curious observation concerned the hybrid offspring of the *Anopheles atroparvus* x *melanoon* cross. The males of this hybrid were quite sterile, but the females seemed normal, bit freely, and laid large numbers of eggs even though not fertilized. We almost never secured eggs from unfertilized females of either of the parent species.

Various authors have given statistics on the number of blood meals required by different species of mosquitoes for the development of a batch of eggs. Such statistics are always based on mosquitoes kept under laboratory conditions, where egg development may be influenced by other factors, particularly the fertilization rate. It seems very likely that in most cases in nature a single blood meal is sufficient for the development of a particular complement of eggs, especially in anophelines, where a large number of eggs are developed simultaneously.

DIGESTION OF BLOOD

Relatively little is known about the processes of digestion in mosquitoes. The presence of anticoagulins and haemagglutinins in the

salivary secretions of some mosquitoes has already been mentioned, and it is probable that such substances assist in the preliminary breakdown of the blood. De Buck (1937) found that the secretion of the stomach of *Theobaldia annulata* contained a strong coagulin which acted on blood which had been previously treated with salivary secretions from the same or other species of mosquitoes. He did not find such a coagulin in the stomach secretions of the male. It has been observed that the blood taken up by mosquitoes is passed directly to the stomach whereas sweet juices or exudates are stored in the crop and passed on to the stomach at intervals (De Boissezon, 1930; MacGregor, 1930).

Huff (1934) made serial sections of the stomachs of *Culex pipiens* killed at various intervals after feeding, in the course of a study of the behavior of malaria parasites. He found that digestion began at the stomach wall and proceeded inwardly, the stomach contents showing a clear stratification. Twenty-four hours after feeding he found an amorphous, serouslike layer just within the stomach wall, followed by a layer of pigment varying in thickness in different mosquitoes and in different parts of the same stomach, and after this a layer of partially digested red blood cells, and finally in the center of the stomach a mass of blood looking quite normal histologically. By the end of 27 hours no normal-appearing red blood cells were left, and cellular blood elements had completely disappeared at the end of 37 hours (temperature condition not stated).

Dr. M. C. Balfour (unpublished observation) found in Greece that blood digestion in *Anopheles superpictus* was more rapid than in *A. maculipennis* or *A. sacharovi*, judged by the length of time that stomach blood would give a reaction with the precipitin test. Two hours after a blood meal, 100 per cent of *sacharovi* gave a reaction, 96 per cent of *superpictus;* twelve hours after the meal 91 per cent of *sacharovi*, 72 per cent of *superpictus;* fourteen hours after the meal, 79 per cent of *sacharovi*, and only 39 per cent of *superpictus*. The behavior of *maculipennis* was similar to that of *sacharovi*. It has been suggested that such specific differences in the digestive process might be a factor in determining the susceptibility of a mosquito to plasmodium invasion.

OVARIAN STAGES

Christophers has studied the development of the ovaries in anophelines in considerable detail, with the object of developing a method of determining the probable age of a specimen when it is caught. His descriptions have been published several times, for instance by Christophers, Sinton and Covell (1936), and are summarized in the malaria textbooks (Boyd, 1930; Russell, West, and Manwell, 1946). Five stages are distinguished, with the following characteristics (as summarized by Boyd):

Stage 1. Follicle consists of a small group of undifferentiated cells, in which the follicle and central cells later become differentiated. The follicle cells then form a distinct layer like cubical epithelium, and the follicle assumes an oval shape. The nucleus of the ovum now becomes distinguishable from those of the nurse cells, and this event terminates the first stage.

Stage 2. In the protoplasm of the ovum appear fine yolk granules, which become coarse and greatly increase in number. The ovum increases relatively to the nurse cells, until it occupies half the follicle.

Stage 3. At the beginning of this stage the nucleus is obscured by yolk and the ovum occupies from one-half to three-quarters of the follicle.

Stage 4. This is initiated by elongation of the follicle, while the nurse cells become less and less conspicuous, and at the termination, the shape of the mature egg is assumed by the follicle.

Stage 5. The floats and other surface characters are differentiated and the egg is ready for laying.

At Amritsar in July (a period of high temperatures) Christophers found that the various stages in *Anopheles subpictus* lasted as follows: 1st stage to 12 hours after emergence; 2nd stage to 36 hours; 3rd stage to the 4th day; 4th stage to the 5th day; and 5th stage to the 6th day. This cycle is repeated with each batch of eggs, except that the first two stages are telescoped after the first laying; the determination of the age of a mosquito thus depends on estimating not only the stage of ovarian development, but also the probable number of egg batches that have been matured. Occasionally one or two eggs remain in the ovaries after the first oviposition, which of course clearly indicates that the ovaries are not in their first cycle. Mer (1932) found that there was an increase in the size of the oviduct with each batch of

eggs, and that the size of this could be used in estimating how many batches had been laid.

MANNER OF LAYING EGGS

Mosquitoes have two quite distinct methods of laying eggs: the eggs may be laid singly or they may be fixed together to form what is called a "raft." In general the species of *Culex*, *Mansonia*, and *Theobaldia* lay their eggs in rafts, while the aëdine and anopheline mosquitoes lay their eggs singly. The possession of a distinctive habit like raft formation would seem almost surely to indicate a common ancestry for all of the species with the habit, just as much as the possession of some distinctive morphological character. It is thus surprising to find both types of habit in the South American genus *Trichoprosopon*. One species, *T. compressum*, lays its large eggs (looking very much like small, uncooked grains of oatmeal) singly on the water surface, while another species, *T. digitatum*, forms an upright raft of eggs, like the raft of the common *Culex*, only with the eggs not so tightly glued together. These two species are very similar in appearance as adults, being distinguishable only by very slight morphological characters. It is interesting that Edwards (1932, p. 64) considers *Trichoprosopon* to be one of the most primitive of living mosquito groups on morphological grounds. Perhaps it represents the stem form in which the raft-laying habit first appeared.

Mosquitoes that lay their eggs in rafts select a free water surface for oviposition, the mosquito resting on the water during the act and carefully placing the eggs against one another as they are laid. These eggs are covered with a mucilaginous material which quickly hardens, binding the eggs together in the raft form. Réaumur wrote a description of raft formation in *Culex pipiens* which has become a mosquito classic; it is quoted by Howard, Dyar, and Knab (1913). The mosquitoes that lay their eggs singly have various methods of oviposition. Some drop the eggs while hovering over water, others rest on the water surface as does *Culex*, while still others apparently lay their eggs in moist situations at times when there is no free water.

Perhaps the best way of illustrating the diversity of egg-laying habits in mosquitoes is by the description of a few cases. Several groups of aëdine mosquitoes breed in tree holes, and in all of these the eggs are apparently laid on the side of the tree hole somewhat above the water line, and they do not hatch until an increase in the amount of

water has caused them to be submerged. The species of the genus *Haemagogus* have this habit, and the following observations on *Haemagogus spagazzinii* illustrate some of the behavior patterns involved:

A female mosquito was placed in an upright glass vial with a filter-paper disc in the bottom, moistened with distilled water. The mosquito was observed making exploratory movements over the disc with the tip of the abdomen, but no eggs were deposited. A slip of filter paper was inserted into the vial until it touched the wet disc in the bottom, and then appressed to the side of the vial, making a moisture gradient. The mosquito crawled onto this, continuing the exploratory movements of the abdomen. Finally one egg was laid, at about the point on the filter paper where the moisture ceased to be visible. Then seven more eggs were laid in about five minutes. Between each egg the female explored the paper carefully with the tip of the abdomen, and usually laid another egg when she chanced to touch a previous egg—in other words, responding to irregularities in the surface. The movement when the egg was laid was notably different from the nervous exploratory movements: the tip of the abdomen was placed in close contact with the surface of the paper and drawn slowly and steadily forward (the mosquito remaining motionless except for the abdominal movement), leaving the egg adhering to the paper. This movement took perhaps two seconds. After laying these eight eggs, the female rested on the cotton plug of the vial and showed no more movements during thirty minutes of further observation. By next morning, many more eggs had been laid, many of these fitting neatly into the groove where the edge of the filter paper met the glass.

The species of *Aëdes* that breed in ground pools apparently lay their eggs among leaves or on the moist ground, where they remain in a state of suspended development until submerged or washed into pools by rains. Wesenberg-Lund (1921, p. 75) described the oviposition of *Aëdes communis* as follows:

For three years I vainly tried to see the process of egglaying. Not until the summer of 1919 on 3/VII did I have an opportunity to observe it in one of the wholly dried up *Mochlonyx*-ponds. The females were sitting under the dry leaves; here, as many times before, I had placed myself on one of the Carex-tufts eagerly observing every grass-stem and every leaf, hoping that here I should see something of the process. Once after having

turned over the withered leaves, I found, in a layer of leaves below those rolled up by the sun, some mosquitoes which slowly flew away. Looking at these leaves with a lens I found them sprinkled with the well-known black mosquito eggs. Later on, leaves which were cleaned were brought into the laboratory and placed in a vessel together with several females: the next day I found the same black eggs scattered over the bottom under the leaves. Therefore we can now take it for granted that this species probably, like all our other *Ochlerotatus* species, lay their eggs on dry earth and singly.

In the laboratory many anopheline mosquitoes, when ready to oviposit, will light on the water and drop their eggs one by one, but there is considerable reason for believing that this habit is abnormal, due to the conditions of confinement (Bates, 1940b). We were able, on several occasions, to observe oviposition in a very large outdoor cage in Albania, and in these cases the eggs were dropped by mosquitoes which were hovering over the water in a sort of "oviposition dance." Dr. John S. Kennedy, who was working in the laboratory, had an excellent chance for observing this dance in caged mosquitoes one day when something went wrong with the stove that was used for heating the room where we kept the mosquito colonies: the room became filled with smoke, and all of the mosquitoes started "ovipositing furiously." Apparently the smoke served as a stimulus. The ovipositing mosquitoes, during this smoke episode, seemed quite undeterred by having a flashlight directed on them, so that it was possible to watch them much more closely than usual. Dr. Kennedy's observations were:

1. Females were never seen to start an oviposition dance without touching the water, and they touched it again every now and then during the dancing, although they sometimes remained hovering without touching it for as much as 30 to 60 seconds.

2. Contact was made with the second and third pairs of legs, not with the first pair, the proboscis, or the abdomen.

3. Dances were frequently executed without dropping eggs.

4. The same female would lay some eggs in a close batch while sitting on the water, then move to another place and lay some more, then take to the air and lay some hovering, and then even sit down in a new place and lay yet more. This may have been due to "smoke nervousness."

5. Before and after laying eggs on the water, many females made random flights near the bottom of the cage, dipping and hesitating in flight frequently.

6. Having laid some eggs while hovering over the water, one female was seen to continue hovering in exactly the same way, and presumably still laying eggs, after it had shifted right away from the dish and was over the bare floor.

7. "Hovering" is a better term than "dancing" for the mode of flight of ovipositing females, for lateral movements are much more restricted than those of swarming males.

In Egypt we found the eggs of *Anopheles multicolor* in nature stuck together in long ribbons; in such a case it seems likely that the eggs have been laid by a mosquito resting on the water. The "oviposition dance" is thus probably not universal among anophelines, though the exceptions may be rare.

A very curious oviposition habit has been observed in the Oriental genus *Armigeres*. Strickland (1917) reported finding a specimen with an egg mass attached to the hind leg, each egg with a protruding larva. When the mosquito was placed in a bottle with some water, it flew down and methodically dipped its leg into the water, whereupon the larvae all emerged and swam away. Barraud (1934) states that these egg masses on the leg have been observed on several occasions on *Armigeres flavus*, and that it is thought to be a method of introducing the larvae through small holes into the bamboo internodes which are their habitat.

SELECTION OF OVIPOSITION SITE

It is well known that each species of mosquito has rather characteristic breeding habits, including a sufficient range so that some species of mosquito larva is able to inhabit almost every type of terrestrial water accumulation, from lake margins and tidal swamps to the thin films of water that accumulate at the leaf bases of certain tropical plants. The larvae frequently show physiological and structural adaptations to their particular habitat: adaptations to the chemical conditions of the water, to the presence of current, to unusual temperature conditions, and so forth. It is often, however, difficult to demonstrate special adaptations in larvae that may in nature be found in highly restricted habitats; and mosquito larvae of many species from diverse natural habitats may often be bred in the laboratory in the same culture medium, showing that the natural distribution is not necessarily caused by peculiar requirements on the part of the larvae.

It is conceivable that the adult mosquitoes might scatter their eggs in all types of water, the differential distribution of the larvae resulting from a differential mortality in different types of breeding place. In the laboratory, gravid female mosquitoes will often oviposit in anything containing water, even in solutions lethal to the eggs or larvae, or on moist paper or cloth; and selection of oviposition site is, as will be shown later, demonstrable only with difficulty. Yet it seems quite clear that the ecological distribution of larvae in nature is largely a result of the different oviposition habits of adult mosquitoes of different species. Many mosquito species can be identified readily from the eggs, and in all cases where studies have been made of the species composition of eggs and larvae in particular breeding places, a close correlation has been found (Lewis, 1939; Bates, 1941c; Thomson, 1940a; Russell and Rao, 1942c).

The general question of the classification of mosquito breeding places, and of the factors that seem to control the ecological distribution of larvae, can best be discussed later in connection with the characteristics of the larval environment. Here we can only review briefly experiments dealing with the oviposition reactions of the adults. In mosquitoes, as in all insect groups with complete metamorphosis, there is a close relation between the oviposition habits of the adult and the physiological requirements of the larva: a relation with a mechanism of instinctive behavior hard for the human observer to understand, but nevertheless fascinating.

Laboratory experiments with oviposition behavior have in general given unsatisfactory results. The reactions of mosquitoes in the restricted and highly simplified environment of a cage often seem quite unrelated to the reactions that we know or suspect to exist in nature, and as was remarked above, almost all mosquitoes will lay eggs in almost any substrate containing water under such cage conditions. When selection from a series of oviposition sites can be demonstrated, the basis of the selection may be difficult to demonstrate, and the result may seem quite unintelligible. As an example, we have repeatedly observed in Colombia that caged females of *Psorophora ferox* will lay many more eggs on the under side of a wet filter paper on top of the cage (the eggs being inserted through the screened top of the cage) than on a similar paper on the bottom of the cage—seemingly as senseless a type of selection as could be imagined.

Many studies have been made of oviposition in water with different

salt content, since salinity is often a clear and obvious defining character of larval breeding places. Kligler and Theodor (1925), for instance, tested *Anopheles sacharovi, sergenti,* and *superpictus,* and found that all three species showed a preference for fresh over saline water, although *sacharovi* is usually found in nature in brackish water and the other two in fresh. De Buck, Schoute, and Swellengrebel (1932) found that *Anopheles atroparvus,* a brackish-water breeder, definitely avoided even weak sodium-chloride solutions, while *messeae,* a fresh-water breeder, seemed to be indifferent to salt content of the water used for oviposition. Similarly, Woodhill (1936, 1941) found that *Aëdes concolor,* a species that lives naturally in sea water, preferred fresh water to sea water for oviposition in the laboratory. We made a series of experiments with oviposition selection by *Anopheles atroparvus* in Albania (Bates, 1940b), and found that while this species was indifferent to the sodium-chloride content of the water, or even showed a slight preference for distilled water over slightly saline water, it showed a definite preference for water containing a small amount (one part per thousand) of calcium sulphate over distilled water. It seemed possible from this that contradictions in laboratory experiments might come from the selection of the wrong factor for testing: the controlling factor, with breeders in saline water, may be some other chemical rather than the obvious sodium salt.

Laboratory experiments with chemicals other than sodium chloride have, however, not been much more satisfactory. Lund (1942) tested the reactions of *Anopheles quadrimaculatus* to a variety of chemical factors without being able to demonstrate any selection. Mehta (1934b) tested *Anopheles culicifacies* and *A. subpictus* with natural waters containing different amounts of free ammonia and with solutions of ammonium carbonate. *Culicifacies,* which is found in nature only in relatively pure waters, oviposited indiscriminately in the laboratory in waters with up to 6.6 parts per million saline ammonia; *subpictus,* which sometimes breeds in quite foul waters in nature, showed a definite preference in the laboratory for waters with a relatively high ammonia content. Thomson (1941a, 1942) found that *Anopheles minimus* in the laboratory was very sensitive to organic pollution of water, refusing to oviposit even in slightly polluted water, although the larvae would grow perfectly normally in such water. He found that *Anopheles hyrcanus* females were much less sensitive to

pollution. Many culicine mosquitoes that breed in highly polluted waters show a strong preference for such water for oviposition in the laboratory; Thomson (1941a) observed this in the case of *Armigeres kuchingensis*, and in Colombia we found that *Trichoprosopon digitatum* would refuse to ovipost unless provided with a rich organic infusion.

A few experiments have been made with effect of water temperature on ovipositing females. Hecht (1930) found a difference between *Anopheles maculipennis* and *claviger*, the former avoiding water below 20° and above 30° C., while the latter preferred water below 20° (which would be in agreement with the usual breeding of *claviger* in cold water). Temperature selection was more difficult to demonstrate with *Aëdes aegypti*. Thomson (1940c) found, in laboratory experiments, that *Anopheles minimus* showed a general avoidance of water with high temperatures for oviposition, but that it showed no choice within the range of temperature likely to be encountered in water in nature at night, when the mosquito would be ovipositing.

Few experiments have been made with the effect of water current on ovipositing females. Thomson (1940b) found that *Anopheles minimus* preferred to oviposit in still water rather than in water with even a slight current (0.05 foot per second), despite the fact that the species in nature usually breeds in association with running water. Ovipositing females seemed unaffected by a continuous surface ripple (caused by allowing a steady drip of water into one of the ovipositing dishes).

The most clear-cut results in laboratory experiments with selection of oviposition site have been obtained with light and background color, suggesting that visual stimuli may play an important role in this reaction. Detinova (1936) found that *Anopheles messeae* showed a clear preference for ovipositing in water colored orange with a tasteless and odorless dye. Bates (1940b) found that *Anopheles atroparvus* regularly selected the pan with the darkest background of a series for oviposition, and the same behavior was found by Lund (1942) with *Anopheles quadrimaculatus*. Thomson (1940a) found that *Anopheles minimus* showed a strong preference for ovipositing in the shaded part of a cage, even when the whole cage was kept in an area of low illumination, approximately equivalent to one-fourth of starlight. At even lower illuminations—lower than normally would

be encountered in nature (one-tenth of starlight)—the pereference disappeared, even though the difference between the shaded and unshaded portions of the cage could still be appreciated by the human eye.

The most interesting field experiments with the behavior of ovipositing mosquitoes have been made in India, by Thomson (1940a, 1940b, 1941a, 1942) with *Anopheles minimus*, and by Russell and Rao (1942c) with *Anopheles culicifacies*. The experiments of Russell and Rao are particularly neat, and form an example of the type of field experimentation that might, if more widely applied, yield valuable information on the factors governing the habitat distribution of various mosquitoes. *A. culicifacies* breeds abundantly in rice fields before and immediately after planting, disappearing after the plants have grown to a height of 30 cm. or more. Russell and Rao showed clearly that this was the result of the oviposition habits of the adult, which was inhibited from laying eggs by the presence of the rice plants. The same effect could be obtained by a variety of devices simulating the obstruction offered by the rice plants: by glass rods, by test tubes containing rice plants (to demonstrate that the effect was not due to the influence of the plants on the water or soil), by barriers of vertical bamboo strips, or by covering areas with wooden or wire-screen boxes. First-stage larvae placed in fields where *culicifacies* had ceased to oviposit grew to maturity normally, showing that the absence of the species was due entirely to the adult oviposition behavior, not to an increased mortality in the larvae. It seemed probable that the mechanical obstruction interfered with the oviposition dance of the adult and that other species (for example, *hyrcanus*) that continued to breed in the fields had different types of oviposition behavior.

CHAPTER VII

MOSQUITO EGGS

> In the development of any organism, we have to distinguish between the internal factors which are at work inside it and the external factors which constitute its environment. Now the internal factors were present in the fertilized egg, and so they can also be regarded as the transmitted factors, passage of which from parent to offspring constitutes heredity. . . . Each ontogeny is a fresh creation to which the past contributes only the internal factors [whose] action is to ensure that if the external factors are normal . . . the animal will develop along the same lines as its parent.
>
> —G. R. DE BEER

THE egg stage of mosquitoes is of biological interest from several rather diverse points of view. For the taxonomist, the eggs may furnish specific recognition characters of considerable importance. The diversity of anopheline eggs has long been recognized, and figures of different egg types have been given by many authors; but little importance was attached to the differences until Martini, Missiroli, and Hackett (1931) discovered that the eggs provided the easiest and only reliable method of separating the various populations related to *Anopheles maculipennis*. It has since been found that many other species closely similar as adults may have strikingly distinct eggs, and the study and description of these egg characters has received increasing attention.

The physiologist often finds the egg stage convenient for various types of experiments, particularly for studies of the effect of temperature on development, since other environmental factors, particularly nutritional factors, are easily ruled out. Many mosquito eggs provide a special and fascinating physiological problem in the nature of the hatching stimulus, and in the factors that control the diapause or state of suspended development. This subject has received a great deal of attention and will be reviewed at some length in the present chapter.

For the naturalist, the study of mosquito eggs has many types of appeal. The eggs show structural and physiological adaptations to the special environments in which they are placed: adaptations that in some cases seem obvious, but that in other cases are difficult to understand. The ecological role of the suspension of development that occurs in the egg stage of many types of mosquitoes is especially interesting, since it provides a sort of buffer mechanism whereby the total species population is protected against regular or irregular periods of adversity. While the scope of "egg behavior" is limited, there are nevertheless characteristic differences among the various species that must be taken into consideration in any study of the natural history of any given species or of the group as a whole.

EGG STRUCTURE

The egg shell is built up of three distinct layers, an inner, thin vitellin membrane which surrounds the yolk and growing embryo, an intermediate endochorion, hard, thick, and opaque, and an outer exochorion, usually thin and transparent. In a newly laid egg, the endochorion is also transparent, making the whole egg appear white; but under normal conditions this layer rapidly darkens and hardens. The micropyle is located near the anterior extremity; its structure in *Anopheles maculipennis* has been described in detail by Nicholson (1921).

The exochorion usually has a complex structure, often forming minute protuberances which give a characteristic appearance to the egg according to the species. Hackett and Missiroli (1935) describe the structure which results in the specific designs of the eggs of the *Anopheles maculipennis* group as follows:

> The design on the egg is produced by the type and arrangement of the *columellae* with which the outer coat (*exochorion*) is thickly studded. Where these are high, with rough or facetted tops, the light is refracted, giving them a gray, and in some types a frosty-white appearance. But in certain spots these protuberances are smooth on top, reduced in size and at times almost suppressed, lending a transparency to the outer coat and allowing the endochorion to show through and produce the characteristic surface markings of the egg. These markings will be described as they appear to the eye, i.e. as though they formed a dark pattern on a gray ground, but it should always be kept in mind that it is the exochorion which, by its light-refracting areas, forms a gray pattern against the uniform brownish-black ground of the endochorion underneath.

In the majority of anopheline eggs, the exochorion is drawn out on the sides to form a frill which completely encircles the egg at "water line." This frill is often modified at the sides into large, hollow, cellophane-like "floats" which are supposed to serve as stabilizers. These floats vary greatly in size and shape in different species, and may be present or absent in closely related species, and may vary considerably in some respects in what is apparently a homogeneous species, or even within a batch of eggs laid by a single female. The frill may be modified in other ways, for instance, to form a stiff collar at one end of the egg in some South American *Nyssorhynchus*. The inflated floats are sometimes formed independently of the continuous frill (for example, in *Anopheles gambiae*) and there may be a system of multiple floats, as in *Chagasia*. The exochorion is sometimes drawn out into long filaments (as in *Anopheles peryassui*) which probably also serve as stabilizers for the egg on the water surface.

Marginal frills are also found on the eggs of other mosquito genera. In *Orthopodomyia*, for instance, the lateral frill is developed into a prominent, complex flange (described as "membranous" by Marshall, and as "gelatinous" by Howard, Dyar, and Knab). In this case the flange is said to serve to hold the egg above the water line in the tree hole where it is laid, instead of serving to increase buoyancy as in the other cases.

Mosquitoes exhibit three main types of egg-laying habit: eggs may be laid singly on the water surface; they may be laid in the form of floating "rafts"; or they may be laid out of the water. Structural modifications reflect these differences in habit. The simple floating eggs have a boatlike shape with accessory devices, such as frills and floats for buoyancy. The eggs of *Megarhinus* are described as "floating buoyantly by means of air bubbles which form among the tubercles or spines present on the eggshell" (Barraud, 1934). In floating eggs (at least in *Anopheles*), the ventral side of the developing larva is uppermost, so that the use of the terms "ventral" and "dorsal" in describing such eggs may be confusing (Christophers and Barraud, 1931); occasionally, as in the plates of Howard, Dyar, and Knab, the upper side of the egg may be termed "ventral," but this seems pedantic.

The eggs of raft-laying species are (as described by Marshall, 1938) "of circular cross-section and taper slightly—the larger (anterior) end being rounded, and the other end bluntly pointed. When ovipositing, the female stands on the water surface with her hind

legs crossed near their extremities, and 'stacks' her eggs (which are covered with the necessary cementing substance) within the V-shaped 'dock' thus formed. The eggs are deposited with their larger ends downwards, so that the upper surface of the raft is noticeably concave." The egg rafts of *Culex* float on the surface of the water, while those of *Uranotaenia* and *Lutzia* are said to be half submerged. The form of the raft may be characteristic: in *Culex* and *Theobaldia* it is usually oval, while in *Mansonia* it is usually long and narrow, consisting of only two rows of eggs. In *Trichoprosopon digitatum*, the eggs do not form a compact mass as in *Culex* rafts, but are loosely stacked to form a reticulate pattern; the raft can thus lie flat on the water surface (not concave), and it is usually round rather than oval. The bottom end of each egg of *Culex* has a curious cuplike corolla, which may have a hydrostatic function.

Eggs laid out of the water must either be placed so that the hatching larva can readily reach the water (as is the case, for instance, with *Orthopodomyia*), or must have adaptations that enable the egg to resist desiccation until such time as it may become submerged. The eggs of *Aëdes*, *Psorophora*, and related genera belong to this class. The egg is typically black, with a heavy shell. Kalmus (1941) has noted that this dark coloration of eggs adapted to resist desiccation is a general rule: "Hibernating and aestivating insects are dark. This is not limited to imagines. The eggs of many Phasmodea which are dropped haphazard in the open and take considerable time to hatch are dark and hard. The eggs of Tipulidae laid in a similar manner are also dark, whereas the eggs of other Diptera which are deposited on a moist substrate are pale and soft." The exochorion of such eggs may be bossed or sculptured, generally showing a hexagonal pattern; the shape is usually fusiform, though there is considerable variation among the different species.

VARIATION IN EGGS

The increasingly widespread use of egg structure as an index of specific differences in the genus *Anopheles* gives special interest to the study of egg variation. It seems at first sight odd that the egg stage should show such striking specific distinctions, since we are accustomed to expect specific characters to develop late in the ontogenetic history of an individual. The *egg shell*, however, is a character of the mother, its structure and pattern depending on the ovarian cells that

lay it down. The diversity of egg shells, then, is really but another manifestation of the endless specific diversity of structure and function shown by the reproductive organs of animals and plants.

These egg characters, like everything else about an organism, are subject to variation, and this variation within a species population may reflect inherited, genotypical differences, or may be the result of a varied response by the same genotype to varying environments. As an example of this latter, "spring eggs" of all of the *maculipennis* forms tend to be darker than "summer eggs"; and in the case of *Anopheles sacharovi*, "spring eggs" have small floats, while "summer eggs" usually have no floats. Mer (1931) first showed that floats would be present on eggs laid in the summer, if the parent mosquitoes were kept at low temperatures during the period of egg development. The North American *Anopheles walkeri* also shows morphological differences between "winter" and "summer" eggs (Matheson and Hurlbut, 1937; Hurlbut, 1938).

Considerable intraspecific variation has been found to exist among the eggs of tropical American *Nyssorhynchus*. Rozeboom (1938) has, for instance, described three "types" of eggs for *Anopheles strodei* in Panama. *Anopheles pessôai*, like the European *sacharovi*, may lay eggs with no floats, or with very small floats.

Size seems to be fairly constant in eggs of a given species, and Sweet and Rao (1937) distinguish two "races" of *Anopheles stephensi* on this basis; they have also (1938) studied variation in size in eggs of *A. culicifacies*.

The eggs of anophelines are now usually figured and described in general faunal papers. Special studies of the egg characters of species in a given fauna have also been published, such as the work of Causey, Deane, and Deane (1944) on the eggs of Brazilian species, and of D'Abrera (1944) on Ceylon species. Eggs of culicine mosquitoes have received less attention. Species of several genera have been figured in the plates of the second volume of the monograph by Howard, Dyar, and Knab.

FIELD STUDIES OF EGGS

The eggs of many anophelines are sufficiently distinct so that they can be identified with a hand lens, particularly by workers thoroughly familiar with a local fauna. It is thus possible to study the oviposition habits of the adult and compare the species of eggs found

in a given habitat with the species of larvae, as was discussed in the preceding chapter. Barber (1935) developed a method of studying eggs in the field by skimming the water surface with a pan and straining the water through a muslin glove on which the eggs would be stranded. Bates (1941c) found that muslin or bolting cloth, stretched taut over an embroidery hoop, was more convenient. The cloth could be marked off in squares for convenient counting. Thomson (1940a) found it necessary to use a different method for Indian anophelines, since the eggs were on an average of much smaller size than European species and difficult to locate on cloth. He used a white-enameled basin for skimming the water surface, and searched for the floating eggs over this white background; the eggs were removed with a wire loop and carried back to the laboratory on damp filter paper in a collecting tube for examination.

Gjullin (1938) has described a machine for separating *Aëdes* eggs from soil and detritus.

EMBRYONIC DEVELOPMENT

No special studies seem to have been made of the embryology of mosquitoes, and it is unlikely that the family would present any peculiar morphological features. A few studies, however, have been made of the effect of environmental factors, especially temperature, on the rate of development as measured by the time elapsing between oviposition and hatching.

From the point of view of development, mosquito eggs fall into two main types: those in which under normal circumstances development is a direct function of temperature, the egg hatching when the embryo is fully developed; and those in which a special stimulus is necessary for hatching. Eggs laid directly on the water surface, including all anophelines and the culicine species that lay floating rafts of eggs, belong to the first type; the eggs of *Aëdes*, *Psorophora*, and related genera, and perhaps of the tropical sabethines, belong to the second type.

The length of time required for development of eggs of the first type at a given temperature is probably a specific characteristic, but in most species the period is short—two or three days at moderate temperatures. Thomson (1940c) found the following times between oviposition and hatching with *Anopheles minimus* at various constant temperatures: 16°, 7 days; 20°, 3½ days; 25°, 2½ days;

30°, 2 days; 35°, 2 days. At alternating temperatures, development seemed to occur at about the same speed as would be expected at a constant temperature corresponding to the mean of the alternations.

Most anopheline eggs are killed by freezing and by temperatures in excess of 40°. There is probably some specific variation in susceptibility to both extremely low and extremely high temperatures. Sergent and Catanei (1935) even report that the eggs of *Anopheles maculipennis* in Algeria are more sensitive to cold than those of Corsica. Sautet (1936) found that eggs from hibernating *maculipennis* and *sacharovi* were killed when exposed for an average of four months at 4°, while summer eggs of the same species hatched in 18 to 20 days at this same temperature. Hatching can be considerably delayed in most anopheline eggs by maintaining them at low temperatures. Boyd, Cain, and Mulrennan (1935) routinely store the surplus eggs of *Anopheles quadrimaculatus* in a refrigerator for two weeks as an insurance against possible accidents to their supply of growing larvae.

The question whether anopheline eggs can resist desiccation has been investigated by a number of people, with conflicting results. It is probable that the eggs of some species, at least, can resist desiccation for short periods. Martini (1923) found that *Anopheles claviger* eggs laid on damp (not wet) earth and kept at 22° or 19°–20° delayed hatching for six weeks. Lamborn (1922), working in Malaya, studied the results of desiccation of anopheline ova of various species. He found that at a very early stage of incubation, the eggs of most species could not withstand even short periods of drought. The only exception was *Anopheles barbirostris* which, even when first laid, could withstand drying for 24 to 48 hours. Eggs of *A. vagus* and *A. fuliginosus*, in which incubation had begun, were able to retain their vitality up to 72 hours when dry. Dessication always checked the development of the ova to some extent, and in no instance did hatching take place until a considerable number of hours after the restoration of the eggs to water. Horsfall and Porter (1946) found that the eggs of *Anopheles punctulatus* and *A. farauti* remained viable as long as 14 days on a moist surface in the laboratory, hatching within a few minutes after being floated on water. Unti (1943a) obtained similar results with various South American *Nyssorhynchus*. Other experiments with the resistance of anopheline eggs to drying

have been reported by Brumpt (1925a), Mayne (1926), and Gebert (1937).

It thus seems well established that the hatching of the eggs of various species of anophelines can be delayed to a greater or less extent under laboratory conditions by partial desiccation. It is not clear, however, to what extent this mechanism may serve for the carry-over of populations through the dry season in the field. Stone and Reynolds (1939) report obtaining larvae of several species of mosquitoes, including *Anopheles albimanus* and *A. punctimacula*, by immersing soil taken from an area in Panama where no water had been standing for at least a month. They deduce that these species normally pass the dry season in the egg stage, but this opinion seems not to have been confirmed by other workers.

HATCHING STIMULUS

A very large number of mosquitoes lay eggs in which the embryo develops normally up to the point of hatching, and then remains in a state of suspended development, or diapause, until some stimulus reactivates the embryonic larva, leading to immediate hatching. This phenomenon occurs in the common laboratory mosquito, *Aëdes aegypti*, as well as in other species of *Aëdes* and in several related genera, and it has been the subject of a great deal of investigation. The eggs may lie dormant for many months or even for more than a year (Bacot, 1918), and the diapause is obviously normally a mechanism for hibernation or aestivation. The phenomenon is also found, however, in many tropical species in areas with climatic conditions that would not seem to require any special aestivating mechanism. In such cases the egg diapause appears to be an adaptation to the peculiar conditions of special breeding places, such as tree holes and transient ground pools, where the water level may be subject to large and abrupt fluctuations. It is interesting to note that species that show an obligate egg diapause are thereby prevented from breeding in stable water accumulations, since the necessary hatching stimulus would not occur. This difference in behavior explains, for instance, the fact that species of *Anopheles* and *Aëdes* only exceptionally occur in the same habitat. The effect of egg diapause on the seasonal curve of adult abundance has been mentioned in Chapter III.

Laboratory studies of the hatching stimulus have lead to diverse,

and in part conflicting, results. Perhaps the most detailed and interesting of these studies are by Buxton and Hopkins (1927), Shannon and Putnam (1934), and Gjullin, Hegarty, and Bollen (1941). Shannon and Putnam worked with the eggs of *Aëdes aegypti* in Brazil. They stressed the importance of the "conditioning of eggs" as a preliminary to hatching. They found that it required from two to three days at a temperature between 25° to 27° for the embryo to develop and reach a stage that would permit immediate hatching. Below 25° the time was longer—at 23.5°, the eggs had to be left moist for at least four or five days. They divided their eggs into four classes according to the nature of the "conditioning" as follows:

Class I. "Properly conditioned eggs" kept on moist filter paper in open air until enclosed larvae are fully developed. Approximately 90 per cent hatch in ten minutes.

Class II. Eggs less than twelve hours old. When placed in water containing food, these do not undergo as rapid or uniform a development as eggs of Class I.

Class III. Eggs air-dried while less than 24 hours old, kept in this state a week or longer. When placed in water, few hatch.

Class IV. Eggs ripened on moist paper and then dried. These may be stored for six months without showing high mortality. About 5 per cent will survive a year or more.

The influence of proper "conditioning" on the length of time required for hatching is nicely shown in the experiment summarized in Table VII.

TABLE VII LENGTH OF ECLOSION PERIOD OF EGGS OF *Aëdes aegypti* IN RELATION TO PREVIOUS "CONDITIONING" PERIOD (SHANNON AND PUTNAM, 1934)

LOT *	HOURS AIR-CONDITIONED		HOURS REQUIRED FOR FIRST HATCHING		ADDITIONAL HOURS REQUIRED FOR FINAL HATCH	TOTAL PERIOD IN HOURS
	Range	Mean	Additional Hours	Cumulated Hours		
1	0–24	12	144	156	216	372
2	12–36	24	52	76	72	148
3	33–57	45	31	76	54	130
4	40–64	52	18	70	48	118
5	60–81	70	8 min.	70	24	94
6	87–111	99	2 min.	99	10 min.	99

* One hundred eggs in each lot, all oviposited less than 24 hours before the beginning of the experiment.

Buxton and Hopkins made extensive experiments with egg hatching in *Aëdes aegypti* and *A. scutellaris* (*variegatus*) in Samoa. Their final conclusion illustrates well the difficulties that attend this type of experimental work. They say:

> The large amount of experimental work which we performed has on the whole added to the pre-existing confusion. But from it, and from a consideration of all the literature there emerges one definite conclusion; after maturation the larva in the eggshell is in a sensitive condition and its emergence may be precipitated by a great variety of influences; some of these are known, for instance, any form of mechanical agitation, the sinking of the eggs, the presence of traces of a diversity of unfamiliar chemicals, and of microörganisms; it is possible that the organisms behave as an unfamiliar chemical by producing enzymes.

Many of the experiments of Buxton and Hopkins were performed before they discovered the necessity of "conditioning" eggs. In the technique that they finally adopted, the eggs were collected from the field containers when less than 24 hours old and were placed on filter paper with a camel's hair brush. The pieces of paper were put in wide-mouthed jars and were carried to the laboratory without becoming dry. They were left to mature for 24 hours in these bottles and were then allowed to dry and were kept dry until used. The mean temperature in their laboratory was 27°. The "conditioning" period which they used was thus about a day less than Shannon's, and this may account for some of the differences between the two sets of experiments.

In the case of eggs put directly into water (Class II of Shannon), it is possible to make direct comparisons between the experiments of Shannon and Putnam and Buxton and Hopkins. Buxton found that 80 per cent of his eggs of *Aëdes aegypti* hatched in the first day, that is, when 24 to 48 hours old, under these conditions. This differs sharply from the results of Shannon, who found that such eggs require on an average of 144 hours for hatching. This possibly indicates a behavior difference between Samoan *Aëdes aegypti* used by Buxton and the Brazilian colony used by Shannon. It is quite possible that this common and widespread mosquito shows physiological differences in different parts of its range, which should be kept in mind in comparing the results of experiments made in different parts of the world.

It was early observed (Bacot, 1917) that the presence of yeast and

bacteria stimulated hatching in the eggs of *Aëdes aegypti*, and it has been thought that the readiness with which eggs hatch in rich organic infusions (Buxton and Hopkins) may be due to secretions of the microflora of such infusions. Gjullin, Hegarty, and Bollen investigated the nature of this hatching stimulus with *Aëdes vexans* and *A. lateralis* and came to the conclusion that hatching was due directly to a reduction of the dissolved oxygen of the medium in which the eggs are emersed. They found that any method that they used to reduce the oxygen content of the water caused hatching: the addition of reducing agents, such as glutathione and thioglycolic acid; reduction of atmospheric pressure; bubbling hydrogen through a sterile solution. Tests of organic solutions which caused hatching, such as leaf infusions, showed that such solutions invariably had a low dissolved oxygen content. These authors thus consider that bacteria, yeast, or other organisms stimulate hatching in *Aëdes* eggs by reducing the oxygen content of the waters. They point out that this type of regulatory mechanism might contribute to the survival of the species, as the natural water with a low content of dissolved oxygen would have a relatively high bacteria content and thus a ready supply of food material. In a few experiments with *Aëdes aegypti*, they found that the effect of lowering the oxygen content was not as striking as it was with the other two *Aëdes* species with which they worked.

Other studies on the effect of various infusions and microorganisms on the hatching of *Aëdes* eggs have been made by Fielding, 1919; Roubaud, 1927; Roubaud and Colas-Belcour, 1927; Magrou, Magrou, and Roubaud, 1931; Rozeboom, 1934; Connell, 1941; Thomas, 1943.

Baker (1935) reported a case in which length of day apparently influenced the hatching of *Aëdes triseriatus* eggs. A mass of eggs was divided in two equal parts.

> One half was laid upon the bench at a distance of about two feet from two 100-watt blue Mazda bulbs with ventilated reflectors. The other half was placed immediately under the bench where it received indirect daylight and hardly any artificial light. The dry halves with their egg deposits were left in these two illumination environments for five weeks. The air moisture ranged from 60 per cent to 90 per cent R.H. Following this treatment each half was submerged in a dish of filtered tree-hole water. At the end of 18 hours two larvae had hatched from the egg mass on the short day

half. No more hatched. The water containing the half which received supplemented daylight swarmed with first stage larvae.

From the literature it is clear that various aëdine mosquitoes vary greatly in the stimulus required for hatching. It is likely that there are also strain differences, and Hovanitz (1946) has produced evidence to show that the hatching response may be in part a characteristic of the individual parent in *Haemagogus spegazzinii*. Marshall (1938, p. 19) has commented on the probable existence of species, strain and individual differences in response to hatching stimulus. The possible range of specific differences is nicely shown by trials that we have made in Colombia with *Psorophora cingulata*, *P. ferox*, and *Haemagogus spegazzinii*. The eggs of *cingulata* hatch at once on immersion in plain rain water; only a few *ferox* eggs will hatch under these conditions, but an immediate hatch may be obtained by adding strong organic infusions. The hatch of *spegazzinii* in such organic infusions as we have tried is irregular and uncertain, but a very complete and prompt hatch may be obtained in a freshly prepared suspension of brewer's yeast.

Desiccation, in the sense that the word is used in these egg-hatching experiments, is a relative term, and it seems probable that various mosquito species differ greatly in the amount of drying that the eggs can stand. Marshall (1938) has pointed out that in nature the eggs normally lie dormant in moist situations, and that failure to obtain hatches in the laboratory may often be due to death caused by too complete desiccation. Our experience in Colombia furnishes an example of this. In experiments with *Aëdes aegypti* reported in the literature, the eggs are often said to have been maintained on dry filter paper. With *Haemagogus spegazzinii* we found that eggs laid on filter paper died if the paper were allowed to dry out completely in room atmosphere. Eggs laid on wood (on the inside of cups made of bamboo segments, for instance) would remain viable for long periods with no special attention, but eggs laid on filter paper had to be stored in Petri dishes where the paper could be kept moist by periodically sprinkling it with water. The moisture content of the substrate, which might be very difficult to measure or to maintain at a constant and known figure, is thus with some species of great importance, and probably a frequent source of the discrepant results reported by different authors from apparently similar experiments.

CHAPTER VIII

THE LARVAL ENVIRONMENT

> And an ingenious Spaniard says, that rivers and the inhabitants of the watery element were made for wise men to contemplate, and fools to pass by without consideration . . . for you may note, that the waters are Nature's storehouse, in which she locks up her wonders.—IZAAK WALTON

Mosquito larvae are, without known exception, aquatic in the sense that they must live in water; though their adaptation to the aquatic environment, like that of all water insects, is secondary and imperfect. Some aquatic insects have developed mechanisms that enable them to use oxygen dissolved in the water, but mosquito larvae are essentially dependent on air for breathing. Typically they live suspended from the surface film, with the spiracles in constant contact with the air; they leave the surface only rarely, usually as an escape reaction or, with some species, in search of food. A few types of larvae spend a larger proportion of time below the surface, coming up only at intervals for respiration. One genus (*Mansonia*) has developed adaptations for securing air from plant tissues, and the larvae remain attached to such plants.

Thus for purposes of ecological generalization, mosquito larvae can be considered as inhabitants of the surface layer of water accumulations. This means that they are largely limited to situations in which some type of protection is available at the surface—protection against the action of waves and currents, and against predators. No mosquitoes have been found in the open waters of lakes, seas, and rivers, but with this outstanding exception, members of the family have become adapted to almost every conceivable type of terrestrial water accumulation. Mosquito larvae are found in a wide variety of niches associated with lakes, marshes, and streams; in transient rain pools and puddles; and, particularly in the tropics, in a great variety of small water accumulations in association with special plants: in tree holes, leaf bases, flowers, fallen leaves and fruits, and so forth.

The study of the life of inland waters (for the most part of fresh

water) has been fenced off as a separate science with the name "limnology" and can be taken with "oceanography" as forming a general field of "hydrography." Limnology has been most vigorously cultivated in Central Europe and the United States, so that the literature is largely concerned with the types of terrestrial water accumulations that are found in those geographical areas. Most mosquitoes are tropical, and our knowledge of the biological, chemical, and physical conditions of tropical fresh-water accumulations—even of major lakes—is woefully limited. Again, a large proportion of mosquitoes inhabit very special types of water accumulations, and these have been relatively neglected by the limnologists, even in the temperate zone. The student of mosquitoes is thus apt to be disappointed by the results of an excursion into the general field of fresh-water biology, though certainly an understanding of the behavior, physiology, and ecology of mosquito larvae is dependent on a general study of the fresh-water environment. Perhaps the best general perspective of the problems of the fresh-water environment is given in the book by Hesse, Allee, and Schmidt (1937); useful general texts on fresh water, often consulted in the preparation of the present manuscript, have been written by Carpenter (1928), Needham and Lloyd (1915), Ward and Whipple (1918), and Welch (1935). It is interesting that despite the generally tenuous connection between limnology and mosquito biology, one of the most interesting and useful books on mosquitoes was written by an outstanding limnologist, Wesenberg-Lund (1921).

The faunal constituents of fresh water, by analogy with those of the sea, are generally classified as *benthos* (bottom inhabiting), *nekton* (free swimming), *plankton* (floating or drifting), and *neuston* (directly associated with the surface film). A great many insects belong to the neuston, including the bugs, beetles, and spiders that stride or skate on the surface (supraneuston) and, outstandingly, the mosquito larvae that hang below the surface (infraneuston). The terms "benthos," "nekton," and "plankton" have become widely adopted; "neuston" is more rarely used, but it may serve a purpose in the present connection in emphasizing that mosquito larvae do not really belong to any of the other three generally recognized aquatic habit types.

The analysis of environmental characteristics is simpler in aquatic than in terrestrial habitats, because the complicating factor of humid-

ity is absent, and because the aquatic habitats are more sharply defined, due to the discontinuity of fresh-water accumulations. Even so, the subject is bewilderingly complex. "Environment" is generally defined as the sum of all external forces or conditions acting on an organism; and the disentangling of particular forces or conditions, and the study of the interplay of various forces and conditions, seems almost hopelessly difficult. The conventional assortment of environmental characteristics into the categories of physical, chemical, and biological is in itself highly arbitrary and perhaps even misleading, since factors belonging to such diverse categories may show various cause-and-effect relationships. There seems, however, to be no other method of handling the subject, at least with our present knowledge of mosquito biology. In the present chapter the physical and chemical characteristics of the aquatic environment are considered, and in the next chapter the physiology and behavior of the larvae themselves are treated. The relation of the larvae to the aquatic community (the biological characteristics of the environment) is then discussed. Finally, an attempt will be made to give coherence to these various single factors in a chapter on the classification of larval habitats.

TEMPERATURE

Temperature conditions in aquatic environments are, in general, much more stable than in aerial environments: the great specific heat of water results in what has been aptly called "thermal conservatism." This generalization, however, loses force when applied to mosquito larvae, because the larvae are apt to be inhabitants of small accumulations of water which show temperature changes closely corresponding to changes in air temperature; or, in large bodies of water, the larvae are inhabitants of the water surface, where temperature changes may be both extensive and abrupt. As always in ecological studies, the significant climate is the "microclimate" of the niche in which the individual lives, and this climate may be strikingly different from the conditions measured by standard meteorological procedures.

The study of temperature effects in the aquatic environment is greatly simplified by the absence of the confusing factor of humidity. The problem of moisture retention has been automatically solved by aquatic animals, and the consequences of temperature changes per se can be studied much more readily than with animals in the aerial

environment. Many studies of this sort have been made with aquatic insects, and the general literature on heat effects has been summarized by Uvarov (1931) and more recently by Wigglesworth (1939). The effect of temperature on mosquito larvae has been studied from two rather distinct points of view: first, its effect on rate of development, essentially a laboratory problem usually considered from the point of view of general physiology; and second, its effect as a limiting factor in the ecological or geographical distribution of particular species, essentially a field problem, though one that cannot be resolved without reference to laboratory experimentation.

The relation between temperature and rate of development (growth) in insects is, of course, direct. At medial temperatures the relation is constant—that is, if the length of time required for development is plotted against the temperature, the resulting curve is a hyperbola and its reciprocal a straight line. There is considerable evidence to show that this relation does not hold near the upper and lower limits of effective temperature: that the so-called "velocity of development" curve is not a straight line, but sigmoid. Both Uvarov and Wigglesworth have summarized the literature on the mathematical description of temperature effects. Little of the general theory of this relation has been based on mosquito work, and the published data on mosquitoes seem in general not complete enough to test the mathematical theories. Studies of mosquito larval growth from this point of view have been published by Headlee (1942), Hurlbut (1943), and Huffaker (1944). Huffaker in particular has made a rather elaborate analysis of data based on the growth of *Anopheles quadrimaculatus*, tending to show that growth rates conform to the "catenary formula of Janisch,"

$$t = \frac{m}{2}\left(a^{T} + a^{-T}\right)$$

where t = time, m = developmental time at the empirically determined optimum, a is an empirically established constant which determines the slope of the curve, and T is the temperature in degrees above or below the optimum. The present author is inclined to agree with Wigglesworth, who points out that the growth curve of insects "represents the sum or resultant of an immense number of chemical and physical reactions, many of which must be differently affected by changes of temperature." He adds that "mathematical formulae can be made to fit particular cases, and to that extent they have some

descriptive value, but none of them is of sufficiently general application to be regarded as embodying any rational principle."

In much of the literature on the effect of temperature on speed of larval development, little account is taken of the composition of the medium in which the larvae were maintained. Yet the nature of the culture medium may have a very considerable effect on rate of growth (as well as on mortality). This can be seen from tables given by Trager (1937) and Bates (1941a). Thomson (1940c) found that the discrepancy in *Anopheles minimus* between growth rate in the laboratory and growth rates in the field at corresponding temperatures was so great that he used only the egg and pupal stages (not subject to the food factor) for temperature studies. Differences in growth rates for the same species reported by different authors (for example, Huffaker, 1944, and Hurlbut, 1943, for *Anopheles quadrimaculatus*) may be a reflection of differences in the culture media used.

The temperature scale for any given species of mosquito might well be considered to include a lower fatal limit, a zone of unfavorable low temperatures, a zone of favorable temperatures, a zone of unfavorable high temperatures, and an upper fatal limit (the thermal death point). Such a terminology avoids the much used term "optimum." The literature contains frequent references to the optimum temperature for this or that species, developmental stage, or developmental process, and the meaning of the term is sometimes considered to be so obvious as to require no definition. Three different concepts of optimum may in fact be applied to larval developmental temperatures; these might be called the "developmental optimum," the "survival optimum," and the "biotic optimum." The developmental optimum, used by Huffaker (1944) and implicit in the application of the Janisch formula, is defined as "the temperature at which the rate of development is highest"; the survival optimum would be the temperature at which mortality is lowest. The two may be combined, as by Mosna (1937), who considered the optimum to be the temperature at which maximum rate of development coincided with minimum mortality. The present author would prefer to think of a biotic optimum, the temperature condition most favorable for the species in the sense of promoting survival and dispersal. The calculation of the precise conditions that would, in this sense, be optimal is no

simple task and is perhaps out of the question with our present knowledge of mosquito ecology.

The concept of a zone of favorable temperatures seems much more practical than the concept of an exact specific optimum. The use of mortality in determining an optimum would in general be difficult, because with any species there is a rather wide range of temperature conditions under which mortality (ideally) is zero, and under which, in fact, mortality is random and obviously unrelated to temperature. Where mortality shows a clear relation to temperature, it seems more likely that the effect is exercised through influence on the culture medium. If the temperature at which development proceeds at maximum speed is taken as the optimum, there is an implicit assumption that mere speed of growth is highly favorable. Fast larval growth may, however, result in a relatively small and weakened adult (data in Bates, 1947b, for *Haemagogus spegazzinii*), and the effect, from the point of view of the species, may be definitely unfavorable.

Effect of low temperatures. The larvae of most species of mosquitoes are killed if frozen, and those that hibernate as larvae, such as the European *Anopheles claviger*, are normally found in water or mud below the ice. An exception is the North American *Wyeomyia smithii* which hibernates frozen in blocks of solid ice in the leaves of the pitcher plants (Howard, Dyar, and Knab, 1913, p. 120). Bliss and Gill (1933) found that larvae of *Aëdes aegypti* could withstand freezing (at — 2° C.) for 2 to 10 hours, but that they were killed by an exposure of 11 hours. Trofimov (1942) found that the larvae of *Anopheles claviger* and *A. pulcherrimus* kept in test tubes suspended above cooling mixtures in thermos flasks, were killed if the water in the tubes froze, but that they survived if the water was supercooled and did not freeze, resisting temperatures of from — 5° to — 9° C. for 20 to 210 minutes. They also survived if the water was only partially frozen, provided they were not in immediate contact with the ice. Sautet (1936) reported that first-stage larvae of *Anopheles maculipennis, A. sacharovi, Theobaldia annulata,* and *Culex pipiens* were killed rapidly by exposure to 4° C., but that the larvae became progressively more resistant in later stages.

In theory each species (and each stage) should have a "threshold of development": "the temperature at which, on the descending scale, the development definitely ceases and at which, on the ascending

scale, the development is initiated" (Uvarov). The determination of such a threshold is difficult. Hurlbut (1943) assumed the threshold for *Anopheles quadrimaculatus* to be 10° (50° F.). Huffaker (1944) observed definite development in this species at a temperature of 8.5°, but this was not measurable since the first stage was never completed. At 6° no observable development occurred. Huffaker thus judged the threshold to be about 7°.

Many temperate-zone mosquitoes show definite adaptations to life at low temperatures. Thus larvae of 16 of the 29 species of British mosquitoes have been collected in January (Marshall, 1938), and in at least 11 of these species the midwinter months are characteristically passed in the larval stage. In the case of *Aëdes rusticus*, the chief larval development occurs in the winter months, first-stage larvae being found in October and the majority of the adults appearing in April and May. *Anopheles claviger*, which hibernates in the larval stage, is found in cold-water environments (such as spring pools) in the summer months, especially in the southern part of its range. The special phenomena of larval hibernation will be discussed in the next chapter.

Favorable temperatures. The difficulties of laboratory study of favorable (optimal) temperatures have been mentioned above. Relatively few laboratory studies in which various species are compared have been made, though the demonstration of specific differences in temperature requirements would seem to depend on such studies. One such study was made by Mosna (1937), comparing *Anopheles labranchiae* and *atroparvus;* he concluded that the most favorable conditions (using survival optimum as a criterion) for *labranchiae* were slightly warmer (alternation of 25° and 35°) than for *atroparvus* (alternation of 25° and 30°). Field records of the temperature characteristics of mosquito breeding places have frequently been made, though the interpretation of such studies is not always easy. Thomson (1940c) has pointed out that temperature records are generally made in the daytime, whereas the actual selection of the habitat is probably made in the evening or night by the ovipositing female, at a time when temperature conditions may be quite different. He found, for instance, that the day temperature conditions in the breeding places of *Anopheles minimus* were distinctive, the water being much cooler than in neighboring habitats of other anophelines; but in the evening hours, when oviposition took place, this temperature difference had disappeared. Symes (1932) came to the conclusion that in Kenya tem-

perature was not a limiting factor in determining anopheline breeding places, since he found diverse species in habitats with similar temperature ranges. The breeding places of *Anopheles plumbeus* and *A. claviger* in southern Europe are certainly characterized by low and relatively constant temperatures (Missiroli, 1936), and temperature may in some such cases be the controlling factor in determining habitat distribution.

Effect of high temperatures. Thomson (1940c) has reported a very interesting set of experiments on the effect of high temperatures on the various stages of *Anopheles minimus* and other Indian anophelines. Eggs and first-stage larvae were more resistant to high temperatures than fourth-stage larvae. The effect of the temperature also depended on the length of exposure: with 39°, for instance, fourth-stage larvae of *minimus* survived exposures of up to 30 minutes; but with longer exposures a certain number of larvae were killed, the proportion depending on the length of exposure; after 24 hours all were killed. Thomson tested the thermal death point of the fourth-stage larvae of various anophelines. He defined the thermal death point as the lowest temperature at which all larvae were killed by an exposure of five minutes, the temperature being raised slowly over a period of one and a half to two hours. The thermal death point must always be defined thus in terms of time of exposure. In bacteriological usage, ten minutes is the conventional exposure period, but the shorter period would perhaps be more convenient for mosquito work if it were generally adopted as a standard. The thermal death point is a convenient and easily applied index of specific physiological characteristics, and it would be useful if some standardized method of making such determinations could be adopted in mosquito laboratories. Thomson arrived at the following figures for the species he tested: *Anopheles insulaeflorum*, 40.0°; *A. minimus*, 41.0°; *A. hyrcanus*, 43.0°–43.5°; *A. barbirostris*, 43.5°; *A. culicifacies*, 44.0°; *A. vagus*, 44.5°–45.0°.

Comparisons among mosquito species are difficult because various authors have used different criteria in studies of high temperature effects. Wright (1927) found that all larvae of *Anopheles claviger* were killed by 5 minutes' exposure to 37°. De Meillon (1934) found a clear-cut difference between *Anopheles gambiae* and *funestus* in ability to withstand high temperatures: *gambiae* larvae were unaffected by one hour at 45° (113° F.), while all *funestus* larvae were killed. It seems

to be generally true that species with a high thermal death point are apt to be found breeding in relatively warm water. For the most part, however, the thermal death point would serve merely as an index of the physiological adaptations of the larva, since such extremely high water temperatures are rather rarely encountered in nature, even in the tropics.

Brues (1939) has summarized records of insects living in hot springs. A species of *Anopheles*, probably either *subpictus* or *ludlowi*, has been reported as breeding in springs with temperatures from 39° to 40°. De Meillon (1934) has found *A. gambiae* breeding in small pools with water temperatures as high as 39°. In Egypt we found *A. pharoensis* breeding in rice fields where the thermograph often recorded 40° at midday. The larvae seemed to be breeding normally in this situation, though in the laboratory we found 40° to be definitely unfavorable (30 per cent of fourth-stage larvae killed by an exposure of one hour to 40°). It seemed likely that in this case the temperature of the surface layer of the water, containing the larvae, might be appreciably lowered by evaporation, as compared with the temperature of the underlying water that would be reached by the thermograph bulb.

The accelerating and stimulating effect of alternating as compared with constant temperature conditions on insects is well known, and the effect on mosquito larvae has been studied by various authors. Periodic short exposures to high temperatures may be definitely favorable (in the sense of speeding up growth or lowering mortality), while constant exposure to the extreme temperature may be fatal. This has been commented on by Huffaker (1944).

LIGHT

Since mosquito larvae are, in general, inhabitants of the water surface, the transparency of the water (that is, the depth to which light penetrates) is not an important environmental factor, as it is with most aquatic organisms. The extent to which the breeding place is shaded is, however, a very important factor, and the mosquito fauna of shaded pools or streams is quite different from that of sunny pools and streams. This may well be in part a function of the adult habitat, forest species quite naturally laying their eggs in forest habitats, and so forth. Thus in the tropics, at least, one could safely make the generalization that diurnal mosquitoes breed in shaded situations, be-

cause the diurnal species are all forest inhabitants, the day climate of open country being unfavorable to mosquito activity.

Even in open country, however, the extent of the shading of a particular larval breeding place may be an index in its fauna. It has been found, for instance, that *Anopheles albimanus* avoids shaded habitats, and Carr (1938) has advocated the planting of trees as a method of controlling breeding. On the other hand, *Anopheles darlingi* seems to prefer a partially shaded situation (Komp, 1942). The question arises, in such cases, as to whether the presence or absence of sunlight has a direct influence on the larva, or an indirect influence through effect on algal growth or other food organisms; or whether the larva is indifferent to the amount of light, the ecological distribution being controlled by the egg-laying habits of the adult.

Laboratory experiments with the effect of light on larval development seem to show, in general, that it is not an important direct factor. Thus Fielding (1919) found that the larvae of *Aëdes aegypti* grow equally well in the presence or absence of light; Jobling (1937) got similar results with *Aëdes aegypti*, *Culex pipiens*, and *C. fatigans*. Danilova and Zubareva (1932) found that light had no effect on the growth of *Anopheles maculipennis*. Trenz (1934), on the other hand, found that when larvae of *Aëdes mariae* were raised in the laboratory without sunlight, the adults were mostly sterile. Frost, Herms, and Hoskins (1936) concluded that small daily doses of ultraviolet light speeded development in *Theobaldia incidens*, though larger amounts were harmful. Most workers have the impression that sunlight is helpful in the laboratory culture of anopheline larvae, and some provision either for sunlight or ultraviolet light is commonly made in laboratories; the demonstration of this favorable effect of sunlight is not, however, easy, and the effect may be indirect. Certainly most algae require sunlight, and algae are frequently favorable in larval culture media, either as food for the larvae or as an aid in maintaining a balance of dissolved gases and in utilizing organic materials unfavorable for the larvae. The direct effect of sunlight on water temperature must also be taken into account.

Mosquito larvae, even of species found in nature in sunny pools, generally show an avoiding reaction to sunlight in the laboratory. If a pan containing larvae is partially shaded, the majority of the larvae will soon be found in the shaded portion. Transplant experiments (such as those reported by Russell and Rao, 1942c) generally show

that larvae normally found in sunny situations will grow perfectly well if planted in shaded ones, and vice versa. Oviposition experiments, on the other hand, show that the egg-laying female may be quite sensitive to light conditions, and this is likely the key to the differential larval distribution (Thomson, 1940a; Russell and Rao, 1942c).

MOVEMENT

The limnologists, in the classification of aquatic environments, make a fundamental distinction between running water (lotic environments) and standing water (lenitic environments). Many mosquito species, especially anophelines, are found breeding typically or exclusively in stream associations, and one might expect in such cases to find special adaptations to life in running water. Actually, however, the water in the microhabitat of stream-breeding species is still, and no mosquitoes are truly adapted to the lotic environment in the sense of being able to maintain themselves and develop in a microhabitat where the water is in perceptible movement. Thomson (1940b) has shown this nicely in a detailed study of the effect of water movement on a typical stream-breeding species (*Anopheles minimus*). The adults in the laboratory showed a definite preference for still over running water for oviposition. The larvae of *A. minimus* showed about the same ability to resist being washed away by current in laboratory experiments as did those of *A. aconitus*, *A. maculatus* (also stream breeders), and *A. hyrcanus* (a marsh breeder). He judged from this that Iyengar (1922) was incorrect in ascribing to the "tail hooks" of larvae such as *A. minimus* and *A. maculatus* the special function of enabling the larvae to cling to boulders at the sides of the stream. The adaptations of the stream-breeding larvae seem to be not so much to life in running water, as to the avoidance of running water. That is, stream-breeding species may show a strong "thigmotropism" (tendency to remain close to objects such as boulders, where water movement is incidentally at a minimum) and a strong avoidance of light, which keeps them in the shade of grass clumps and so forth (where water movement is also at a minimum). Thus, while water movement may be a fundamental factor in defining the general habitat of a mosquito species, it is apparently an unimportant factor in the environment of the individual larva.

SURFACE CHARACTERS

Since mosquito larvae are members of the neuston, the character of the water surface is of great importance to them. It is a common laboratory observation that anopheline larvae are easily killed by a surface scum, and the maintenance of a clean surface is an important factor in the culture of such larvae. There may be specific differences in adaptation to surface characters, since Eckstein (1936) found that *Anopheles atroparvus* was able to survive better than *A. messeae* in water with the surface covered with talc or pollen. Renn (1941) found that the method of feeding of larvae of *A. quadrimaculatus* and *A. crucians* depended on the amount of surface tension of the water. When the surface tension was very low (35.4 dynes/cm.) larvae were unable to remain at the surface. The larvae themselves have a considerable effect on the surface tension. Renn found that when six fourth-stage *A. quadrimaculatus* larvae were placed in an aquarium 22 cm. in diameter, the surface tension rose from 53.0 dynes/cm. to 63.4 dynes/cm. in 90 minutes, the tension in a control aquarium remaining constant. Few field studies of surface tension in relation to mosquito breeding have been made. Renn found that the tension in various natural breeding places of *A. quadrimaculatus* ranged from 65.0 to 73.0 dynes/cm.

DISSOLVED GASES

Several authors have made studies of the amounts of dissolved oxygen and carbon dioxide in larval breeding places, generally finding no correlation between these factors and breeding (Boyd, 1929; Howland, 1930a; Morin and Bader, 1933). Unti (1943b) found that if the oxygen content of water from natural breeding places was slowly reduced from the original amount (5 to 7 parts per million), fourth-stage larvae of *Anopheles argyritarsis* died when it reached 3 to 4 ppm, and *A. albitarsis*, *A. strodei*, and *A. noroestensis* when it reached 1.5 ppm. Anopheline larvae died in 15 to 45 minutes in water that had been boiled and thus contained no oxygen. Larvae of *Culex fatigans* were unaffected by oxygen reduction. Wang (1938) also found that anopheline larvae required more oxygen than larvae of *Culex* or *Aëdes*.

HYDROGEN-ION CONCENTRATION

Many studies have been made of the hydrogen-ion concentration in mosquito breeding places: partly because *p*H is an important factor in many biological processes, but also partly because it is the most easily measured of the physicochemical factors of the aquatic habitat. Early studies seemed to confirm the importance of *p*H, and MacGregor (1927, p. 227) was able to make the following generalizations: "There are definitely acidophile and alkalinophile species. There are also a few species which, to some extent, tolerate both acidity and alkalinity. The majority of pond-, swamp- and river-breeding anopheline species are alkalinophile. A lesser number of anopheline larvae have, however, been found to occur in acid waters and are acidophile. Nevertheless there does not appear to be any record of what is generally an acidophile anopheline species being found also in alkaline waters, or vice versa." The other extreme is perhaps the conclusion reached by Symes (1932) from a study of *p*H in relation to anopheline breeding in Kenya: "Anopheline larvae appear to be able to tolerate practically the whole range of values recorded for natural waters in the Nairobi district. . . . For practical application in the field, the usefulness of *p*H determination has yet to be demonstrated."

Hopkins (1936, p. 5) has given a very interesting and fair summary of the literature on *p*H. He emphasizes the importance of *p*H readings both in mosquito breeding places and in water accumulations where mosquitoes do not breed. For instance, Kirkpatrick (1925) has given a rather extensive series of readings of the *p*H of mosquito breeding places in Egypt, which are all alkaline: but this means little, since the waters of Egypt are in general alkaline, whether they breed mosquitoes or not. Within the normal *p*H range of Egyptian waters (7.5 to 9.0) Kirkpatrick found no correlation between *p*H and species of mosquitoes breeding in the water.

Representative field studies of *p*H in relation to mosquito breeding are those by Senior White (1926), MacGregor (1929), Boyd (1929), Howland (1930a), and Unti (1942). In the cases where a clear correlation between *p*H and mosquito breeding has been found (such as the preference of *Anopheles crucians* for acid waters noted by Boyd), there is no way of determining whether the relation to *p*H is direct or indirect. The latter seems more likely, since acidity certainly influences the microbiota, and is often correlated with the landscape

type of the breeding place—both factors much more directly related to anopheline breeding than *p*H itself.

The influence of *p*H on the microbiota makes laboratory experimentation difficult. Wigglesworth (1942) took advantage of this to grow *Aëdes aegypti* in a practically bacteria-free medium by adjusting the *p*H to 4.0—apparently with no ill effect on the mosquito larvae. Woodhill (1938) was able to grow *Culex fatigans* in a *p*H range from 4.2 to 9.0; he found that in the very acid or very alkaline media development was slower, but he pointed out that it would be impossible to say whether this was a direct effect, or indirect through the retardation of bacterial development. Other laboratory experiments with *p*H have been reported by MacGregor (1921), Kligler and Theodor (1925), Pruthi (1931) and Frost, Herms and Hoskins (1936).

NITRATES AND ORGANIC MATERIALS

It was early observed that the mosquito fauna of polluted and unpolluted waters was quite different, and in particular that anopheline larvae were rarely found in polluted waters. The most obvious characteristic of polluted waters is the high content of nitrates, or even nitrites; and several authors have made studies of the correlation between such nitrogen compounds and mosquito breeding. The total organic content of water and the nitrate content are generally related, and so the two are most conveniently considered together. It seems clear that both are important from the point of view of mosquito breeding, but our knowledge of the whole subject is very fragmentary. About all that is accomplished by a review of the literature is to reveal the existence of a promising field for investigation. Again the problem is to distinguish between the effect of the various dissolved compounds themselves and their effect through controlling the nature of the microbiota. In the case of anophelines, for instance, there is an obvious inimical effect from the bacterial scum that is apt to form on water with a high organic content, and where a definite scum does not form, the surface tension of the water may be affected by the bacterial growth.

Field studies have generally shown that anopheline larvae are not found in water with an appreciable nitrate content. Beattie (1930) found no correlation between organic nitrogen and larval abundance in the English ponds that she studied, perhaps because all were below

the limiting threshold. Senior White (1926) found that saline ammonia in amounts of even less than 1 ppm was inhibiting to anopheline breeding; Buxton (1934) found that larvae of "*Anopheles tarsimaculatus*" (perhaps *A. aquasalis*) in Trinidad were rarely found in water with an ammonia nitrogen content above 0.3 ppm; Williamson (1936) found that in India and Malaya anopheline breeding was largely correlated with a low nitrogen content of the soil. De Jesús (1936) found that the breeding of *A. minimus* var. *flavirostris* was correlated with low concentration of nitrogen. Williamson (1928) found that larvae of *A. maculatus* were most abundant, and those of *A. kochi* least abundant, when the albuminoid content of the water was least. He suggested that the condition unfavorable to *A. maculatus* was defective oxidation in the presence of excess albuminoid matter.

Thomson (1941a, 1942), in a general study of the influence of the composition of the water on the breeding of *Anopheles minimus*, found that free and saline ammonia was rarely present in more than a trace in different anopheline breeding places, and he consequently considered this test useless for distinguishing different types of breeding places. He found the albuminoid ammonia and the "Tidy figure" (oxygen absorbed from permanganate) much more useful indexes. The Tidy figure is an indication of the amount of carbonaceous organic matter present, serving consequently as a measure of pollution. According to the *Standard Methods* of the American Public Health Association, the proportion of carbon measured by the Tidy figure varies in different waters, since the carbon in nitrogenous matter is not so readily oxidized as that in carbonaceous organic matter. Thomson's results, however, indicate that wider studies of this type of factor would be warranted. He found, as was pointed out in Chapter VI, that the avoidance of water with a high organic content was a result of the oviposition behavior of the adult female of *A. minimus*, rather than an inability of the larvae to develop, since larvae could be raised in the laboratory in water with a degree of pollution thirty times greater than that avoided by gravid females.

Relatively few laboratory experiments have been made with nitrogen compounds and organic pollution. Brink and Das Chowdhury (1939) found that fourth-stage larvae of *Anopheles stephensi* all died in concentrations of ammonium sulphate higher than 0.5 per cent, while larvae of *Culex fatigans* pupated normally in a concentration of 1.5 per cent. First-stage larvae of C. *fatigans* were more sensitive,

dying in a concentration of 1 per cent. Bates (1941a) found that potassium nitrate had an unfavorable effect on larvae of *Anopheles atroparvus* when one part in ten thousand was added to an otherwise favorable medium, but the injurious effect was not as marked in the case of *A. superpictu*

INORGANIC SALTS

In coastal areas the difference between the mosquito faunas of fresh and brackish waters may be striking; since in several areas of the world important malaria vectors are brackish water breeders, the general question of the salinity tolerance of larvae has received a great deal of attention. Kirkpatrick (1925) made analyses of the NaCl content of the water of a large number of breeding places of Egyptian mosquitoes. He classed the 22 species concerned as: (1) purely fresh or nearly fresh-water breeders (14 species); (2) fresh water definitely preferred but sometimes found in brackish water (4 species); (3) salt water definitely preferred but sometimes in fresh (1 species); (4) purely salt water (1 species); (5) more or less indifferent (2 species). He was able to plot the frequency of occurrence in water of different salinities of 15 of the commonest species. From an inspection of his figure, it seems that all but two species were more commonly found in fresh water (less than 5 parts per thousand NaCl) and that the chief difference between species was in *range of tolerance*. Twelve of the 15 species were at times found in water of more than 5 parts per thousand NaCl; 7 were at times found in more than 10 parts per thousand; and 3 were found at times in water with more than 35 parts per thousand (greater salinity than sea water). Kirkpatrick's Egyptian results seem to hold true rather generally: that is, that the difference among mosquito species in the upper limit of salt tolerance may be great, but that relatively few if any species are strictly confined to waters of high salinity.

Beadle (1939) has published a detailed study of the mechanism of the regulation of osmotic pressure in the haemolymph of a salt-water mosquito, *Aëdes detritus*. He found that the larvae could regulate both the total osmotic pressure and the chloride content of the haemolymph in water of varying salinity. The salt exchange with the environment took place through the gut, the body surface being impenetrable to salts and water, and the control mechanism seemed to be through excretion of salt by the Malpighian tubes. The larvae

of this salt-water species were not able to concentrate chloride from hypotonic solutions as readily as larvae of fresh-water species. This difference among species in ability to utilize dispersed ions was noted by Wigglesworth (1938).

Attention in field studies of mosquito habitats has been directed almost exclusively to the sodium-chloride content of the water, though it seems possible that other mineral salts may be important in defining specific habitats. Laboratory studies have been made with a fairly wide range of salts, but mostly from the point of view of the nutritional requirements of the larvae, which will be considered in the next chapter.

CHAPTER IX

THE PHYSIOLOGY AND BEHAVIOR OF LARVAE

> All cold-blooded animals . . . spend an unexpectedly large proportion of their time doing nothing at all, or at any rate, nothing in particular.—CHARLES ELTON

THE object of the previous chapter was to review the general physical and chemical characteristics of the aquatic environment in so far as they are known to affect the distribution of mosquito larvae. In the present chapter the food requirements, physiology, and behavior of the larvae themselves are considered.

FOOD REQUIREMENTS

The normal food of most mosquito larvae seems to be microorganisms, particularly bacteria, yeasts, and protozoa, as well as any detritus that may be small enough to be picked up by the action of the mouth brushes in filtering the water. Great difficulty has been experienced in raising larvae in sterile media, which would be almost the only method of judging the basic food components really required by the larvae for growth. The experiments of Trager (1936) indicate that *Aëdes aegypti* is able to grow on materials in solution only, and there is a general impression that most larvae depend in part, at least, on food materials in solution in the water, even though their main diet may be particulate material.

Laboratory experiments with nutritive materials must be interpreted with caution. Delayed growth and high mortality, for instance, may be due to the unfavorable effect of some toxic material in the medium rather than to a dietary deficiency. This is particularly true with sterile media, since waste products may accumulate that would be removed from the medium under more balanced conditions. The larvae of some species of anophelines are difficult to grow under laboratory conditions; Bates (1941a) found that the addition of sand, mud, or charcoal to the medium had a favorable effect on survival and

growth, with some evidence that this effect might be due to the adsorption of toxic materials. Various investigators have found that frequent changes of water may be necessary with such larvae.

General papers on the food requirements of larvae have been written by van Thiel (1928), Hinman (1930, 1933), Rozeboom (1935), Frost, Herms, and Hoskins (1936), and Buddington (1941). The general subject of insect nutrition has been reviewed by Trager (1947).

Proteins. The experiments of Wigglesworth (1942), although aimed primarily at studying food storage rather than nutritional requirements, are also of great interest in this latter connection. Wigglesworth worked with larvae of *Aëdes aegypti* that were kept on a normal diet up to the fourth stage; within an hour of molting to the fourth stage, the larvae were transferred to a 0.01 *M* acid potassium phthalate in tap water adjusted to *p*H 4.0 with phosphoric acid. They were kept in this solution at 28° C. until their reserves were used up—in 10 to 14 days. The larvae were used for experiments when all fat droplets had disappeared from the fat body, as observed by transmitted light under the microscope; the larva becomes sluggish and generally dies within 48 hours or so after this stage is reached. By keeping the larvae in this acid medium, and transferring them to fresh solutions every 12 or 24 hours, the growth of bacteria was inhibited; while the cultures were not sterile in the bacteriological sense, bacteria were so rare as to be of no nutritional importance to the larvae. The larvae were killed after being fed on the substances under investigation, and the distribution of protein, fat, and glycogen studied by various selective staining methods.

Wigglesworth states that "when the larva is fed on casein alone, the reserves in the fat body and elsewhere do not differ from those formed in larvae feeding on the normal diet of microorganisms: abundant protein, fat and glycogen are laid down. This confirms the evidence of other authors that protein in insects may serve as a source of both fat and glycogen."

Fat and carbohydrates. Wigglesworth summarized his experiments with these substances as follows: "Starch and other carbohydrates lead to very large accumulations of glycogen and, to a lesser extent, of fat. Fat, on the other hand, although it can obviously serve as a source of muscular energy, does not lead to the deposition of visible glycogen."

Other organic compounds. The classic studies on the accessory food

requirements in mosquito larvae are those by Trager (1935a, 1935b, 1936, 1937), Trager and Subbarow (1938), and Subbarow and Trager (1940). Trager found that it was possible to get normal growth of *Aëdes aegypti* in a bacteriologically sterile medium consisting of heat-killed yeast and autoclaved liver extract.

> A proper quantity of living yeast suspension in distilled water supports good growth of the larvae, but this same quantity of heat-killed yeast suspension under sterile conditions supports only a very slight growth. If, however, it is supplemented with an adequate amount of autoclaved liver extract, growth proceeds at a maximum rate. Liver extract without yeast gives no growth. Very large amounts of heat-killed yeast suspension (30 to 40 times as much as required in the presence of liver extract) support fair growth even in the absence of liver extract. Thus at least two substances or groups of substances are required by the larvae. Only one of these (designated as growth factor A) is present in liver extract. Both are present in heat-killed yeast, but much more yeast is needed to furnish adequate amounts of the one present in liver extract than of the other. (Trager and Subbarow, 1938).

Trager's final conclusion seems to be that two of the growth factors required by *Aëdes aegypti* are riboflavin and thiamin, but that for completely normal development a third factor, of unknown chemical nature but present in a "flavin-purine complex," is necessary. A similar conclusion was reached by Buddington (1941) in work with *Aëdes aegypti* and *Culex pipiens* in different types of media. There seems to be no question but what the accessory growth factors required by mosquito larvae for normal development are ordinarily supplied by living microorganisms; hence the difficulty of raising larvae in sterile media.

Inorganic materials. Information on the mineral requirements of mosquito larvae—indeed of insects in general—seems to be meager. Wigglesworth (1938) in a study of *Aëdes aegypti* and *Culex pipiens* in dilute sodium-chloride solutions found that "*Aëdes aegypti* is more efficient than *Culex pipiens* in absorbing and retaining chloride in dilute media, and *Culex* is perhaps a little better at keeping chloride out in more concentrated media." The ability of larvae to pick up chloride ions from the medium seems to be directly related to the size of the anal gills. Trager (1936) found that calcium was necessary for growth in *Aëdes aegypti;* normal growth did not take place in solutions containing various combinations of the salts NaCl, KCl,

and $MgSO_4$, but did take place if $CaCl_2$ was added: and growth in solution containing only calcium chloride was as good as in solutions containing this salt in combination with others. In experiments with Albanian anophelines (Bates, 1939b), we found that calcium salts were a necessary part of any culture medium. Calcium seemed particularly necessary for survival in solutions of magnesium sulphate, and by growing larvae in media containing five parts per thousand magnesium sulphate, and various low concentrations of calcium sulphate, we were able to show that the anopheline species tested varied greatly in the amount of calcium needed to permit survival. The importance of calcium to mosquito larvae has been noted by several authors (for example, Woodhill, 1936; Frost, Herms, and Hoskins, 1936).

METHODS OF FEEDING

Mosquito larvae have several different methods of feeding. Beklemishev (1930) recognized four methods: (a) gnawing hard submerged objects; (b) scraping off the periphyton on the surface of such objects; (c) swallowing comparably large floating bodies, such as Ostracods and Cladocera; and (d) filtering small suspended particles from the water. Such a list could probably be somewhat enlarged if all possible cases were taken into consideration. As has been shown, mosquito larvae may feed on dissolved material in the water, and at the other extreme a few species are predacious, killing and eating mosquito larvae of other species that may be as large as the predator larva itself.

The "filter" method of feeding has been the most often described and is, perhaps, the characteristic method used by mosquito larvae; it is certainly the commonest method among anophelines. Renn (1941) has shown that there are really two quite distinct methods of gathering food by filtering. The more commonly observed method he calls "eddy feeding":

> When larvae feed in infusions of bacteria, protozoa, and algae, in suspensions of yeast and algae, or under conditions where the water surface bears islets of floating oily materials, it will be observed that the vibrating mouth brushes stir the water about the head in two large eddies that rotate in such a way as to drive the common converging current toward the mouth. These eddies may extend for considerable distances beyond and to the sides of the head; their diameters often exceed the total lengths of the

larvae. Suspended materials are swept from their peripheries into the incurrent stream and pass to the synchronously moving mandibles and maxillae, where they are sifted or broken into acceptable fragments. Very fine materials escape in the efferent water that flows at right angles across the posterior margins of the antennae. Large unacceptable masses are grasped by the maxillae, drawn below the surface, and cast downward out of the eddy paths by a quick rotation of the head.

The volume of water exploited in this manner may be surprisingly large, ranging from 0.5 to 2.0 cc. per day for third- and fourth-stage larvae of various species (Senior White, 1928).

The second method, interfacial feeding, is described as follows by Renn:

If the larvae of *Anopheles quadrimaculatus* or of *A. crucians* are examined when they are feeding in their natural breeding waters or in aquaria containing fresh pond water, it will be observed that the movement of fine floating materials in the zones of action about their heads does not

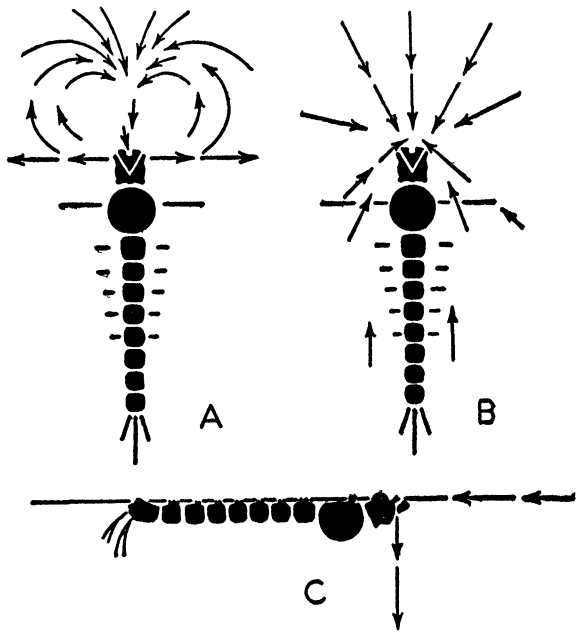


FIG. 7. DIAGRAM TO SHOW PATTERN OF WATER CURRENTS IN "EDDY" FEEDING OF LARVAE (*A*), AND IN "INTERFACIAL" FEEDING (*B*, *C*) (FROM RENN, 1941).

correspond to the "eddy" pattern commonly described as characteristic of anopheline larvae. Instead of sweeping in converging helical eddies, the fine floating particles approach the mouth along straight lines. Moreover, they come from all directions with approximately equal velocities. The movement of particles from the tail region is slightly slower; their path is broken by various emergent platelets, hairs, and bristles that distort the water surface. There materials flow forward around the tips of the antennae, and enter the mouth from the front. Otherwise, there is no evidence of eddies such as appear when larvae feed in infusions.

The "interfacial feeding" may be readily demonstrated by dusting air-floated talc upon aquaria containing feeding larvae. Talc is acceptable to larvae, and they remove it rapidly from the surface. It may be noted that the general configuration of groups or patterns of dust particles undergoes lateral compaction and axial stretching as it moves toward the vibrating mouth parts. The distortion of the dust figure on the surface is in no sense "eddywise."

All the larvae in a given aquarium will show the same type of feeding pattern. Fine floating particles, or groups of particles, approach the upturned mouth in straight lines. As they enter the zone of brush activity they move forward with increasing velocities and with a pronounced jerkiness. This intermittent advance is of the same period as the brush and maxillary vibrations.

If larvae showing the "interfacial" mechanism of feeding in pond water are transferred from clean water aquaria to infusions or to yeast suspensions, the above pattern disappears, and the "eddy" or "free" feeding habit takes its place. Hence it is concluded that the type of feeding employed is determined by the physical properties of the medium in which the larvae are active. Several experiments to be described later bear out this conclusion.

When larvae feed in clean pond water, particles upon the surface are carried in fixed relations to one another by the contracting air-water interface upon which they rest or in which they may be imbedded. Any turbulent movements that may be present in the subsurface strata do not extend into the interface. For this reason the term "interfacial feeding" is used to describe the mechanism; the term "film feeding" has been applied earlier to a variety of surface-feeding habit in which thin mats of bacteria and other organisms are dragged from the water surface. In interfacial feeding, food materials are brought within reach of the larval mouth much as a child might augment his radius of food gathering by pulling the tablecloth.

That the interfacial film itself undergoes contraction and flow may be shown by the following simple experiments. If a single larva is placed in

a large drop of water on a microscope slide, a second drop placed a few millimeters to one side, and the two drops connected by a thin filament of water drawn along the surface of the side, fine talc particles blown upon the preparation will move rapidly from the unoccupied droplet, across the bridge, and directly into the mouth of the feeding larva. This movement takes place only when the larva feeds, and there is no comparable flow of suspended materials within the droplets.

Shipitzina (1935) attempted to determine the size of particles that are swallowed by the larvae of *Anopheles messeae* by scattering quartz sand of varying degrees of fineness on the surface of the water; after exposure for several hours, the larvae were dissected and the largest particles of sand found in them measured. The size of the particle swallowed by the first instar larvae varied from 22.8 to 34.2 microns, the diameter being about 20 per cent of the width of the head of the larva. This percentage increased with each molt, and larvae of the fourth instar swallowed particles varying from 68 to 165 microns in diameter—up to 31.2 per cent of the width of the head. Coggeshall (1926) made a series of dissections of anopheline larvae with the object of determining whether they showed any preference for certain types of food. He came to the conclusion that in the three species studied, there was no discrimination in selection of food: the plankton organisms found in the digestive tract of the larvae included all of the types found free in water in which the larvae were living. Howland (1930a and 1930b) came to a similar conclusion with regard to various mosquito larvae found in English ponds.

PREDACIOUS LARVAE

It is interesting that the predacious habit has developed independently in the larvae of a number of mosquito groups, involving distinct adaptations both of structure and behavior. In a sense, all mosquito larvae are predacious, since their chief food seems to be microorganisms, living or dead. Mostly, however, these are caught by a passive filtering of the water.

The literature contains many references to "cannibalism" among larvae, especially anophelines, not generally considered as being predacious. The cannibalistic habit is generally attributed to overcrowding or inadequate food under conditions of laboratory culture. Such cannibalism probably arises from the gnawing type of food behavior: live larvae might thus be injured, killed, and eventually eaten by their

companions. Probably in many cases the larvae eaten are those that have died as a result of the unfavorable environment. It is interesting in this connection that cast larval skins are often eaten. Christophers and Chand (1916) describe the larva of the tree-hole-breeding *Anopheles culiciformis* as being actively predacious, and the same observation has been made on the North American *barberi* (Howard, Dyar, and Knab, 1913). Christophers (1933) remarks of the related *A. barianensis* of India that the "larvae greedily attack and gorge themselves on fragments of crushed insects thrown on the water." These tree-hole-breeding species thus probably represent the closest approximation to active predacity found in *Anopheles*.

The distribution of active predacity, involving obvious structural and behavioral modifications, through the rest of the mosquito family is very interesting. It may be sketched as follows, using the classification of Edwards (1932):

Tribe Megarhinini

This includes the single tropicopolitan genus *Megarhinus*, the larvae of which are highly modified for predacity, the mouth brushes being prehensile and the teeth of the mandibles very strong. The larvae are usually found singly in "container" habitats (tree holes, bamboo sections, special plants) and feed primarily on other mosquito larvae. Numerous references to the larval habits may be found in the taxonomic literature. They are generally rather slow growing, and Schwetz (1930) records survival for ten weeks without food; Wigglesworth (1929) kept a *Megarhinus* larva alive for five months at 24° C. by limiting its food supply.

Tribe Culicini

The "Sabethes" group. Predacity has been attributed to many sabethine genera, often by inference from the strongly developed larval mandibles and maxillae, and in certain cases there is direct evidence, as in some species of *Goeldia* (Komp, 1936).

Zeugnomyia. This Malayan genus is placed in the *Uranotaenia* group by Edwards (1932); he states that "the larvae occur in water collected in large fallen leaves in jungle, and are usually associated with larvae of *Uranotaenia obscura* and *Aëdes jugraensis*, upon which they feed."

Psorophora. The predacious habit has apparently developed inde-

pendently several times in the *Aëdes* group of genera. The subgenus *Psorophora* includes some eight American species, all with predacious larvae inhabiting temporary ground pools. The larvae are very voracious and grow very rapidly; notes on their habits were included in the monograph of Howard, Dyar, and Knab, and in other taxonomic papers on the American fauna.

Aëdes. The species of the African and Oriental subgenus *Mucidus* seem to play the same role as the American *Psorophora*, feeding voraciously on other mosquito larvae in temporary ground pools.

Eretmapodites. The predacious habits of the larvae of this aëdine genus described by Haddow (1946) have generally been overlooked; apparently all are predacious, and all breed in small water accumulations, such as in fallen leaves or leaf axils. Haddow reports experiments with the food habits of various species. He found, for instance, that third-stage *E. chrysogaster* larvae would, on an average, devour six similar sized larvae of *Aëdes simpsoni* each in a twenty-four-hour period; they frequently attacked *A. simpsoni* pupae, but usually unsuccessfully. *E. chrysogaster* larvae were found to be relatively immune to attack by their own kind, even small larvae escaping large larvae, probably because of their tough integument and strong swimming habits.

Haddow found *Eretmapodites ferox* to be the most actively predacious species of all. "If an *A. simpsoni* larva is placed in a small dish with a large *E. ferox* larva, the latter will in many cases cross the dish straight to its prey, swimming by means of its mouth-brushes (a peculiar, rapid, gliding motion) and, seizing it immediately, will begin to shake and worry it much as a dog shakes a rat. The savage nature of the larva is shown by the fact that it almost always occurs alone in an axil." *E. ferox* larvae consumed an average of nine A. *simpsoni* larvae in a twenty-four-hour period.

Armigeres. Of this Oriental aëdine genus Edwards (1932) states that the "larvae of many species, if not all, are very cannibalistic."

Culex. The larvae of the tropicopolitan subgenus *Lutzia* are predacious on other mosquito larvae; they inhabit ground pools.

GROWTH

All mosquito larvae molt four times: that is, there are four larval instars between the egg and the pupa. No exceptions to this seem to have been observed, although in other insects the number of molts

is sometimes variable. The relative length of the various instars may be a specific characteristic, since the figures given by various authors vary (Bates, 1941c); but in general the second and third instars are shorter than the first, and the fourth is longest of all. Thus in *Haemagogus spegazzinii*, the first instar occupied 26 per cent of the larval growth period, the second 14 per cent, the third 17 per cent, and the fourth 43 per cent. Huffaker (1944) has pointed out that the growth of the fourth instar involves by far the greatest increase in mass, as well as basic tissue transformations, so that it is not surprising that it should require the longest time.

The rate of growth of mosquito larvae seems to depend largely on three general classes of factors: the environmental temperature, the inherent (genetic) characteristics of the species, and the nature of the culture medium (availability of food). The effect of environmental temperature on growth was discussed in the preceding chapter. The effect of genetic factors within a species population is difficult to evaluate with the data available. Huffaker (1944) has pointed out that differences in growth rates between the "Boyd" and "Wilson Dam" strains of *Anopheles quadrimaculatus* may have a genetic basis, and certainly speed of development in other insects is known to be under genetic control. Among species, the differences in normal rates may be enormous. Species that breed in transient water accumulations, such as rain pools, are apt to show very rapid growth rates. Rapid growth may also be important in stable habitats, such as ponds, where there is a well developed predator fauna. The slowest growth seems to occur in groups that live in small but relatively stable accumulations of water, such as are found in bamboo internodes, where speed of growth in itself loses its advantages in escape from predation or environmental adversity. Rate of growth may vary characteristically within a group of closely related species, as Hovanitz (1946) has shown for *Haemagogus*.

The composition of the culture medium has a very marked effect on growth as well as on mortality. This is in part dependent on the amount of food available, as can be seen from the effect of overcrowding, shown nicely in experiments reported by Shannon and Putnam (1934) summarized in Table VIII.

Trager (1937) developed a formula for expressing the results of growth experiments in which both survival and speed of growth would be taken into account. He used the numerical result of the expression "N x 1/T," in which "N" is the percentage of larvae to

TABLE VIII EFFECT OF OVERCROWDING ON PUPATION PERIOD AND MORTALITY OF *Aëdes aegypti* LARVAE AT 27° C (SHANNON AND PUTNAM, 1934)

NUMBER OF EGGS PER LOT	DAYS TO FIRST PUPATION	DAYS TO FINAL PUPATION	NUMBER OF PUPAE	PER CENT EGG AND LARVAL MORTALITY
100	5	7	95	5.00 ± 1.47
400	5	10	338	15.50 ± 1.22
1000	7	16	790	21.00 ± 0.87
4000	10	60	2096	47.60 ± 0.53

reach the fourth larval stage within ten days and "T" the time in days required by these larvae to reach this stage. Under optimum conditions at 28° C. the larvae of *Aëdes aegypti* reached the fourth stage in four days, so that the numerical expression of this rate of development would be 100 x ¼ = 25. An application of the formula is shown in Table IX, copied from Trager's paper. Trager points out that while the time limit used in his formula is arbitrary, a shift in the length of time used would have no effect upon the result. In working with species with slower development than *Aëdes aegypti*, it might be necessary to use a longer standard-time interval.

TABLE IX RELATION OF RATE OF GROWTH TO CONCENTRATION OF LILLY LIVER EXTRACT 343 IN *Aëdes aegypti:* 25 LARVAE FOR EACH LINE, 0.1 CC. YEAST SUSPENSION PER LARVA (TRAGER, 1937)

CONCENTRATION OF EXTRACT 343 PER CENT	T	N	N × 1/T	ADULTS ♀	ADULTS ♂	DAYS TO ADULT
1.00	4.08	100	24.5	8	15	8.5
0.90	4.12	100	24.3	12	11	9
0.80	4.08	100	24.5	15	9	8.5
0.70	4.16	100	24.0	10	13	9
0.60	4.36	100	22.9	10	12	9.5
0.55	4.28	100	23.4	8	14	10
0.50	4.76	100	21.0	15	9	11
0.45	5.50	96	17.4	11	12	13.5
0.40	5.43	84	15.5	8	10	13
0.35	5.79	76	13.1	7	10	15.5
0.30	6.69	52	7.8	5	7	19.5

HIBERNATION IN THE LARVAL STAGE

The number of mosquito species that hibernate in the larval stage is rather small, but the mechanism of this method of hibernation is

very interesting. Probably the best studied case is that of *Anopheles claviger* in Europe. The larvae of this species pass the winter in mud on the bottom of the ponds in which they breed. These ponds are usually frozen over, but laboratory experiments show that if the larvae are frozen in the ice, they die. It has frequently been assumed that these hibernating larvae must be in a peculiar inactive state—a state of "diapause." Various authors have reported that such larvae brought into the laboratory and placed in favorable temperatures fail to grow, and various studies have been made of methods of "reactivating" such larvae. Sautet (1933) found that growth was resumed in these larvae when a small quantity of an oxidizing agent (*eau de Javelle* or potassium permanganate) was added to the water, and he concluded that the natural reactivation of the larvae was due not to the rise in temperature alone, but to the accompanying release of oxygen by water plants owing to their increased chlorophyll production. Roubaud gives a special name to this cessation of growth in overwintering larvae, calling it "asthenobiosis." He considers that it is related to an alternation of generations and that it is quite independent of external environmental influences. He has developed the theory especially with regard to *Anopheles plumbeus* (Roubaud and Colas Belcour, 1933), which shows the same type of hibernation as *Anopheles claviger.*

In Albania, we were not able to confirm the presence of a special diapause state in the larvae of *Anopheles claviger.* Larvae collected under winter conditions and brought into the laboratory seemed to show normal growth, and it seemed to us that the suspension of growth in nature was purely a temperature phenomenon. This is also the experience of Marshall in England. He states (1938, p. 128): "Larvae of *Anopheles claviger* furnish an example of a quiescent period, since specimens collected at any time during the over-wintering interval and kept at the ordinary temperature of the laboratory complete their development up to the adult stage without further delay."

Baker (1935) has made an interesting set of experiments with the effect of length of day on hibernating larvae of *Anopheles barberi* (closely similar in structure and habits to the European *A. plumbeus*) and *Orthopodomyia signifera*—both larvae that breed in tree holes in the northeastern United States. Larvae were placed in two bamboo containers with filtered tree-hole water. One container was subjected to sixteen hours' illumination a day (normal winter daylight

supplemented by seven hours of artificial light) while the other container was left with normal winter light only (about nine hours a day). He notes that the anopheline larvae in the container with the long light period became positively phototropic toward the end of the first week and were cruising about over the surface of the water. They pupated in from three to four weeks after the beginning of the experiment. The larvae in the short-day container showed no signs of growth; they remained photophobic and revealed no evidence of feeding throughout the duration of the experiment and for several weeks thereafter.

RESPIRATION

Mosquito larvae are adapted to breathing air, and the majority of the species come to the surface regularly for this purpose, or remain at the surface breathing through the respiratory tube, or "siphon." It is clear that a certain amount of respiration continues even when the larva is deprived of access to the surface; such respiration is probably a function of the general integument since the so-called "anal gills" seem to serve for the regulation of osmotic pressure, rather than for respiration, as is shown by the work of Wigglesworth. The length of time required for "drowning" larvae, if denied access to air, varies with the species of larva, its stage of growth, and the amount of dissolved oxygen in the water (Costa Lima, 1914, 1916; Kalandadse, 1933). Survival without free air depends also on temperature, and at very low temperatures where metabolic processes are at a minimum, larvae can survive for very long periods by cuticular respiration alone. This is shown by larvae that hibernate in the larval stage in water under ice (for example, *Anopheles claviger*).

Hopkins (1936) noted that larvae that breed in small container habitats (tree holes) spend much less time at the surface of the water than larvae that live in open habitats; he noted also that such larvae have more highly developed anal gills. The larva of the North American *Psorophora discolor*, which breeds in rain pools, is said never to come to the surface for air, resting on the bottom, back down (Smith, 1904); it also has very large anal gills. While the development of the gills in such larvae is probably correlated with the low concentration of mineral salts in container or rain-water habitats, it seems very likely that such larvae may also depend on cuticular respiration to a greater extent than larvae in open terrestrial habitats.

Larvae of the genera *Ficalbia, Mansonia* (*Taeniorhynchus*), and *Aëdomyia* show highly specialized respiratory behavior. The *Mansonia* larvae have been best studied, as species are found both in America and Europe. Larvae of these genera obtain oxygen from the surface of plants, or from plant tissues. The habit of respiration by attachment to plants was apparently first described by J. B. Smith (1908) for the American *Mansonia perturbans;* his discovery was confirmed and amplified by Grossbeck (1908) in the same year. Further observations were published by Dyar and Knab (1910) and by Howard, Dyar, and Knab (1913). The structure and habits of the European *Mansonia richiardii* have been described by Edwards (1919), Wesenberg-Lund (1921), and Marshall (1938), where references to various other papers will be found. Hopkins (1936, p. 19) has reviewed the general subject of modification of respiratory structure and habit in African larvae.

Edwards (1932, p. 115) summarizes the habits of the species of the *Mansonia* group as follows:

> The larvae of all species of this genus occur in overgrown swamps or ponds, and derive their air from the roots of water plants, which they pierce by means of the saw-apparatus in the siphon; they attach themselves firmly to the roots of the plants and seldom if ever come to the surface to breathe. In the case of the subgenera *Mansonia* and *Mansonioides,* floating aquatic plants are effected, but in *Coquillettidia* and perhaps *Rhynchotaenia* the larvae are found in the mud among the roots of grasses, bulrushes and other plants. Pupae also derive their air from the same source, piercing the rootlets with the spine-like tips of the respiratory organs and remaining below until ready for emergence, when they rise to the surface. In the case of *Coquillettidia* the tip of the trumpet breaks off and remains in the plant, but in *Mansonioides* it is withdrawn.

LARVAL BEHAVIOR

Larval behavior has received much less attention than adult behavior, probably because the subject is primarily of academic interest, whereas adult behavior is directly involved in the process of disease transmission. Studies of larvae should be simpler to make, since the aquatic environment is more easily subject to laboratory control than the aerial environment, and since the range of movement of larvae is more limited. An analysis of larval behavior will surely be necessary for an understanding of the ecological relationships of the various

species; and the subject would thus seem to be a fruitful field for research.

Movement. Locomotion in mosquito larvae seems to be of two main types: by jerks of the body, and by propulsion of the mouth brushes. The movements of anopheline larvae at the surface are generally of the first type: movement is tail foremost, and generally accomplished in a series of short spurts. The ventral tail brush and various setae may serve as paddles in such movements.

> If a culicine larva which is feeding at the surface of the water is carefully watched it will be noticed to be moving slowly but steadily in a forward direction, but without any motion of the body. This effect is apparently produced by the movements of the mouth-brushes, but it is difficult to understand why, if this be the case, larvae are sometimes observed to lie motionless at the surface of the water, although the mouth-brushes are in use. On the other hand, larvae are able to change their course without any apparent movement except of the brushes, and this cannot readily be accounted for except as due to independent action of the mouth-brushes of one side (Hopkins, 1936).

Some culicine larvae describe continuous circles as they feed at the water surface, surely caused by a differential rate in the movement of the mouth brushes. "Crawling" under water over the bottom is also generally attributed to the propulsive action of the mouth brushes.

There are various accounts of mosquito larvae crawling out of water. Nikolsky (1924) reports that the larvae of *Anopheles maculipennis* can crawl on any surface that does not absorb moisture too quickly; if they encounter a surface absorbing water, they suddenly alter their direction. In the case of water collected in hoofprints, Nikolsky has observed the larvae to crawl from one to another as each dried up, covering a maximum distance of 75 cm. in this fashion. O'Connor (1923) states that the larva of *Aëdes kochi*, which lives in leaf bases, can leave the water and "creep like a caterpillar," thus being able to adapt itself to life in small transient water accumulations. Ability to crawl seems also to be a characteristic of the larvae of the American sabethine mosquitoes, which normally breed in flowers, leaf bases, and other small water accumulations.

Stance. As has so often been pointed out, anopheline larvae characteristically lie parallel to the surface of the water, while other mosquito larvae hang head down, with only the caudal siphon attached to the surface film. The angle of the body varies greatly with different

culicine species, and some (such as *Uranotaenia*) have a normal stance not strikingly different from that of *Anopheles*. Anopheline larvae at rest are almost invariably found touching surface debris or vegetation, and Renn (1943) has shown nicely that this is simply a mechanical result of surface factors, rather than a "tropistic" reaction. The anopheline larva at the surface produces both positive and negative menisci: parts of the submentum, the palps, the thoracic hairs, the palmate hairs, and the spiracular plate are unwetted, making negative menisci; the posterior borders of the spiracular plate wet readily and form a positive meniscus. The orientation of such a system of menisci can be demonstrated by the use of a model:

> If a small larva-sized, boat-shaped piece of metal foil or cellophane is bent sharply upward at one end and downward at the other and floated carefully at the water surface, a negative meniscus will be formed at the depressed end, and a positive meniscus will be produced under the upturned end. Now, if the boat is coaxed into the vicinity of a wetted stem, the end supporting the positive meniscus will move toward the stem. It will appear to be strongly attracted and will climb the meniscus as a live larva would. Only the upturned, positive meniscus bearing end of the boat will do this. The end surrounded by a negative meniscus will be forced away with equal vigor. When the boat approaches a waxy stem with a negative meniscus, opposite relations apply; the depressed end is attracted and the upturned end with a positive meniscus is strongly repelled (Renn, 1943).

Alarm reactions. Most mosquito larvae drop from the surface at once if "alarmed." If a shadow passes over a dish of larvae, or if the dish is jarred, every larva will frequently disappear from the surface. The strength of this reaction seems to vary with the species, and in some anopheline larvae the habit is so strongly developed that it renders their laboratory manipulation difficult. In some cases the larva remains at the bottom in a rigid, "death-feigning" position, called by the psychologists "letisimulation." This trait is highly developed in the South American *Anopheles darlingi*, so that it is often difficult to distinguish dead larvae from individuals feigning death. In some other species, the specific gravity of the larva seems to be such that it cannot maintain itself on the bottom except by constant or frequent movement; such for instance, is the case with *Culex fatigans*.

Robert Gordon, Jr., tested the "alarm reaction" of a variety of mosquito species while working in the Villavicencio laboratory. He found

that the larvae were disturbed by a diminution of light, but not by an increase. *Anopheles darlingi* larvae, if left in a pan under an electric light, would dive to the bottom if stimulated by a sudden but very slight decrease in the light intensity, the threshold of the reaction being a difference in light intensity hardly detectable by the human eye. They were, however, undisturbed by the turning on of a bright light. Different species showed different behavior, apparently related to the habitat of the larva. Thus *Anopheles* (*Stethomyia*) *nimbus*, which breeds in forest streams, showed no alarm reaction to light changes. The reaction would obviously be harmful for such larvae, since if they were disturbed by every shadow, they would be in danger of being swept away in water currents. Species from bamboo internodes also showed no alarm reaction, perhaps because light would be dim or absent in this habitat. The most sensitive of all of the species tested was *Culex* (*Carrollia*) *urichii*, collected from water in palm-flower bracts on the forest floor; yet a closely similar species, *Culex* (*Carrollia*) *metempsytus*, found breeding in bamboo internodes, show no alarm reaction at all to light changes.

Orientation reactions. Larvae show tropistic reactions to various stimuli: light, current, temperature, and probably also to contact and chemical stimuli. Light reactions are the most obvious and probably play a dominant role in larval behavior. Miller (1940) has shown that in *Culex pipiens* rising to the surface is a light reaction: larvae die if kept in a container with only the bottom illuminated. Larvae of many species will crowd to the bottom if illumination is from below, but the surface orientation is probably normally based on reactions to gravity and water pressure as well as to light. Thus Jobling (1937) was able to grow *Culex pipiens*, *C. fatigans*, and *Aëdes aegypti* in complete darkness, which would not be possible if light reactions were essential for surface orientation.

The normal light reactions of larvae are of several sorts. Thomson (1940b) has analyzed the behavior of *Anopheles minimus*, finding three types of light reactions (using the terminology of Fraenkel and Gunn, 1940): *photokinesis*, greater activity in sunlight than in shade; *photofobotaxis*, avoiding reaction when moving from a shaded to an unshaded area; and *phototelotaxis*, spontaneous direct movement toward shade near at hand, quite apart from photokinetic reaction. Thomson also experimented with the larvae of *Anopheles maculatus* and *A. hyrcanus*. He found that the reactions of *A. maculatus* were

very similar to those of *A. minimus*, while *A. hyrcanus* showed quite different behavior, being quite uninfluenced by light and shade. Both *A. maculatus* and *A. minimus* are found in running-water habitats, while *A. hyrcanus* breeds in still water. Thomson reached the conclusion that the reactions binding the *maculatus* and *minimus* larvae to shade were adaptations to this running-water environment, since the result would be to keep the larvae confined to areas shaded by vegetation where the hazards from current would be minimized. Rudolfs and Lackey (1929) found that the light reactions of *Culex pipiens*, *Aëdes vexans*, and *A. canadensis* varied depending on the type of diet on which the larvae were maintained.

Mosquito larvae generally react to a current by swimming against it, but Thomson was not able to detect any direct evidence of avoidance of flowing water in the species with which he experimented.

Relatively few experiments with larval reactions to temperature gradients seem to have been made. Ivanova (1940) tested the behavior of *Anopheles maculipennis* in a container about 4 feet long in which the temperature was varied from 15° to 18° at one end to 37° to 40° at the other. Most of the larvae congregated where the water had a temperature that approached the optimum (23° to 28°); larvae in cooler or warmer water were stimulated by the temperature and moved about in jerks until they accidentally entered the optimum zone, where thermokinesis ceased, locomotor behavior giving place to feeding behavior.

The tendency of larvae to remain attached to vegetation or debris at the water surface has generally been ascribed to thigmotaxis. Renn (1943) has shown that the behavior may result from a purely mechanical action of surface forces, though it is possible that a positive contact orientation of the larva is also involved. Mosquito larvae would surely also show reactions to chemical stimuli, but this seems not to have been investigated.

CHAPTER X

THE BIOLOGICAL ENVIRONMENT OF THE LARVA

> Some artists tell us that in order to appreciate a landscape one should stand upon one's head, or in some other way contrive to see it upside down: familiar details gain a fresh significance from the new viewpoint. In the same way, to a biologist trained in the school of anatomy, and used to thinking first of the type, then of its circumstances, the opposite line of approach may bring new comprehension, though at first, perhaps, it may induce a little giddiness.
>
> —KATHLEEN CARPENTER

THIS chapter might, with equal appropriateness, be headed "The Relations of the Larva to the Aquatic Community," since the study of the biological environment involves essentially the description of the community and the study of intracommunal relationships. Our knowledge of mosquito larvae, from this point of view, is essentially fragmentary. A certain amount of study has been given to the question of what organisms serve as larval food, and whether the presence of particular organisms may be a controlling factor in larval distribution. A great deal of attention has been given to larval predators, partly in the hope of thereby finding a key to biological control. The relation between larvae and major aquatic vegetation also has been given considerable attention, since the aquatic vegetation often serves as an obvious and convenient guide to specific types of anopheline breeding places. But these various aspects of communal relations are far from sufficient for building up an adequate picture of the aquatic community and of the relation thereto of the various types of mosquito larvae.

The study of communities has come to form a major part of the science of ecology, and certain aspects of this study have acquired a well developed methodology with special techniques and a special vocabulary for the recording of observations. The major developments in this field have been made by botanists, and the vocabulary

and methods are thus largely concerned with plant communities and their succession. This is logical, because the plant community always forms the background against which the animals must be studied. The task of the botanists is also simpler, because their organisms stay put under conditions where they are always readily available for continued or interrupted observation. The progress of ecological botany would have been much less spectacular if the botanists had had to trap their plants every time they wanted to make an observation on them! Despite this handicap, the zoologists have also made considerable progress in the study of community relationships; but here again the vocabulary and the techniques have largely been developed in connection with studies of vertebrates. This means that when the student of mosquitoes surveys the literature of ecology, he is apt to find many suggestive ideas and methods, but little that is of direct help in interpreting the situation that he is attemping to study.

Partly this is a result of difference in point of view. The ecologist (or the limnologist) is studying total situations—communities as such, their history, and their interrelations. The mosquito student is concentrating on a particular type of organism, trying to determine its relations to the various aspects of the world in which it lives. Usually the mosquito larva is a relatively trivial, or subordinate, member of its community. The removal of a particular mosquito species or of all mosquito larvae would probably make no difference to the total ecological situation, since the role of the larva as predator on microorganisms or as prey for other organisms would presumably rapidly and inconspicuously be taken over by some other member of the community. Mosquito larvae, in other words, do not form a controlling element in determining the community type. Such a control may be exercised by certain insects, such as chironomid larvae when they are present in great numbers, by major vegetation, by microorganisms of certain types, and perhaps by key predators or parasites, such as certain fish or pathogenic organisms.

In short, mosquito larvae, in most situations, are not "dominant" organisms. The idea of the "dominant" has played a conspicuous role in ecological thinking. In the list of definitions of "community" given by J. R. Carpenter (1938), there occurs, for instance, the quotation: "Communities must be determined by dominants rather than habitats; the limits of dominants as such are the limits of the community." The

first definition of dominant, in turn, is "an organism which controls the habitat." The emphasis on dominants seems to me to be a result of the development of ecology in the temperate zone, where forests and grasslands are in fact often dominated and controlled by one particular species of plant. The search for a single dominant or even for a group of dominants in a tropical situation may be quite futile, especially in climax forests where the idea of dominant got its start in the temperate zone. In a particular aquatic community some plant, such as a species of pond lily or sedge, may really exert a controlling influence over the entire habitat, and thus correspond to the concept of dominant. But more often it seems to me that the concept of dominant may be an oversimplification of little help in interpreting the complex of intracommunal relationships. More useful, perhaps, would be an attempt to estimate the extent to which various species and types of organisms control and modify the success with which other organisms survive in the same community.

Such a study, in a community of numerous species, may seem almost hopelessly difficult. Many mosquito larvae, however, form parts of relatively simple communities, such as those found in tree holes, temporary rain pools, or (in the tropics) in a host of special situations associated with growing plants. In such communities mosquito larvae may be controlling members; certainly they are often dominant both in numbers and in bulk. These restricted, highly specialized, and faunally simple communities have, however, not been studied from this point of view of communal ecology. One big difficulty in such a study would be the intermittent or transient character of most of the inhabitants: like mosquitoes, many of the other community members belong to quite different associations as larvae and as adults, or possess special mechanisms for carry-over from one favorable period or place to another. The study of the dynamics of the populations of such communities could have little significance without taking also into consideration the communities occupied by the same animals in other life-history stages, which might remove all of the advantage of the simplicity of the special aquatic habitat.

Since a truly logical discussion of the communal relations of mosquito larvae seems to be impossible with the information available, the material of the present chapter is lumped, quite arbitrarily, under the headings of Microbiota, Vegetation, Parasites, Predators, and Com-

petition. The most that can be said for this scheme is that it covers a fair sample of the major types of relationships in which mosquito larvae are probably involved.

MICROBIOTA

This is an awkward word, but it seems the easiest label to use for the relations of larvae to the microorganisms found in the same community. Direct relations (food chains) probably mostly involve plankton, but larvae are undoubtedly influenced in many ways by microorganisms that do not form part of the plankton, particularly by sessile algae that may serve as food for species of larvae that graze, and by the flora of the bottom deposits of shallow pools. The microorganisms of the larval community are of ecological interest from three distinct points of view: directly, as a source of larval food; indirectly, because of their influence on the physical and chemical characters of the environment; and more indirectly, as indicators of the general environmental conditions with which particular species of mosquitoes are associated.

The feeding behavior and food requirements of larvae were discussed in a previous chapter. Whether particular types of organisms are preferred or are necessary as food for larvae, or whether the type of microorganism available in the habitat ever serves as a limiting factor in mosquito breeding, has been the subject of a great deal of controversial discussion. An idea of the nature of the literature on the subject can be obtained from the following quotation from Hinman (1930):

Metz (1919) found that food could not be considered a limiting factor in the distribution of anopheline larvae. Lamborn (1922) believes that Chironomidae inhibit Anophelines by collecting all the floating algae, for the purpose of forming their larval cases, thus starving the Anophelines. Bradley (1924) did not consider larval food as an important factor in the increase or decrease of Anopheline breeding. Rudolfs (1924a, 1924b) determined that the food supply was the main requirement for breeding and that the *kind* of food supply might induce certain species to breed in certain places. The same worker (1928) concludes, "That insufficient food supply for bacteria might be responsible for the absence of mosquito breeding. Again, the food supply for mosquito larvae, consisting of microscopic animals and plants, is directly correlated with breeding." Bradley (1926) observed that an increase in the number of larvae present in selected areas did not cause a proportionate increase in the rate of emergence. He

believes this may have been caused by a scarcity of food in the concentrated areas, or by the activities of certain enemies of the larvae. Harold (1926) states that the *p*H of the water is of minor importance to the developing larvae and that the factor of prime importance is food. Sebentzov (1926) and Adova, Nikitinski and Sebentzov (1927) in a study of the plankton of peat bogs reached the conclusion that *Cloeon* and *Agrion*, in particular, with *Daphnia*, *Bosmina* and numerous rotifers are indicative and *Epitheca* and *Libellula* contra indicative of the presence of *Anopheles*. Hamlyn-Harris (1927, 1928) also agrees that food is the main determining factor in the choice of preferential breeding places. He found that the presence of algae leads to the appearance of *Anopheles annulipes*. Swellengrebel, de Buck and Schoute (1927) found that the size of *Anopheles maculipennis* was affected to a certain degree by their food. Beklimishev, Mitrofanova, Beklemischev, and Mitrophanova (1928) in a study of *Anopheles maculipennis* found that insufficient food only retards development and decreases the percentage of the survivors, but some larvae develop, at least up to the fourth stage. Boyd and Foot (1928) in a careful study of the alimentation of Anopheline larvae and its relation to their distribution in nature concluded that *A. quadrimaculatus* and *A. punctipennis* appear to ingest the same plankton elements but no positive correlations were found to exist generally in nature between the distribution of larvae and the distribution of the plankton. They further concluded that the distribution of *A. quadrimaculatus* and *A. punctipennis* is not controlled by nutritional factors. This last view seems most reasonable to the author and would be supported by his dissection results. Yet Galli-Valerio (1929) observed that with the disappearance of a green alga *Anopheles maculipennis* became scarce in a lake but *Culex pipiens* developed in numbers.

The conclusion reached by Hinman in his own studies, and which seems now to be well established, is that mosquito larvae in general show no discrimination in feeding, ingesting any material of the right particle size. Much of this material may be indigestible and pass through the gut unchanged; but the rest, including a wide variety of microorganisms, furnishes the basic food of the larvae. To what extent, if ever, this food supply is a limiting or controlling factor in breeding is the question. In normal situations the plankton is probably sufficiently abundant, and mosquito larvae are relatively sufficiently scarce, to make it unlikely that food supply would ever be the limiting factor in determining the abundance of any given species. And the correlation between the occurrence of larvae of particular species of mosquitoes and particular types of microorganisms is prob-

ably always indirect, through the operation of other environmental factors.

The possible indirect influence of microorganisms on mosquito larvae through their action on the chemical and physical environment seems to have received almost no attention, though it is surely important. The influence of nitrates, nitrites, and pollution in general on mosquito breeding, discussed in a previous chapter, would seem to indicate an important relation between bacteria and mosquito larvae. Bacterial scums, which form in many situations, seem to be directly inimical to anopheline breeding. In the laboratory culture of mosquito larvae, one often gets the impression that certain types of microorganisms are "favorable" when they appear in the culture medium, and others "unfavorable"; and there is a widespread belief, for instance, that green algae in the culture medium are favorable for anopheline larvae because of their effect on dissolved gases in the water, aside from their possible value as food.

The possible use of plankton as indicator organisms of the suitability of a particular habitat for the breeding of given mosquito species was mentioned in the quotation from Hinman above, where the work of Sebentzov (1926) and Adova, Nikitinski and Sebentzov (1927) on Russian peat bogs was cited. Matheson and Hinman (1930) made a detailed investigation of the plankton of two pools: one a *Chara* pool in which no mosquito breeding occurred, the other a woodland pool in which mosquitoes bred abundantly (*Aëdes stimulans*, *A. fitchii*, and *A. excrucians*). The plankton fauna of the *Chara* pool was much more abundant and varied than that of the woodland pool, and the authors concluded that plankton studies would be of little use as an indication of suitability for mosquito breeding.

The use of plankton as index organisms for mosquito breeding would seem in any event an awkward expedient, since the plankton would be more difficult to collect and identify than the mosquitoes themselves. However, many types of plankton are undoubtedly more sensitive than mosquito larvae to slight environmental differences, and the requirements of plankton organisms would likely form a better basis on which to study the classification of fresh-water habitats than would the requirements of mosquito larvae. This is the thesis of Frohne (1939), who made a thorough study of the desmid fauna of certain ponds in South Carolina and Georgia, making a classification of the ponds on the basis of the desmids, and then correlating anoph-

eline breeding with the pond classification thus achieved. A wider application of this method of study would certainly seem to be justified.

VEGETATION

The larvae of almost all mosquitoes are associated in one way or another with vegetation, with the so-called "higher plants." About the only exceptions would be species that breed in rock pools, in gravel seepage, in crab holes, in artificial containers, or other such situations. The larval association with higher plants is of two main types: either the plants grow in the larval habitat, or the plants form the larval habitat. The first would include the growth of reeds, water lilies, and so forth in ponds and marshes; the second, tree holes and special water-collecting plants such as bromeliads, bamboo, and *Nepenthes*. The association between the larva and the plant is particularly intimate in the second case, since as a general rule a particular species of mosquito is associated with a particular species of plant, undoubtedly showing highly specialized physiological adaptations to the peculiar chemical and physical conditions produced by the habitat-forming plant. Since the association between particular plants and particular mosquitoes is so close, the subject is perhaps best discussed in the following chapter, where the classification of larval habitats is treated. This type of plant-larva association seems unimportant in the temperate zone, where it is limited to a few species breeding in tree holes or to ecological curiosities like the *Wyeomyia-Sarracenia* association in North America. In the tropical forest, however, the major part of the mosquito fauna may breed in plant containers, and the association can hardly be dismissed as unimportant, or of interest only as a curiosity.

Most investigations of larval association with vegetation, however, are concerned with the first situation: with plants growing in the larval habitat. Since the anopheline vectors of malaria, with very few exceptions, are found breeding in association with aquatic plants, the subject has received a considerable amount of attention from malariologists.

Conspicuous aquatic plants would seem to offer the most serviceable type of index organism for distinguishing different sorts of larval habitats. Studies with this object in view have, however, generally given rather unsatisfactory results, in that there seems rarely, if ever,

to be a working correlation between the incidence of a particular species or complex of plants and particular mosquito species. Frohne (1939, p. 1,376) has pointed out that the distribution of higher aquatic plants ("macrophytes") may be in part accidental, chance determining which species or types become established as dominants, and that, in any event, relatively little is known about the factors controlling the ecological distribution of such plants. Ecological succession is also generally fairly rapid in fresh-water habitats, and the species composition of plants occurring in a particular location may change over the years without any correspondingly striking change in the general environmental characteristics or in the mosquito fauna.

While the species of plant may not, in general, be a useful index in determining mosquito distribution, the type of plant is certainly important. The experiments of Russell and Rao (1942c) with the effect of vertical obstructions on the oviposition of *Anopheles culicifacies*, discussed in an earlier chapter, show clearly that the adult mosquito, in egg laying, may be influenced by whether the vegetation is horizontal or vertical; and the amount of shade, determined by the type and distribution of vegetation, is also known to influence the ovipositing habits of the adult. These influences have long been recognized by malariologists in working out classifications of breeding places, and in drawing up habitat descriptions.

Hess and Hall (1945) have given a general review of the relation between plants and anopheline breeding. They propose the following classification of aquatic plants from this point of view:

1. *Emergent.* Typically growing with some of the vegetative parts extending above the water surface.
   a) *Erect.* Standing erect with relatively firm stems and leaves.
      (1). *Leafy.* Possessing an abundance of well defined leaves.
      (2). *Naked.* Usually lacking well developed blades, or if blades are present, they are linear and erect.
   b) *Flexuous.* Having stems which are relatively weak and flexuous, or if firm and erect, possessing lax leaves.
   c) *Carpet.* With relatively short shoots forming dense attached mats.
2. *Floating.* Typically having the entire plant or some of the vegetative parts floating on the water surface.
   a) *Pleuston.* Minute plants floating free at the water surface.
   b) *Floating Mat.* Composed of relatively large plants forming a mat which floats at the water surface.

c) *Floating Leaf.* Consisting of attached plants bearing floating leaves with relatively long flexuous peticles.

3. *Submerged.* Typically growing with the vegetative parts largely submerged.

The correlation that they found between the abundance of larvae of *Anopheles quadrimaculatus* and these various types of vegetation is shown in the accompanying table, copied from their paper. They attribute the larval frequency in the various vegetation types directly to the "intersection line values" (see below). It would be interesting to have comparative figures for larval distribution among such vegetation types for various anopheline species.

TABLE X. RELATION BETWEEN THE BREEDING OF *Anopheles quadrimaculatus* AND TYPE OF VEGETATION (HESS AND HALL, 1945)

TYPE	NUMBER OF SPECIES	NUMBER OF SAMPLES	NUMBER LARVAE PER SQ. FT.	
			Plot Maximum	Mean
Flexuous	11	1748	49.9	7.9 ± 1.8
Submerged	8	473	37.0	6.7 ± 2.0
Carpet	3	155	11.2	6.5 ± 2.7
Floating mat	1	680	26.0	6.2 ± 1.9
Erect leafy	7	1113	15.2	4.2 ± 0.9
Coppice	3	160	19.6	3.9 ± 1.4
Pleuston	3	28	6.0	3.2 ± 0.6
Woods	Several	390	5.6	1.9 ± 0.6
Erect naked	4	438	6.2	1.6 ± 0.5
Floating leaf	5	2271	7.5	1.1 ± 0.7
Totals	45	7456		

The "intersection line" is a term proposed by Hess and Hall (1943). It is defined as "the line of intersection between three interfaces, water-air, water-plant, and plant-air. Thus for a floating leaf the intersection line is the perimeter of the leaf where it intersects the water surface; for an emergent stem, it is the perimeter of the stem at the water surface; etc." The "intersection value" is then defined as "the number of meters of intersection line per square meter of water surface." The authors found a direct relation between *A. quadrimaculatus* breeding and the intersection value in a series of samples taken in lotus (*Nelumbo*), whose large floating leaves would be comparatively simple to measure. The idea was extended in a paper by Rozeboom and Hess (1944) in which results with various other types of aquatic

vegetation were summarized. Since this line, in most types of vegetation, would be very difficult to measure, these authors recorded the amount of intersection line as "high, medium, low or zero."

The idea of the intersection line is probably useful in so far as it emphasizes the importance of the extent to which the water surface is broken as a measure of the amount of resting place available to anopheline larvae; it is, in this sense, a corollary to the meniscus reactions observed by Renn (1943). It is a factor that in most situations, however, would be impossibly difficult to measure (for example, in grass), and it fails to take into account the type of vegetation, or the situation of the vegetation (marginal, part of a large mass, isolated clump), surely as important with most anophelines as the actual amount of break in the water surface. For most culicines the concept is quite meaningless, although their occurrence too may be related to density of vegetation.

Various types of plants have been at one time or another reported as directly inimical to larval breeding, or as indicators of conditions unfavorable for breeding. Earlier references on this subject have been summarized by Covell (1931b). He lists three groups of such plants: (*a*) those which act by covering the water surface so thickly as to prevent respiration of the larvae; (*b*) those that act by entrapping the larvae; and (*c*) those which are actively poisonous to larvae. Various authors have included such plant genera as *Lemna*, *Azolla*, *Wolffia*, *Anacharis*, and *Trapa* in the first group. The outstanding example of the second group is the bladderwort (*Utricularia*), whose ability to capture larvae has been described by several authors (for example, Brumpt, 1925b). The literature on plants said to be actively poisonous to larvae is very extensive, most of it concerned with a controversy over the inimical effect of *Chara*, first observed by Caballero (1919). Covell gives many references in his book. Typical papers are those by Matheson and Hinman (1931), who found that *Chara* was unfavorable to larvae, and by Hamlyn-Harris (1932), who found that *Chara* had no inimical effect. Recently Hess and Hall (1945) have found that the water shield (*Brasenia*) either exerts an unfavorable effect on *Anopheles quadrimaculatus*, or else is an indicator of unfavorable conditions, since larvae have almost never been found in association with the plant in a large series of samples in different localities.

PARASITES

Mosquito larvae are remarkable, in the parasite-ridden insect world, for their freedom from this particular type of biological relationship. The list of microorganisms detected in the gut or body cavity of larvae is fairly extensive, but the organisms are mostly of doubtful or unknown pathogenicity. An anomalous group of fungi found in many parts of the world seems to be specialized for parasitism in mosquito larvae; certain hydrachnid mites have been reported from larvae; and in many situations larvae accumulate a fairly extensive growth of epizoons and epiphytes such as *Vorticella* and various types of algae, but such fortuitously acquired organisms can scarcely be classed as parasites. That about completes the parasite list. An extensive compendium of the parasites found in mosquitoes, both larval and adult, was published by Speer (1927). The major contribution since that date has been the description of the fungi of the genus *Coelomomyces*, and the biological role of these is still very imperfectly understood.

The genus *Coelomomyces* was proposed by Keilin (1921) for a fungus of doubtful affinity found in the body cavity of a single larva of *Aëdes albopictus* (*scutellans*) from Malaya. Two similar species parasitizing *Anopheles* were described by Iyengar (1935) from India, and Walker (1938) recorded four forms of *Coelomomyces* from *Anopheles* in West Africa. The genus was reviewed by Couch (1945), who described five more species from North America, parasitic on *Anopheles*, *Psorophora ciliata*, *Culex erraticus*, and *Uranotaenia sapphirina*. Walker made various attempts at experimental infection with these fungi, with inconsistent and apparently inexplicable results. Couch has suggested that Walker's results would be understandable if *Coelomomyces* behaved like the distantly related fungus, *Catenaria*. "In this species the resting bodies germinate only after they have been dried and then, when flooded, only a few germinate at any one time. The rest must be dried again and flooded again before they are ready to germinate." The similarity between this behavior and that of aëdine mosquito eggs is striking. Further experimental work with *Coelomomyces* infections would certainly seem to be warranted because of the biological interest of the relationship, even though such fungi are probably of no practical importance. Muspratt (1946) has reported a considerable mortality in *Anopheles gambiae* larvae in certain situations, caused by *Coelomomyces* infections. Walker (1938)

has reviewed the various other records of fungi of different sorts found infecting mosquito larvae.

Hydrachnid mites are probably best considered as parasites of adult mosquitoes. Although there are various records of hydrachnids on larvae, they are much more commonly found on adults. Brown (1936) found that the mites were rarely able to establish themselves on larvae, but that they frequently infested pupae, passing from the pupal skin to the adult as it emerged.

The literature on the various microorganisms that have been found associated with insects (including larval and adult mosquitoes) has been reviewed at some length by Steinhaus (1946).

PREDATORS

The comparative paucity of known parasites of mosquito larvae is more than counterbalanced by the abundance of predators. It has been fashionable to publish records of mosquito predators ever since the appearance of the famous prize essays sponsored by Dr. R. H. Lamborn in 1889 on dragonflies versus mosquitoes (see Howard, Dyar, and Knab, 1913, p. 165), and the literature on the subject is enormous. Hinman (1934a) in a summary of the predators of mosquito larvae, exclusive of fish, quotes from over a hundred papers. Hinman refers to a paper on "The Use of Fish for Mosquito Control," published in 1924 with a bibliography of 217 titles. Covell (1931b) lists 21 papers dealing exclusively with fish as larval predators. The rate of publication on fish, particularly on *Gambusia*, has surely increased considerably in the period since 1930. With these papers by Hinman and Covell, and the well indexed abstracts published in the *Review of Applied Entomology*, it should be easy to draw up and maintain a list of the animals known to have ingested mosquito larvae at one time or another. The significance of such a list is somewhat dubious, since we know remarkably little about the quantitative relations between mosquito larvae and their predators.

It is possible that the significance of predation in the dynamics of animal populations has been greatly overemphasized in the past, perhaps because of the influence of catch phrases of the post-Darwin period, such as the "struggle for existence," the "survival of the fittest." Predation is most strikingly shown and most easily studied in the vertebrates, and Errington (1946) in a recent review of the whole subject of vertebrate predation has given good reasons for presuming

that population abundance may be more directly due to intraspecific factors, such as territorial adjustments, than to interspecific predation. The concept of territory, of course, does not enter into the biology of mosquitoes, but it is striking that there seem to be no published studies of mosquito-predator cycles, whereas there is abundant evidence of the importance of mosquito-climate cycles.

It has often been pointed out that the fact that a predator eats mosquito larvae under laboratory conditions is merely an indication that it may feed on larvae in the field, and not in any way proof that mosquito larvae form its natural prey. This is illustrated by the controversy over dragonfly nymphs. These feed readily on mosquito larvae in laboratory aquaria, but Smith (1904) pointed out that in nature "they are bottom feeders, as a rule, and usually way below the range of even *Culex*, while *Anopheles* is in no danger from them whatever." In the case of some predators, such as fish, that swallow prey whole, it is possible to get an idea of the types of organisms that serve as food in nature by an examination of stomach contents. With most insect predators this is not possible, and the only recourse ordinarily is field study of the habits of the various organisms concerned.

Brooke and Proske (1946) have suggested a method of determining the prey of insect predators by the use of a precipitin reaction. They found that it was possible to prepare an anopheline antiserum by intraperitoneal inoculations of macerated anopheline pupae in a rabbit, and that this antiserum gave a precipitin reaction when tested against the homologous antigen. The anopheline antiserum reacted with all stages of both anopheline and culicine mosquitoes, but there were no cross-reactions with six species of predators that were tested when in a starved state. The predators did, however, give a reaction when they had recently fed on mosquito larvae. Brooke and Proske made only one test under field conditions, with inconclusive results. Whether the method is practical or not can hardly be judged until extensive field trials have been made.

The nature of the prey of fish can be determined by an examination of the stomach contents, and Hess and Tarzwell (1942) have made a very interesting study from this point of view of the food habits of the well known North American top minnow, *Gambusia*. To measure the food preferences of this fish, they used the "forage ratio," which is obtained by dividing the percentage of a given kind of organism in the stomachs by its percentage in the environment. As

a formula, the forage ratio $= \frac{n}{N}/\frac{n'}{N'}$ where n = number of any organism in the stomachs, N = total number of organisms in the stomachs, n′ = number of the same organism in the environment inhabited by the fish, and N′ = total number of food organisms in the environment. By this system a forage ratio of 1 would indicate that an organism was being taken at random according to its relative abundance in the environment, a higher ratio would indicate preference or greater availability, and a lower ratio the opposite.

They found that "the size of the forage ratio for both anopheline and culicine mosquitoes was directly correlated with their population densities, but when they were present in equal numbers, the greater preference was shown for culicines. As mosquito densities increased, the number of fish eating them and the number eaten per fish also increased. The forage ratio for anopheles was 1 when the larval density was about 2 per square foot of water surface; below this point it decreased and above it increased, reaching a high of over 14 when the larval density was 16 per square foot."

The major animal groups that have been noted as including predators on mosquito larvae are listed below. This list is based on the review by Hinman (1934a) and specific records with supporting references, when not given here, can be found in his paper.

Coelenterata

Various records of Hydra ingesting larvae.

Platyhelminthes

A record of the flatworm, *Planaria*, devouring larvae.

Rotifera

Hinman mentions rotifers destroying larvae.

Annelida

A record of leeches preying on larvae.

Mollusca

Certain snails apparently excrete substances toxic to larvae under laboratory conditions; at least, Gasanov (1938) records *Limnaea* as destroying young larvae and eggs of *Anopheles maculipennis* under both field and laboratory conditions.

Arthropoda

Crustacea

Records of entomostraca and crayfish.

Arachnida

Water spiders are recorded as predators.

Insecta

Ephemeroptera

Several records of predacious May-fly larvae.

Odonata

The controversy over the importance of dragonfly nymphs has been mentioned above.

Neuroptera

Hinman remarks that "little specific information exists."

Hemiptera

The various predacious water bugs are undoubtedly important mosquito predators. Hinman lists records for the following families: Notonectidae, Corixidae, Nepidae, Belostomidae, Naucoridae, Veliidae, and Hydrometridae.

Coleoptera

The aquatic beetles are undoubtedly also important predators on mosquito larvae. Hinman lists records for Dytiscidae, Gyrinidae, Hydrophilidae, and Cicindelidae.

Diptera

Mosquito larvae as predators of mosquito larvae have been discussed in another chapter. Other families with species recorded as larval predators are Chironomidae, Tipulidae, Calliphoridae, Anthomiidae, and Dolichopodidae.

Vertebrates

Pisces

The number of fish recorded specifically as larval predators must be very considerable, though most attention has centered on the North American species of *Gambusia.*

Amphibia

Records include various species of newts and tadpoles.

Reptilia

There are two records of turtles as predators on larvae: one by Hinman for the North American *Pseudemys elegans,* the other by Lewis (1942) for the Sudanese *Pelomedusa galeata.*

Aves

Various records of ducks and shore birds.

COMPETITION

This heading is included as an indication of ignorance, rather than with the object of summarizing any known body of facts. Two illustrations may be cited as a strong indication that interspecific competition does exist among mosquito larvae, even though nothing is

known about it. *Anopheles labranchiae* in the Mediterranean is normally pretty well restricted to breeding in the large brackish coastal marshes. But in Sicily, where no other species of the *maculipennis* group occurs, *labranchiae* has invaded a wide variety of fresh-water habitats (Hackett and Missiroli, 1935). In the New Hebrides only one anopheline is known, *Anopheles farauti* (sometimes called *A. punctulatus*). Here, according to Daggy (1945), the larvae are found in an amazing range of habitats:

> It has been found in almost every type of fresh and brackish water inhabited by mosquitoes, the only exceptions on Espiritu Santo being tree-holes, axils of water-holding leaves, and halves of coconut shells and cacao pods. Its breeding places range from margins of large lakes to hoofprints, from swift streams through all gradations to stagnant swamps, from densely turbid water to crystal clear springs, from relatively cool water to hot springs, from pools and streams with dense vegetation to puddles with no apparent plant growth whatsoever, from open sunlit areas to shaded pools and even the dim interior of flooded buildings and deep, shaded wells.

Both of these cases surely sound as though the normal highly restricted breeding habits of mosquitoes might be in some way related to the problem of avoiding interspecific competition. The succession of species that sometimes occurs in a given habitat with seasonal changes in environmental conditions may also be caused by slight differences in the efficiency of various species in meeting the conditions —differences that would not be significant in the absence of competition. We really know nothing, for instance, about favorable and unfavorable larval densities under various sorts of conditions. The problem is perhaps not easily attacked, but its study would certainly seem warranted.

CHAPTER XI

THE CLASSIFICATION OF LARVAL HABITATS

> Investigators are always divided into those who are looking for rules and those who are looking for exceptions.—L. W. HACKETT

IN ANY faunal list of mosquitoes, the larval habitats of the various species are indicated by brief descriptive phrases, such as "tree holes," "grassy pools," "forest streams," and so forth. The breeding places of each species of mosquito are characteristic, in some cases limited to a single highly specialized and peculiar type of habitat, in some cases including a wide variety of aquatic situations. Probably no two species have exactly the same breeding-place characteristics, for while many species of larvae may be found together in a particular pond or tree hole, the association between two or more species seems rarely to be universal or invariable. The problem of defining these breeding-place characteristics is not, however, simple, as can be inferred from the preceding chapters.

The classification of larval breeding places involves two somewhat different, though essentially related, problems: first, that of the terms or characteristics to be stressed in describing or defining the habitat of a particular species; and second, that of the criteria to be used in studying the relationships among habitats, in building up a classificatory scheme. These problems are in part academic, in part practical. Man simply cannot face the chaos of nature without attempting to introduce some sort of an orderly classification—this is as true of the mosquito habitats as it is of the mosquitoes themselves. Habitats, however, seem to be even less susceptible of orderly classification than the mosquitoes, probably because the criteria defining habitats are less tangible. Yet the study of the development and evolution of habitat adaptations, surely essential to an understanding of mosquito biology, can hardly be undertaken without first achieving some definition and classification of the habitats themselves.

From the practical point of view, it is important to have a classification of the various mosquito breeding places in a region for the intel-

ligent application of control measures. Such a classification may, however, be purely empirical, since its chief purpose is to provide a basis for making records of operations. Practical classifications of anopheline breeding places have, in fact, received far more attention than theoretical classifications.

CLASSIFICATIONS OF HABITATS

Boyd (1930) has given a summary of various schemes used by malariologists for the classification of anopheline breeding places. These mostly have been designed for the practical purposes of mosquito control, and are well exemplified by the system that he himself has used in the southern United States:

A. Rain water or surface drainage:
   a) Artificial containers
   b) Tree holes, leaf bases, and so forth
   c) Overflow, or pools left by receding flood wave
   d) Temporary pools of rain water collected *in situ* (ruts, excavations in areas of low ground-water level).
B. Receiving both surface and ground water:
   a) Bogs
   b) Swamps
   c) Marshes
   d) Ponds
   e) Lakes
   f) Lime sinks
C. Seepage or underground water:
   a) Shallow wells, springs, or seepage outcrops
   b) Pools in areas of high ground water: excavations old or new; intermittent streams pooled.
   c) Dry-weather flow of ditches and small streams
   d) Permanent streams at normal levels (including canals); currentless (at tidal levels); with perceptible current.

Larval surveys have very generally been recorded with some "punch-card" system, and the categories of breeding places are thus most conveniently arranged so that they will fit the available spaces on the recording cards. An excellent example of a larval survey carried out by such a method has been described by Russell and Jacob (1942). They were interested in determining the larval frequency of various anophelines in different types of breeding places in the Madras Presidency, and they used punch cards with the following categories

of habitats: tree hole; artificial container; pit; cart tracks; hoofmarks; ditch; rice field fallow; rice field growing; rice-field channel; swamp; hill stream; river edge; river pool; river-bed pool; rain-water pool; spring pool; irrigation channel; tank or pond; well.

It is notable that half (9 of 18) of the categories used by Russell and Jacob are man-made. Malaria seems often to be a concomitant of human engineering works, and malariologists are always very much preoccupied with roadside ditches, irrigation channels, rice fields, borrow pits, and so forth. These human constructions often differ markedly from the natural habitats of a region, and their invasion by local species thus represents special adaptations on the part of the species concerned. A study of the factors in which the man-made breeding places of a region differ from the natural breeding places might yield very interesting results from the point of view of the adaptability of different mosquito species. Species that become readily adapted to human alterations of the environment are, in general, the dangerous disease vectors.

Shannon (1931) was among the first to become preoccupied with the natural classification of larval breeding places. He observed that "the larvae of each species are more or less restricted to a special type of habitat; and further, the natural classification of habitats is in accord with the natural classification of the family as based on larval and adult characters." Shannon divided the factors governing selection of larval habitat into two classes, one based on "location" of the water, the other on "condition" of the water. His classification based on "location" was as follows:

A. In depressions in the ground.
   1. Natural: lakes, ponds, streams, marshes (tidal and fresh), springs, rock pools, and so forth.
   2. Artificial: reservoirs, ditches, wells, excavations (for example, borrow pits), road ruts, and so forth.

A minor class produced by animals, may be noted, for example, hoofprints, crab holes, wallows, and so forth. The importance of these from the standpoint of mosquito biology is indicated by the fact that one genus, *Deinocerites*, and several species of *Culex* breed exclusively in crab holes.

B. In containers on or above ground.
   1. Natural: water-holding plants (tree holes, bromeliads, and so forth), fallen leaves and nuts, and so forth.

2. Artificial: tanks, barrels, tins, bottles, unused boats, and so forth.

A minor class, consisting of animal remains, may also be noted: egg shells and sea shells, skulls and horns of cattle, and so forth. An accumulation of these is at times an important source of stegomyia production.

Shannon did not attempt to build a detailed classification based on the "condition" of the water, noting only that important factors would be "temperature; whether flowing or stagnant, shaded or sunlit, fresh or foul; presence or absence of salt or other inorganic compounds and dissolved gases; whether with or without plant life; presence or absence of enemies."

Hopkins (1936) has developed a classification of breeding places based on that of Shannon, using "location" and "condition" as the major dichotomy. His major subdivisions are as follows:

I. Location.
   a) Ground pools (including small lakes and weedy edges of larger ones, swamps, springs, rivers, ditches and hoofprints).
   b) Rock pools. "These form a quite distinct class intermediate between (*a*) and (*c*), but with a fauna differing from both."
   c) Small containers. "This class has a very constant fauna, most of the members of which are found nowhere else." Subdivisions of the container class include: tree holes, artificial containers, fallen leaves, plant axils, crab holes.

II. Condition.
   a) Salts or other dissolved inorganic substances.
   b) Dissolved organic matter.
   c) Suspended mud.
   d) Presence or absence of plants.
   e) Temperature.
   f) Light and shade.
   g) Hydrogen-ion concentration.
   h) Food.
   i) Natural enemies.

In a natural classification of mosquito habitats, the factors of "condition" and "location" should surely be considered together. "Location," really, is merely another of the "conditions" defining the larval habitat. More logically, perhaps, one could divide the limiting factors defining breeding places into three classes of "visual" (landscape fac-

tors, size and appearance of breeding place), "physical" (current, wave action), and "chemical," as was done in a field study of Albanian anophelines (Bates, 1941c).

The larval habitat of a particular mosquito species depends primarily on the oviposition habits of the adult, as was pointed out in Chapter VI. The characters defining the habitat, then, are presumably of a sort perceptible to the sense organs of the adult. There are probably four classes of stimuli to which the adults may respond (vision, smell, taste, and touch) and the description and classification of breeding places might well be undertaken in terms of possible reactions to these classes of stimuli.

It seems likely that the visual stimulus is most important. Reaction to visual stimuli is most readily demonstrable under laboratory conditions, and the prominence of "location" among factors defining different species habitats seems likely to be a result of visual orientation. Smell and taste are, of course, difficult to differentiate, but probably there is both a distant reaction (smell) and an immediate reaction (taste) in many situations. The tactile reaction is most clearly demonstrated in the observations on *Haemagogus spegazzinii* quoted in Chapter VI, and it may well be important with many aëdine and sabethine species.

OUTLINE OF A SCHEME FOR BREEDING-PLACE CLASSIFICATION

Various authors (particularly Dyar) have pointed out that the most primitive types of living mosquitoes, such as *Megarhinus*, *Orthopodomyia*, *Trichoprosopon*, live in tree holes or similar container habitats, and consider that this may indicate that tree holes form the original, primitive type of mosquito habitat, and that the colonization of ground pools and streams has been comparatively recent. It seems more logical to me, however, to consider ponds or marshes to represent the most generalized mosquito habitat, even though this habitat may be occupied at present by the most specialized mosquitoes. Primitive mosquito types may have survived in container habitats more often than in marsh habitats because of the reduced competition in such habitats. Thus, by analogy, the most primitive mammals are now found in South America and Australia, but no one takes this as an indication that South America and Australia represent the original home of mammals. Similarly primitive mammals may have very spe-

cialized habits—the living South American sloths, for instance—but no one supposes that these represent the original mammalian habits.

For the purposes of habitat analysis, then, it seems most convenient to consider ponds or marshes as the most generalized habitat of mosquito larva. In protected areas of such bodies of water (that is, where there is a good growth of vegetation), some mosquito larvae can almost always be found, and probably more species are found in such locations than in any other general type of habitat. Specialization may take the form of adaptation to marshes with distinctive chemical properties of the water (salt marshes, peat bogs), or of adaptations to special niches within the marsh (*Mansonia* larvae attaching themselves to grass roots).

If this is the primitive situation, adaptations to other habitat types have occurred in several directions. One line would be to smaller and smaller water accumulations: ponds to puddles to hoofprints and so to transient accumulations of water. Another line would involve increasing modifications of behavior and structure to permit life in various degrees of running water or in pools associated with streams. The specialized container habitat may have been derived from the puddle habitat. Tree holes seem to be the most generalized container habitat, and there is unquestionably a close connection between the habit of breeding in tree holes and breeding in small rock pools, some species (like the tropical American *Anopheles eiseni*) being found in both habitats.

The major division in mosquito breeding places is perhaps between *permanent* and *transient* accumulations of water. The fundamental nature of this division is brought out by the fact that the whole group of mosquito genera related to *Aëdes* require that the eggs be dried before hatching, so that the species all necessarily breed in fluctuating accumulations of water. From the limnological point of view, the difference between transient and permanent water is also of basic importance, since the first type of habitat always requires special adaptations on the part of the inhabitants for the survival of drought periods. The distinction has not received much limnological attention, perhaps because of the tendency to neglect the study of transient water faunas altogether.

The limnologists make their basic division between *standing* and *running* water, calling the first "lenitic environments," and the second "lotic environments." The adaptations among mosquitoes to standing

versus running water are not, in general, very profound. This is true chiefly because no mosquitoes have invaded truly lotic environments: the mosquitoes that are found in streams and stream associations are adapted to life in areas of quiet water in the streams—in stream pools, back eddies, seepage areas, and so forth. Their habit adaptations are aimed at keeping them out of the current, rather than at enabling them to live normally under the force of the current. Thus there is no large taxonomic group of mosquitoes adapted to running-water habitats; the ability to inhabit stream situations has rather arisen repeatedly at the specific level.

Both Shannon and Hopkins have emphasized the importance of ground versus container habitats. The adaptations to special water containers are certainly among the most striking and interesting of the habit modifications shown by mosquitoes, and container-breeding is generally a characteristic of genera or subgenera, not of isolated species. Containers thus surely form one of the major subdivisions of larval habitats.

From these various considerations it seems logical to divide larval habitats into four major groups: A, permanent or semipermanent standing water; B, running water; C, transient ground pools; and D, containers. How to arrange a hierarchy of specific habitats under these major headings is another, and more difficult problem. For the purpose of discussion in the present book the following scheme is used:

A. Permanent or semipermanent standing water
  Fresh water:
    1. Large marshes or marshy zones in lakes
    2. Small ponds or marshy areas in the open
    3. Special situations, such as spring-fed pools
    4. Special plant associations
    5. Special chemical conditions, such as peat bogs
    6. Swamps
    7. Forest pools
  Brackish water:
    8. Brackish marshes and swamps
    9. Small accumulations of brackish water
B. Running water:
    10. Open streams in association with vegetation
    11. Open gravel stream beds
    12. Forest streams

C. Transient ground pools:
   13. Transient ground pools in the open
   14. Transient forest pools
D. Container habitats:
   15. Rock holes
   16. Tree holes
   17. Ground containers
   18. Special plant associations
   19. Crab holes

A. *Permanent or semipermanent standing water*

"Permanent" with regard to fresh water is at best a relative term, since the prime biological characteristic of all fresh water is its impermanence, at least in geological time. In the present connection the difference between permanent and transient water may perhaps best be based on vegetation: water that remains in place long enough to develop a characteristic aquatic vegetation may, from the mosquito point of view, be considered as permanent; and water which does not remain long enough to result in modification of vegetation as transient. Tropical marshes which disappear in the dry season but contain water for the rest of the year would, by this system, be classed as permanent, and their mosquito fauna is, in fact, similar to that of neighboring marshes that, because of greater depth or greater size, fail to dry. Transient waters in the tropics dry up several times during the year.

Rigid distinction between standing and running water is just as difficult as the distinction between permanent and transient water. The difference between a mountain stream and a lake seems clear, but in practise the ecologist is constantly encountering situations that from one point of view are "standing water," and from another "running water." Generally, of course, "perceptible flow" is an adequate criterion.

Fresh water

The vast majority of mosquito species occur in fresh water—in water with a chloride content so low as not to be perceptible to taste. Adaptations to brackish and salt water occur, however, in many genera, and the major dichotomy under "permanent standing water" is thus perhaps most logically made between fresh and brackish habitats.

1. *Large marshes or marshy zones in lakes.* This, as was pointed

out above, may be regarded with some reason as the most generalized of larval habitats. It is occupied in every part of the world by many species of *Anopheles* and *Culex*. It seems doubtful whether, from the mosquito point of view, there is any important distinction between a lake and a marsh. The criterion of a lake is open water, and mosquito larvae do not occur in such water, since they require the protection of vegetation. Marshes, for the most part, represent a stage in lake succession where shoal conditions permit the general growth of vegetation: this may represent only a part of the lake area, or the whole lake area.

Size of the body of water apparently is important from the point of view of mosquito ecology. Thus in Albania, we found that *Anopheles subalpinus* seemed to be restricted to habitats associated with large accumulations of water, while typical *maculipennis* showed no such restriction (Bates, 1941c).

Limnologists have given a deal of attention to the classification of lakes, and there is a considerable literature on the subject which has been summarized in such books as those by Pearse (1926), Hesse, Allee, and Schmidt (1937), and Welch (1935). These lake classifications again seem to be largely irrelevant from the mosquito point of view, though little attention has been given to the subject by entomologists.

The type of vegetation is probably important in defining specific mosquito habitats within this general class, especially whether the vegetation is emergent or horizontal. Adaptations to rice fields represent a modification of the marsh-breeding habit, and Russell and Rao (1942c) have shown the importance of the type of vegetation in determining the species composition in such a case.

2. *Small ponds or marshy areas in the open.* The distinction here is primarily of size of the body of water. Most ditches, borrow pits, and similar artificial breeding places represent this general class, which is inhabited by a large and varied mosquito fauna. These smaller habitats, although essentially permanent from the limnological point of view—that is, characterized by special aquatic vegetation, and by the presence of purely aquatic animals such as fish—are more apt to dry up in whole or in part at some time during the year, and thus may at times include species of *Aëdes, Psorophora,* and related genera, as well as the more usual *Anopheles* and *Culex.*

Specific subdivisions of this habitat would again probably be based largely on type of aquatic vegetation, perhaps also on the physical

and chemical condition of the water, and on the amount of shade. Shade seems always to be an important factor in defining mosquito breeding, and for that reason shaded and open habitats are classed apart in the present scheme; yet in the case of small ponds and marshy areas there is every intergrade from those that are completely open to those that are completely shaded, forming part of a forest or swamp. The tropical American *Anopheles darlingi*, for instance, seems to breed typically in small partially shaded ponds.

3. *Special situations, such as spring-fed pools.* Peculiar types of small ponds and pools are apt to occur in different parts of the world, and are apt to have a special fauna. They are thus logically classed apart from the more generalized small ponds and marshes. Spring-fed pools are perhaps the commonest example of this type: they are usually characterized by unusually clear and cool water, and although very small, may have an open center with marginal vegetation. Spring pools usually have a relatively high turnover in water, and thus pertain to the type of habitat that is somewhat intermediate between standing and running water. Division into specific habitats would again mostly depend on the physical and chemical conditions of the water—thermal characteristics (cold springs, hot springs) and unusual mineral contents. *Anopheles claviger* in southern Europe is characteristically an inhabitant of cool spring-fed pools, and in this case temperature seems to be the determining factor, since the species is found in a wider range of habitats in southern Europe in early spring and in northern Europe throughout the year.

4. *Special plant associations.* In some cases the mosquito fauna of permanent standing water seems to be associated with some specific plant rather than with the more general character of the marsh, pond, or pool. One such plant, widely found in the American tropics, is the water hyacinth (*Piaropus*). Komp (1942) reports water lettuce (*Pistia*) to be a favored habitat of *Anopheles triannulatus*. Where the association with a particular plant is clear, it is probably better to use a separate habitat category.

5. *Special chemical conditions, such as peat bogs.* Brackish waters could, of course, be considered as representative of "special chemical conditions," but they are so widespread that they warrant separate treatment. Peat bogs, with their highly acid conditions, represent a special habitat that has been much studied in the northern hemisphere (Irwin, 1942).

6. *Swamps.* In the present scheme, a "marsh" is considered to be an extensive area of inundated open country (savannah), while a swamp is considered to be an inundated forest. Shade is a very important factor in defining mosquito breeding, and thus the faunas of marshes and swamps are fundamentally different.

7. *Forest pools.* The great swamps, of course, are apt to include areas of open water where conditions approach those of lakes and ponds; on the other hand, they grade into semipermanent forest pools and into temporary forest pools—the latter a particularly important type of mosquito breeding place under tropical conditions. The distinction between swamps and forest pools, primarily a matter of size, would seem, however, to be important.

Brackish water

As was pointed out in Chapter VIII, the majority of mosquito species known to breed in water with a high sodium-chloride content seem to be characterized by a *tolerance* for this condition, rather than by a *preference* for it. Species which are able to tolerate a high salinity, however, become dominant in maritime areas, and constitute an important special fauna. If a major dichotomy is made between fresh and brackish habitats, the detailed classification of the brackish habitats should probably parallel that of the fresh ones, since the whole gamut of conditions of size and shade may be found with both types of water. Once salinity is used as a criterion, however, there is also the question of amount of salt, since some species can tolerate a slight salinity, while others may be found even in water with a salt content higher than that of the sea.

A complex classification of brackish habitats would, however, probably defeat its purpose, and the simplest possible subdivision of major significance from the mosquito point of view is perhaps based on size.

8. *Brackish marshes and swamps.* Here would be included such notorious mosquito habitats as the salt marshes of New Jersey and the mangrove swamps of the tropics—large areas of brackish water, with specific habitats depending on amount of salinity, amount of shade, and on special climatic and geographical features.

9. *Small accumulations of brackish water.* All of the other brackish habitats could be lumped under this heading. Probably again shade and salinity would be the primary criteria for subdivisions.

B. *Running water*

Perhaps again it should be emphasized that no mosquitoes are known to be adapted to life in running water in the sense that species of *Simulium* or of caddis flies, for instance, are so adapted. Yet many species, particularly many unrelated groups of *Anopheles*, have become adapted to situations associated with running water, and the presence or absence of current in the general environment is undoubtedly the limiting factor in defining the habitat.

10. *Open streams in association with vegetation.* This habitat merges imperceptibly with the category of "small ponds or marshes." Islands of *Potamogeton* in large rivers, for instance, may be inhabited by typical marsh mosquitoes. Even though the river itself has considerable current, surface water in the island of vegetation will be still. Again, the fauna of the grassy margins of streams may be the same as the fauna of the grassy margin of a pond. A great many species typically associated with streams are, however, rarely or never found in association with apparently similar accumulations of still water.

11. *Open gravel stream beds.* A few species of mosquitoes, especially of *Anopheles*, in various parts of the world have adopted the gravel beds of braided streams as a characteristic habitat. *Anopheles superpictus* of southern Europe and *A. pseudopunctipennis* of tropical America represent this group. The larvae live in areas where the water seeps through the gravel, and in small areas of still water. There is frequently also a growth of filamentous algae in such situations. Since these stream species are greatly affected by floods, they have a characteristic peak of abundance during dry periods.

12. *Forest streams.* The fauna of forest streams is quite distinct, including many characteristic species of anophelines in various parts of the world, such as the species of *Chagasia* and *Stethomyia* in tropical America and *Anopheles marteri* in southern Europe. In general each species occupies a special sort of a niche: *Anopheles marteri*, for instance, in quiet pools among large rocks below waterfalls; *Chagasia* among the roots of plants like tree ferns; *Stethomyia* among dead leaves and other debris along stream margins.

C. *Transient ground pools*

The main justification for treating transient pools as a major subdivision of mosquito habitats is, as was pointed out above, the adapta-

tion of aëdine genera to life in such situations through suspension of development in the egg stage. Such mosquitoes are, in fact necessarily inhabitants of transient or fluctuating water accumulations in cases where desiccation is a necessary preliminary to egg hatching. In many cases the larvae, once hatched, develop with extraordinary rapidity —another necessary adaptation for a transient habitat. The basic subdivision of temporary ground pools would be into shaded and open —pools in the forest, and pools in grassland.

13. *Transient ground pools in the open.* Temporary pools in meadows and savannahs are apt to be inhabited by different species of *Aëdes* and *Psorophora* from pools in forest. They are also very frequently utilized by species of *Anopheles* and *Culex* if they remain for more than a few days without drying. Further division of this category into specific habitats would probably vary in different geographical areas. A widespread special class consists of hoofprints, cart tracks, and similar very small temporary puddles, which are often invaded by certain species of anophelines: except for hoofprints in muddy areas such as pond margins or seepages, this class of breeding place is pretty much a by-product of human activity.

14. *Transient forest pools.* This is an important category of breeding places, especially in the tropics, and in many places accounts for a large proportion of the mosquitoes—in bulk if not in numbers of species. Various species of *Aëdes* and *Psorophora* are often found in different pools, but no study seems to have been made of differences that might underlie subdivisions of this general habitat.

D. *Container habitats*

This class of larval breeding place could be subdivided endlessly, since in the tropics almost every type of water container has its peculiar species of mosquito. Under natural conditions, container habitats are a purely forest phenomenon since no types of natural containers occur in the open, with the possible exception of small shells. Man-made containers in such situations, such as tin cans, are apt to be invaded by adaptable species from a variety of natural habitats.

For present purposes containers may conveniently be grouped under four headings: rock holes, tree holes, ground containers, and special plant associations. A fifth category, crab holes, is added here because it is a specialized habitat that seems to fit nowhere else.

15. *Rock holes.* Rock holes seem to form a transition between pool

habitats and container habitats. A few species, such as the tropical American *Anopheles eiseni*, may include rock pools, rock holes, and tree holes within the range of habitats; other species, such as some tropical American *Aëdes* (as *fluviatilis*), seem to be restricted to rock holes, especially holes in the large rocks in mountain-stream beds. Such rock holes when deep and narrow are also occasionally invaded by tree-hole species.

16. *Tree holes.* These form the most widespread class of container habitats, with a specialized fauna in the temperate zone as well as in the tropics. Different tree holes may contain different species of mosquitoes, but little work seems to have been done on the possible basis of this selection. The genus *Orthopodomyia* and certain subgenera of *Culex* and *Aëdes* characteristically inhabit tree holes, with, in addition, a wide variety of sabethine species in tropical America. *Anopheles* has in general failed to invade the tree-hole habitat except for a small group of closely related species including *plumbeus* in Europe, *barberi* in North America, and *barianensis* in Asia. In tropical Africa and America no species seems to have become fixed in this habitat, though *eiseni* is occasionally found in tree holes in tropical America.

17. *Ground containers.* All kinds of things capable of holding water fall to the ground in a tropical forest, and have become the habitat of a variety of characteristic mosquito species. Fruit husks, snail shells, flower sheaths, dead leaves, trunks rotting on the ground, all may hold water and breed species of *Eretmapodites*, *Trichoprosopon*, *Wyeomyia*, and so forth. Forests in the temperate zone are less productive of such water containers, and this mosquito habitat seems to be unknown there. Perhaps the chief factor determining which mosquito species occurs in which container is the condition of the water: some species may breed in containers with very foul water, while others are found only in clear rain water.

18. *Special plant associations.* The association of the larvae of certain species of mosquitoes with water collected by certain species of plants forms one of the most fascinating sections of all mosquito biology. Yet it has been subject to relatively little study, perhaps because it is primarily a phenomenon of the tropical forest. One association, that of *Wyeomyia smithi* with water in the leaves of the pitcher plant, *Sarracenia*, occurs in temperate North America and has been the subject of considerable study.

Three large groups of plants should perhaps be segregated because

they retain collections of water that are utilized by many different species of mosquitoes, often occurring in association. These are the bamboos of various parts of the world, the bromeliads of the American tropics, and *Nepenthes* of the Old World tropics. The conditions governing species distribution in bromeliads have been studied by Pittendrigh in Trinidad (mostly unpublished, but some data in Downs and Pittendrigh, 1946): important factors are the height of the plants on trees, the kind of plant, and the amount of shade. Similarly in bamboo, the species of mosquitoes found in water in a particular internode may depend on the size of the opening, the height above ground, and the condition of the water (foul or clean).

In addition to these habitats occupied by a number of mosquito species, there are many cases in tropical forests in which a water accumulation in a particular type of plant is inhabited by a single and peculiar species of mosquito. Thus in the vicinity of Villavicencio in eastern Colombia one species of *Wyeomyia* has been found only in the leaf bases of a species of *Xanthosoma;* the leaf bases of another type of aroid are occupied by another species of *Wyeomyia;* water in the bracts of *Heliconia* flowers by still another *Wyeomyia;* water under the petioles of the giant banana-like *Ravanela* by another very characteristic *Wyeomyia,* and so forth. Every imaginable water accumulation in the forest seems to have acquired some species of mosquito adapted to its peculiar conditions.

19. *Crab holes.* Crab holes, as the habitat of the mosquitoes of the genus *Deinocerites,* seem to form a habitat class by themselves. This specialization may have had its origin from temporary ground pools or from some container habitat.

All of this adds up to a demonstration that, despite the accumulated verbiage, we still lack the information necessary for a sharp definition of the characteristics of the habitats of individual mosquito species, or for the construction of a logical classification of such habitats. In particular, enough attention has not been devoted to the *limiting factors* of the ecological distribution of larvae: a subject that will probably have to be approached through a study of the ovipositing habits of the adults. There is also a great lack of *quantitative data* of the sort that would be necessary for the elucidation of the environmental relationships of mosquito larvae. A great deal could probably be learned through the analysis of the frequency of association of differ-

ent larval species in particular situations, as was done, for instance, by Russell and Rao (1940) in their study of anopheline habitats in south-eastern Madras. The study of larval density offers great possibilities, as does the study of the rate of survival of different species in given habitats. Above all, we need a closer integration of field observation and laboratory experiment, and the development of new techniques, especially for the accumulation of quantitative data. Such studies are not easy, but the possible rewards in the uncovering of biological principles underlying the environmental relations of organisms would seem to warrant the effort.

CHAPTER XII

THE PUPAL STAGE

> A developing organism is thus a system struggling with the help of its ancestral tendencies to survive and to convert itself into successive viable shapes.—G. R. DE BEER

INSECT metamorphosis provides a fascinating field of study: a field that has been well exploited at the descriptive level, but that has not provided the contribution to biological speculation and theory that one would expect. The general subject of the evolution of the insect larva has been reviewed by Chen (1946), who points out the inadequacies of the theory of Brauer, who regarded the diversity of larval forms as the result of secondary and adaptive modifications of a primitive type, and of Berlese, who considered that larvae are precociously emerged embryos whose diversity of types is primarily a consequence of variations in time of emergence. Chen considers that all insect larvae are derivable from a common ancestor, and that existing types of larvae have probably arisen in two ways: by specialization and (in the case of certain parasitic hymenoptera) by precocious embryonic emergence or "progenesis." He points out that specialization of larval types has taken place chiefly through the reduction of appendages, proceeding from the posterior part of the body to the anterior, and governed by a progressive degree of inhibition of imaginal growth. This inhibition of imaginal growth is initiated in the egg, and the pupal stage serves to restore the original line of development by the removal of the inhibitive control. He considers that the earliest larvae were probably aquatic.

As Wigglesworth (1939) has pointed out, the holometabolous insect (that is, the insect with complete metamorphosis) may be looked upon as made up of two organisms, the larva and the adult, existing in a single individual. Various experiments have shown that in *Drosophila* there is "a wave of imaginal determination spreading throughout the egg, quite separate in point of time from the larval determination,

and occurring at a stage of development when histological differentiation of both larval and imaginal structures is entirely wanting." Actual metamorphosis, the change of the animal from the larval form to the adult form effected during the pupal stage, might therefore be regarded as the progression of this adult determination to the stage of visible differentiation. "This conception would make metamorphosis, what many have considered it, a return to embryonic development."

If we combine the ontogenetic conception of Wigglesworth with the phylogenetic theory of Chen, we must regard the larva not as a perambulating embryo, but as a specialized, free-living organic form which carries with it, in a more or less suspended state, the embryonic material of the adult form. The larva and the adult are subject to quite different environmental pressures, especially where they form parts of distinct biological communities, and as a result may have independent, though necessarily parallel, evolutionary histories. For this reason generalizations concerning ontogenetic development, such as the "laws of von Baer," often seem quite irrelevant when one attempts to apply them to insects with complete metamorphosis.

The laws of von Baer have been restated by de Beer (1940) as follows:

1. In development from the egg the general characters appear before the special characters.
2. From the more general characters the less general and finally the special characters are developed.
3. During its development an animal departs more and more from the form of other animals.
4. The young stages in the development of an animal are not like the adult stages of other animals lower down on the scale, but are like the young stages of those animals.

Of these four generalizations, only the last has direct application to holometabolous insects such as mosquitoes; but the others acquire meaning if we consider the larva and adult as two different organisms. Thus in larval development, the first-stage larva is apt to show more general characters than the later stages, a situation that is utilized by morphologists, for instance, in attempting to work out the homologies of larval setae. But the larval form may develop a considerable degree of specialization without this having any reflection in the adult form.

This is brought home to the student by the frequency with which the *larvae* of different mosquito species may be readily distinguishable by morphological characters, although *adults* may seem identical—or, perhaps more rarely, vice versa.

We shall return to this problem later in considering the basis of the classification of mosquitoes. It has relevance, however, to physiological and ecological, as well as morphological studies. In all work on insect biology one must perform a sort of sustained operation of mental juggling, considering the characteristics of the larva and adult as separate and independent phenomena, the products of different environments and different pressures; yet remembering always that these two organic forms are simply aspects of the same organism, and that any change or influence affecting the one form must inevitably also involve to some extent the other form.

The pupal stage, with this concept, provides the mechanism for the resumption of the embryonic development leading to the adult form. Its function, however, is not only to provide for the morphological and physiological changes incident to the transformation of the larva to the adult: it must also serve as a bridge whereby the organism can pass from the larval environment to the adult environment. This ecological function is particularly important where the two environments diverge as widely as they do in the case of mosquitoes. The structure and behavior of mosquito pupae are only intelligible if this is kept in mind.

THE ECOLOGICAL FUNCTION OF THE PUPA

The pupal stage of insects is generally motionless. In most flies the pupa is formed inside of the last larval skin (the so-called "coarctate pupa"), which becomes hard and rigid so that no movement at all is possible. In other flies and in the lepidoptera, the wings and legs of the pupa are firmly soldered down to the body by the molting fluid after the final larval molt, and movement is limited to a wriggling of the abdominal segments (the "obtect pupa"). Such movements usually serve for little more than to turn the pupa over, or perhaps to work it into a position favorable for the adult emergence. Mosquito pupae, although belonging to this type with power of movement limited to the abdomen, manage to be very active—to move speedily and for considerable distances. They are undoubtedly the most active of all insect pupae.

Wesenberg-Lund (1921) has discussed this peculiarity of mosquito pupae at considerable length. In the first place, he observes that while mosquito pupae can be very active, the power of locomotion is generally highly overrated. "If we keep mosquito pupae in aquaria, it can be shown that voluntarily they never leave the point of support which they have once acquired, that is to say: if the aquarium is never moved and if it always stands in the shade. I have in my aquaria reared imagines from pupae which most probably have never made a single somersault movement. I suppose that even in nature it may happen that many pupae moult without having made more than very few movements." He considers that stimuli leading to pupal movement in nature are probably chiefly sunbeams (pupae are very sensitive to light changes), raindrops, and disturbance caused by possible predators. The total energy liable to be used by a pupa in movement is probably small, however, because of the short duration of the pupal stage—only two or three days with most species under natural conditions, and in some cases even less.

It is notable that adaptations for the survival of prolonged periods of adverse conditions have not been found in any mosquito pupae. With most insect groups hibernation is commonly a function of the pupal stage, and diapause phenomena (periods of suspended development) are often found. Mechanisms of hibernation or aestivation are found in the egg, larval, and adult stages of mosquitoes, but not in the pupal stage. This is probably related to the mobility of the pupa, it being impossible to provide for a suspension of development in an active stage (such as the pupal mosquito) in which there is no mechanism for the intake of nourishment.

The peculiarities of the mosquito pupal stage, then, are short duration and power of movement. Wesenberg-Lund places these characteristics in perspective by a review of the various mechanisms which exist in other aquatic insects for providing for the transition from the aquatic to the terrestrial environments. Many aquatic insects leave the water as larvae and pupate in terrestrial situations: the transition here is accomplished by the prepupal larva. In May flies there is a "highly peculiar subimago state . . . in which the legs and wings are often covered by a coating of minute thorns which hinder the wings from getting wet." In the Trichoptera the mature pupae leave the larval cases and become free-swimming organisms, reaching the water surface through swimming movements with the specialized meso-

thoracic legs (or in some cases crawling to the surface over vegetation). Once attached to the surface film, the pupal skin, when it has burst, serves as a support for the emerging adult. Chironomids and mosquitoes use this same mechanism, the floating pupal skin serving as the first support of the adult in the terrestrial environment.

Black flies and some aquatic lepidoptera ascend to the surface as adults: the black flies using "air from the pupal tracheal system as air-bubbles, wrapped up in which they ascend as imagines to the surface with extraordinary rapidity; the moment the surface is reached the bubble bursts and the fly stands dry upon the waves." In aquatic lepidoptera, the ascent to the surface is made with an air bubble held between the wings and the thorax, and the adult when it leaves the pupa "is covered with a wax-like substance which prevents the body from getting wet."

Thus the problem, with aquatic insects, of how to launch the adult into the terrestrial world dry-shod has been met in various ways. Since with mosquitoes the larval life is passed at the surface, it is to be expected that pupal adaptations would be for surface existence, where the pupal skin can conveniently serve as the launching platform for the adult. The two adaptations of speedy development and power of movement, then, are related to this exposed surface position.

PUPAL BEHAVIOR

The pupa in most mosquito species is considerably lighter than water, its buoyancy due to a large air space between the wing cases on the underside of the cephalothorax. By vigorous movements of the abdomen, which terminates in two large paddles, the pupa can propel itself with considerable speed, though without control of direction, through the water, gaining depth by a series of somersaults. When movement stops, the pupa rises directly to the surface where its position ("stance") is determined by the subcutaneous air bubble which forces the dorsum of the cephalothorax against the surface film, where orientation is further governed by the spiracles ("air trumpets") and the two large stellate hairs of the first abdominal segment—making four points of attachment to the film. Cases in which the pupa can remain submerged without active movement may be rare, though we have observed such behavior in various tropical container breeders; *Megarhinus* pupae, for instance, will sometimes lie quiescent on the bottom of a dish for considerable lengths of time.

Pupal behavior has received little attention from students. In general the pupae lie motionless, usually touching some emergent object (vegetation), an orientation that is probably governed by surface tension forces as described by Renn (1943) for larvae. They respond to visual or tactile stimuli as described by Wesenberg-Lund, but apparently no one has studied this behavior in detail. One gets the impression, from handling various kinds of mosquitoes in the laboratory, that there may be considerable differences in pupal behavior among species.

It is probable that each species of mosquito has a characteristic time of day for pupation, though the data on this point are meager. Sen (1935) found that the eight common species of *Anopheles* of Bengal all pupated most commonly during the day. Lamborn (1922), on the other hand, found that the Malayan anophelines mostly pupated during the night. Neither author has attempted to determine whether there was a definite cycle in pupation characteristic of a given species and correlated with the daily environmental cycle. Rozeboom (1936) found that pupation with *Anopheles albimanus* in Panama occurred most actively at midday. We have found the same to be true with *Anopheles darlingi* in Colombia. With other species, however, such as *Anopheles mediopunctatus,* we have found that pupation generally takes place at night.

The length of the pupal stage in a given species is probably purely a function of temperature. Thomson (1940c) found with *Anopheles minimus* that the pupal stage lasted 4½ days at 16°, 2¾ days at 20°, 1¾ days at 24° and 1¼ days at 30°; at 35° the pupae did not develop. In a series of experiments with *Anopheles darlingi* in Colombia, we found the mean length of the pupal period at 30° to be 28.9 hours, with a minimum period of 27 hours, and a maximum of 30 hours. Since checks were made every hour, there is a probable observational error of two hours in the extreme cases, so that it is likely that the length of pupal life in a given species under constant temperature conditions is extremely regular. The precise length of the stage varies greatly with different species. With *Anopheles benarrochi,* a species very similar to *darlingi* but with a slightly slower rate of larval growth, the mean length of the pupal stage at 30° was found to be 32.5 hours. With many container-breeding mosquitoes, such as species of *Wyeomyia,* the pupal stage may last for several days under comparable temperature conditions. With *Haemagogus spegazzinii* we found that

the pupal stage lasted 6.9 days at 20°, 4.9 days at 25° and 3.0 days at 30° (Bates, 1947b).

Both Sen (1935) and Rozeboom (1936) have observed that anophelines of a given species are apt to emerge from the pupa at a given time of day. It seems likely from the foregoing that this is controlled by the time of pupation, and that the significant point for study would be the factors of the daily environmental cycle controlling time of pupation rather than time of emergence.

Various authors have described the emergence of the adult from the pupa. The following account is from Marshall and Staley (1932, quoted in Marshall, 1938):

When the straightening of the pupal abdomen signifies that the time for emergence has arrived, the scales of the adult become visible through the pupal skin, and rhythmical movements of the pharyngeal pump commence. The air from the "vescicle" begins to creep towards the head, forming a bubble at the base of the proboscis before spreading generally under the pupal skin. Before the air has entirely surrounded the adult, a portion of it is aspired through the oral aperture and passed into the mid-gut, where it forms a long, narrow bubble reaching as far back as the fourth abdominal segment and having a diameter about half that of the abdomen. At the same time a further straightening of the adult's abdomen occurs (due in part to muscular action and in part to the increase of internal pressure caused by the swallowed air) with the result that the thorax is pushed forwards until it ruptures the pupal skin and thereby establishes direct communication between the oral aperture and the external air. Air is now swallowed more rapidly, further extending the abdomen and increasing its rigidity, the legs and abdomen meanwhile making alternate movements to facilitate the act of emergence. By this time the air within the mid-gut has extended back as far as the sixth segment, the wall of the stomach being now very tenuous and in close contact with that of the abdomen. As soon as the fore-legs are entirely (and the mid-legs nearly) withdrawn from the pupal skin, the body of the adult, which has so far maintained a vertical position, bends forward until the fore-legs rest upon the water surface. The hind-legs are soon freed in their turn, the fully emerged adult then resting for a brief period, either upon the discarded pupal skin or upon adjacent vegetation.

CHAPTER XIII

MOSQUITOES IN RELATION TO OTHER ORGANISMS

> When an ecologist says "there goes a badger," he should include in his thoughts some definite idea of the animal's place in the community to which it belongs, just as if he had said "there goes the vicar."—CHARLES ELTON

THE difficulty of analyzing the interrelations of organisms in communities has been touched on in the chapter on the biological environment of the larva. In the case of adult mosquitoes the difficulties are increased by the mobility of the animals—the mosquito can be kept under observation only for short periods of time, and then only uncertainly and with difficulty. Yet the adult mosquito does not live isolated in the physical environment; its life is controlled and conditioned not only by temperature and humidity, but by the host of other living organisms with which it is associated. And some of these associations, such as the vector relationship with the causative organisms of human disease, are of surpassing and direct interest to us—the human animal.

All ecological textbooks emphasize the difficulty and complexity of the relationships within biotic communities. These relationships are then illustrated with concepts like that of the food chain, such as grass to grasshoppers to frogs to snakes to hawks. There is a great deal of discussion of "key-industry animals" who convert the vegetable material into the first of the animal predator links; and of the dominant predators who end the chain and lord it over the rest of the community.

One wonders where to fit mosquitoes into these schemes. They seem never to be key-industry animals, nor lordly terminal links: in fact it is hard to assign them any position in an abstract chain. The adults, like the larvae, are always subordinate and incidental parts of their community, despite their nuisance value to even the most lordly

member of this community. They probably get blood from all sorts of sources; they in turn are probably preyed upon by all sorts of middling predators; from this point of view they are inconspicuous members of the vast biotic proletariat, equally ignored by hawks and by ecologists.

The only method of attack on this problem that I can see is to distort the community by focusing on the mosquito and then attempting to determine how the lines of relationship run. There seem, in this case, to be four major types of relationship, for each of which a label is handily available: we find mosquitoes acting as *parasites* on other animals; mosquitoes as *prey* for numerous types of predators; as *hosts* in turn for various parasites; and in a special form as *vectors* of certain parasites whose major interest comes from their relations with a third party such as man. Besides these four outstanding relationships there are all kinds of vague and special lines: mosquitoes getting protection from vegetation, males getting sustenance from plant products, females searching out particular plants as part of the oviposition behavior, perhaps mosquitoes resting on cobwebs and then becoming inaccessible to ants. Then there are the relationships that always attract attention because they are so outstandingly queer, like the *Harpagomyia* mosquitoes that titillate the *Cremastogaster* ants into giving them food.

These miscellaneous and vague relationships can be deduced, in so far as we know anything about them, from the general discussion in the preceeding chapters of this book. Their neglect here is not due to an underestimation on the part of the author of their importance to biological theory: it is due to his complete failure in attempts to organize all community relations of mosquitoes into a neat and logical scheme, which again may be blamed perhaps partly on the inadequacy of the information available. Hence the remainder of this chapter will be concerned only with mosquitoes as parasites, as prey, as hosts, and as vectors. The two following chapters are then dedicated to a more detailed analysis of two groups of vector relationships.

MOSQUITOES AS PARASITES

The adult bloodsucking mosquito may be considered as a parasite. This involves us in the problem of the definition of "parasite"—a legalistic problem that soon becomes entangled in fine distinctions among parasitism, predatism, and commensalism. Wheeler (1928, p.

52) has remarked that "it would be easy to show by the citation of many examples that parasitism is an extremely protean phenomenon, one which escapes through the meshes of any net of scholastic definition in which we may endeavor to confine it." Wheeler himself considers "parasitism" to include "any complex of vital processes, which maintain themselves at the expense of other vital processes, in the same or other organisms, without reacting on these processes in an equivalently sustentative manner."

More simply, Wheeler points out that "predatory animals kill other animals and devour them wholly or in part; parasites put other organisms in the position of 'hosts' by living directly on their tissues in such a manner as not to cause their immediate death." Stunkard (quoted by Herms, 1939, p. 21) has simplified the definition even more: "A parasite is an organism which lives at the expense of its host, giving nothing of value in return." This, of course, fails to distinguish sharply between parasitism and predatism, but most definitions break down at this point. By predatism, of course, we mean a big animal killing and eating a little animal—the lion and the sheep. By parasitism, we mean a lot of little animals annoying a big animal—the fleas on the lion. The distinction in ordinary practice seems clear enough, whatever the difficulties presented by the "parasitoid" hymenoptera and similar borderline cases.

It is interesting to note the direction of adaptation in parasites and hosts: the adaptation of the parasite is to the host, while host adaptations involve avoidance of, or protection against, the parasite. In cases where the relation between the two organisms is a partnership—and the line between parasitism and mutualism or symbiosis is not always easy to draw—the adaptations are converging. Hence the emphasis on the definitions of both Wheeler and Stunkard on absence of reciprocity in parasitism.

Parasites can be classified in all sorts of ways, depending on the kind of host, on the behavior of the parasite, on its life cycle, and so forth. Many words have been coined for particular parasitic types: ectoparasites, endoparasites, phytoparasites, facultative parasites, obligate parasites, and so forth. Herms (1939) classes mosquitoes as "intermittent parasites, which prey on the host at times only."

The ancestors of mosquitoes were probably phytophagous or saprophagous, perhaps with the food habits that persist in the males today. But the bloodsucking habit must be an ancient one to have

permitted the development of complex parasite histories like those shown by *Plasmodia,* and the loss of the bloodsucking habit in some contemporary mosquitoes may well be a secondary development. Such loss of a parasitic habit is notably rare in animals, and shows how little fixed the intermittent parasitism of the adult mosquito has become, despite the presumed age of the habit. It is also notable that mosquitoes do not show great specialization for particular hosts. Species and groups of mosquitoes may differ broadly in their host preferences, but they have not developed that sharp host specificity that characterizes more fully developed parasites—fleas, for instance.

The characteristics of the parasitism of adult mosquitoes have been discussed in Chapter V, since an account of the food habits is essentially a description of the parasitism. The subject is introduced here again only to emphasize the fact that mosquitoes themselves are essentially parasites, as well as being, in turn, hosts and vectors of other parasites.

MOSQUITOES AS PREY

A considerable number of different kinds of predators have been observed to capture adult mosquitoes, as can be seen from the records compiled by Hinman (1934b). It is even more true of adults than of larvae, however, that we have really no data on the relative importance of different types of predators in the control of natural populations. The list of observed predators is so haphazard that there seems no point in quoting or summarizing it.

Mosquitoes would undoubtedly form part of the diet of any of the great host of predators that live at the expense of the even greater host of medium-sized insects. The importance of a given predator would depend on how far its habits coincided with the habits of mosquitoes: in the case of mosquitoes in flight, the predator would have to be active at the time and place of the mosquito flight; in the case of resting mosquitoes, the predator would have to occur in the sort of situation used by mosquitoes for shelter. Thus in studying mosquitoes and predators we come, as always, against the problem of our ignorance of the resting and flight habits of the vast majority of mosquitoes species.

Bats and dragonflies have attracted much notice as predators of mosquitoes in flight (Howard, Dyar, and Knab, 1913; Hinman, 1934b). Unfortunately most of the discussion of these predators is concerned

with their possible importance in control, not with any attempt to estimate their biological role in the maintenance of the balance of mosquito populations, though a study of this natural balance would seem a useful preliminary for control projects. Bats, in the cases where their crepuscular flight habits correspond with the flight habits of certain mosquito species, would certainly seem likely agents of considerable destruction, and Vlasov (1927) has estimated that under favorable conditions a bat may kill 700 mosquitoes per day. The habits of adult dragonflies less often correspond with adult mosquito habits, but crepuscular species may again be important predators. This would probably also be true of many species of insect-eating birds.

Enemies of resting mosquitoes probably include all insectivorous predators in the resting habitat—whatever that may be. In the case of domestic species, spiders and ants are probably both important; certainly both may cause great destruction in caged mosquitoes. In the case of *Anopheles maculipennis* in southern Europe, spider webs seemed often to be the favored resting place, and we had the impression not only that the mosquitoes were relatively immune to the web-building spiders, but that the web-resting habit protected the mosquitoes against other possible enemies, such as ants or jumping spiders.

MOSQUITOES AS HOSTS

A very considerable number of microorganisms have been found associated with adult mosquitoes. Mostly these have been discovered and described incidentally, in the course of studies of the mosquito cycles of organisms causing human disease, and as a result we know remarkably little about their biology from the point of view of mosquito economy. Microorganisms found in a mosquito are presumably living at the expense of the mosquito, and can thus be considered as parasites, but in very few cases do we have direct evidence that the organisms are pathogenic or harmful to the mosquitoes. In some cases the microorganisms may play a symbiotic role, aiding, for instance, in the digestive process; but of this too there is no direct evidence. The whole field of the relations between insects and microorganisms has been reviewed at length by Steinhaus (1946).

About all that can be done here is to present a synoptic review of the types of organisms that have been found associated with mosquitoes, making a more detailed report in the following chapters only

on the two types that have been subject to the most intensive study, viruses and plasmodia.

Viruses

In general we can only recognize the presence of a virus by some indirect effect, and thus the viruses that are known from mosquitoes have all been discovered because of their pathogenicity for some vertebrate. The number of such viruses is, however, considerable, as can be seen from the summary in the following chapter; and one is led to wonder whether there may not also be viruses adapted solely to life in mosquito tissues. The recognition and isolation of such a virus would, at the present time, be very difficult, if not impossible, because of limitations of technique.

Rickettsiae

Hertig (1936) has reported on a rickettsia-like microorganism, which he named *Wolbachia pipientis*, invariably found in the ovaries of *Culex pipiens*. (Specimens from Massachusetts, Minnesota, and China were examined.) Dr. Julian de Zulueta tells me that students in Tate's laboratory in Cambridge, England, have confirmed the presence of this organism in both *Culex pipiens* and *C. molestus*. Steinhaus (1946, pp. 325–328) has summarized what is known of this rickettsia. Brumpt (1938) has described an organism, which he named *Rickettsia culicis*, found in the stomach epithelium of *Culex fatigans*.

Bacteria

No bacteria pathogenic for adult mosquitoes seem to have been described. Apparently, also, no study has been made of the bacteria associated with mosquitoes. In the course of virus studies in Colombia, we inoculated suspensions of tissues from many species of wild mosquitoes into mouse brain, and we were interested to observe that death from brain abscesses in the mice was much more frequent with some species of mosquitoes than with others, indicating differences in bacterial contamination. The inoculations were made by grinding 25 mosquitoes in 2.0 cc. of 10 per cent normal human serum in water, and injecting 0.03 cc. of the resulting suspension in each of six mice. Mortality in the first 24 hours might be caused by the trauma from the injection or by toxic materials; after 24 hours it seemed generally

to be caused by bacterial contamination. Mortalities with different types of mosquitoes in one series were as follows:

SPECIES	NO. MICE INOCULATED	PER CENT MOUSE MORTALITY, 2–14 DAYS AFTER INOCULATION
Haemagogus spegazzinii	1080	5
Psorophora cingulata	144	16
Mixed sabethine species	168	7
Mansonia spp.	60	16
Aëdes serratus	66	5
Culex spp.	84	17
Anopheles rangeli	600	4

It seems that *Psorophora*, *Mansonia*, and *Culex* were much more frequent carriers of bacteria pathogenic for mice than *Haemagogus*, *Aëdes*, or *Anopheles*.

Fungi

Various records of fungi associated with adult mosquitoes have been listed by Speer (1927), and Howard, Dyar, and Knab (1913) quote the very interesting account of Pettit of adult mosquitoes killed by an epidemic of a species of *Entomophthora*. Observations of fungi pathogenic for adult mosquitoes are, however, infrequent.

Yeasts

Various authors have described yeasts found in the digestive tract of mosquitoes; early accounts have been summarized by Howard, Dyar, and Knab (1913), and records up to 1927 listed by Speer. There seems still to be considerable doubt, however, whether such yeasts are parasitic, symbiotic, or merely accidentally ingested (Hecht, 1928).

Protozoa

Flagellates. Quite a few trypanosomes have been described from mosquitoes, though mostly little or nothing seems to be known of their life histories or pathogenicity. Insect trypanosomes in general are classed in three genera, *Leptomonas*, *Crithidia*, and *Herpetomonas*, and species of all three types have been described from mosquitoes; reviews of the literature have been written by Thompson and Robertson (1925), Speer (1927), Galvão and Coutinho (1941), and Wallace (1943).

It is generally assumed that these flagellates are purely mosquito parasites, infection being perhaps hereditary. Galvão and Coutinho noted that in anophelines with *Herpetomonas pessôai* the ovaries failed to develop, and they consider that the parasite is definitely pathogenic for the mosquito. Wallace (1943) made detailed laboratory studies with *Crithidia fasciculata* in *Aëdes aegypti* and *Culex pipiens*. He was unable to transmit infection from adults to larvae, but adults were readily infected by feeding on cotton swabs that had been dipped in *Crithidia* cultures. When infected and uninfected adults were confined together in a lantern chimney, the infection was transmitted, presumably through feeding on faeces.

Many types of vertebrate trypanosomes have insect vectors, and when one considers the host of trypanosomes found in tropical animals, it seems not unlikely that life histories with mosquitoes as vectors or alternate hosts may yet be described. Some of the flagellates described from mosquitoes may belong in this category.

Spirochaetes. Spirochaetes have been reported from mosquitoes several times, but their significance is obscure and there seems to be no evidence of mosquitoes transmitting species pathogenic for vertebrates. Records of mosquito infestations with spirochaetes are summarized by Sinton and Shute (1939) and Steinhaus (1946). Sinton and Shute found a heavy infestation of a species resembling *Spirochaeta culicis* in the salivary glands of a specimen of *Anopheles atroparvus*. The theory that *Leptospira* was the causative organism of yellow fever stimulated attempts to transmit these organisms with *Aëdes aegypti*, but with negative results (Gay and Sellards, 1927).

Sporozoa. Gregarines have been reported from mosquitoes, as from most other insect groups, fairly frequently. The relationship between gregarines and their hosts—whether symbiotic or purely parasitic—seems still to be obscure.

The various haemosporidia reported from mosquitoes can best be considered in relation to the problems of mosquitoes as vectors of malaria, since the mosquito represents merely one stage in the life history of the parasite.

Various parasites belonging to the heterogeneous group of "Neosporidia" have been described from mosquitoes. A species described as *Nosema stegomyiae*, parasitic in *Aëdes aegypti*, was studied by the French Yellow-Fever Commission (summarized in Howard, Dyar, and Knab, 1913); the larvae may be killed, and transmission

was considered to be hereditary through the adults that survived infection; larvae could also be infected through spores mixed with the food. A few other species of *Nosema* have been reported from mosquitoes. It may be noted that this genus includes the causative agent of the famous pébrine disease of silkworms, studied by Pasteur. Several genera of Microsporidia, in addition to *Nosema*, have been found in mosquitoes, but little is known of their biology. Swellengrebel (1920) has described a Haplosporidian (*Myobium myzomiae*) from the intestinal tract of *Anopheles indefinitus*.

Nematodes

Mosquitoes are hosts during part of the life cycle of numerous filarial worms: as such they may be considered as vectors of the filaria, since human interest is primarily concentrated on the disease in the vertebrate hosts. Many mermithid nematodes have also been reported from mosquitoes, but little is known of their biology. Most of these mermithids are, in fact, assigned to the genus "Agamomermis," "a collective group to contain immature Mermithidae not developed to a stage which permits a determination of the genus" (Speer, 1927).

Both the filaria and the mermithids often cause considerable injury to their mosquito hosts.

Trematodes

Various distomes have been reported from mosquitoes, but again little is known of the life history of the parasites, and most are assigned to an artificial genus "*Agamodistomum*," corresponding to the *Agamomermis* of the mermithids. Van Thiel (1930) has described the life history of one species of which the ultimate form occurs in the lung of a frog, and earlier stages in a snail (*Planorbis*) and in the larvae and adults of the mosquito *Anopheles maculipennis*. This parasite furnishes a nice example of the nomenclatorial problem of these distomes, since the form in the frog was described as *Pneumonoeces variegatus*, the form in the mosquito as *Agamodistomum anophelis*, and the form in the snail as *Cercaria anophelis*.

Mites

Adult mosquitoes are often observed to be parasitized by hydrachnid mites, a single adult mosquito often having many of the bright-red mites attached to its abdomen. In a given region it is

usually notable that certain mosquito species will be frequently parasitized, others rarely or never. Shapiro, Saliternik, and Belferman (1944) have suggested that mite infestations might be used as an index of the breeding place, since the hydrachnids are not universally distributed in aquatic habitats. Mosquito larvae are apparently not as often infected as the adults, and in many cases the mites may establish themselves on the pupa, transferring to the adult as it emerges (Brown, 1936). There seems to be no report of measurable injury to the mosquitoes because of the mite parasitism.

Marshall and Staley (1929; also in Marshall, 1938) have described dark serpentiform tubes in the abdominal integument of mite-infested mosquitoes, originating at points where the mouth parts of the mites had been inserted; they consider that these probably represent some kind of a protective reaction on the part of the mosquitoes to the effect of the bites.

Midges

Ceratopogonine midges of the genus *Culicoides* have several times been observed attacking recently engorged mosquitoes. The records, which have been listed by Hinman (1934b), would seem to be chiefly of interest as curiosities.

MOSQUITOES AS VECTORS

Various more or less restrictive definitions have been proposed for the term "vector," but it would seem most useful if used for any organism acting as a transmitting agent for a parasite. Paraphrasing the definition of Leach (1940), a vector is an agent of dissemination or inoculation or both of the causative organism of a disease. The use of the term "vector" thus implies a particular point of view—the medical point of view—in which interest is centered on the disease of a vertebrate host. From a broad biological point of view such a term may be misleading, since in emphasizing the infection of the vertebrate host it may obscure the complicated life-history cycle of the parasite. Yet the concept of vector provides a very useful shorthand that it would be pedantic to discard. It is very convenient to speak of *Anopheles gambiae* as an "efficient vector of malaria," or of *Aëdes aegypti* as the "vector of urban yellow fever," and of *Haemagogus spegazzinii* as the "vector of sylvatic yellow fever."

When primary interest is centered on the parasite life history rather

than on the vertebrate disease, some other terminology is preferable. It is common practice to speak of the animal harboring the adult or sexually mature form of the parasite as the "definitive host," and of the animal in which only immature parasite forms occur as the "intermediate host." By this system, man is the definitive host of *Filaria*, the mosquito the intermediate host; in the case of *Plasmodium*, man is the intermediate host and the mosquito the definitive host. In the case of many parasites—the viruses for instance—it is impossible to apply this system because of the absence of sexual forms, or at least the absence of knowledge of such forms.

From another point of view, one may be interested in the mechanism of maintenance of the parasite—in the determination of the "reservoir" of infection. Here too the arthropod-vertebrate roles may be reversible. In the case of malaria, the "reservoir" maintaining the parasite population would seem to be man, since the parasitism may persist indefinitely in that host, but is limited to a single cycle in the insect host. In the case of yellow fever, however, the infection in the vertebrate host seems always to be short and self-limited, while it persists in the mosquito for the life of the insect, which is thus probably the chief agent for the maintenance of the parasite population.

Inevitably, however, we look upon mosquitoes chiefly as vectors of disease, as agents of transmission of vertebrate infections. Various classifications of transmission phenomena from this point of view have been proposed (reviewed in Leach, 1940), and the most widely adopted is that of Huff (1931). He divided arthropod vectors into four groups:

1. Cyclopropagative, the parasitic organisms undergoing cyclical change and multiplying within the vector, exemplified by plasmodia in mosquitoes.
2. Cyclodevelopmental, the organisms undergoing cyclical change within the vector, but not multiplying, as in the case of filaria in mosquitoes.
3. Propagative, the organisms multiplying within the vector but undergoing no cyclical change, exemplified by yellow-fever virus in mosquitoes.
4. Mechanical, the organism neither changing nor multiplying within the vector, as in the case of mosquito transmission of the virus of equine infectious anemia.

Mosquitoes act as vectors to four distinct groups of vertebrate parasites: viruses, haemosporidia, filarial worms, and the botfly *Derma-*

tobia. A few trematode species might be included as a fifth group (see above), but the life history of the mosquito-infesting forms is very little known. There are also a few other types of parasites that may at times be transmitted mechanically by mosquitoes, though this seems not to be the usual transmission mechanism; tularaemia (Philip, Davis, and Parker, 1932) may be cited as an example.

The phenomena of mosquito transmission are quite distinct in each of these four groups, and each undoubtedly represents a separate evolutionary development. The relations between mosquitoes and viruses and malarial organisms have been the subject of a great deal of study, warranting summary in some detail in separate chapters. The vector relationship with *Dermatobia* and filaria will, however, be reviewed briefly first.

MOSQUITOES AS VECTORS OF DERMATOBIA

The tropical American botfly, *Dermatobia hominis,* which goes through larval development in the integument of various mammals, including man and his domestic animals, more especially cattle, has long been known to have a curious indirect method of oviposition. The adult female fly when ready to lay eggs is zoophilous, in that she is attracted to warm-blooded animals. She rests on a potential host until a mosquito or small fly alights near her; the mosquito or fly is pounced upon, and if captured, the *Dermatobia* female in the course of a hovering flight, lays a group of eggs on the victim, generally plastered against the abdomen. After a suitable incubation period, the eggs hatch and the young larvae hang suspended from the egg shells, ready to drop off when their carrier again alights upon a warm-blooded vertebrate.

The adult *Dermatobia,* by reason of its zoophilous habit when ready to oviposit, selects an egg carrier that is also zoophilous and consequently likely to return to a suitable host when the eggs have completed their incubation period. Various species of mosquitoes and small flies show very different infestation rates with *Dermatobia* eggs (Bates, 1943). The controlling factors in determining vector species seem to be: (a) zoophilous habit and diurnal flight period, corresponding to the habit and flight period of the *Dermatobia;* (b) moderate size (most Tabanids, for instance, would be too large for the *Dermatobia* to handle, flies like *Hippelates* too small); and (c) moderately active habits, since very sluggish insects would not stimulate the

pouncing behavior of the *Dermatobia*, and very active ones would escape. Among mosquito species in the Villavicencio area, *Aëdes serratus* and *Psorophora ferox* show the highest infestation rates with *Dermatobia* eggs, most nearly fulfilling these various requirements.

This, it seems to me, is a simple example of a vector relationship. It is sometimes called "phoresy," a term "proposed by P. Lesne in 1896 to designate the carriage of certain insects by others for purposes not associated with direct feeding" (Clausen, 1940), but phoresy seems to be a rather unnecessary term for the occasional incidence of vector relationships among parasitic insects—a phenomenon that gains added interest if it is related to the general problem of the indirect transfer of parasites from host to host. In the classification of Huff, the *Dermatobia*-mosquito relationship is "cyclodevelopmental," since embryonic development occurs on the carrier, though there is no multiplication.

MOSQUITOES AS VECTORS OF FILARIA

The vector relationship of mosquitoes to human filaria is notable because it was the first instance discovered of an arthropod acting as intermediate host to an organism causing vertebrate disease. Patrick Manson, in 1878, observed the development of filarial worms in mosquitoes, though he failed to determine how the worms got from the mosquito back to man. Influenced by the current belief that mosquitoes bit but once and died after a short period, he presumed that the worms must be liberated in water, perhaps getting back to man in drinking water. Details of the life history of the filaria in mosquitoes and of the method of transmission were not worked out until much later, through the combined efforts of many investigators—a history that has been summarized in the book by Howard, Dyar, and Knab (1913).

Contemporary parasitologists seem generally agreed that human mosquito-transmitted filariasis is caused by only two species of parasites, *Wuchereria bancrofti* and *W. malayi*, though there are several other types of human filariasis transmitted by other types of blood-sucking insects. The transmission phenomena of *bancrofti* and *malayi* seem to be essentially similar, though different vector species may be involved.

The sexually mature filaria inhabit the lymph glands and produce immense numbers of microfilaria which circulate in the blood stream,

usually with a characteristic diurnal periodicity. The microfilaria develop no further unless they are picked up by a mosquito, in which they undergo a characteristic metamorphosis. The development in the mosquito, as described by Feng (1936) for *malayi* in *Anopheles hyrcanus* at a temperature of about 30° C. is as follows: the microfilaria casts off its "sheath" in the gut of the mosquito in about three hours and penetrates into the abdominal cavity, migrating toward the thorax, which it reaches after about ten hours. The larva molts twice in the thorax, once after about four days, and again after about six days, showing different morphological characteristics with each molt. After the sixth day (in the third larval stage), the majority of the larvae migrate to the head, taking up position in the interior of the labium. When the mosquito bites a warm-blooded animal, larvae break free from the labium and crawl out onto the skin of the host, penetrating through the mosquito bite or some other abrasion: a process that can be completed only under favorable circumstances, since the larvae die quickly if they remain exposed on the skin.

Filaria, like the *Dermatobia*, thus belong to the cyclodevelopmental category in Huff's classification, since there is a larval development in the mosquito host, but no multiplication. The filaria larvae are definitely injurious to the mosquitoes, and various observers, like Menon and Ramamurti (1941), have found that patients with a heavy microfilarial rate are unsuitable for infection experiments because of the high mosquito mortality caused by the parasites.

From laboratory experiments made by many workers, it appears that mosquitoes vary considerably in their susceptibility to filarial infection, though results by different authors with a given species of mosquito are not always consistent. *Culex fatigans* seems always to be an efficient vector in the laboratory, and this species is very generally considered to be the most important natural vector, though some species of *Anopheles* are important in certain parts of the world (Edwards, 1922). *Aëdes aegypti* has generally been found to be resistant to infection.

The experiments of Newton, Wright, and Pratt (1945) and Newton and Pratt (1946) show nicely the varying susceptibility of a series of different mosquito species to filarial infection. Of particular interest is their finding that *Psorophora confinnis* from Puerto Rico and the United States differed in susceptibility; 12 per cent of the former developing infective larvae as compared with 80 per cent of

the latter in parallel experiments, though the two strains seemed to be identical in larval and adult morphology. Eyles and Most (1947) tested the susceptibility of a number of North American mosquitoes to a "nonperiodic" type of *bancrofti* filaria from the South Pacific. Their infection rates (based on dissections 10 days or more after feeding) were as follows: *Culex pipiens* 84 per cent positive; *C. fatigans* 35; *C. erraticus* 23; *C. salinarius* 3; *Anopheles walkeri* 36; *A. punctipennis* 2; *A. quadrimaculatus* 0; *A. freeborni* 0; *Aëdes triseriatus* 7; *A. aegypti* 3; *Psorophora ferox* 0; *Mansonia perturbans* 4.

A vast number of species of filaria are known to be parasitic in vertebrates, and as far as they have been studied these seem generally to be transmitted by bloodsucking insects. Aside from the human *Wuchereria*, the best known species with mosquito vectors is *Dirofilaria immitis* of dogs, which has been the subject of considerable study because of the convenience with which the host can be handled in the laboratory. Summers (1943) in a study of the relative efficiency of various mosquitoes as vectors of this filaria, found that the species could be divided into four groups: I, those that refused to feed (*Culex apicalis*); II, larvae that failed to develop because of some inhibitory factor (*Culex salinarius, Aëdes aegypti, A. vexans, Psorophora ferox*); III, larvae whose development was retarded (*Culex territans*); IV, larvae whose development was normal (*Aëdes infirmatus, A. sollicitans, Anopheles crucians*).

Higby (1943a and 1943b) found that *Dirofilaria scapiceps* of the snowshoe rabbit and *Dipetalonema arbuta* of the porcupine were transmitted by various species of *Aëdes*.

Abbott, Roden, and Yoeli (1946) have reported that *Anopheles sacharovi* and *A. maculipennis* in Greece seem to be the vectors of a species of filaria causing "equine dermal filariasis." They found simultaneous infections in *Anopheles sacharovi* with filarial larvae and plasmodial oöcysts.

CHAPTER XIV

MOSQUITOES AS VECTORS OF VIRUSES

> Nature makes so gradual a transition from the inanimate to the animate kingdom that the boundary lines which separate them are indistinct and doubtful.—ARISTOTLE

A "VIRUS" is generally defined as an ultramicroscopic infectious agent capable of passing through the usual porcelain bacterial filters, and not susceptible of cultivation except in the presence of living cells. In other words, the two basic criteria used in limiting the concept are size and obligate parasitism. The very small size and the obligate parasitism may be related, since the reduction in size may be possible because the pathogen depends on the host cell for most of the metabolic functions.

Any detailed discussion of the nature of viruses is beyond the scope of this book. The subject is, in any event, still a field for speculation, with little definite evidence available. Basically there are two schools of thought: one considering that the viruses have evolved from the plasma components of their hosts; the other that they are descended from a line of originally free-living microorganisms, showing ever greater specialization toward parasitism, until they have become completely dependent on the host cells. It has been suggested that viruses are genes that have lost their cytoplasm; that they are essentially complex enzyme-like chemicals and not "organisms" in any sense of the word; that they are highly specialized bacteria.

The first virus to be recognized was a plant pathogen (tobacco mosaic by Ivanovski in 1892), and a great deal of basic virus work has been carried out by plant pathologists. Stanley, in 1935, made one of the great strides in virus study when he announced the production in crystalline form of this same tobacco-mosaic virus. Two excellent general books on viruses have been written by plant men—by Smith (1940) and by Bawden (1943)—and these authors have summarized much of the speculation concerning the nature of these pathogens.

The best summaries on animal viruses are those by Rivers (1948) and van Rooyen and Rhodes (1948). The bacterial viruses, or bacteriophages, have been reviewed by Delbrück (1946).

Perhaps various kinds of things have been caught within the size limits set for viruses. It is possible to make reasonably accurate determinations of the size of virus particles by various methods, including filtration through membranes of known pore size, rate of sedimentation on centrifugation, and electron microscopy. By such methods it is found that viruses show a tremendous range in size, from the Rickettsial particles which might well be small bacteria, to the particles of foot-and-mouth disease, which approach molecular dimensions, the one being fifty thousand times as large as the other. The only common denominator through this range of size is obligate parasitism.

Our knowledge of viruses is too limited to permit the construction of any logical classification, and no attempt at classification has gained any general acceptance. Viruses are known mostly by the symptoms they produce in their hosts, and the animal viruses are commonly named for the disease of which they are the causative agent. The recognition of a given virus depends first on the symptoms of the host, and more precisely on immune reactions of the host. Animal viruses in general call forth a highly specific immune response in vertebrate hosts, and immunological methods must always be resorted to for the identification of any newly isolated strain. By this means it is relatively easy to recognize different kinds of virus, but no clue is given as to the possible interrelationships among the various kinds.

Mosquitoes were first linked with animal viruses by the discovery in 1900 by Reed, Carroll, Agramonte, and Lazear of the transmission of yellow fever by *Aëdes aegypti*—though the causative agent of yellow fever was not positively identified as a virus until the publication in 1930 of the studies carried out by Sawyer and his coworkers. The story of the early work with yellow fever has often been told; Howard, Dyar, and Knab (1913) include a rather detailed summary with complete references. Details of the transmission mechanism could not be worked out until a laboratory animal had been found that could serve as host. The discovery of suitable laboratory hosts for yellow fever, and the discovery of the mosquito transmission of the various viruses causing human encephalitis, led to the many investigations of

the mosquito-virus relationships in the period after 1930 that are reviewed in the present chapter.

A fairly extensive list of mammalian viruses are now known to have mosquito vectors, or to be capable of surviving in mosquito tissues. It may be well to review this list of viruses before taking up the general aspects of virus behavior in mosquito hosts.

YELLOW FEVER

It was long thought that yellow fever depended for its maintenance on a single species of mosquito, *Aëdes aegypti*. Early experiments with other types of mosquitoes gave negative results, and the control of *Aëdes aegypti* proved to be a very effective measure in eliminating yellow fever from American cities, so that experimental and epidemiological results were in accord. Experiments with yellow fever were necessarily very limited as long as man was the only known host, but with the discovery in 1927 of the susceptibility of the rhesus monkey, new fields of experimentation were opened up. Among other things, tests with various kinds of mosquitoes were made both in Africa (Bauer, 1928) and in America (Davis and Shannon, 1931a, 1931b) and it was found that many species of mosquitoes were capable of transmitting the virus.

The fact that other mosquitoes as well as *Aëdes aegypti* could transmit the virus remained purely a matter of academic interest until the discovery of the sylvatic or "jungle" epidemiological form of the disease in America in 1932 (Soper and others, 1933). The search for the vectors and hosts of the virus under forest conditions has led to the publication of a considerable volume of literature, summarized in papers by Bugher and others (1944), Bates (1946), Taylor and Da Cunha (1946) and Laemmert and others (1946). A corresponding sylvatic situation has been found to exist in Africa (Mahaffy and others, 1942) and a series of papers dealing with mosquito habits in connection with African yellow-fever studies has been published by Haddow (1945a, b, c, and 1946).

Laboratory experiments with the transmission of yellow-fever virus by mosquitoes other than *Aëdes aegypti* are summarized in the accompanying table. The results are grouped in three categories: those that were completely negative, those in which at least some of the mosquitoes were found to have retained virus for long periods (9 or 10 days or more), though no successful transmissions were obtained,

and those in which the virus was transmitted by bite. These experiments are of somewhat uneven value, since in some cases large numbers of mosquitoes were used and tested after varying incubation periods, while in other cases only two or three specimens were available for testing.

TABLE XI SUMMARY OF LABORATORY EXPERIMENTS WITH INFECTION OF MOSQUITOES WITH YELLOW-FEVER VIRUS

SPECIES	REFERENCE	NEGATIVE ON INJECTION	POSITIVE ON INJECTION	TRANS. BY BITE
Anopheles albitarsis	Davis & Shannon, 1931b	×		
Anopheles tarsimaculatus	Davis & Shannon, 1931b	×		
Anopheles gambiae	Philip, 1930a	×		
Trichoprosopon digitatum	Davis & Shannon, 1931b	×		
	Kumm & Frobisher, 1932	×		
Wyeomyia bromeliarum	Davis & Shannon, 1931a	×		
Wyeomyia oblita	Davis & Shannon, 1931a	×		
Limatus durhami	Davis & Shannon, 1931a	×		
Mansonia africana	Philip, 1930			×
Mansonia albicosta	Whitman & Antunes, 1937		×	
	Davis & Shannon, 1931b		×	
Mansonia chrysonotum	Davis & Shannon, 1931b		×	
	Whitman & Antunes, 1937		×	
Mansonia fasciolata	Davis & Shannon, 1931b		×	
	Whitman & Antunes, 1937		×	
Mansonia juxtamansonia	Whitman & Antunes, 1937		×	
Mansonia titillans	Kumm & Frobisher, 1932		×	
Mansonia uniformis	Kerr, 1932		×	
Psorophora cingulata	Davis & Shannon, 1931b		×	
Psorophora ferox	Whitman & Antunes, 1937			×
	Davis & Shannon, 1931b		×	
Aëdes				
(Ochlerotatus) nubilus	Whitman & Antunes, 1937		×	
(Ochlerotatus) scapularis	Whitman & Antunes, 1937			×
(Ochlerotatus) serratus	Davis & Shannon, 1931b		×	
(Ochlerotatus) taeniorhynchus	Whitman & Antunes, 1937		×	
	Davis & Shannon, 1931a			×
(Finlaya) fluviatilis	Davis & Shannon, 1931a			×
	Whitman & Antunes, 1937			×
(Finlaya) geniculatus	Roubaud, Colas-Belcour, & Stefanopoulo, 1937			×
(Finlaya) terrens	Davis & Shannon, 1931b		×	
	Whitman & Antunes, 1937		×	

SPECIES	REFERENCE	NEGATIVE ON INJECTION	POSITIVE ON INJECTION	TRANS. BY BITE
(Howardina) fulvithorax	Davis & Shannon, 1931b	×		
	Whitman & Antunes, 1937		×	
(Stegomyia) africanus	Philip, 1929			×
(Stegomyia) albopictus	Philip, 1930b			×
(Stegomyia) apicoargenteus	Bauer, 1928	×		
(Stegomyia) luteocephalus	Bauer, 1928			×
(Stegomyia) metallicus	Lewis, Hughes & Mahaffy, 1942			×
(Stegomyia) simpsoni	Philip, 1929			×
(Stegomyia) vittatus	Philip, 1929			×
(Aëdimorphus) apico-annulatus	Bauer, 1928			×
(Diceromyia) taylori	Lewis, Hughes & Mahaffy, 1942			×
Haemagogus equinus	Waddell & Taylor, 1945			×
Haemagogus spegazzinii	Antunes & Whitman, 1937			×
	Bates & Roca, 1945			×
Haemagogus splendens	Anderson & Osorno, 1946			×
Haemagogus uriartei	Antunes & Whitman, 1937		×	
Eretmopodites chrysogaster	Bauer, 1928			×
Culex fatigans	Davis & Shannon, 1931b		×	
Culex nigripalpus	Whitman & Antunes, 1937		×	
Culex thalassius	Kerr, 1932			×

Results with anophelines and sabethines have been completely negative, though the few species of sabethines tested are hardly an adequate sample of the group. Laemmert and others (1946) mention unpublished experiments with *Goeldia frontosa* in which transmission by bite occurred; and virus was isolated from a mixed group of wild-caught sabethines by Shannon, Whitman, and Franca (1938). It is curious that both the African and American *Mansonias* retain virus for long periods of time, though transmission has been obtained with only one species. The only *Aëdes* species to give completely negative results is *apicoargenteus*. In a series of experiments in which positive results were obtained with other species (Bauer, 1928), a rhesus injected with 11 mosquitoes 37 days after the infectious meal remained normal, and another injected with 16 mosquitoes after 17 days also remained normal. It seems probable that the species of *Aëdes* that were found to retain virus but that failed to transmit by bite would

be found capable of transmitting if maintained for a sufficiently long incubation period at favorable temperatures.

The retention of virus by occasional specimens of species of *Culex* for long periods seems very interesting. Kerr (1932) obtained transmission by bite in several instances with *Culex thalassius* after a prolonged incubation period (27 days or more).

Field studies seem in general to indicate that yellow fever is transmitted by a comparatively few species of mosquitoes, despite the wide range of types that have been found capable of retaining virus under laboratory conditions. *Aëdes aegypti* is still the only known natural vector under urban conditions, and under forest conditions in America the evidence in most regions indicates a single species, *Haemagogus spegazzinii*, as the probable vector. Virus has been recovered on seventeen different occasions from wild-caught material of this species in Colombia and Brazil. Vast numbers of other mosquitoes have been tested in the course of epidemiological investigations, and virus has been recovered on three occasions from *Aëdes leucocelaenus*, once from a mixed collection of 88 specimens of various sabethine species, and once from *Haemagogus lucifer* and/or *equinus* (reference in Laemmert, Ferreira, and Taylor, 1946). The peculiar importance of *Haemagogus spegazzinii* as a vector may be due to its abundance, to an unusually great longevity under natural conditions, to close association with susceptible mammalian hosts, or perhaps to a combination of such factors.

In Africa, virus has been recovered from wild-caught *Aëdes simpsoni* (Mahaffy and others, 1942) and from a mixed group of 12 species of *Aëdes* (not including *aegypti* or *simpsoni*) (Smithburn and Haddow, 1946). Many lines of evidence indicate that *Aëdes africanus* is probably the most important vector in the forests of East Africa. Various other species have been suspected on epidemiological grounds of being vectors, particularly *Aëdes taylori* and *metallicus* in the Sudan (Lewis, Hughes, and Mahaffy, 1942) and species of *Eretmapodites* in Uganda (Haddow, 1946).

DENGUE

Mosquitoes were suspected as vectors of dengue soon after the discovery of their role in yellow fever, and in 1906 Bancroft, in Australia, published observations and experiments incriminating *Aëdes aegypti* as the vector. Experimental work with dengue has been greatly

handicapped by the lack of suitable experimental animals. Certain monkeys have been found to be susceptible, and successful infection of white mice by intracerebral inoculation was obtained in 1945, but techniques for using these animals in transmission experiments have not been worked out, and infection experiments have thus necessarily been made with human volunteers. Lumley (1943) has published a detailed summary of the literature on dengue, including the transmission experiments.

Aëdes aegypti seems to be the universal vector in urban epidemics of dengue. *Aëdes albopictus* and *Armigeres obturbans* have also been found to transmit the virus under experimental conditions. A considerable number of experiments with *Culex fatigans*, undertaken because this species was early suspected as a vector, have given consistently negative results.

WESTERN EQUINE ENCEPHALITIS

A group of viruses that produce similar or identical symptoms in mammalian hosts but that have been found to be immunologically distinct entities are known collectively as the "encephalitides." Three viruses have been found to cause this type of disease in the United States, known as the eastern equine, the western equine, and the St. Louis types, and all three appear to be mosquito transmitted. Another immunologically distinct type, called Venezuelan equine encephalitis, is found in South America.

Western equine encephalitis (or "encephalomyelitis") was first recognized as a clinical entity and the virus isolated in 1930, and the virus was first experimentally transmitted by mosquitoes three years later (Kelser, 1933). The disease has since been the subject of extensive field and laboratory investigations, which have been summarized from the entomological point of view by Reeves (1945). Apparently the virus is normally maintained as an infection of birds (particularly the domestic fowl), with *Culex tarsalis* as the most important vector; infections of horses and man are incidental in the normal cycles of the virus.

Extensive field investigations of the disease have been carried out by W. McD. Hammon, W. C. Reeves, and their associates, and virus has been recovered from wild-caught *Culex tarsalis* 83 times, twice from *Aëdes dorsalis*, and once each from *Culex pipiens*, *Culex stigmatosoma*, *Theobaldia inornata*, and *Anopheles freeborni*. Norris (1946)

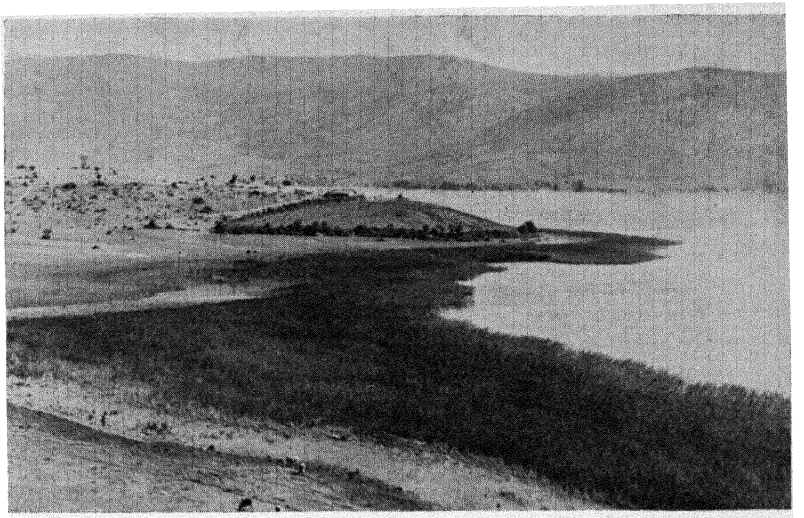
has recovered the virus from *Culex restuans* in Canada. Reeves (1945) points out that the 83 isolations of virus from *Culex tarsalis* were made from a total of 21,833 specimens tested; while from over 25,000 specimens of *Aëdes* tested at the same time and from the same areas, only the two *dorsalis* isolations were made, although in the laboratory various species of *Aëdes* have been found to transmit the virus readily. Reeves considers this difference to reflect the food habits of the mosquitoes: *Culex tarsalis* feeds predominantly on birds, which are the common host of the virus, while *Aëdes* characteristically bite mammals (98 per cent postive for mammalian blood in precipitin tests).

Laboratory experiments with virus transmission have been summarized by W. A. Davis (1940) and Hammon and Reeves (1943a). Transmission has been obtained with *Aëdes aegypti, A. sollicitans, A. taeniorhynchus, A. vexans, A. albopictus, A. nigromaculis* and *A. dorsalis, Culex tarsalis, Theobaldia incidens*, and *T. inornata*. The results of a single experiment with *Culex stigmatosoma* were inconclusive. Virus persisted in *Psorophora confinnis* for seven days only, and experiments with *Culex pipiens, Anopheles freeborni*, and *A. punctipennis* gave negative results. The field-virus isolations from *Culex pipiens* and *Anopheles freeborni* may thus represent "dead ends"—mosquitoes that had recently fed on an infected host, but that would be incapable of transmitting the virus.

EASTERN EQUINE ENCEPHALITIS

The serological distinctness of eastern and western equine encephalitis was recognized in 1933. The western type has been found as far east as Alabama, and the eastern type as far west as Michigan and Texas (Hammon, 1945). Human infections with virus of the eastern type appear to be much rarer than with the western type. The epidemiology of the eastern encephalitis has not been studied as intensively as that of the western, but it is assumed that the two viruses have closely similar mechanisms of maintenance and spread. Virus has not been isolated from wild-caught mosquitoes in the case of the eastern type.

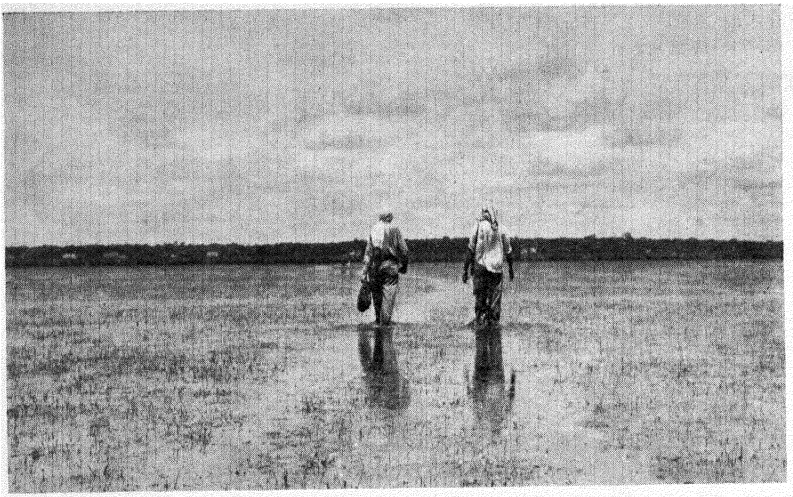
It is notable that Merrill and Ten Broeck (1935) found that *Aëdes aegypti* transmitted the western virus much more readily than it did the eastern virus. This may, however, have been due to peculiarities of the virus strains or mosquito strains used in their experiments.



Marshy zone in lake. Mosquito larvae in lakes occur in zones where they are protected from wave action by vegetation (Lake Ochrida, Albania).

Marsh. The mosquito fauna of marshes may depend on the type and density of vegetation. *Anopheles hyranus* occurred in this area of rushes (*Phragmites*) at Lake Terbuf, Albania.

PLATE 2. LARVAL HABITATS



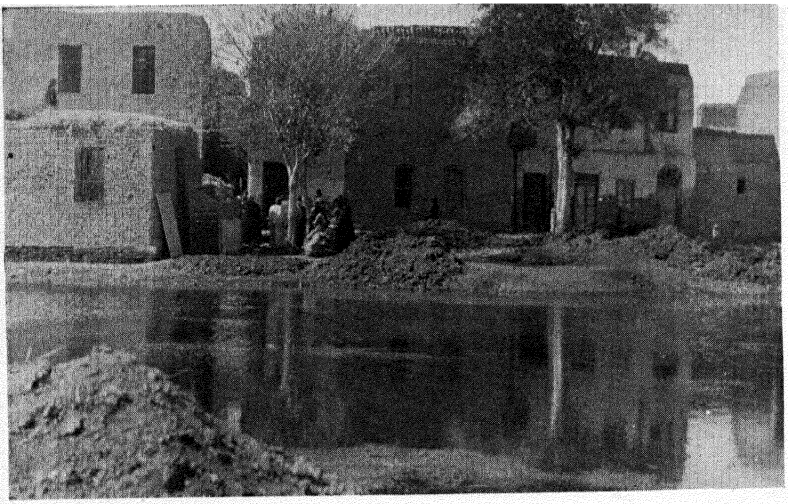
Marsh. The flooded savannahs of interior South America breed immense numbers of mosquitoes of many species. The common anopheline in this marsh near Orocüe, Colombia, was *Anopheles albitarsis.*

Open pond. Larvae in ponds are generally most numerous in the marginal vegetation. *Anopheles rangeli* was the dominant species in this pond at Villavicencio, Colombia.

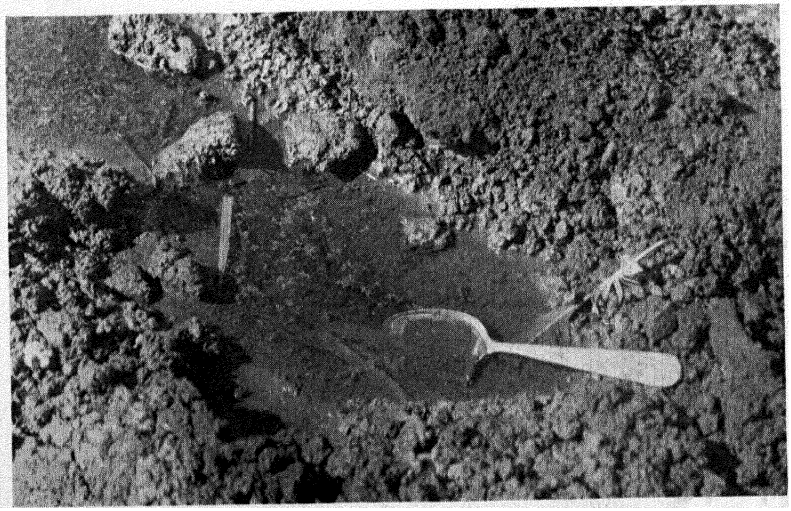
Brackish water. Coastal waters, with appreciable salinity, have a special mosquito fauna in various parts of the world. These marshes on the Adriatic coast at Shën Gjini, Albania, form the habitat of *Anopheles sacharovi* and various culicines.

Brackish water. This shows one of the pools in the coastal area pictured in the upper photograph. A gasoline tin, with top and bottom removed, serves to fence off an area of one-tenth of a square meter, in which a total count of larvae can be made.

PLATE 4. LARVAL HABITATS



Pond. The breeding places of an intensely cultivated area, such as Egypt, are almost all man made, often, like this village pond at Halaba, Egypt, through carelessness.



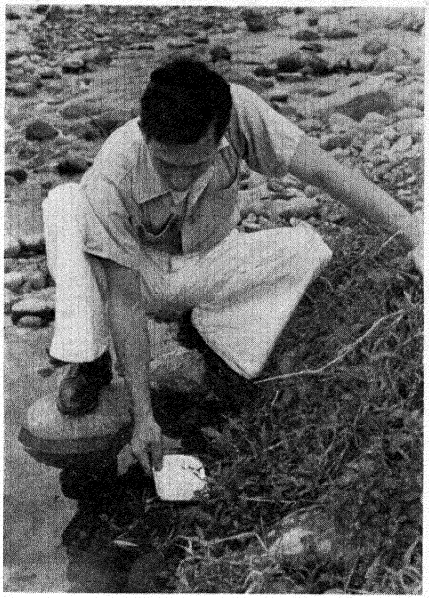
Pond margin. This detail of the margin of the village pond at Halaba, Egypt, shows the niche in which *Anopheles multicolor* larvae were found.

Forest pool. This particular pool, an oxbow from a small stream at Villavicencio, Colombia, contained larvae of *Anopheles mediopunctatus.*

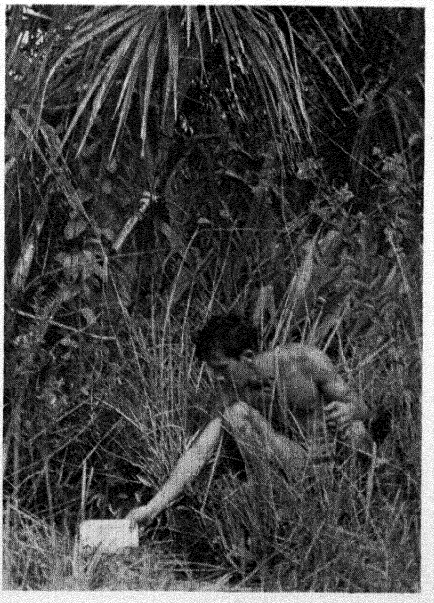
PLATE 6. LARVAL HABITATS

Open stream. Seepage in the gravel beds of streams forms the special habitat of several species of anophelines in various parts of the world. Larvae of *Anopheles superpictus* were found in this stream at Tirana, Albania.

Shaded stream. Pools in mountain streams in the Mediterranean region, like this one near Elbasan, Albania, form the special habitat of *Anopheles marteri.*

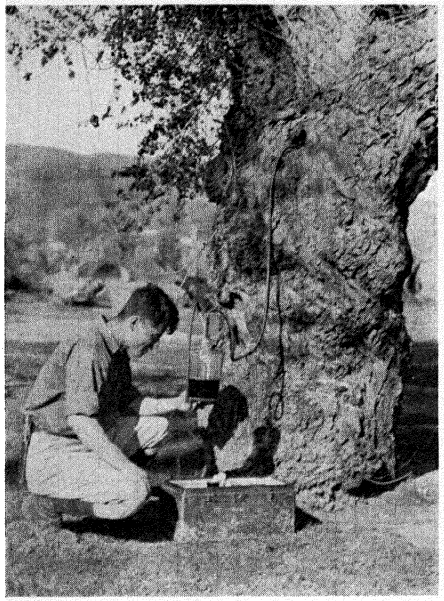


Open stream. Quiet side pools form another niche utilized by mosquito larvae. *Anopheles pseudopunctipennis* was breeding here at Villavicencio, Colombia.

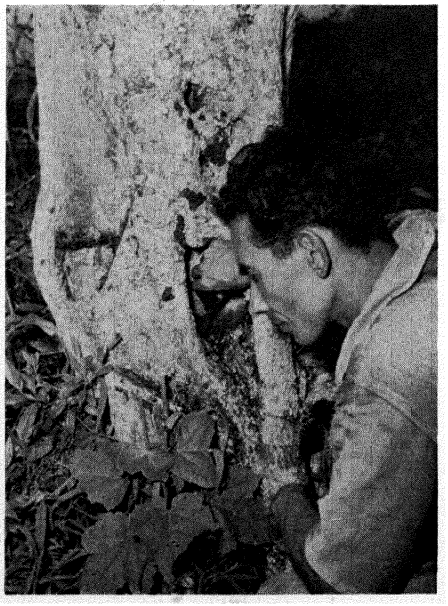


Open stream. Small streams in the grassy savannahs of the upper Orinoco region of Colombia are lined by Mauritia palms; they form the larval habitat of *Anopheles peryassui.*

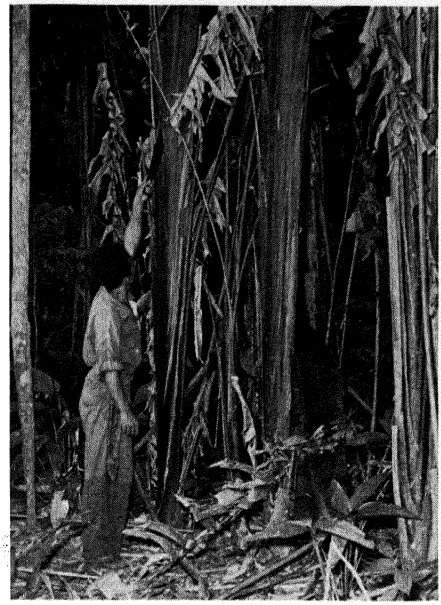
PLATE 8. LARVAL HABITATS



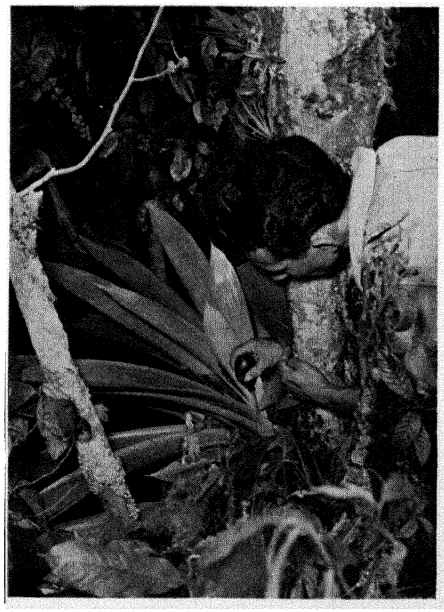
Tree hole. Tree holes are the only common container habitat in temperate latitudes; to collect the larvae, it is necessary to remove all of the water, for which purpose a siphon arrangement is convenient (near Scutari, Albania).



Tree hole. The mosquito fauna of tree holes in tropical forests is both large and varied (Villavicencio, Colombia).



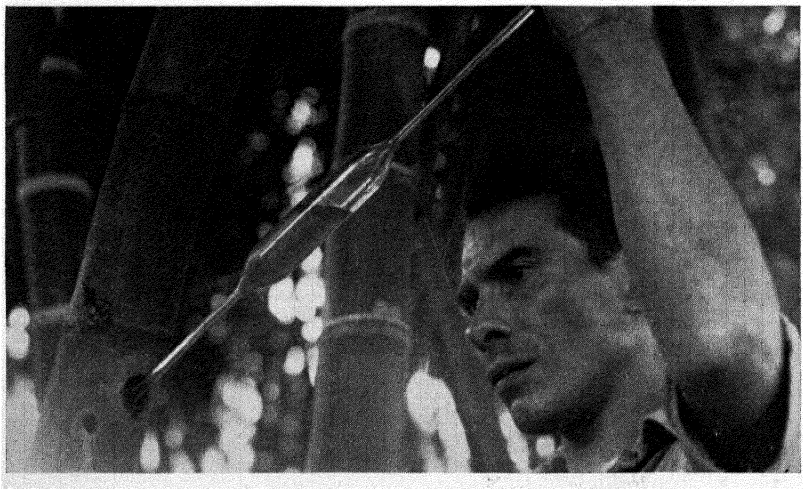
Plant container. Water which accumulates under the leaf-bases of *Ravanela* (a plant of the banana family) forms the specialized habitat of several species of *Wyeomyia* and of a predacious *Megarhinus* (Villavicencio, Colombia).



Plant container. The bromeliads form a large family of Neotropical plants, including many species that retain water at the bases of the leaves. These provide the habitat of a large and well studied assemblage of mosquitoes (Villavicencio, Colombia).

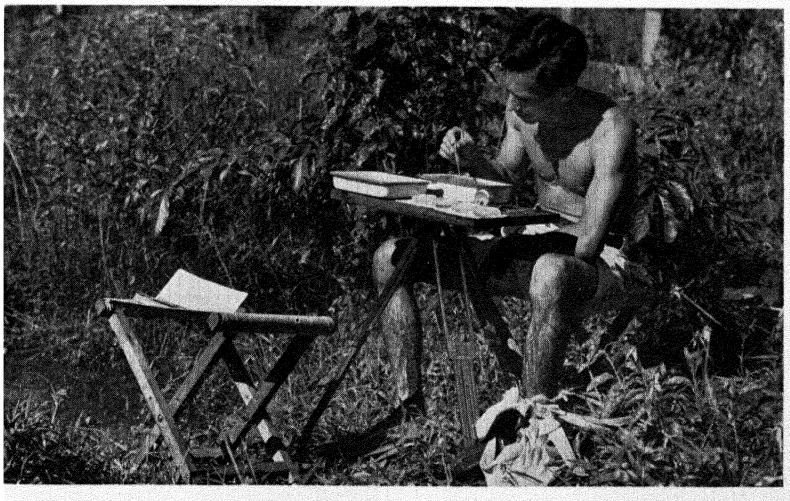
PLATE 10. LARVAL HABITATS

Ground container. The stems of fallen palm leaves form a common type of ground container in the tropical forest, the special habitat of several mosquito groups, including species of *Carrollia*, *Limatus*, and *Culex* (Villavicencio, Colombia).



Plant container. Internodes of growing bamboo, punctured by insects or birds, collect water and form the specialized larval habitat of a large number of South American mosquitoes of several genera (Villavicencio, Colombia).

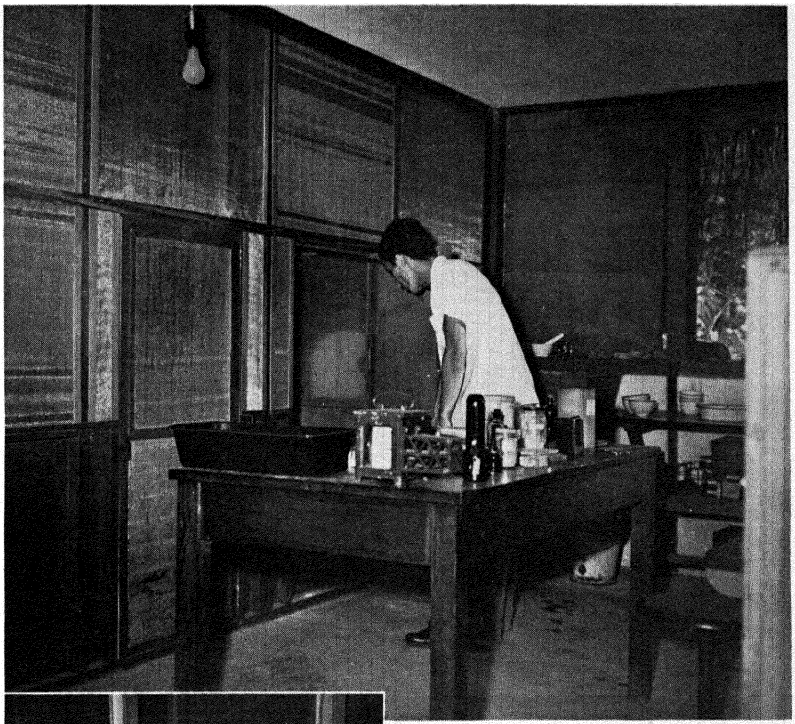
A pond for larval study created by damming a small stream: such artificial habitats permit considerable control over environmental conditions (Villavicencio, Colombia).

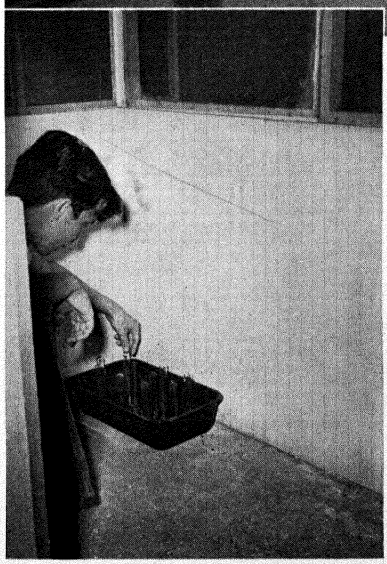


The counting of larvae by stage of growth furnishes a convenient index of breeding conditions. Such counts may be made in the laboratory or (as here) with portable equipment in the field (Villavicencio, Colombia).

PLATE 12. MOSQUITO TECHNIQUE

For studies of mosquito stratification in the forest, parallel captures at ground level and at different heights were made; the platform here is only 6 meters above the forest floor, but the mosquito captures in the two stations were significantly different (Villavicencio, Colombia).

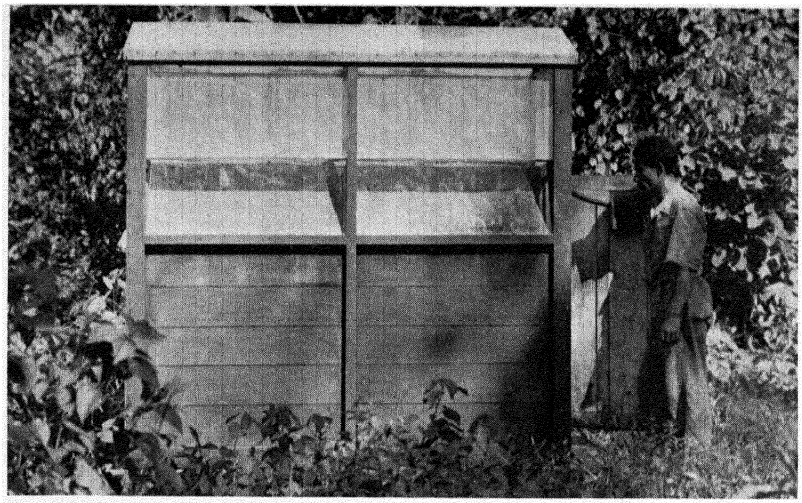




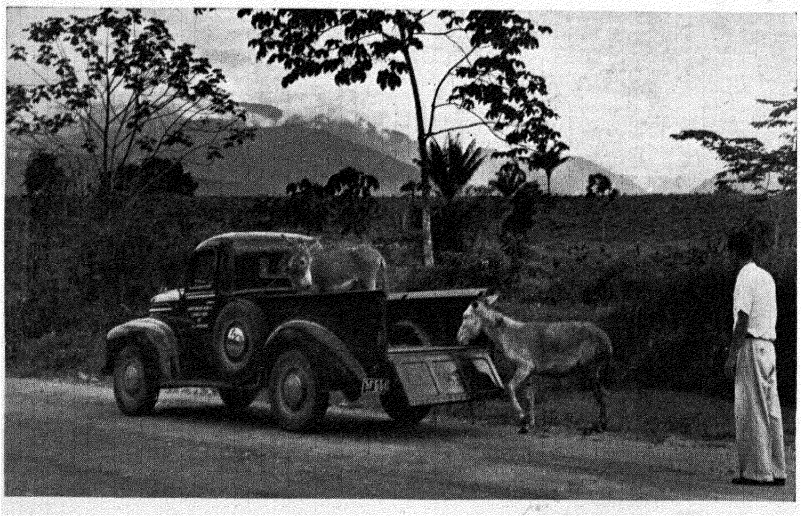
Cages for mosquito colonies were built across this room; each cage is two meters square, a convenient size for observation (Villavicencio, Colombia).

This shows the interior of a 2 meter square cage, with a pan arranged for an oviposition experiment with test-tubes as "obstacles" (Villavicencio, Colombia).

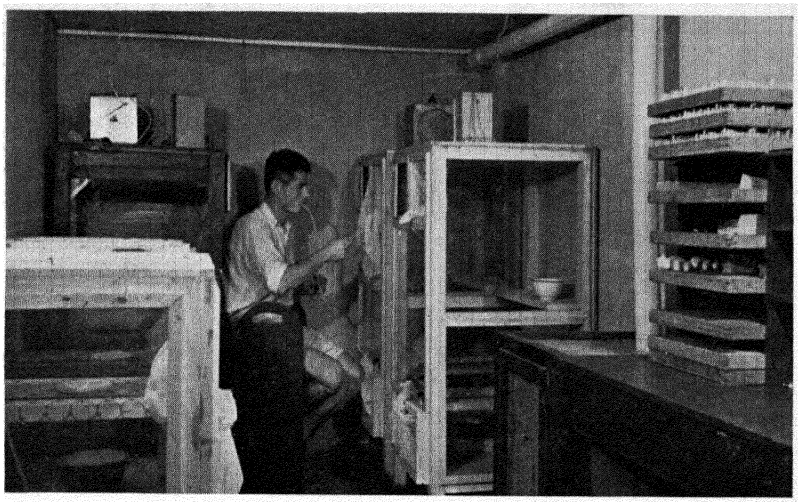
PLATE 14. MOSQUITO TECHNIQUE



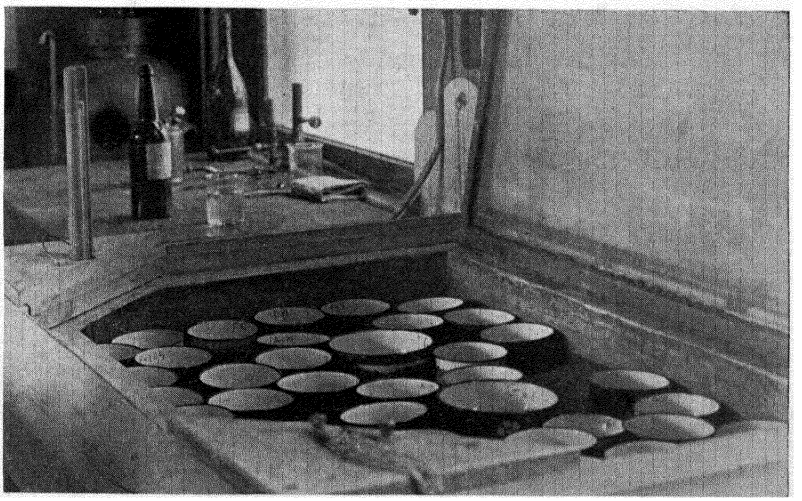
Stable traps form a convenient method of catching large numbers of adult mosquitoes (Villavicencio, Colombia).



Donkeys make good bait for mosquito traps because they are easily handled, learning to enter traps and to ride to location in trucks (Villavicencio, Colombia).

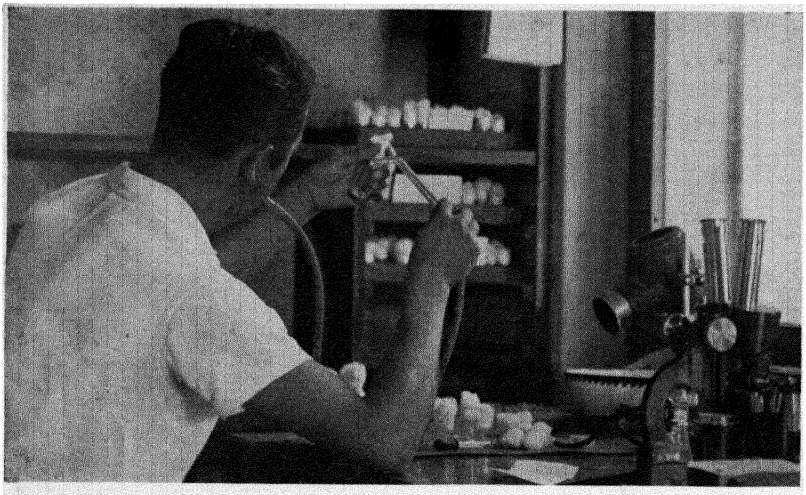


A cellar makes a convenient room for mosquito experiments, since temperature and light can be easily controlled (Tirana, Albania; photo by L. W. Hackett).

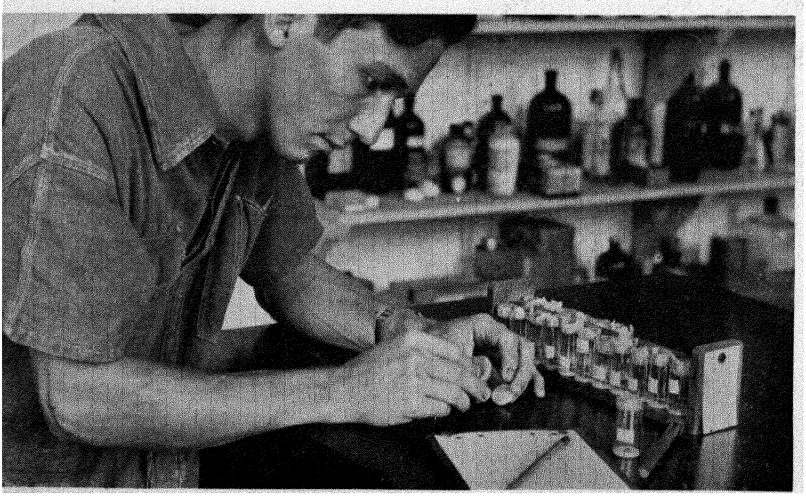


This type of water bath, modeled after a design by Boyd, furnishes a convenient means of raising numbers of larvae at a constant temperature (Tirana, Albania).

PLATE 16. MOSQUITO TECHNIQUE



A bent glass tube forms a useful instrument for handling live mosquitoes; the end of the tube is stoppered with a piece of bolting cloth, so that the mosquitoes can be sucked in and blown out with the breath (Tirana, Albania).



Individual mosquitoes can conveniently be kept in vials stoppered with cotton plugs or wire cups. Such vials serve for oviposition or for infection experiments (Villavicencio, Colombia).

Laboratory transmission experiments with the eastern virus have been summarized by W. A. Davis (1940). Transmission has been obtained with *Aëdes aegypti*, *A. cantator*, *A. sollicitans*, *A. taeniorhynchus*, *A. vexans*, *A. atropalpus*, and *A. triseriatus*. Virus persisted in *Anopheles punctipennis* as long as the insects remained alive, but no transmissions were obtained. Completely negative results were obtained with *Culex pipiens*, *C. salinarius*, and *Mansonia perturbans*.

VENEZUELAN EQUINE ENCEPHALITIS

This virus was first recognized as immunologically distinct in 1938; it has since been reported from Colombia, Ecuador, and Trinidad (Hammon and Reeves, 1945). *Mansonia titillans* has been found naturally infected in Trinidad, and experimental transmission has been demonstrated with this species (Gilyard, 1944). Gilyard was able to infect mosquitoes by feeding them on a donkey, which indicates a considerable difference in virus behavior from that of the eastern- and western-type viruses which seem rarely or never to circulate in the blood stream of large mammals in sufficient quantity for mosquito infection (Hammon and Reeves, 1945).

ST. LOUIS ENCEPHALITIS

St. Louis encephalitis was first recognized as a clinical and etiologic entity in the course of an epidemic in St. Louis county in 1933, and the virus has since been found to be widely distributed in the western United States. In most respects the epidemiology and clinical manifestations (in man) appear to be practically identical with those of western equine encephalitis (Hammon and Reeves, 1945).

Virus has been isolated in the field eight times from *Culex tarsalis*, once from *Culex pipiens*, and once from *Aëdes dorsalis* (references in Hammon and Reeves, 1945). Hammon and Reeves (1943b) have reported an extensive series of laboratory transmission experiments with this virus. They obtained transmission with *Culex tarsalis*, *C. coronator*, *Aëdes lateralis*, *A. taeniorhynchus*, *A. nigromaculis*, *A. vexans*, *Theobaldia incidens*, and *T. inornata*. Virus persisted for long periods in *Culex stigmatosoma*, *C. fatigans*, *Psorophora ciliata*, and *Anopheles maculipennis*. Tests were completely negative with *Culex erraticus*, *Aëdes aegypti*, *Psorophora confinnis*, and *Anopheles punctipennis*. In the case of *Culex fatigans*, the negative results were obtained with

a strain from Texas; a strain from Southern California was subsequently found to transmit the virus (Hammon and Reeves, 1943b, footnote).

JAPANESE B ENCEPHALITIS

Much of the literature on this disease has been published in Japanese and Russian periodicals, where it is hardly available to Western workers. Hammon and Reeves (1945) have summarized what is known, pointing out that it is widespread in Japan, Siberia, China, and that there is serological evidence of its presence in Central Africa. It is the most serious of the mosquito-transmitted encephalitides, with high human morbidity and mortality rates in many epidemics.

Reeves and Hammon (1946) have obtained laboratory transmission with *Culex pipiens*, *C. molestus*, *C. fatigans*, *C. tarsalis*, *Aëdes nigromaculis*, *A. dorsalis*, *Theobaldia incidens*, and *T. inornata*. Virus was found to persist in *Aëdes varipalpus* and *Anopheles freeborni*, though no transmissions were obtained. Four experiments with *Aëdes vexans* gave completely negative results. Japanese and Russian workers have isolated the virus in the field from *Culex pipiens* var. *pallens* and *C. tritaeniorhynchus*, and have reported laboratory transmissions with *Aëdes togoi*, *A. albopictus*, and *A. japonicus*.

WEST NILE VIRUS

This virus was isolated in Uganda by the intracerebral inoculation of mice with serum from a woman with a mild febrile illness. It was found to be immunologically distinct from the previously known encephalitides, and from immune surveys it appears to be a widely distributed disease in Central Africa. Philip and Smadel (1943) demonstrated that the virus could be transmitted in the laboratory by *Aëdes albopictus*, using hamsters as hosts.

LYMPHOCYTIC CHORIOMENINGITIS

Coggeshall (1939) found that the virus agent of this disease could be transmitted from guinea pig to guinea pig by the bite of *Aëdes aegypti*. Experiments by Milzer (1942) show the importance of temperature in this transmission mechanism; experiments were successful when the mosquitoes were kept at 28°, 30°, and 32° during the incubation period, while no virus could be demonstrated in mosquitoes kept at 25°. Transmission experiments with *Culex pipiens* and *Aëdes*

albopictus failed, but the failures lack significance since the mosquitoes were maintained at temperatures below 25°. Various other arthropods have been reported to be capable of transmitting this virus. It is generally considered to be unlikely that mosquitoes are involved in the epidemiology of the disease.

RIFT VALLEY FEVER

This is a virus disease of young sheep and calves named for the Rift Valley of Kenya where it was first observed. The virus has been subjected to considerable laboratory study, and a number of accidental human infections in laboratory workers have resulted in a disease clinically similar to dengue or mild attacks of yellow fever. The virus may be handled by the same manipulative techniques as yellow fever in the laboratory, differing in that adult white mice show circulating virus and are susceptible on peripheral as well as intracerebral inoculation. With yellow fever and many of the encephalitides, adult mice are susceptible to virus only on intracerebral inoculation, and the virus remains localized in nerve tissue. Laboratory studies of the virus have been summarized by Kitchen (1934).

Laboratory transmission experiments with this virus are incomplete but suggestive. Daubney and Hudson (1933) found that *Mansonia fuscopennata*, *M. versicolor*, and *M. microannulata* all retained the virus for at least 9 days: transmission by bite was not obtained, perhaps because of difficulty in inducing the mosquitoes to feed. Mulligan (1938) recovered virus from wild-caught specimens of *Aëdes durbanensis* by inoculation in lambs and mice, but again failed to obtain transmission by bite. Smithburn and Haddow (1946) in the course of attempts to isolate yellow-fever virus from forest mosquitoes in Uganda recovered Rift Valley fever virus from a mixed lot of species of *Eretmapodites*, and in another case from species of the *Aëdes tarsalis* group.

OTHER NEUROTROPIC VIRUSES FROM MOSQUITOES

Extensive attempts have been made to discover the relative importance of various mosquito species as vectors of yellow fever in South America and Africa, and of encephalitides in North America, by testing wild-caught mosquitoes for virus. Normally, pools of mosquitoes are ground up together in a diluent and the resulting suspension inoculated intracerebrally in white mice, since these animals are

highly susceptible to such viruses by this inoculation route. The animals are observed daily, and any that show signs of illness are killed, and brain suspension passaged to new mice so that an attempt can be made to discover the nature of the infectious agent. If filtration through the standard Seitz or Berkefeld filters shows the agent to be a virus, specific identification is attempted by immunological procedures. For example, if the virus is inactivated by incubation with serum known to contain yellow-fever antibodies, in suitably controlled experiments, the infectious agent can be assumed to be yellow-fever virus, and so forth.

In the course of these attempts to determine the natural vectors of the encephalitides and yellow fever, several viruses have been discovered capable of causing encephalitis on intracerebral inoculation in mice, but immunologically distinct from the causative agents of any of the known human or animal diseases. These viruses form a fascinating, and by no means easy, field of study. The presence of many such infectious agents in widely separated parts of the world surely indicates a special relationship between mosquitoes and an extensive group of agents pathogenic for vertebrates, of which perhaps only a small fraction that happen to cause serious disease in man or his domestic animals have previously come to our attention.

The description and demonstration of the specific distinctness of a supposedly new virus is a tedious process, and the publication of detailed studies of these various viruses isolated from mosquitoes has consequently been delayed. The following list is at least suggestive of the fruitfulness of this line of investigation.

Semliki Forest Virus, described by Smithburn and Haddow (1944), was isolated from a batch of 130 mosquitoes of the *Aëdes* (*Aëdimorphus*) *abnormalis* group by the inoculation of a filtrate of the mosquito suspension intracerebrally in white mice. Specific humoral antibodies for the virus were subsequently found in humans and six species of monkeys, and it seems likely that the monkey *Cercopithecus nictitans* may be the natural host of the virus. Attempts at laboratory transmission of the virus with *Aëdes aegypti* and *Mansonia fuscopennatus* failed (Smithburn, Mahaffy, and Haddow, 1944).

Bunyamwera Virus was also isolated in the course of yellow-fever studies in Uganda (Smithburn, Haddow, and Mahaffy, 1946). It was recovered from a rhesus monkey that had been inoculated with the

unfiltered supernate of a suspension of 4,114 specimens of 14 different species of *Aëdes*. Neutralizing antibodies for the virus were later found in a forest monkey and in several humans. Smithburn and his associates mention that still a third, as yet undescribed, neurotropic virus has been recovered from mosquitoes in the course of their work.

Anopheles A Virus (Roca, 1944) was recovered in the course of yellow-fever studies in eastern Colombia by the intracerebral inoculation in white mice of a suspension of 59 specimens of *Anopheles (Kerteszia) boliviensis.*

Anopheles B Virus (Roca, 1944) was recovered six months later from a suspension of 21 specimens of *Anopheles boliviensis* from a slightly different locality. It proved to be immunologically distinct from the previous *Anopheles* virus and from other neurotropic viruses.

Wyeomyia Virus (Roca, 1944) was recovered from a group of 160 *Wyeomyia melanocephala* in the course of the same epidemiological study of yellow fever.

Ilheus Virus (Laemmert and Hughes, 1947) was isolated in the course of yellow-fever studies near Iheus on the Brazilian coast from a group of mosquitoes containing various species of *Psorophora* and *Aëdes*. Various native mammals, including marmosets, were found to be somewhat susceptible to the virus, and laboratory transmission was obtained with *Aëdes aegypti, Aëdes serratus,* and *Psorophora ferox,* using baby mice as mammal hosts.

Hammon and Reeves (1945) mention the isolation of a virus (BFS-91) which they have been unable to identify from *Aëdes dorsalis* in Kern County, California.

EQUINE INFECTIOUS ANEMIA

This virus disease of horses is believed to be commonly transmitted by bloodsucking arthropods. Stein, Lotze, and Mott (1943) report transmission by *Psorophora columbiae* by interrupted feedings: the mosquitoes were allowed to start feeding on an infected horse and then to finish feeding on a normal animal, which subsequently became infected. This is "mechanical transmission" in the classification of Huff, and is thus a quite different phenomenon from mosquito transmission of the neurotropic viruses, which become definitely established in the insect tissues.

FOWL POX

This virus disease of poultry is believed normally to be transmitted by direct contact, but various laboratory experiments have indicated that mosquitoes may also be vectors. The transmission again is probably mechanical, since there seems to be no definite incubation period in the mosquito, but in this case the mosquitoes may remain infectious for periods as long as 14 days (Kligler, Muckenfuss, and Rivers, 1928). This long survival may be related to the great resistance of the virus to desiccation: crusts removed from the epithelial lesions of chickens are known to retain virulence for many months.

RABBIT MYXOMA

Another virus disease which can be transmitted mechanically by mosquitoes is a South American infection of rabbits known as "rabbit myxoma." Aragão (1943) obtained transmission with *Aëdes scapularis* and *A. aegypti,* and transmission by *Culex fatigans* has also been demonstrated. The mosquitoes may remain infectious for as long as 17 days, but there is no evidence of an incubation period in the mosquito or of invasion of the mosquito tissues.

ADAPTATIONS AND RELATIONSHIPS AMONG THE MOSQUITO TRANSMITTED VIRUSES

Green (1942) has pointed out that the diverse adaptations of a given virus can conveniently be classified as of three distinct types: cytologic, histologic, and zoologic. Experiments with the modification of virus strains in the laboratory normally involve only the last two classes of adaptations—to types of tissue and to species of host. These adaptations seem at times to be highly restricted. The susceptibility of the white mouse to yellow-fever virus, for instance, depends on the virus being introduced into particular tissues, and varies with the age of the mouse and the genetic composition of the mouse strain. In other cases the adaptations of viruses may seem exceedingly broad. Yellow-fever virus, again, is adapted to an extraordinary range of mammal hosts, including very primitive ones (certain species of marsupials).

Perhaps the greatest contrast in virus adaptation is that offered by the alternation in insect and mammal hosts. The two environments,

from the point of view of the virus, must be totally distinct; yet the adaptations must be highly specific, since the virus is adapted only to certain tissues in certain species of mammals, and only to certain highly circumscribed groups of insects. Tissue specificity has not been demonstrated in the insect host, but there seems to be a high degree of "species specificity," as can be seen from an inspection of the table of experiments with yellow-fever virus in different mosquito species.

In the case of certain plant viruses, it has been found that the virus may become established in a normally nonsusceptible insect species if the virus is introduced directly into the tissues by puncture (experiments reviewed by Storey, 1939). Merrill and Ten Broeck (1935) found that this was also the case with an animal virus. From this it has been concluded that the factor differentiating a vector from a nonvector species of mosquito may be some property of the intestinal mucosa of the mosquito. Many cases, however, have been described in which virus persists in a mosquito species for long periods without the mosquito being able to transmit the virus by bite (for example, the species of *Mansonia* tested with yellow fever by Whitman and Antunes, 1937). In such cases the virus has surely passed the intestinal mucosa of the mosquito, and it seem likely that the rate of growth of the virus in the mosquito tissue is simply much slower than is the case when the virus is established in a vector mosquito species. Mosquito susceptibility to virus invasion is probably a matter of degree, with every gradation between a highly susceptible species (relatively rapid virus multiplication and short incubation period) and a completely nonsusceptible species (no evidence that virus is able to invade the insect tissues). Mosquito susceptibility, in this sense, parallels mammal susceptibility which, with regard to a given virus, also varies greatly from species to species.

The virus-insect adaptations may thus be complex, involving various characteristics of the virus strain and of the insect host. The tissue adaptations of viruses are commonly called "tropisms," and the ability of a virus, pathogenic for mammals, to invade insect tissue and thus to depend on insect vectors for transmission may perhaps conveniently be termed "entomotropism." The viruses that show this adaptation may then be grouped as the "entomotropic viruses." This would include all of the viruses described in the preceding section, except the last three (equine infectious anemia, fowl pox, and rabbit

myxoma), for which mosquitoes seem to be merely mechanical agents of transmission.

These entomotropic viruses may form a related group of pathogenic agents. Some of them have been grouped by Hammon, Reeves, and Gray (1943) as the "arthropod-borne virus encephalitides," but this excludes yellow fever and dengue, which show points of resemblance to the encephalitides aside from the phenomenon of mosquito transmission. It is particularly notable that all of the entomotropic viruses have neurotropic adaptations in the mammalian host in the special sense that they are capable under some circumstances, at least, of causing a fatal encephalitis on intracerebral inoculation in mice, and one is led to wonder whether there is some special connection between entomotropism and neurotropism.

There are, of course, many viruses that cause a fatal encephalitis in white mice on intracerebral inoculation that are not known to be capable of infecting mosquitoes or other arthropods, but the correlation is none the less striking. A special instance of the relationship is the number of new viruses that have been found in different parts of the world by the technique of inoculating wild mosquitoes into mouse brain tissue.

The neurotropism of these viruses may be increased by serial intracerebral passage in mice in the laboratory until the strain so passaged assumes "fixed" characteristics for this tissue, losing many of its other properties. There are many indications that such treatment results in a loss of entomotropism in the particular virus strain so treated. Thus Davis, Lloyd, and Frobisher (1932) found difficulty in infecting *Aëdes aegypti* on rhesus monkeys circulating yellow-fever virus of a fixed neurotropic strain. Sabin and Schlesinger (1945) found with dengue virus after mouse passage that "*Aëdes aegypti* . . . became infected with difficulty since large numbers of mosquitoes and an extrinic incubation period of more than three weeks were required to transmit the virus." Bates and Roca (1946b) failed completely in their attempts to infect haemagogus mosquitoes with neurotropic yellow-fever virus. Hammon and Reeves (1943b) attribute their success in obtaining laboratory transmission of St. Louis encephalitis by mosquitoes, in contrast with previous failures, to the "use of a virus freshly isolated from mosquitoes, not brain-passage 'fixed,' "

Thus while the entomotropic viruses all share a characteristic neurotropic property, the two adaptations are probably quite independent,

since an increase in the neurotropism through laboratory manipulation seems to be accompanied by a loss in the adaptation of the virus to the tissues of its insect vector.

MOSQUITO INFECTION WITH THE ENTOMOTROPIC VIRUSES

The transmission of the entomotropic viruses seems to involve a true infection of the mosquito vector by the virus. There is no evidence of pathological lesions in the tissues, or of a measurable deleterious effect on the survival of the mosquito; but the prime criterion of virus infection is multiplication, and it seems clear that the virus multiplies in the mosquito. Merrill and Ten Broeck (1934) were able to maintain the virus of western equine encephalitis in *Aëdes aegypti* by serial passage through ten successive lots of mosquitoes, each new lot feeding on a suspension of the ground bodies of the mosquitoes of the preceding passage. The virus must thus have multiplied sufficiently in the mosquitoes to compensate for the tremendous loss at each transfer. Whitman (1937), by means of titrations of pools of mosquitoes at regular intervals after infection, showed that the amount of virus of yellow fever in *Aëdes aegypti* drops considerably immediately after the infectious meal, and then begins to build up until the mosquito becomes infective. The same phenomenon was demonstrated by Bates and Roca (1946a) with yellow fever in haemagogus mosquitoes. Trager (1938) demonstrated the multiplication of western equine encephalitis virus in mosquito tissue by the *in vitro* cultivation of the virus in cultures of mosquito tissue.

Whitman and Antunes (1938a) demonstrated that *Aëdes aegypti* could be infected with yellow-fever virus by the immersion of the larvae in virus suspensions, the virus persisting through metamorphosis to the adult mosquito. All attempts to demonstrate hereditary infection of viruses in mosquitoes by transmission through the egg from one generation to another have failed, however.

It may be noted that the evidence for infection in the case of insect vectors of plant viruses is not so clear. The evidence has been reviewed by Storey (1939), Leach (1940), and Bawden (1943). It is probable that in most cases with plant viruses the vector insect ingests a certain amount of virus, which is subsequently redistributed in the course of feedings on other plants. The incubation period, or perhaps better "latent period," in this case would represent the time required for the virus to enter the insect body fluid and reach the saliva. At-

tempts to demonstrate increase in amount of virus in the insect have in general failed, and various authors have found that while an insect may remain infective for long periods, it is by no means infective for life. The length of time that an insect remains infective may depend on the length of time that it has fed upon the source of the virus. In the case of animal viruses, the mosquito seems always to remain infective for life, and while the mosquito infection may depend on the virus dosage ingested, this seems to be a threshold phenomenon.

It is convenient to classify the factors governing mosquito infection —infection by the normal route of feeding on a host with virus circulating in the blood stream—into four groups. These are: (1) the characteristics of the virus, (2) the characteristics of the mosquito, (3) the virus dosage ingested, and (4) the environment of the mosquito.

Characteristics of the virus. If we consider adaptation to growth in mosquito tissue to be a distinct virus property which can be labeled "entomotropism," mosquito infection depends in the first place upon the presence of entomotropic adaptations in a particular kind of virus, and in the second place on the degree of entomotropism shown by the particular strain of the virus. Virus adaptations, like those of bacteria, seem at times to be very labile, subject to considerable modification through passage under different conditions—modification that sometimes is easily reversed and that sometimes seems to be irreversible. There is some evidence in the literature to the effect that mosquito infection may vary with different strains of a given virus, and in particular that modification through an increase in neurotropism by brain passage may result in a decrease in entomotropism.

Characteristics of the mosquito. Infection with a given virus clearly depends on the species of mosquito, and to a large extent also upon the species group (genus or subgenus). The most extensive experiments have been made with yellow-fever virus, and the correlation between susceptibility and generic grouping (shown in Table XI) is striking. The species of the subgenus *Stegomyia* of *Aëdes*, and of the genus *Haemagogus*, seem to be particularly efficient vectors for yellow fever. The frequency with which the various entomotropic viruses may be transmitted by *Aëdes* seems to reflect some special susceptibility of this group to virus infection. The single species, *Aëdes aegypti*, has been found to be capable of transmitting a large propor-

tion of these viruses, and there seem to be no reports of completely negative results with this insect, though experiments are lacking in the case of many of the viruses. *Anopheles*, on the other hand, seems to be resistant to infection with entomotropic viruses, and it is clearly associated with such pathogens only in the case of Roca's Anopheles A and B viruses.

In most cases it is uncertain whether a given mosquito species may include different genetic strains of varying virus susceptibility. In many experiments only a certain proportion of the individual mosquitoes will be found to be infected, and this may reflect genetic characteristics of these individuals, or it may be the result of some accident such as the amount of virus ingested. Hammon and Reeves (1943b) found that a strain of *Culex fatigans* from Southern California would transmit St. Louis encephalitis, while a strain from Texas failed to transmit.

Virus dosage ingested. Regular mosquito infection clearly depends on the ingestion of a certain minimal infectious dose of virus, probably varying with the kind of virus, kind of mosquito, and circumstances of infection. Infection experiments with many viruses, however, have been found to be unsatisfactory when source animals were used with very small amounts of virus in circulation. Merrill and Ten Broeck (1935) have shown the importance of dosage in governing infection of *Aëdes aegypti* with virus of western equine encephalitis, and an analysis of experiments from this point of view with yellow-fever virus and haemagogus mosquitoes has been reported by Bates and Roca (1946a).

Environmental factors. The length of the virus incubation period in mosquitoes is clearly a function of temperature, and there is also a certain amount of evidence that the original establishment of the virus infection in the mosquito may depend in part on temperature. Milzer (1942) found that *Aëdes aegypti* did not become infected with the virus of lymphocytic choriomeningitis at temperatures below 26°, and Bates and Roca (1945, 1946a) found that haemagogus became infected with yellow fever at temperatures below 25° only when they had ingested very large amounts of virus. Reports of conflicting experiments in the literature with transmission by a given species of mosquito may often reflect differences in the temperature conditions under which the experiments were made.

THE EXTRINSIC INCUBATION PERIOD

The period between the infectious feeding and the first infective feeding of a mosquito is known as the "extrinsic incubation period." Its length depends on the same factors as those that control infection itself: kind of virus, kind of mosquito, dosage ingested, and temperature. That strain of virus may be a factor is shown by the experiments of Whitman and Antunes (1938b), who found that the incubation period of yellow-fever virus in *Aëdes aegypti* was longer in the case of South American "jungle" strains than in the case of the African Asibi strain. With a given virus, the length of the incubation period seems to differ somewhat with each species of mosquito. In the case of yellow fever, for instance, *Aëdes aegypti* is normally infective after an incubation period of nine or ten days under "room-temperature" conditions; with *Culex thalassius* under similar conditions, Kerr (1932) found an incubation period of at least 27 days to be necessary before the mosquitoes became infective. The effect of virus dosage, which is not easily evaluated, has been discussed by Bates and Roca (1946a).

Most interest has centered on the effect of temperature on the length of the extrinsic incubation period. Davis (1932a) found that with *Aëdes aegypti* and the African Asibi strain of yellow-fever virus, mosquitoes became infective after 4 days at 37°, 5 days at 36°, and 6 days at 31°. At room temperature averaging 25.1°, the mosquitoes became infective at the end of 8 days; when the room temperature averaged 23.4°, they were infective after 11 days but not after 9 days. At a constant temperature of 21°, the mosquitoes were infective after 18 days, but at 18° they were not infective even after 30 days.

With *Haemagogus spegazzinii* and a Colombian strain of yellow-fever virus, Bates and Roca (1946a) found the minimum incubation period to be 28 days at a constant temperature of 25°, 23 days when kept for 20 hours daily at 25° and 4 hours daily at 30°, 12 days when kept for 20 hours daily at 25° and 4 hours daily at 35°, and 10 days at a constant temperature of 30°. The effect of the short daily exposure to 35° in shortening the incubation period is striking, since the mean temperature under these conditions (calculated by hours) would be only 26.6°. A constant temperature of 35° was found to be very unfavorable to the mosquitoes, and no transmissions were obtained.

Whitman (1937) attempted to follow the course of the virus in mosquitoes by the titration of pools of mosquitoes at regular intervals after infection, and Bates and Roca (1946a) attempted the same thing by the inoculation of single mosquitoes into groups of mice. From this work it appears that there is an initial period of virus loss in the mosquito, lasting for 2 days at 30°, 3 to 4 days at 25°, and 5 days at 20°; this is followed by a period of virus gain, during which the amount of virus demonstrable in the mosquitoes gradually increases, the rate of increase depending on the temperature. At a constant temperature of 20°, no increase in the virus content of the mosquito was demonstrable, the virus remaining at the level established at the end of the "period of virus loss." It looks as though transmission were a threshold phenomenon, the mosquito becoming infective when its total virus content had reached a certain level—a level marked by the appearance of a minimum infective dose of virus in the salivary secretions.

Various experiments (for example, those of Davis and Shannon, 1930) indicate that virus is very generally distributed through the mosquito tissues during the incubation period.

VIRUS TRANSMISSION

Once a mosquito becomes infective with yellow-fever virus, and presumably also with the other entomotropic viruses, all of the evidence indicates that it remains infective for life. Bates and Roca (1946a) were able to keep records of the transmission of individual mosquitoes by having them feed on baby mice, which were very susceptible and available in large numbers, and the instances in which an individual mosquito that had once infected a mouse failed to be infective on a subsequent occasion were so rare that they could be classed as experimental accidents. In some cases a mosquito could be induced to probe or feed on five or six successive days, transmitting virus on each occasion.

From the behavior of the infections in mice and monkeys infected by the bite of single mosquitoes, the virus dosage injected by the mosquito must be considerable. Davis (1934) attempted to estimate the amount of virus thus injected by the extraction and titration of the total virus content of baby mice immediately after being bitten by mosquitoes. He came to the conclusion that the virus injected was

of the order of magnitude of 100 minimum infective doses (for rhesus), and that it might represent about 1 per cent of the total virus content of the mosquito.

Most transmission experiments with virus have been made by allowing groups of several infected mosquitoes to feed on susceptible hosts. In experiments in which mosquitoes are tested individually, as was done by Bates and Roca, it becomes evident that individual mosquitoes of the same lot show considerable differences in behavior, some becoming infective much earlier than others. This great individual variation is shown nicely in the experiment summarized in Table XII.

TABLE XII TRANSMISSION OF YELLOW-FEVER VIRUS TO BABY MICE BY FEEDINGS OF INDIVIDUAL MOSQUITOES: *Haemagogus spegazzinii* MAINTAINED AT 25° FOR 20 HOURS DAILY AND AT 35° FOR 4 HOURS DAILY (BATES AND ROCA, 1946A)

DAYS	NUMBER OF FEEDINGS	NUMBER OF TRANSMISSIONS	PER CENT TRANSMITTING
11–15	20	3	15
16–20	17	2	13
21–25	12	7	58
26–30	11	6	55
31–35	12	10	83
36–60	12	11	92

CHAPTER XV

MOSQUITOES AS VECTORS OF PLASMODIA

> Malaria, an entomological rather than a social problem, exists where certain anophelines with peculiar feeding habits can maintain themselves in sufficient numbers to ensure transmission.
>
> —L. W. HACKETT

MALARIA is a very important disease. Over wide areas of the earth's surface it is the most serious problem of public health, and any textbook of tropical medicine includes some marshaling of statistics on the morbidity and mortality caused by malaria. The statistics, with their trails of ciphers, involve quantities that pass beyond the zone of easy human comprehension and have little relevance to the measurement of the impact of malaria on the human community. The force of malaria should not be judged by the number of people that it kills, or by the number of man-days of human energy lost in the course of clinical attacks. It is reflected more staggeringly in the accumulated misery of the populations in which it holds full sway. Here, of course, we reach one of those perhaps insoluble cause-and-effect problems: whether the miserable living conditions are the result of the ever present malarial infection, or whether the dominance of malaria is made possible by the miserable condition of the human community. Whichever the cause, the association is clear, and any alleviation of the lot of mankind in the lowland tropics would seem to be contingent on the control of the malaria.

The importance of malaria is very generally recognized, and it would be possible to compile imposing statistics on the amount of scientific energy that has gone into its study, on the accumulated verbiage of the books, articles, and pamphlets that have been written about it, on the number of millions of dollars that are spent annually in efforts at control. And the result of all of this expenditure of energy, verbiage, and money has been very considerable progress, by any criterion. Malaria, as a global problem, has not been conquered in

the sense that yellow fever or smallpox have been conquered, but many competent authorities consider that we have all of the essential tools, in insecticides, techniques, and drugs, that are necessary for the conquest. The major difficulty according to this school of thought, is not lack of tools, but lack of organization, money, and knowledge in the areas where these are most needed.

I must admit that I subscribe to this school of thought, even though at first glance this opinion would seem to involve the corollary that further "malaria research" was unnecessary and hence a waste of time and money. From an immediately practical point of view, perhaps the corollary is justified: better insecticides, better techniques, and better drugs may well be found, but the great practical need seems to be for the organization and application of the resources that are already available.

This is, however, not a valid argument for the abandonment of malaria research: it is an argument for the planning of this research with theoretical rather than practical aims. The practical work of past years has resulted in an impressive accumulation of information about the parasite, the vector, and the host relationships in malaria, which provides an almost unique background for further studies. Studies of malaria, released from the immediate practical objective, may serve to throw light on the general phenomena of parasitism and disease epidemiology, may serve to explain the origin and biological significance of this fascinating relationship between plasmodium, mosquito and mammal, bird or reptile.

The subject of "mosquitoes as vectors of plasmodia" is too large for any significant review within the confines of this chapter, but the subject has been well covered by other authors, so that there is no real need for another review. The object here is primarily to place the mosquito-plasmodium relationship in perspective with the other aspects of the natural history of mosquitoes, and the emphasis is thus somewhat different from that usually found in textbooks of tropical medicine or medical entomology. For an introduction to "malariology" in all of its ramifications, the books by Boyd (1930) or Russell, West and Manwell (1946) should be consulted. The books by Hackett (1937) and Swellengrebel and de Buck (1938) tell the fascinating story of the study of the epidemiology of malaria in Europe, a special case, but one that illustrates the problems of malaria everywhere.

TYPES OF PLASMODIA

The genus *Plasmodium* is generally defined as including parasites which undergo asexual reproduction in the red blood cells of vertebrates (though there may also be "exoerythrocytic" stages), the asexual forms containing pigment granules derived from the blood cells, and at times forming sexual forms (or gametocytes) which undergo further development only in an alternate arthropod host (mosquitoes). The genus is usually placed in a family by itself, the Plasmodiidae, belonging to the order Haemosporidia of the Protozoan class Sporozoa. Similar haemosporidia are the genera *Haemoproteus* which, like *Plasmodium*, has sexual forms (gametocytes) in the red blood cells, but not asexual forms; and *Leucocytozoon*, which has the sexual form in leucocytes. *Babesia* which, like *Plasmodium*, is intracellular in red blood cells, has no pigment granules. As far as is known, all plasmodia have mosquitoes as alternate hosts, going through a sexual cycle in the insect. The other haemosporidia have similar life cycles, but with other types of artropods as alternate hosts.

There is a general agreement among parasitologists that human malaria may be caused by four different species of *Plasmodium*. With regard to the plasmodia found in other types of vertebrates, there is no such agreement. Russell, West, and Manwell (1946) in a review of the animal plasmodia consider that there are at least a dozen species in birds, five species in monkeys, and thirteen species in reptiles. In addition several species have been described from bats in various parts of the Old World, and from other mammals, including squirrels, dormice, buffalo, and antelope. Plasmodia have also recently been described from amphibia. Mosquito studies have been made only with the plasmodia of reptiles, birds, monkeys, and man.

Reptilian plasmodia. Thompson and Huff (1944) have given an interesting account of what is known about the malarial parasites of lizards. Huff once observed an oöcyst of *Plasmodium floridense* in *Aëdes aegypti*, but subsequent attempts to infect *Aëdes aegypti* and *Culex pipiens* with lizard parasites failed. In one experiment in which these species fed on lizards with gametocytes of *Plasmodium rhadinurum* in circulation, all of the mosquitoes died within 24 hours after feeding, though other specimens that had fed on uninfected lizards at the same time, and that were kept under similar conditions,

showed good survival. This suggests that the parasites may be lethal for these mosquitoes.

Avian plasmodia. Hewitt (1940) has written an excellent, book-length summary of the literature on bird malaria. The fact that relatively little space in his book is devoted to the phenomena of mosquito transmission reflects the general preoccupation of parasitologists with the behavior of plasmodia in the vertebrate rather than the mosquito hosts. Hewitt has summarized successful attempts at mosquito infection with bird plasmodia: nineteen species of mosquito at that time had been found to be susceptible to one or more of six species of avian plasmodia. *Culex pipiens* had been successfully infected with five of the six plasmodia: *P. relictum*, *P. cathemerium*, *P. elongatum*, *P. circumflexum*, and *P. rouxi*. The sixth plasmodium, *P. gallinaceum*, was infective only for three species of *Aëdes*.

Most experimental work has been carried out with *Plasmodium relictum* and *P. cathemerium*, both originally isolated from English sparrows, and occurring in Europe and North America. Both species have been found to be pathogenic for a wide variety of passerine birds, and laboratory studies are generally carried out with canaries as hosts. Hewitt lists the following mosquitoes as susceptible to infection with *P. relictum: Aëdes aegypti*, *A. communis*, *A. mariae*, *Culex fuscanus*, *C. hortensis*, *C. fatigans*, *C. pipiens*, *C. salinarius*, *C. trasalis*, *C. territans*, and *Theobaldia longiareolata*. *Plasmodium cathemerium* has a similar range of mosquito hosts.

Hewitt has not summarized the mosquito experiments that gave negative results, though such experiments are very important in judging the degree of host specificity shown by a parasite. Huff (1927) reported on an extensive series of attempts to infect various mosquito species with these plasmodia, especially *P. relictum*. He encountered technical difficulties, mainly in inducing the mosquitoes to feed, so that he was able to complete transmission experiments only in three cases. He failed to obtain stomach or gland infections, however, with five species of *Aëdes*, with *Anopheles punctipennis* and with *Psorophora ferox*, while positive results were obtained with *Aëdes aegypti* and various species of *Culex*.

In recent years extensive experiments have been made with two species of plasmodia pathogenic for gallinaceous birds, *P. gallinaceum* and *P. lophurae*. Both may be transmitted by *Aëdes aegypti;* and *Culex pipiens*, the common laboratory vector of the plasmodia of

passerine birds, is completely insusceptible to both. Jeffery (1944) has reported on a series of parallel experiments with *Plasmodium lophurae*. He found *Anopheles quadrimaculatus* to be the most susceptible of the four mosquitoes tested, with *Aëdes albopictus* and *A. aegypti* following in that order; no infections at all were obtained with *Culex pipiens*.

Species of the genus *Culex* have generally been supposed to be the chief natural vectors of avian malaria, because many species apparently have birds as preferred hosts, and because they are ready vectors of the passerine plasmodia under laboratory conditions. Our knowledge of the vector relationships of these parasites, however, is incomplete, to say the least.

Primate plasmodia. Aberle (1945) has made a very complete compilation of the literature on primate malaria. He lists a total of fifty attempts by different workers to infect mosquitoes with various species of monkey plasmodia: *P. brasilianum*, *P. cynomolgi*, *P. gonderi*, *P. inui*, *P. knowlesi*, and *P. kochi*. Attempts to infect mosquitoes other than *Anopheles* with these parasites all failed (except for one instance in which a specimen of *Culex vishnui* showed oöcysts in the stomach), though the number of experiments is perhaps too small to be given much weight. Only thirteen attempts to infect mosquitoes other than *Anopheles* seem to have been made. These include attempts to infect *Culex pipiens* with *P. cynomolgi*, *P. knowlesi*, and *P. kochi; Culex fatigans* with *P. inui; Aëdes aegypti* with *P. cynomolgi* and *P. kochi; Aëdes albopictus* with *P. inui; Mansonia africanus* with *P. kochi* and *Armigeres obturbans* with *P. inui*.

Experiments with anophelines have by no means been universally successful. Negative results with mosquito infections with plasmodia are, however, notoriously difficult to evaluate and the number of experiments is quite inadequate for judging possible differences in susceptibility among anopheline species. It is clear, however, that the different species of plasmodium found in primates have different vector relations. Thus Coggeshall (1941) had no difficulty in infecting *Anopheles quadrimaculatus* with *Plasmodium cynomolgi*, obtaining numerous and heavy infections (as many as 1080 oöcysts on one stomach) and successfully transmitting the parasite from rhesus monkey to rhesus monkey by mosquito bite. He failed completely, however, in comparable attempts to infect these mosquitoes with *Plasmodium knowlesi* or *P. inui*.

Attempts to infect anophelines with *Plasmodium cynomolgi* have quite generally been successful, and positive results have been reported with several species: *A. maculipennis*, *A. maculatus*, *A. culicifacies*, *A. splendidus*, and *A. annularis*, as well as *A. quadrimaculatus* (references in Aberle, 1945). It thus seems very likely that this plasmodium has the same vector relations as the human plasmodia. In the case of the other primate plasmodia, the evidence is not so clear, despite the occasional positive results with anophelines.

Human plasmodia. Malaria in man is generally considered to be caused by four morphologically distinct species of parasites, commonly known as *Plasmodium falciparum*, *P. vivax*, *P. malariae*, and *P. ovale*. Each of these "species" is apparently made up of numerous "strains" which may show considerable differences in behavior. All are highly host specific for man; attempts to infect other primates with human parasites have very generally failed, although some of the monkey plasmodia are morphologically hardly distinguishable from the human forms.

Studies of the vector relationship between mosquitoes and human plasmodia are covered in a literature that is so vast that adequate summary from a particular point of view is almost impossible. Direct mosquito-infection studies have generally been of two types: the dissection of wild-caught specimens in an effort to determine natural vectors, and laboratory tests to determine species susceptibility. Parasites found by the dissection of wild mosquitoes cannot be specifically identified, so that it is not possible by this means to get data on the relative importance of different anopheline species as vectors of the different plasmodia. Laboratory transmission experiments with human plasmodia are subject to many technical difficulties, and a synthesis of data derived from various experiments carried out by different workers under different conditions would be hazardous, to say the least. In general, however, no mosquito other than species of *Anopheles* (in the wide sense) has been found to be susceptible to infection with human parasites; and all species of *Anopheles* that have been adequately tested by various workers under different environmental conditions have been found to be susceptible in some degree to infection by some strain or other of the human plasmodia.

Most experimental work has been done with *Plasmodium vivax* and *falciparum*. Infection experiments with *P. malariae* are so difficult that really adequate laboratory reproduction of a transmission cycle was

not obtained until 1933 (Siddons, 1944a). Studies of *P. ovale* are inadequate largely because of the comparative rarity of this species of parasite.

A "critical review of the data regarding the transmission of malaria by the different species of *Anopheles*" was published by Covell (1927) with a later supplement (1931a). Weyer (1939) has made a similar compilation. In addition there are various reviews for limited regions, such as those by Covell (1944) for India and the Far East, and Simmons (1941) for North and Middle America. The booklet by Russell, Rozeboom, and Stone (1943) on the anophelines of the world contains notes on the relation of various species to malaria as far as is known.

DEVELOPMENT OF THE PLASMODIUM IN THE MOSQUITO

Infection of the mosquito depends on the ingestion, with the blood meal, of sexual forms of the parasite: microgametocytes, or male forms, and macrogametocytes, or female forms. These are present in varying abundance in the peripheral blood of infected vertebrates, and the factors that govern their time of appearance and density are obscure.

"Gametogenesis," or the transformation of the gametocytes into "gametes," mature sexual cells, occurs in the mosquito stomach. The process in the male cells can be seen under the microscope if a drop of blood with gametocytes, mixed with a little saline-citrate solution to prevent clotting, is observed for a few minutes under a cover slip. "The process of exflagellation may be completed within ten or fifteen minutes, fertilization ensuing soon thereafter. The change in the male cell involves leaving the host erythrocyte, and the extrusion of about eight microgametes, resembling long flagella. These remain attached to the parent cell for a few minutes, whipping about actively until they are at last liberated and swim away, seeking the female gamete" (Russell, West, and Manwell). The gametogenesis of the female cell is much less conspicuous.

Fertilization, or the union of the micro- and macrogametes, results in the production of a motile, elongate zygote, called the "oökinete." The process up to this stage can be observed *in vitro*, and occurs in any mosquito, whether susceptible to infection or not. The next step, penetration of the stomach wall by the oökinete, seems to be crucial in determining whether an infection will be established in the mos-

quito or not. Huff (1934) has studied this process in great detail, and reached the conclusion that penetration is extracellular, and not a boring process.

When the oökinetes are first found in the vicinity of the stomach wall they are lying parallel to it and in the serous mucoid layer adjacent to the cells of the stomach wall. As the parasite grows it becomes relatively thicker and gradually forces two of the stomach cells apart. It gradually becomes more spherical and forces the stomach cells apart nearer and nearer the outside of the stomach wall. The stomach cells now begin to come back to their original positions on the inner side. Finally the parasite, now an oöcyst, comes to lie under the outer envelope of the stomach.

Once established on the outside of the stomach wall, the oöcyst grows rapidly, the rate of growth depending on the temperature, the parasite species, and perhaps also on the mosquito species. Huff (1940) has made a statistical study of oöcyst growth with several strains of avian plasmodia. When mature, the oöcysts may have a diameter of 50 to 60 microns. The parasite cytoplasmic material undergoes repeated division during this process of growth until, at maturity, the oöcyst contents are several hundred to several thousand spindle-shaped "sporozoites." The oöcyst bursts, and the sporozoites are liberated into the body cavity of the mosquito, finding their way to the salivary glands where they become lodged either within the cells or in the ducts, ready to infect the next vertebrate host.

The vector relationship between mosquitoes and plasmodia is thus *cyclopropagative*, in the classification of Huff (1931), since the parasites undergo both cyclical change and multiplication. Since the sexual stage of the parasite cycle occurs in the mosquito host, this is considered to be the "definitive host," with vertebrates as "intermediate hosts." The vertebrate in this case, however, would seem to serve as the "reservoir" for the maintenance of the disease, since the infection may persist indefinitely, while in the mosquito it is limited to a single cycle. This contrasts with the relationship in viruses, such as yellow fever, where the vertebrate infection is limited to an acute attack followed by immunity, while the mosquito infection is limited only by the life of the insect. Plasmodial sporozoites in mosquito glands may apparently remain infective for very long periods, but even so in malaria the insect seems to act largely as a transmitting agent, not as a means for the maintenance or geographical dispersal of the parasite.

FACTORS GOVERNING MOSQUITO INFECTION

A great deal of attention has been given, in the study of the epidemiology of human malaria, to the problem of the factors that make one species of mosquito a dangerous vector, while other species apparently are unimportant. Hundreds of species of anophelines have been described, and in so far as these have been tested in the laboratory, all seem to be more or less susceptible to infection with human plasmodia. Yet it is increasingly clear that in any given region it is usually a single species that is primarily responsible for the transmission of the disease. Study has centered on the human disease since nothing is known of the natural vectors of the primate and avian plasmodia, and little of the epidemiology (or epizoology, if you will) of the diseases they cause. The primate and avian plasmodia have, however, been extensively used for laboratory studies of the factors governing mosquito susceptibility, so that any synthesis of our knowledge of the vector relationship of mosquitoes and plasmodia would have to take into account studies with all three groups of parasites.

It seems to me that the factors governing vector efficiency are most easily considered as belonging to two general classes, which might be called ecological and physiological. The division is perhaps arbitrary, like the division between the sciences of ecology and physiology, and depends chiefly on whether the factors are most readily studied by field or laboratory techniques.

As *ecological factors* I would include the elements of the life history of the vector that determine its association with the vertebrate host and its ability to sustain the parasite. It is axiomatic that an efficient vector must have habits that lead to a close association with the vertebrate host: in the case of human malaria, the mosquito species must bite man readily, and (for "civilized" man, at least) must have biting habits that lead it to haunt human dwellings. It must be an abundant species, and a significant proportion of the species population must live long enough for the completion of the parasite cycle. The importance of these various ecological factors has long been recognized, and they have served as the stimulus for a great deal of the investigation of mosquito biology that has been summarized in the earlier chapters of this book.

The *physiological* factors in plasmodial infections can perhaps be most conveniently discussed under the same four general headings

that were used in the case of virus infections: (1) characteristics of the parasite; (2) characteristics of the mosquito; (3) the parasite dosage; and (4) the environment of the mosquito.

Characteristics of the parasite. The problem of mosquito susceptibility to parasites has been studied from two angles: the susceptibility of a given mosquito species or strain to different species and strains of parasites, and the susceptibilty of different strains and species of mosquito to a given parasite strain. The present section is devoted to studies of the first sort.

Presumably each species of *Plasmodium* has a characteristic range of mosquito hosts, as we attempted to show in the section on types of plasmodia earlier in this chapter. Among the three common human species (*vivax, falciparum,* and *malariae*), however, no sharp differences in vector susceptibility have been demonstrated: it seems that "strains" of a given human plasmodia species may show greater differences than are shown among the species themselves. This was first observed by James, Nicol and Shute (1932), who found that they could not infect their English *Anopheles atroparvus* with an Indian strain of *falciparum* malaria, though these mosquitoes could be infected with Italian strains. Boyd, Carr, and Rozeboom (1938) carried these studies further by testing *Anopheles quadrimaculatus* and *A. punctipennis* from Florida, and *A. albimanus* from Panama, with strains of *vivax* and *falciparum* from Florida and Panama. They found that the Florida mosquitoes were easily infected with both the Florida and Panama strains of parasites, but that the Panama mosquitoes were distinctly refractory to the Florida parasite strains that were tested.

This suggested that there might be some relation between the strains of plasmodia and the species of anophelines found in a given locality. Further studies, however, have failed to show any very clear-cut geographical relationship in mosquito susceptibility. Thus Boyd (1940) remarks that "local strains of parasites may or may not show a high degree of adaptation to anophelines which are coindigenous to their own faunal regions, and, conversely, anophelines may or may not show a high degree of susceptibility to exotic strains of the parasites." Other studies of this sort have been reported by Watson (1945) and Young and others (1946).

Boyd, Stratman-Thomas, and Kitchen (1935) found that to infect *Anopheles quadrimaculatus* with *Plasmodium falciparum* a higher density of gametocytes should be in circulation in the host than was

needed to infect the same mosquito with *P. vivax:* but it is difficult to be sure whether this is a property of the two plasmodium species, or of the particular strains used.

Laboratory strains of plasmodia are commonly maintained by blood inoculations from host to host, so that the normal alternation of vertebrate and mosquito cycles is short-circuited. In the course of such passages several strains of different types of plasmodia have been observed to lose the property of producing gametocytes, thus becoming completely uninfectious for mosquitoes. These cases have been reviewed by Huff and Gambrell (1934). In some instances when gametocyte production has become low in the course of constant direct blood passages, the ability to produce large numbers of gametocytes may be regained after mosquito passage (Huff, 1941a).

Characteristics of the mosquito. It has long been known that different species of mosquitoes differ greatly in their susceptibility to infection with a given species or strain of plasmodium, and it has more recently been found that susceptibility may be a characteristic of different genetic strains of the same mosquito species. Comparisons of susceptibility to laboratory infection among different anopheline species have been reported by many authors. Thus Boyd and Stratman-Thomas (1934b) found that *Anopheles quadrimaculatus* was more susceptible than *A. crucians* to infection with all three human plasmodia in parallel experiments. Barber and Rice (1935b) in a long series of experiments obtained laboratory infections in 20 per cent of the specimens of *Anopheles sacharovi*, 20 per cent of *A. maculipennis* (presumably a mixture of typical *maculipennis* and *subalpinus*), and in 55 per cent of *A. superpictus*. Boyd and Kitchen (1936) found that *Anopheles quadrimaculatus* and *A. punctipennis* were about equally susceptible to two strains of *Plasmodium vivax*, but that *A. punctipennis* varied from high susceptibility to a probable refractoriness to different strains of *P. falciparum*, while *A. quadrimaculatus* showed some degree of susceptibility to all of the *falciparum* strains tested.

Huff (1929, 1934) discovered that strains of *Culex pipiens* (probably the species now known as *Culex molestus*) susceptible and insusceptible to infection with *Plasmodium cathemerium* and *P. relictum* could be established by laboratory selection. Natural immunity to the plasmodium seemed to be a hereditary characteristic, behaving as a Mendelian dominant. Attempts to demonstrate differences in susceptibility among strains of a given anopheline species have not been

so successful. Thus Boyd, Kitchen, and Mulrennan (1936) found both the "inland" and "coastal" varieties of *Anopheles crucians* to be relatively poor hosts for *Plasmodium falciparum.* Russell and Mohan (1939a) were not able to find any differences in susceptibility between their two "races" of *Anopheles stephensi.* Boyd (1941) found no difference between two strains of *A. quadrimaculatus,* one from Florida and the other from Alabama. Russell and Mohan (1939b) tried a slightly different approach by testing the susceptibility of *A. stephensi* bred from larvae kept in different types of environments; they failed to detect any significant difference in susceptibility to *P. falciparum.*

Hovanitz (1947) made an extensive series of experiments with the susceptibility of *Aëdes aegypti* to *Plasmodium gallinaceum* with the object of determining whether this had a genetic basis comparable with that found by Huff in *Culex pipiens.* He was unable to demonstrate any genetic effect in six generations of selection, though he did find considerable individual variation in susceptibility. He observed that mosquitoes that developed more oöcysts also laid more eggs, which he considered might indicate an individual difference in the absorption of blood after digestion.

The parasite dosage. "Dosage" in the case of mosquito infections with plasmodia, means the number of male and female gametocytes ingested with the blood meal. Gametocyte densities should always be calculated at the time mosquitoes are given an infectious meal, but unfortunately, no standard method of expressing such density has been adopted. Some authors give the number of gametocytes per cubic millimeter of blood, some the number per 100 leucocytes, some the number per 10,000 red blood cells. Ten gametocytes per 100 leucocytes would very roughly be the equivalent of 500 gametocytes per cubic millimeter, or of one gametocyte per 10,000 red blood cells. The exact relations would, of course, vary considerably with the species and physiological state of the animal. The method of counting gametocytes per 10,000 red blood cells, commonly used in studies of bird malaria, is probably the most accurate and satisfactory, though tedious.

It is clear that within a certain median range of gametocyte densities, the percentage of mosquitoes infected is dependent on the number of gametocytes in circulation. This is shown, for instance, in the table given by Simmons (1941) for his experiments with *Anopheles punctimacula* and *Plasmodium falciparum* in Panama. In experiments

in which the host showed 0.4 to 3 gametocytes per 100 leucocytes, 11 per cent of the mosquitoes became infected; with 2.5 to 5 gametocytes, 25 per cent; with 6 to 12 gametocytes, 34 per cent; with 13.2 gametocytes, 86 per cent. Another example, with a different type of plasmodium and mosquito, may be found in Table 3 of Jeffery (1944), in which he summarizes the results of infection experiments with *Aëdes albopictus* fed on successive days on ducks. When the gametocyte count was 22 per 10,000 red blood cells, 14 per cent of the mosquitoes became infected; with 68 gametocytes, 20 per cent; with 94 gametocytes, 40 per cent; with 157 gametocytes, 42 per cent.

It seems also that there is a *minimum infectious density* for each particular species (or strain) of parasite and each particular species (or strain) of mosquito. This concept has been discussed by Boyd, Stratman-Thomas, and Kitchen (1935), who found that one gametocyte of each sex per 100 leucocytes would consistently infect about two thirds of the specimens of *Anopheles quadrimaculatus* with *Plasmodium vivax*, while 11 gametocytes of each sex per 100 leucocytes were necessary to achieve a similar percentage of infection with *falciparum.*

There is also, apparently, what might be called an *effective infectious density* at which all susceptible specimens of a given mosquito strain become infected, so that further increases in gametocyte density do not influence the result of the experiment. This concept has been utilized by Huff (1934), who carried out his experiments with the infection of *Culex pipiens* (or more probably, *molestus*) with *Plasmodium cathemerium* and *P. elongatum* using hosts with more than 70 gametocytes per 10,000 red blood cells: at this density he calculated that each mosquito ingested at least 53,200 gametocytes. He was able to show by a series of double feedings that infection in such experiments depended on individual susceptibility rather than on gametocyte density: mosquitoes that acquired oöcysts from the first feeding also became infected at the second feeding, while those that failed to become infected at first were not infected later.

The environment of the mosquito. It was early discovered that environmental temperature played an important part in mosquito infections with plasmodia: that the length of the extrinsic incubation period was a function of temperature, and that infections failed to become established at low temperatures. Stratman-Thomas (1940) has made a careful study of the effect of different temperature con-

ditions on the course of infection of *Anopheles quadrimaculatus* with *Plasmodium vivax*, and his results provide an example of the sort of phenomena that may be expected. He found that the development of the parasite in the mosquito could be completed at constant temperatures between 15°–17° and 30° C., the extrinsic incubation period being 38 days at 15°–17°, 14 days at 22°–23°, 10 days at 26°, and 8 days at 28° and 30°. He considered 28° to be the optimum, since it was "the lowest temperature at which the cycle was completed in the shortest time." Development was never completed at temperatures below 15° or above 30°.

He found that

> shortly after feeding on a gametocyte carrier, *A. quadrimaculatus* was completely sterilized of its plasmodial infection at 37.5° in 2 to 3 hours; at temperatures of 1° to 10°, 2½ days were necessary. During the growth of the oöcysts (7 to 13 days after the infective feeding) exposure of the mosquito to 37.5° for 18 to 24 hours aborted its development, only a very small percentage of these mosquitoes ultimately showing sporozoites in their salivary glands. At 1° to 10°, 24 days were required to interrupt oöcyst development. When the sporogenous cycle was completed and sporozoites were present in the salivary glands of the anophelines, 24 hours' exposure to 37.5° had a marked inhibitory effect on the infectivity of the sporozoites in human inoculation. This effect was not observed in anophelines kept at 1° to 7° until after 50 days at this low temperature.

The parasite was thus found to have much narrower temperature requirements than the mosquito host.

It has generally been found that environmental relative humidity has no effect on the development of the plasmodium in the mosquito, though of course it has a considerable effect on the survival of the mosquito itself. References to specific studies of the effect of these environmental factors are given in the reviews of Huff (1941b) and Siddons (1944b).

MOSQUITO TRANSMISSION

The length of the extrinsic incubation period (or "sporogenous cycle") of plasmodia in mosquitoes varies greatly with the species (or strain) of plasmodium, but as far as I am aware, there is no evidence that the cycle with a given strain of plasmodium varies with the species of mosquito host. This is perhaps related to the fact that mosquito infection with a plasmodium seems to be an all or none

phenomenon, controlled by whether the parasite is able to reach the oöcyst stage on the stomach wall of the mosquito. Once established, the course of development of the oöcyst seems to be dependent on the inherent nature of the parasite and environmental temperature. Individual mosquitoes, if infected at the same time and maintained under uniform conditions, become infective at about the same time. This contrasts with the situation in mosquitoes infected with entomotropic viruses, discussed in the preceding chapter. In virus infections, the length of the extrinsic incubation period in an individual mosquito varies greatly, apparently depending on the dosage originally ingested and the specific or individual nature of the mosquito, as well as on the virus strain and the environmental temperature. In plasmodial infections the mosquito is not infective for vertebrates until the sporozoites have been released from the oöcyst: infections cannot be established by the injection of mosquito stomachs containing immature oöcysts (Brooke, 1942). Vertebrate infections with virus, on the other hand, may be established by the injection of the mosquito at any time after the infectious meal.

All of this is strong evidence of a fundamental difference in the two classes of mosquito infections. The alternation of hosts with plasmodia involves an alternation of parasite cycles in which the parasites differ profoundly both in morphological and physiological characteristics. While we cannot examine the virus particles directly, the indirect evidence would seem to indicate that they undergo no cyclical metamorphosis in the mosquito host.

The specific nature of the length of the extrinsic incubation period in plasmodia is nicely shown by the characteristics of the three common human species. Thus in Boyd's laboratory, in a long series of transmission experiments with many parasite strains, the incubation period (at temperatures in the neighborhood of 20°) for *Plasmodium vivax* has been found to be 16 to 17 days, for *P. falciparum*, 22 to 23 days, and for *P. malariae*, 30 to 35 days (Boyd and Stratman-Thomas, 1933).

The infected mosquito has a definitely limited, though very large, supply of sporozoites available for transmission. Many successive infections may result from the bites of such mosquitoes, but it is not clear whether the infectiveness is ever lost under ordinary circumstances. The infectiveness of mosquitoes harboring *Plasmodium vivax* declines appreciably 30 days and more after the completion of the

extrinsic incubation period (Boyd and Stratman-Thomas, 1934a), and with *P. falciparum* somewhat earlier (Boyd, Stratman-Thomas, and Kitchen, 1936), and no infections have been obtained in Boyd's laboratory after 50 days. These mosquitoes, however, were maintained in a refrigerator, and it seems likely that the loss of infectiveness is a cumulative effect of the cold. Degenerated sporozoites have been observed in naturally infected mosquitoes more often in the temperate zone than in the tropics (Barber, 1936). Such effects might be due to unfavorably high as well as unfavorably low temperatures.

CHAPTER XVI

THE SPECIES PROBLEM

> The close genetic ties which bind species together into single bodies bring into relief the problem of their fission—a problem which involves complexities akin to those that arise in the discussion of the fission of the heavenly bodies, for the attempt to trace the course of events through intermediate states of instability, seems to require in both cases a more detailed knowledge than does the study of stable states.—R. A. FISHER

THE major part of this book has been concerned with matters that would be considered to be purely physiological or purely ecological, according to the common methods of subdividing the biological sciences. The material of the three preceding chapters, however, would probably usually be classed as a part of parasitology or medical entomology, since neither ecology nor physiology are normally considered to include the special problems of the relationships between parasites and hosts. The present chapter and the two following are concerned with subjects classified as part of still another biological science, taxonomy.

All of these topics, however, are integral parts of the natural history of mosquitoes. From this point of view it is even difficult to draw a line between the fields of taxonomy and ecology, though since the one deals with the classification of animals and the other with their environmental relations, they would seem to be sufficiently distinct. Yet the taxonomist, in collecting his animals, has always dealt with ecology; and the ecologist, in identifying his animals, has always become involved in taxonomy. In the particular case of the "species problem," the two fields merge completely, since the recognition and definition of natural populations (species) depends inevitably on both the field studies of conventional ecology and the structural studies of conventional taxonomy. The classification of mosquitoes and the distribution of mosquitoes, which form the subjects of the

following two chapters, are similarly involved in any synthesis of the natural history of the group, though the emphasis in treating such subjects from this point of view may be different from the emphasis that would be given in a purely taxonomic treatment.

It is perhaps an oversimplification to speak of the "species problem," since there is really a whole series of problems involved in the concept of species. In the form of questions, these problems might be stated as: What is a species? How do species become distinct, and by what mechanisms are the distinctions maintained? How can different species be recognized? How are different species and different stages in the process of speciation to be named?

The idea of species seems simple enough—it is a "kind" of animal —but attempts at formal definition of the term meet endless difficulties. A precise and universally applicable definition is perhaps impossible, since by the process of organic evolution we are attempting to isolate under the concept "species" a particular stage in a continuous series of changes. Yet animal life, as we observe it at a particular time and a particular place, does not represent a continuum of variations: the individuals, for the most part, seem to be grouped into distinctive and reproductively independent populations—species. Most of the difficulties come when we attempt to decide whether populations in different places, or at different geological times, are "conspecific." Other difficulties arise when we attempt to frame a definition in terms that will cover plants, bacteria, insects, mammals, and other diverse organisms which may have distinct sorts of mechanisms for maintaining the independent populations that we want to include under our universal term.

In sexually reproducing organisms, "species" has come very generally to be applied to "genetically distinctive, reproductively isolated, natural populations" (Emerson, 1938). In the vast majority of cases, such populations are characterized by distinctive morphological traits which serve for their identification. Much of the task of conventional taxonomy is concerned with the recognition of the morphological discontinuities which reflect the genetic discontinuity resulting from the sexual isolation of these natural populations. In some cases the morphological discontinuities may involve very obscure or apparently trivial structures, and there is increasing evidence that in many instances sexually isolated populations may have no discernible morphological recognition characters at all. Cases of this sort

have come to light mostly because of the intensive study of the genetics of natural populations of *Drosophila*, and are best understood by an examination of the *Drosophila* literature reviewed in such books as those by Dobzhansky (1941), Dobzhansky and Epling (1944), and Patterson (1943). Populations of this sort are aptly called "cryptic species."

The most significant work on the "species problem" among insects has probably been that carried out with *Drosophila*, but the subject has forced itself on the attention of mosquito students because of the anomalous behavior of various presumed species of medical importance, most notably *Anopheles maculipennis*. The study of these mosquitoes has contributed somewhat to an understanding of the general problem, and it seems likely that intensive work on mosquito populations could contribute a great deal more, complementing in a way the *Drosophila* studies, since the mosquito work must necessarily be undertaken from a somewhat different point of view and with different methods of approach.

The "species problem" has become a major preoccupation of current taxonomic science and has formed the subject of a number of recent books which effectively bring together the scattered literature and furnish a background against which further studies may be undertaken. Notable among these books are those by Robson (1928), Robson and Richards (1936), Huxley (1940, 1942), Dobzhansky (1941), and Mayr (1942).

The discovery of cryptic species—of sexually isolated populations with few or no tangible recognition characters—has caused a great deal of pain to some conventional taxonomists, since they feel that the whole basis of the system of Linnean nomenclature is endangered in so far as it depends on the assorting and classification of dead museum specimens. The use of genetic or biological procedures for the identification of a species is certainly a tremendous technical handicap, and the author at one time proposed that the term "species" be limited to populations "distinguishable by some heritable morphological characteristic" (Bates, 1935). Such a limitation is, however, untenable, since the presence, absence, or detection of distinguishing morphological characteristics is essentially irrelevant to the question of whether or not two populations are genetically independent.

Fortunately, it seems probable that conventional taxonomic pro-

cedures serve adequately for the recognition of the vast majority of insect species, and are thus sufficient for the overwhelming task of cataloguing organic diversity. The problem of the description of the million or more forms of insect life seems to be endless, and the investigation of the genetic and biological characteristics of each of the forms is obviously out of the question. This is no reason, however, for stopping taxonomic study at the morphological level in a few groups where, because of ease of technical manipulation (*Drosophila*), or because of economic importance (mosquitoes), work at the physiological level is possible. And in the groups where the recognition of populations is based on nonmorphological criteria, it would seem awkward not to reflect this situation in the nomenclatorial system.

The system of Linnean nomenclature is an extraordinary invention. Devised with the conviction that each species was formed by God, and that the object of natural history was to detect and catalogue these various divine creations, it has withstood many storms since 1758. One is tempted to say that it has survived all advances in biological knowledge; but it would probably be more apt to say that, because of its simplicity as a method of cataloguing and indexing the unwieldy chaos of organic form, it has been a major factor in promoting biological advance. Linnean nomenclature made the recognition of the phenomenon of evolution possible, and withstood the effects of this demonstration that the discontinuities that it catalogued were essentially ephemeral. It has withstood the legal minds who codified its principles and made convenience subordinate to precedent. It will undoubtedly withstand the application of its Latin formulas to cryptic species, painful as the process may be to "cabinet naturalists."

Part of the species problem, then, consists in determining what is meant by the term. If the definition "sexually isolated populations" is accepted, the recognition of species in a given locality generally depends on the discovery of morphological discontinuities serving to mark this sexual isolation. Experimental procedures are necessary where there is doubt as to the significance of the morphological discontinuities, or where anomalies in behavior indicate the possibility of cryptic species.

The question next arises: How did the sexual isolation of these species come about? which is, essentially, the problem of the origin

of species. This also involves taxonomy, because we need not only to recognize the end stage of sexually isolated populations, but also to recognize intermediate, partially isolated populations—subspecies—and to deal in some manner with intraspecific variation.

Some of the most intensive taxonomic work has been carried out in birds, mammals, and butterflies, and students of these groups are very generally convinced that "speciation," the cleavage of a single population into two or more sexually isolated populations, generally occurs through geographical isolation. If genetic interchange is prevented by geographical barriers, the course of evolution in the segregated populations may follow different lines, resulting in differences great enough so that when the two populations again come in contact, intermixture will not occur. Upon this theory is based the geographical subspecies of vertebrates and butterflies, and in these groups no other form of intraspecific variation is recognized in the nomenclatorial system.

The application of the system depends on a process of deduction. If two recognizable different kinds of animals live in the same area, they must be sexually isolated in order to maintain their differences, and therefore they must be "species." If populations in two neighboring areas differ, the differences may be maintained by geographical rather than sexual isolation: if the difference is of an order of magnitude or type that in other situations characterizes coexisting species, the two populations are presumed to be species; if the difference is less so that one can presume that mixing would occur if the geographical barrier were removed, or if intermediate areas are actually occupied by intermediate forms, the populations are called "subspecies."

Many types of factors other than geographical barriers can be imagined as causing the preliminary segregation of populations. Habitat associations within a given area might be so distinct that the part of a population in one habitat would rarely or never come in contact with the part in another habitat. A rhythm of seasonal adaptations might cause isolation, one part of a population reaching sexual maturity at one time of year, another at another time. Host adaptations in parasites may cause very effective isolation, the parasites of one species rarely or never coming in contact with the part of the population parasitizing another species. Some genetic factor causing behavior variation—especially affecting time or method of sexual con-

tact—might at once provide a very effective split in a population. All of these possibilities may be classed as "sympatric speciation" in contrast with the better known geographical or "allopatric speciation."

We have little evidence as to the possible mechanism of speciation in mosquitoes; most detailed work has so far been concerned with the problem of recognizing species rather than of determining how the species arose. The studies that have been made, however, indicate that mosquitoes may be excellent experimental animals for the study of the mechanisms of isolation that lead to speciation, especially in the groups where behavior variation is being studied concomitantly with structural variation. The intensive studies of behavior and morphological variation in mosquitoes have stemmed directly from the discovery of cryptic species in various groups, and it may be worth while to review briefly some of these cases.

The vast majority of studies of subspecific variation in mosquitoes have been carried out on the morphological level: variant individuals or groups of individuals being given distinctive names as "varieties," with little or no real attempt to determine their biological status. Many mosquitoes show a very considerable range of variation in adult structure—*Anopheles hyrcanus* and *A. funestus* are outstanding examples—and the varieties described for these mosquitoes sometimes show a limited geographical distribution, so that they may be presumed to be subspecies in the sense used in vertebrates and Lepidoptera. Where biological studies have not been made, however, speculation as to the possible status of a morphological variant seems futile, so that there is no point in reviewing these cases. Two instances of cryptic species have been investigated rather fully—*Anopheles maculipennis* and *Culex pipiens*—and while even in these cases our knowledge is far from adequate for an understanding of the possible method of speciation, they still serve to point the way in which the problem can be attacked by a combination of field observation and laboratory experiment, associated with morphological studies.

ANOPHELES MACULIPENNIS

Roubaud, Wesenberg-Lund, and Grassi, in France, Denmark, and Italy, independently in 1920 and 1921 published speculations on the curious absence of malaria in some parts of Europe where *Anopheles maculipennis* was abundant. Roubaud thought that there were two "physiological races," one adapted to feeding on man, the other on

large domestic animals. Wesenberg-Lund thought that in Denmark the species had changed its food habits, becoming adapted to large domestic animals. Grassi, like Roubaud, thought there was a "biological race" that did not bite man.

The earliest and some of the most thorough experimental work was carried out in Holland by Swellengrebel and de Buck. They found that the Dutch mosquitoes did really belong to two quite independent populations which differed in adult feeding habits, adult mating habits, larval breeding places—two populations differing in everything except morphology. They recognized that by any logical criteria they were dealing with two "species" of mosquito; they considered it inadvisable to give these species Latin names, but referred to them as "shortwings" and "longwings" because of a statistical difference in size in the two populations.

The realization by Hackett, Missiroli, and Martini that the curious egg variations earlier described by Falleroni could be used to identify these biologically different populations made comparative studies very much easier, since it offered a method, however cumbersome, of identifying the mosquitoes. These various key discoveries led to a very great deal of work which has been reported in a voluminous literature partially summarized by Hackett and Missiroli (1935) and Bates (1940a).

One of the *maculipennis* populations, the one called "shortwings" by Swellengrebel and de Buck, "*atroparvus*" by van Thiel, proved easy to raise in captivity, since the males would mate in a very small cage. It was thus possible to attempt crosses by placing males of this type with females of the other forms. It was discovered that the various populations were separated by sterility barriers (de Buck, Schoute, and Swellengrebel, 1934; Bates, 1939a). In one case (*atroparvus* x *messeae*) the F_1 eggs either fail to hatch, or the larvae die soon after hatching. In another case (*atroparvus* x *sacharovi*), the F_1 generation consists entirely of sterile males, the females dying before development is completed. In the cross *atroparvus* x *typicus*, the F_1 generation produces healthy and vigorous adults, but both sexes are completely sterile. In the case of *atroparvus* x *subalpinus* a certain number of the female hybrids are fertile, so that they can be backcrossed with *atroparvus* males, but no normal hybrid males were obtained until the third backcross. Two other crosses, *atroparvus* x *melanoon* and *atroparvus* x *labranchiae*, also produce normal hybrid

females and sterile hybrid males. It is interesting that these crosses have been attempted by various workers in Holland, Italy, and Albania, and the results have been closely similar.

The striking difference between the mating habits of the Dutch "shortwings" and "longwings," the first mating readily in small cages, the second refusing to mate under any laboratory conditions that have so far been devised, is of great significance. It probably indicates that the mating habits of the two populations in nature are so different that individuals would never come in sexual contact with one another. Laboratory experiments in Albania (Bates, 1941b) indicate that each of the known *maculipennis* populations probably has distinctive mating habits.

By every criterion, these *maculipennis* populations seem to be sexually isolated populations—species—except for a few instances where the observed egg variation seems to be geographically limited so that the populations could be called subspecies. The eggs of the different forms are very distinct, furnishing a morphological criterion for the recognition of the populations, and there are various less tangible characters in the larva and adult. Ungureanu and Shute (1947) have found that some of the *maculipennis* populations can be distinguished as adults by characters of the wing scales.

Mosquitoes morphologically very similar to these European *maculipennis* populations occur in North America; the common eastern *Anopheles quadrimaculatus* is probably a member of the same complex, and a number of forms based on trivial morphological characters have been described from the west and from Mexico. What is known about these forms has been reviewed by Aitken (1945). Unfortunately biological studies comparable to the studies of the European populations have not been undertaken, so that interpretation of the status of the American forms is uncertain. Since the American forms, for the most part, inhabit distinct geographical ranges, it seems likely that they are, in part at least, subspecific populations: but the nature of their genetic relationships to each other and to the European populations could only be determined by crossbreeding experiments.

ANOPHELES PUNCTULATUS

The interesting situation presented by this Australasian anopheline has been reviewed at some length by Rozeboom and Knight (1946). They arrived at the conclusion that there are three genetically inde-

pendent populations (species) present in the area—*punctulatus* Dönitz, *farauti* Laveran (*moluccensis* Swellengrebel and Swellengrebel), and *koliensis* Owen (*moluccensis* in part of authors). These three are distinguishable by slight differences in the coloration of the labium of the adult, by details of larval chaetotaxy, and more certainly by characteristics of the setae of the pupae. The forms have almost universally been treated as "varieties" in the literature.

Typical *punctulatus* was readily colonized, but attempts to colonize *farauti* failed because of refusal of the females to feed. Attempts at cross-mating failed, but the authors point out that their experiments were only of a preliminary nature: they indicate, however, rather profound habit differences between the two forms. In some localities pure populations of single forms were found, in others two or all three forms occurred together, sometimes in about equal proportions and in the same breeding places.

From the evidence presented by Rozeboom and Knight, it seems that these mosquitoes behave as independent populations—as true "species." It is easy to imagine that the genetic and behavioral isolating mechanisms might have arisen among populations geographically separated on different islands of the area, reaching a stage enabling the independence of the populations to be maintained after they had again come in contact through changes in distribution. This classic hypothesis of "speciation" would seem adequately to explain the present situation. A detailed study, in a situation like this, of insular variation within a presumed species, taking into account behavioral as well as structural characters, might lead to very interesting results. Rozeboom and Knight found no absolute geographical differences among populations of the same species at different localities, but they found differences in range of variation that in some cases seem significant. The conditions under which their study was made unfortunately precluded any study of behavioral variation.

ANOPHELES STEPHENSI

Two "races" of *Anopheles stephensi* have been described by Sweet and Rao (1937), and further experiments with these races were published by Sweet, Rao, and Rao (1938). These authors were able to distinguish two populations by measurements of the length and breadth of the ova; they called these "type B" and "type M," the former being considered as "typical" and the latter described as

"variety *mysorensis*." Apparently the population in a given locality usually belonged to one or the other of the types, mixtures not being reported, but both types were found in the same general regions, so that there is no geographical barrier between the two. The "type B" was readily colonized and seemed to live longer, mate more readily, and bite man more readily than "type M." The authors report that "there would seem to be a quite definite natural barrier to successful crossbreeding between types B and M, since with crosses in both directions only a small minority of the females laid eggs and a still smaller minority laid viable eggs. From the viable eggs of the very few cross-mated females laying, it was possible to raise successive generations of hybrids, but in each generation there were instances of the laying of sterile eggs." Considerable more information would be needed for an attempt at evaluation of this situation.

ANOPHELES GAMBIAE

Ribbands (1944) has shown that a form described in 1903 as *Anopheles gambiae* var. *melas*, is in reality a distinct, coexisting species. It differs from typical *gambiae* only in slight and inconstant colorational characters of the adult palpi, and more constantly in characters of the egg and larval pecten. The two species have quite different breeding habits, *gambiae* being found in fresh water and *melas* in brackish water. In the laboratory, *gambiae* could not be raised to maturity in more than 37½ per cent sea water, while *melas* could be raised in 150 per cent sea water.

Thomson (1945) has described characters by which the eggs of *gambiae* and *melas* can readily be recognized, making field studies of the oviposition habits possible. He confirmed Ribbands's observation that *gambiae* is predominantly an inhabitant of fresh water, *melas* of brackish. Later, Thomson (1947) found that both *melas* and *gambiae* could be induced to mate in captivity if the cages were "illuminated by a table lamp with an orange colored shade for several hours at night." He was able not only to maintain colonies of both species (no mention is made of any observation of behavior differences between them), but also to undertake cross-mating experiments. Healthy hybrid generations were obtained from both *melas* x *gambiae* and *gambiae* x *melas* crosses. "Attempts to get these hybrids to mate with each other, or with pure *gambiae* and *melas*, were unsuccessful, and examination showed that while the females were apparently normal

sexually, the males were in most cases sterile, with underdeveloped or atrophied testes."

THE SUBGENUS NYSSORHYNCHUS OF ANOPHELES

The tropical American anophelines of the subgenus *Nyssorhynchus* are important vectors of malaria in many regions, and a very considerable literature has grown up dealing with their taxonomy. The various species are quite similar to one another as adults, and for their classification most reliance has been placed on the structure of the male genitalia. The eggs of these species are often strikingly different from one another, and variations of egg forms within a presumed "species" have been given varietal names. Summaries of the literature on the variation of these mosquitoes have been written by Rozeboom and Gabaldon (1941) and Rozeboom (1942a). Since investigation has been entirely on the morphological level, there is no information available that would be of use in evaluating the possible significance of the observed variation. The group, however, would seem to offer exceptional opportunities for experimental taxonomy, as many of the species are readily adapted to laboratory manipulations. Rozeboom (1936) successfully colonized *Anopheles albimanus* in Panama, and three other species (*darlingi*, *strodei*, and *argyritarsis*) have been colonized in the Villavicencio laboratory.

CULEX PIPIENS

Roubaud (1930) first presented evidence that the European *Culex pipiens* consisted of two races characterized most strikingly by ability in the one case to produce eggs without a blood meal, while in the other case a blood meal was necessary. He called the one "autogenous" and the other "anautogenous" with reference to this characteristic. These mosquitoes formed the subject of a series of careful experimental studies by Tate and Vincent (1936), and there seems to be no question but what two genetically independent populations are present in Europe—two *species*. Marshall and Staley (1937) proposed that the autogenous form be called *Culex molestus*, reviving an old name long listed in the synonymy of *pipiens;* their proposal has been very generally followed by subsequent authors. The whole *pipiens-molestus* problem has been reviewed by Marshall (1938) in his book on British mosquitoes. He gives a fairly lengthy series of characters whereby larvae and adults of the two species may be distinguished.

With *Culex pipiens* (in the strict sense) mating is invariably initiated when the mosquitoes are in flight, while with *C. molestus,* males will mate with resting females. *C. pipiens* will not produce eggs unless it has had a blood meal, while *C. molestus* strains have been maintained successfully for years without access to blood—though they bite man readily if given the opportunity. Tate and Vincent obtained cross-mating between the two forms, and found that ability to mate with resting females appeared in the F_1 generation, while ability to lay eggs without a blood meal sometimes did not appear until the F_2 generation.

Marshall maintained a collection of *molestus* strains at the Hayling Island laboratory (from Hull, Westminister, and Hayling in England, and from Paris) and tried cross-mating among these. A total of twelve strain crosses were made, and the females mated readily and laid egg rafts in all cases; in only seven cases, however, did the eggs hatch. The successful crosses were Hayling males with Hull, Paris, or Westminister females; Westminister males with Hull or Paris females; and Hull males with Paris females. This suggests that the genetics of *molestus* strains might form a very interesting field for study. The *pipiens* problem has attracted little attention in America, but apparently both *pipiens* and *molestus* occur there (Richards, 1941).

In the tropics of both hemispheres, *Culex pipiens* is replaced by another species known as *Culex fatigans* (or sometimes as *Culex quinquefasciatus*). These two have always been listed as "good species" by systematists because they may be distinguished by various adult characters, including differences in the male genitalia. Weyer (1936) found that *molestus* and *fatigans* crossed readily, though he failed to obtain *pipiens-fatigans* crosses. *C. fatigans* itself, as a tropicopolitan species, has been reported as having different habits in different places, and may well include strains with different biological characteristics.

SPECIFIC CHARACTERS

The frequency with which structural differences in the egg have been found to characterize otherwise closely similar mosquitoes is interesting. At first sight it might seem an exception to von Bauer's law that "animals are more similar at early stages of their development from the egg than when they are full grown." The exochorion of the egg, however, is a character of the parent female, not of the enclosed embryo; its structure and pattern are the product of the

ovarian cells of the mother. Egg differences are thus characters of the adult reproductive system, just as much as are structural differences in the accessory organs associated with the genitalia of the male. They form but another example of the frequency, throughout both the animal and plant kingdoms, with which the most clear-cut distinctions between species are found to be properties of structure or behavior associated with sexual reproduction.

There is a tendency to regard divergence in these sexual characters as an infallible guide to population divergence, which may be misleading because these characters, like everything else, are subject to variation. Thus recently some authors have been giving names to every variant mosquito egg, without attempting any investigation of the possible significance of such variants. Eggs may vary, however, as a result of purely environmental factors: the eggs of *sacharovi* have small floats if developed by females kept at low (spring) temperatures, and no floats if developed at higher (summer) temperatures. Similarly, the pattern of the eggs of typical *maculipennis* varies with the environmental temperature at which the eggs have developed. In some cases variation in egg structure seems to be random, perhaps dependent on genetic strains within the population rather than on environmental differences—for example, the variation in *Anopheles strodei* described by Rozeboom (1938) for the Panamanian population. In the case of *Anopheles pessôai* in the Villavicencio region, we may have a case where there is genetic diversity dependent on environmental variation for its expression, since with this species about one-third of the females lay eggs with small floats if kept at a constant temperature of 30°, while none lay eggs with floats if kept at 20°.

Structures associated with the genital system of the male are generally regarded as providing the most convenient and the most reliable guide to specific divergence in mosquitoes. The structure of the male genitalia is used almost universally in mosquito taxonomy in defining species, and it is consequently surprising to find how little study has been given to the subject of possible variation in these structures. Yet they surely are subject to individual variation. Lindsey (1939) made a statistical study of structural variation in the genitalia of the butterfly, *Pyrgus communis*, and concluded that "the quantitative variability of details of genitalic structure is greater than that of the size of the whole organism and probably as great as that of other char-

acters of taxonomic value," and that "variability of subordinate structures of the claspers is so great and so erratic that these details must be used in taxonomy with great care." Shute (1935) in a study of the genitalia of several of the species of the *Anopheles maculipennis* group found that the range of variation in the structure of the spines of the harpago was different in the different species, but that the variation was too great for the accurate identification of individual specimens.

Thus the genital structure within a given species may show appreciable variation. Conversely, species known to be distinct on other grounds may have male genital structures that are practically identical. An example is given by Marshall (1938): the two English mosquitoes *Theobaldia morsitans* and *T. fumipennis* are practically identical in external characters of the adult and in the structure of the male genitalia, yet the two may easily be separated in all four larval stages.

Structural recognition characters for species may be found in any stage of the life history, and for the most part such recognition characters involve trivial differences of no apparent adaptive significance. Thus in mosquitoes such characters commonly involve egg pattern or proportions, details of the arrangement of larval or pupal spines, slight differences in the proportions of bands on palpi or legs of the adult, or in the relative size of wing spots. The apparently nonadaptive nature of these differences among species has led to a great deal of speculation (summarized by Robson and Richards) which seems to the author quite beside the point. These trivial morphological characters do not constitute the basic differences among species; they are simply indexes, very convenient when present, but often probably the result of accidental variation spreading through a population. Thus the European species related to *Anopheles maculipennis* and the South American species of *Nyssorhynchus* are distinguishable by very slight and obviously nonadaptive morphological differences, but when subjected to laboratory study, the species are found to show profound physiological differences. Growth rates, tolerance for toxic materials, temperature requirements, sexual and food behavior usually differ greatly from species to species. These, and perhaps even more intangible characters, surely have adaptive significance, and it is the evolution and variation of these, not of the banding of the palpi, that need study if we are to understand the processes of evolution.

POSSIBLE MECHANISM OF SPECIATION

The cases quoted in this chapter shed very little light on the possible mechanism of speciation in mosquitoes. It is striking that geographical variation has attracted almost no attention in mosquitoes; insular races, for instance, have only rarely been described from the archipelagos of the East or West Indies, where restricted geographical populations are so abundantly evident in other groups. On the other hand, "sympatric" varieties, variations occurring together with a supposed type form, have fairly frequently been described.

It is difficult to decide whether the absence of described geographical variation in mosquitoes reflects a real situation, or whether it is a result of failure to study variation from this particular point of view. Mosquitoes are small, rather uniform animals, and must be examined one by one under a microscope so that pattern variation, striking in a drawer of mounted butterflies, might not be so easily correlated with geographical distribution in mosquitoes. In a few cases where morphological variation has been studied in great detail, there is a definite geographical pattern. An instance is the distribution of the American *maculipennis* forms *occidentalis*, *freeborni*, and *aztecus* mapped by Aitken (1945, p. 279). Among the European *maculipennis* populations two pairs, *melanoon-subalpinus* and *atroparvus-labranchiae*, show clear geographical cleavage. It may be that in other cases the apparent absence of geographical subspeciation is due to failure to search for this particular phenomenon.

Yet we have no direct evidence of nongeographical speciation in mosquitoes. The author undertook studies of the European *maculipennis* populations with the hope that these would shed some light on a possible mechanism of sympatric speciation, but he came to the conclusion that the various populations probably represented ancient and well established species whose present geographical distribution probably had little or no relation to the possible place or method of origin. These European *maculipennis* differ so profoundly from one another in every respect except appearance that it is difficult to believe that the similarity of appearance means recent origin. Wheeler (1910, p. 174) has pointed out that some of the ants of the Baltic amber cannot be distinguished from species living today, and if external morphology can be so conservative in one insect group, it may well be equally conservative in others. The mere fact that *messeae*,

typical *maculipennis*, and *sacharovi* all live side by side today is, then, no evidence that they originated in the same locality.

On the other hand, the sexual incompatibility observed by Marshall (1938) among various strains of *Culex molestus* suggests that mosquitoes may have some mechanism by which sexual isolation can arise within a previously interbreeding population. The sharp specificity of sexual behavior among mosquitoes is striking; it forces itself on the attention of anyone who attempts to establish laboratory colonies of a series of similar species. One species will mate readily in small cages, another requires special provision of space or lights, another will refuse to mate under any circumstances that the experimenter can devise. Certainly specific sexual behavior patterns among mosquitoes deserve much more attention from biologists than they have yet received.

In phytophagous insects "biological races" may arise through the association of different parts of a population with different host plants, and one can imagine this isolation being as effective as that brought about by geographical barrier (Thorpe, 1930). There is no basis, however, for an analogy between mosquito populations and populations of animals that pass their entire lives in association with a given host. The adults of mosquitoes like the *maculipennis* populations certainly show distinct host preferences, but the differences are of degree, not absolute, and the host association occupies only the few moments of feeding. Sexual contact, where isolation must occur, may be in a quite different environment from that in which feeding occurs; oviposition and larval growth occur in still another environment, so that close association between a given mosquito species and a given host species is very unlikely. The differing host preferences of adult mosquitoes seem, then, rather a result of the diverging evolution of separate populations than a cause of the separation of the populations.

If the primary isolating mechanism in mosquitoes were ecological, one would expect to find that distinct yet closely similar species would have distinct larval habitats. Yet constantly, in mosquitoes, one is impressed by the similarity of the larval habitats of similar mosquitoes. The range of ecological situation occupied by each species is, to be sure, apt to be characteristic, but the differences between closely similar species are sometimes so slight as to be difficult to describe or define, and there may be many situations where the closely similar species are found together as larvae. This is true of the European

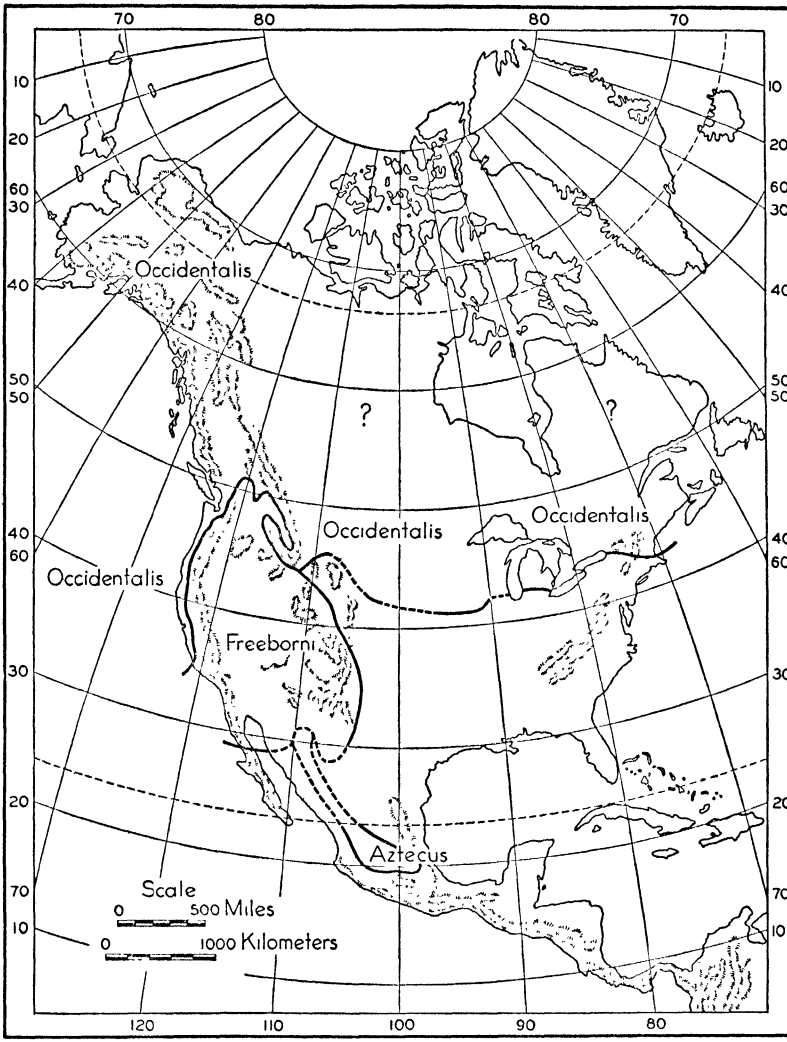


FIG. 8. GEOGRAPHICAL DISTRIBUTION OF THE NORTH AMERICAN POPULATIONS OF THE *Anopheles maculipennis* GROUP (FROM AITKEN, 1945).

maculipennis, it is true of South American *Nyssorhynchus*, and Rozeboom and Knight (1946) mention collecting all three of their *punctulatus* species in the same pool. The most sharply defined larval habitats are those of the container breeders, yet even here different species of *Kerteszia* may occur in the same bromeliads, and closely similar species of *Wyeomyia* have been described as occurring in Heliconia bracts, and so forth. As in the adult host preferences, the differing ecological requirements of the larvae seem to be a result of the diverging evolution of populations that have become independent, rather than a possible primary isolating mechanism of these populations.

CHAPTER XVII

THE CLASSIFICATION OF MOSQUITOES

> "What's the use of their having names," the Gnat said, "if they won't answer to them?"
>
> "No use to *them*," said Alice; "but its useful to the people that name them, I suppose."—LEWIS CARROLL

MOSQUITOES, in the sense in which the word is used in this book, are very generally recognized as constituting a subfamily, the Culicinae, of the family Culicidae. Two other subfamilies are included in this family, the Dixinae and the Chaoborinae. Since the members of these other subfamilies do not suck blood, and are thus not implicated in disease transmission, they have been very little studied and knowledge concerning them is limited to taxonomic descriptions of species. They would probably generally be referred to, along with a host of other nematocerous diptera, as "midges" or perhaps as "gnats." In the English language it seems most sensible to restrict the word "mosquito" to the bloodsucking midges and their immediate relatives in the subfamily Culicinae. It might be pointed out that the word *mosquito* in Spanish generally lacks this special meaning, since by origin it refers to any "little fly": "mosquitoes" in the English sense are more precisely covered by the Spanish *zancudo* (*zanzare* of the Italians).

F. W. Edwards in 1932 published a monumental catalogue of the mosquitoes of the world, in which he recognized 1,400 species belonging to 30 genera, with 59 additional subgenera. Such a catalogue represents a tremendous amount of labor, since the bibliography of the vast literature on mosquitoes is by no means easy to untangle; it may thus be some time before Edwards's work is superceded, and it forms the basis of the mosquito nomenclature used in this book and of the discussion of classification in the present chapter.

Howard, Dyar, and Knab (1915) have reviewed the early history of mosquito taxonomy. Linnaeus proposed the genus *Culex* in the

tenth edition of his *Systema Naturae*, the book which serves as the starting point for zoological nomenclature. Of the six species that he included in this genus, only two (*pipiens* and *bifurcatus*) are considered to be mosquitoes in the modern sense. The interpretation of these old insect names is at best very doubtful, and the problem of the significance of these two Linnean mosquito names has been neatly solved by recent authors, following a suggestion of Martini, by considering them to be the female and male of the same insect, the common domestic mosquito of western Europe. During the nineteenth century entomologists paid remarkably little attention to mosquitoes, and only a few genera and species were described: the genera *Aëdes* and *Anopheles* were proposed by Meigen in 1818; *Sabethes*, *Megarhinus*, and *Psorophora* by Robineau-Desvoidy in 1827, and five more genera were added by Lynch Arribalzaga in 1881.

Interest in mosquitoes developed rapidly after the discovery of their economic importance, and in a catalogue of the species of the world published in 1905 Theobald recognized 62 genera and 450 species; by 1910 this had increased to 149 genera and 1,050 species. The number of genera has been sharply reduced by the current practice of using broad, inclusive generic concepts for mosquitoes—a practice that will be discussed later in this chapter. The rate of description of new species has also fallen off. As remarked above, Edwards recognized 1,400 species in 1932, which is not a large increase over the 1910 figure, and since 1930 about 42 new names have been proposed annually for mosquitoes (data from the *Zoological Record*). Detailed explorations of the Pacific Islands during World War II have yielded a considerable number of new mosquito species, and judging from our experience in eastern Colombia, there must still be many undescribed species in South America. Thus while a "new species" of mosquito is nothing like as great a novelty as a "new species" of bird, yet the vast majority of different kinds have probably already been collected, pinned, and named by someone, and it seems likely that the total world fauna is on the order of magnitude of 2,000 species.

There are, of course, two questions: the number of *species* of mosquitoes, and the number of *names* for mosquitoes. By a random count of one-tenth of the index to Edwards's 1932 catalogue, I find that there are almost twice as many *names* as there are *species* recognized by Edwards: he places 48 per cent of the names as synonyms! Ed-

wards was a very conservative taxonomist, and it may be that many of the names that he treated as synonyms will subsequently be found to apply to distinguishable species. But the number will not be very large, since most of these synonyms represent plain mistakes on the part of the authors of the names.

The problems of mosquito nomenclature and classification are certainly complicated by this superfluous accumulation of names. The complex synonymy of mosquitoes is often blamed on the "amateurs," on the fact that so much of the taxonomic work has been carried out by medical men, for instance, with no "professional" entomological training. I am not sure that this accusation is justified: a statistical analysis of mosquito nomenclature might well reveal that the mistakes had been made as often by professional entomologists as by amateur entomologists, particularly since some of the entomologists who have worked with mosquitoes have been rather careless taxonomists. Furthermore, in many animal groups with which only professional taxonomists have worked, the nomenclature seems to be just as confused as it is in mosquitoes.

I think there has been, among both professionals and amateurs, a rather general failure to appreciate the importance of tools in taxonomic work. The study of viruses, for instance, cannot be undertaken lightly because such studies require large supplies of expensive experimental animals and rather elaborate equipment. It is obvious that studies involving chemical analyses cannot be undertaken without both the necessary reagents and the necessary apparatus. The corresponding tools for taxonomic research are a reference library and a reference collection. For some groups of animals it is possible to assemble adequate library and museum facilities fairly easily, so that taxonomic studies can be undertaken by an isolated individual, though such studies in any animal group would involve frequent and rather prolonged visits to the great museums where the basic general libraries and collections have been assembled. In a group of animals like mosquitoes (or mammals or birds or butterflies), such work by an isolated individual is practically impossible because of the difficulty of assembling the necessary resources. Worth-while taxonomic research (in the classical sense of description of species and revision of classification) is, then, for practical purposes limited to individuals who are connected with institutions where the necessary resources are available, just as virus research is limited to individuals attached

to institutions that have managed to assemble the necessary resources. Work by students who fail to recognize these limitations is apt to be more of a hindrance than a help to the progress of science, and to bring ridicule rather than glory to the student.

There is an undeniable thrill to finding something "new," whether it is a new fact or a new species of *Anopheles;* and in both cases there is a considerable temptation to announce the discovery to the world without going through the tedious process of checking the supposed novelty against the accumulated literature to place it in perspective with what is already known. This may cause other workers considerable inconvenience. It is also hard to remember that the mere description of a new species, or observation of an isolated new fact, is, in itself, work at the lowest level of science. The taxonomist who makes the greatest contribution is not the one who describes the most species, but the one who achieves the most reasonable scheme of relationships among species, who builds a sound classification and contributes thus to the understanding of natural phenomena.

It may be worth while to emphasize that these remarks about the importance in taxonomic research of adequate resources in terms of library and reference collection apply to what is, perhaps, but one aspect of the general field, that of the revision of classification. Regional faunal studies are conventionally also classed as a part of taxonomy, and for this work intimate acquaintance with the animals in nature is far more important than reference facilities. These regional studies constitute one of the most useful types of investigation in building up a general picture of the "natural history" of a group of organisms. Their value depends almost directly on the field experience of the author, though a collaboration with men having conventional taxonomic resources at their disposal is important for such studies, if their nomenclatorial basis is to be sound.

THE PRINCIPLES OF ANIMAL CLASSIFICATION

The classification of animals has two functions: to serve as a filing system for the accumulation and communication of observations, and to show the presumed evolutionary relationships among the various animal groups. These functions at times conflict, in so far as a classification that illustrates most clearly the evolutionary relationships of animal groups may prove to be hopelessly awkward for the purposes of routine identification. In such a case it seems to me that the prac-

tical considerations should receive first attention. Simpson (1945) in his admirable review of the "principles of taxonomy" has put this very clearly:

> It is often stated that the purpose of classification is or should be to express phylogenetic relationships, but, in the first place, no one has ever devised a method of classification that could express phylogeny sufficiently or consistently, and, in the second place, the system that is actually used in zoölogy was not devised for that purpose and is notably incapable of serving it. The basis of this system is phylogenetic, as has been strongly emphasized here, and this means that the groups to be recognized in classification should be as nearly as possible valid phylogenetic entities and that the criteria of definition are to have phylogenetic implications, but this is quite different from expressing phylogeny. The categories and nomenclature of classification are also used to discuss phylogeny and so to express it in quite a different sense, in much the sense in which a dictionary might be said to express the English language.
>
> This is, as I see it, the primary purpose of classification: simply to provide a convenient, practical means by which zoölogists may know what they are talking about and others may find out. It is helpful for this purpose and it is also a secondary but still essential aim that classification should be consistent with the most important thing that evolutionary taxonomists have to talk about, that is, with animal affinities.

The tendency to stress the nonpractical aspects of taxonomy is perhaps a reaction against the general disrepute in which animal classification has at times been held by physiologists, ecologists, and the like who have scorned the "sorting of dead specimens according to a few rather superficial morphological characters." Since the physiologist and ecologist would be unable to work at all without the studies of the taxonomists, this seems a rather silly attitude. The taxonomists have striven mightily to rehabilitate their science, as can be seen, for instance, by the collection of essays on "The New Systematics" in the volume edited by Huxley (1940). The object seems to be to make taxonomy synonymous with evolutionary biology—which is perhaps as farfetched as trying to reduce it to the level of assorting postage stamps. To quote Simpson again, "classification is, above all, a practical problem," and one that fully merits attention for its own sake.

Experimental biologists often complain of taxonomists because they change the names of animals. This name changing, however, arises from two distinct causes. One, which is very generally deplored

even by the taxonomists themselves, is the result of the laws of priority: if, by digging through ancient files of journals a name can be found older than the name in current use for a particular animal, the older name must be substituted. This should, sooner or later, reach an end point, since the supply of old and obscure journals, though large, is definitely limited. The other cause of name changing results from the discovery of new relationships, of new and perhaps more satisfactory methods of classifying a group of animals. Rearranging these animals under different generic concepts may cause temporary inconvenience, but if the end result is an improved classification the inconvenience is a small cost. Complete stability of nomenclature could probably only be achieved through stagnation, which would not be convenient for anyone except possibly the textbook publishers.

Before the experimental biologist throws too many stones at the taxonomists, he might reflect a little on his own efforts at classification—at the nomenclature, for instance, of "orientation reactions," or of enzymes or vitamins, or compile a list of synonyms of such cell inclusions as mitochondria. I doubt whether there is anything in the classification of the animal kingdom as confused as the various attempts of ecologists to classify animal or plant habitats. The formal classification of natural phenomena in terms understandable to the human mind and yet reflecting the observed relationships of these phenomena is not easy. Whatever their defects, the systems of animal and plant classification in current usage are a considerable monument to human achievement and have provided an indispensable foundation for the recording of information in all of the biological sciences.

The basis of zoological nomenclature is the species concept. The application of this concept in mosquitoes has been discussed in the preceding chapter; a few words might be added here concerning the *nomenclature* of species. The rules of zoological nomenclature have been codified by the International Zoological Congress, and published in various books and pamphlets. There is an "International Commission on Zoological Nomenclature," and recently an "International Trust" has been formed which will publish the commission's *Opinions* and the *Bulletin of Zoological Nomenclature* (see *Nature*, Vol. 159, p. 328). Anyone publishing new zoological names or engaged in taxonomic research should be thoroughly familiar with these various publications.

Contemporary taxonomic practice has come to place great reliance

on the use of "types" for names, and as Simpson (1945) has pointed out, "it is one of the peculiar shortcomings of the International Rules that they nowhere mention types of species and that they give elaborate rules for determination of the types of genera without ever saying exactly what the type of a genus is, what it is for, or how it functions." Many mosquito names have been proposed even in recent years without proper type designations, and names have been shifted about in the synonymy without previous study of the type specimens of the names so shifted, so that some discussion of the type concept may not be out of place here. Again I can do no better than to quote Simpson:

> The sole purpose of types is to serve as name-bearers. Every specific and subspecific name is attached once and for all to a certain concrete, objective specimen, an individual animal, usually a preserved dead animal or a part thereof collected, prepared and stored in a museum. (Designated types do not, however, cease to be types if they are not successfully preserved or if they are subsequently lost or destroyed.) . . . When specimens are transferred from one species to another or when two supposed species are merged into one, it frequently happens that two specific types are placed in one species, which thus acquires two names since the names have to follow the name-bearers, the types. (The fate and allocation of other known specimens have no bearing whatever on nomenclature.) . . . If the type attached to the synonymous name is later removed and put in a species without the bearer of an older name, the formerly synonymous name goes with it and is revived. . . . It is a natural but mistaken assumption that types are somehow typical, that is, characteristic of the groups in which they are placed. It is, of course, desirable that they should be typical because then they are less likely to be shifted about from group to group, carrying their names with them and upsetting nomenclature, but there is no requirement that a type be typical, and it frequently happens that it is quite aberrant.

A very complex nomenclature has been proposed for different kinds of types, whether of species or of genera, but most of these names are of dubious value. To distinguish the real type from all of the other kinds of types, it is often called a "holotype"; a corresponding specimen of the opposite sex from the type may be called an "allotype," and all of the other specimens that the author used for basing a new name may be called "paratypes"—but only the single type specimen, the "holotype," has any nomenclatorial significance. Specimens from the same place as the type may be called "topotypes" and have a cer-

tain value when the type is lost if the species is subject to geographical variation. Simpson finds four kinds of names useful for types: the *type* itself, a single specimen; *syntype* (more commonly called *cotype* in entomological practice), one of several coordinate specimens given equal value in the original proposal of a specific name ("in new work syntypes should never be used and only one type should be designated, but syntypes were widely used in the past and so must still be dealt with"); *lectotype*, a syntype selected by a subsequent reviser to serve as sole type; and *neotype*, a substitute proposed when a type has been destroyed.

In nomenclatorial practice, names for categories lower than species are treated as specific names: that is, subspecies, "varieties," "races," and so forth, if they are given formal Latin names, should have types; and if any subsequent reviser decides that this type belongs to a distinct "species," he must use the subspecific name attached to the type for his species. There is also no mechanism whereby a Latin name for a specific or subspecific concept can be "tentatively" proposed: naming an animal is an all or none phenomenon, either it is named or it is not named, and if a name is once proposed and it proves to be synonymous with some older name, the synonym must always be carried along in the catalogue. There seems to be a feeling among some students of mosquitoes that proposing a subspecific or varietal name is a less serious undertaking than proposing a new species, but the nomenclatorial and bibliographic consequences are the same. To avoid the tangle of nomenclatorial law, the author must use some vernacular name, as Swellengrebel and de Buck did with their "long-wing" and "shortwing" forms of *Anopheles maculipennis* in the Netherlands, or some serial designation such as "var. A" and "var. B." Some such procedure as this appeals to me as the most sensible if it seems desirable to publish observations on a mosquito of uncertain nomenclatorial status.

There are a great many mosquito names of uncertain application. If types for such names exist, it is the duty of taxonomists working with the groups to search out the types and thus determine the status of the name from the characters of the name-bearer. But in many cases there are no types; the original specimens have been lost, and the descriptions are so vague that they can be interpreted in various ways. The International Rules provide for the rejection of *nomina nuda*, names which are proposed without a characterization of the

animal designated, but they make no provision for names with inadequate characterization. Simpson has proposed that these latter be called *nomina vana*, and suggests that they likewise be ignored or listed (as is frequent entomological practice) at the end of a catalogue as of *incertae sedis*, names of "uncertain application."

In the case of mosquitoes, Edwards in his monumental catalogue has listed all of the names somewhere, assigning all of them to some specific concept or other, either as names for the species, as synonyms of older names, or as questioned synonyms. Further investigation of these old and inadequately characterized names would seem to me quite futile. A great deal of print has been used in speculating, for instance, about what kind of a mosquito Say had before him in 1823 when he proposed the name *Culex quinquefasciatus*. No one will ever be able to reach any definite decision. No one can ever be sure what Wiedemann meant in 1828 by *Culex fatigans*. Edwards has used Wiedemann's name for the common domestic mosquito of the tropics, and listed Say's name as a questioned synonym. Wiedemann's name has the immense advantage of being shorter, and since the publication of Edwards's catalogue it has been used by almost all workers except those in the United States, who cling to Say's name, I suppose, from a sort of national pride. The easy way out, in handling the *nomina vana* of mosquitoes, would seem to be for everyone to agree to follow the various precedents established by Edwards in his catalogue, making the catalogue a sort of "official list."

GENERIC GROUPINGS IN MOSQUITOES

The species is the closest thing there is to an objective category in the hierarchy of animal classification. The grouping of species into genera, families, orders, classes, and phyla, very necessary if the chaos of organic diversity is to be studied, is a highly subjective process. Species, that is, reproductively isolated populations, are pretty much equivalent concepts in all sexually reproducing animals, so that comparisons, for instance, of behavior or distribution among species in birds, butterflies, mosquitoes, and snails may be made with some confidence that such comparisons involve equivalent units. Comparisons among genera and families in such diverse groups of the animal kingdom, however, would have no meaning whatsoever.

The history of generic usage in mosquitoes illustrates very well the subjective nature of such categories. The maximum of generic "split-

ting" was probably reached by Theobald (1910), who used 149 genera for 1,050 species, or an average of 7 species per genus; and of "lumping" by Edwards (1932), who placed 1,400 species in 30 genera, an average of 47 species per genus. Edwards and Theobald used rather different sorts of morphological criteria for grouping their species, but the difference between their systems represents not so much a matter of the criteria used for defining groups as a different conception of the function of the genus. With Theobald the genus is the most elementary grouping of species; with Edwards it is a higher category. Edwards groups species into "series," these into "groups," these into "subgenera," and these finally into "genera." Thus the genus of Theobald is roughly equivalent to the series of Edwards.

The genera of Theobald probably correspond more closely to the usual practice of zoologists, especially in well studied groups such as the vertebrates and the Lepidoptera, than do those of Edwards. One of the most thorough taxonomic studies with which I am acquainted is the revision by Rothschild and Jordan (1903) of the lepidopterous family Sphingidae, the hawkmoths. The authors paid especial attention to the definition and classification of genera, attempting to make this category as objective as possible. They define the genus as "a classificatory unit one category higher than species comprising one definable group of species." This policy resulted, in the case of the Sphingidae, in the division of 772 species among 166 genera, an average of somewhat less than five species per genus. There has been no attempt to alter generic concepts in the Sphingidae in the 44 years that have elapsed since the publication of their revision, which is a considerable tribute to its soundness: the only changes have been purely nomenclatorial, due to a change in the method of selecting genotypes. The generic category of Rothschild and Jordan corresponds very closely to general usage with birds, mammals, and a few thoroughly studied groups of insects.

The use of the category "genus" for the "least definable group of species" certainly has great advantages, especially in achieving a certain degree of objectivity and, hence, of uniformity through the various classes of the animal kingdom. But there are also great advantages in the inclusive generic concept used by Edwards, which seems to me to correspond rather closely to the generic concept of the botanists. The greatest advantage is the practical one of enabling the names to be recognized. Anyone in a very short while can become familiar with

the thirty generic names used by Edwards, so that he will recognize a mosquito name any time he happens to come across one. The general zoologist can be expected to recognize "*Anopheles gambiae*" not only as a mosquito name, but as a name for one of the group including the vectors of malaria; while only a specialist could be expected to recognize a Theobaldian name like "*Pyretophorus gambiae*" without some explanatory phrases. The general zoologist cannot be expected to recognize all mosquito generic names, and a term like "*Bironella papuae*," for instance, would have to be explained; but such small and aberrant mosquito groups would in any event be of interest only to the specialist. By using the Edwardsian generic concepts we achieve a situation comparable to that achieved by the botanists, who can expect all biologists to recognize a great many of their genera, broad concepts like *Pinus*, *Ribes*, *Quercus*, and *Rosa*, to take a few terms at random. With the broad mosquito genera we have three terms, *Anopheles*, *Culex*, and *Aëdes* that we can expect to form part of the general biological vocabulary, and these three terms cover a very significant portion of the world's mosquito fauna.

Edwards himself has pointed out that "the advantages of employing larger generic concepts are, first, that the wider relationships of the species are more clearly indicated; secondly, that limits can more readily be assigned to the genera than in the case of more numerous and smaller groups; and, thirdly, that it ensures the avoidance of duplication of specific names; the use of subgeneric terms enables those who wish to do so to make use of the smaller divisions." The inclusion of the subgeneric term in the name formula makes for a ponderous label, such as *Anopheles* (*Nyssorhynchus*) *darlingi*, or (when subspecies are referred to) *Anopheles* (*Anopheles*) *melanoon subalpinus*. But in ordinary text the addition of the subgeneric name to the formula seems rather gratuitous. *Anopheles darlingi* serves quite precisely to indicate what particular mosquito is under discussion, and the term *Nyssorhynchus* is useful chiefly when we want to discuss group characters—for instance, to point out that the *Nyssorhynchus* group of *Anopheles* breed characteristically in sunny pools, while the *Kerteszia* group are found in container habitats.

The kind of hierarchy to be used in the classification of mosquitoes is one problem; the kind of morphological characters to be used as criteria for distinguishing the various categories is another problem. As Simpson (1945) has pointed out:

The condition that classification must be consistent with phylogeny has as its most important corollary the requirement that all the animals within a given group, whatever its rank, must have a common origin. A second and scarcely less important corollary is that the animals assigned to a given group are more closely related to each other than they are to the members of other groups of the same rank. Both requirements demand exceptions and qualifications; practical classification can be achieved only by compromise.

Generic, or other supraspecific categories, used in the classification of a group of animals like mosquitoes, should then ideally fulfill three requirements: first, that they have recognition characters for larvae and for both sexes of the adult; second, that the included species appear to have had a common ancestry; and third, that the included species or groups appear to be more closely related to each other than to excluded species or groups. It seems to me that practical classification requires emphasis on the first requirement. The phylogeny of mosquitoes, for instance, can apparently be worked out very neatly by the study of the male genitalia; but groupings based on genital structure are of no use in the practical problem of identifying a female mosquito or a larva, and if such groupings are to be recognized in the nomenclature it would seem necessary that recognition characters, of however "trivial" phylogenetic significance, be found for these other forms.

The mosquito genera proposed before 1900 were based mostly on characters of the palpi, number of joints, and differences in development between the two sexes. In 1901 Theobald published the first volume of his *Monograph of the Culicidae,* and in this he used, in addition to the characters of previous authors, the shape of the wing scales for generic diagnosis, a system that was carried to the extreme in the fourth and fifth volumes of the monograph (1907 and 1910). Theobald's genera were quite impractical, based as they were on secondary sexual characters, which required the examination of specimens of both sexes for proper generic diagnosis, and on the shape of wing and body scales, which were very variable and ill defined. This classification was criticized by Dyar and Knab (1906) in a paper in which they proposed a classification based entirely on the larvae.

The taxonomic ideas of Dyar have had considerable influence in the development of the classification of the Culicidae now in general use. These ideas, first outlined in the 1906 paper, were developed in

the "monograph" of Howard, Dyar, and Knab, in numerous papers published in his journal, *Insecutor Inscitiae Menstruus*, and in his last work (1928), *The Mosquitoes of the Americas*. Dyar placed great stress on the structure of the male genitalia, not only for specific diagnosis but for generic groupings as well, and in the early 1920's he proposed many genera based on such characters. By the time of the publication of his 1928 book, he had returned to the use of inclusive genera with numerous subgenera (subgenera were not used in the Howard, Dyar, and Knab monograph).

F. W. Edwards was largely responsible for the extensive use of the arrangement of the various bristles or setae of the thorax for generic grouping. These setal characters have the advantage of applying to both sexes (which is not true of the characters of the palpi or genitalia) and seem to result in groupings which are "natural," judged by their correlation with larval characters and structural differences in the genitalia.

Practical classification requires that we list our families, genera, and species in a lineal order. Phylogenetic classification pictures these groups as diverging branches of an ancestral tree. It has become customary to reconcile these two requirements by listing groupings in an ascending order from relatively "generalized" to relatively "specialized." Thus in arranging the classification of mosquitoes, the list should start with the groups that seem to have retained the most primitive characters, that seem to represent the types that we might presume to be ancestral to all living mosquitoes. In the original Linnean system, the reverse order was used: Linnaeus started with his idea of the most highly developed organism, man, and gradually worked downward to the worms. This type of arrangement has persisted in a few groups of animals, most notably in the Lepidoptera, and has been carried over into the mosquitoes by Dyar, presumably because his habits of thought were conditioned by his extensive work in Lepidoptera. Such a system is distinctly out of line with the arrangement used in other families of Diptera, however, and its continued use by a few taxonomists is definitely anachronistic.

In actual practice the selection of a given lineal order for genera is, of course, apt to be highly arbitrary, particularly in a group like mosquitoes where there is no fossil evidence to aid in the interpretation of possible phylogeny. Edwards (1932) divided the mosquitoes into three "tribes," the *Anophelini*, the *Megarhinini*, and the *Culicini*.

In common with most recent students of mosquitoes, he has regarded the anophelines and the megarhines as closer to the generalized mosquito ancestor than the culicines, and therefore places them first, though all three tribes include, of course, examples of highly specialized structures or behavior.

Edwards has discussed the problems of the classification of the culicines at some length (1932, p. 63). There are five main groups of genera, the *Sabethes* group, *Uranotaenia* group, the *Theobaldia-Mansonia* group, the *Aëdes* group, and the *Culex* group, which he lists in this order of specialization. These groups illustrate nicely the problems of reducing a diverging phylogenetic tree to a lineal order. The sabethine genera are placed first because one of the group, the tropical American *Trichoprosopon*, seems to include about the most "primitive" of living culicines, though some of the other genera are very highly "specialized." The genus *Culex* (and its close relative *Deinocerites*) is placed last because of the "specialized character of the male genitalia and of the larval siphon, though some other characters of the group could well be interpreted as primitive."

The phylogeny of mosquitoes, like that of other animal groups, offers a fascinating field for speculation, but speculation it must remain because of the completely fragmentary character of fossil material in the group. Significant research on "macroevolution" is likely to depend on work in groups like the mammals and the molluscs, where fossil material is abundant. In such groups the phylogenetic function of classification may be paramount, but in mosquitoes the paramount function is surely the practical problem of the identification of specimens and the provision of as nearly a natural system as possible for the recording and relating of observations and experiments.

CHAPTER XVIII

THE DISTRIBUTION OF MOSQUITOES

> It thus comes to be admitted that a knowledge of the exact area occupied by a species or a group is a real portion of its natural history, of as much importance as its habits, its structure, or its affinities; and that we can never arrive at any trustworthy conclusions as to how the present state of the organic world was brought about, until we have ascertained with some accuracy the general laws of the distribution of living things over the earth's surface.
>
> —ALFRED RUSSEL WALLACE

Mosquitoes are found almost everywhere, abounding as individuals (if not as species) even in the subarctic, and reaching the most remote desert oases. A few oceanic islands form the outstanding exception to this universal distribution, as there is good evidence that some of them were devoid of mosquitoes before the advent of man and modern transportation. The Hawaiian Islands, for instance, have no endemic species, and it is supposed that the mosquito species now present have arrived since 1826 (Russell, West, and Manwell, 1946).

The distribution of mosquito studies has also been almost universal. The faunas of Europe and North America have been studied in some detail because they are convenient to the great centers of research, and the tropical faunas because of the importance of the insects in disease transmission. Studies in the tropics have quite naturally centered on the genus *Anopheles,* but entomologists have usually been unable to resist the temptation to collect other species as well; and yellow fever has provided an important stimulus for studies of the culicine faunas at least in Africa and South America.

Despite these extensive and intensive studies, mosquitoes probably do not form a very good basis for research in animal geography. For one thing, the few fossil mosquitoes that are known are of little help in understanding the past history of the group. For another, the various taxonomic categories are not sufficiently clearly defined to form

a sure foundation for generalization. In particular, the broad generic concepts, useful for practical classification, are probably misleading in making geographical comparisons. And there is still no unanimity among students as to what constitutes a mosquito "species," so that the statement "*species alpha* occurs from Oregon to northern Chile" means nothing unless we are familiar with the author's ideas about species and with the sort of study that has served for determining the specific identity of the Oregonian and Chilean populations. Further, mosquitoes are less easily collected than, say, birds or butterflies. Adults (except of the "voracious" kinds) are often hard to find and difficult to preserve in good condition. Mosquito collecting in the course of a constantly moving expedition is necessarily haphazard and uncertain, and our knowledge is thus apt to be detailed only for the vicinity of laboratories where mosquitoes can be bred and the material of the various stages conveniently preserved. It is particularly true for mosquitoes that a map showing the distribution of various species is apt to reflect the distribution of the collectors more than the distribution of the animals.

The above limitations would apply to almost all insect groups, with the outstanding exception of the butterflies; and with most insect groups there would be the added limitation that probably at least half of the tropical fauna would be completely unknown. Research on the general principles of animal geography, for these reasons, is probably most profitably carried out through the analysis of the data from various groups of vertebrates; and the various faunal regions and subregions are in fact generally defined in terms of vertebrates and higher plants. The geographical distribution of mosquitoes is, however, sufficiently interesting for its own sake to warrant considerable study; and the various limitations listed above will probably be overcome in time, except that there is little hope for ever finding an adequate fossil record for the group.

FAUNAL REGIONS

Zoologists, following the system first proposed by Sclater and developed at length by Wallace (1876), very generally recognize six major faunal regions on the earth: the Palearctic, the Ethiopian, the Oriental, the Australian, the Nearctic, and the Neotropical. The natural boundaries of these regions only in part correspond with political

or continental frontiers, and mosquito studies (like other human activities) are apt to be limited by political rather than natural frontiers. Fairly recent mosquito lists are available for all of the regions except the Oriental. The distribution of anophelines has, of course, received special attention and Russell, Rozeboom, and Stone (1943) have published complete lists of the species known from various parts of the world.

The Palearctic Region

The region is generally defined as including all of Europe, Africa north of the Sahara, and the temperate parts of Asia, including Persia, Afghanistan, Tibet, most of China, and the Japanese archipelago. This is a vast area, but with a remarkably uniform fauna. Growth in knowledge of the fauna is shown by the increase in the number of species in successive recent lists: thus 94 species were recognized by Edwards (1921), 114 species by Edwards (1926), and 125 species by Martini (1930). The number of species will probably be further increased when the central Asiatic area has been adequately explored.

The fauna of northern and central Europe is quite similar to that of North America. Edwards (1921) lists 17 species common to the two continents, and nine others as having representatives in North America that differ only in slight morphological characters. Various characteristically Ethiopian species are found in North Africa, and there is a considerable intrusion of Oriental species in the East. Indeed, Edwards remarks that on the basis of the mosquitoes, the southern islands of Japan at least as far north as Tokyo might be better considered as belonging to the Oriental faunal region.

The subregions of the Palearctic (like those of the Nearctic) are not clearly defined, but it is usual to recognize a "European" subregion extending east to the Urals and south to the Mediterranean peninsulas; a "Mediterranean" subregion, including the Spanish, Italian, and Balkan peninsulas, the Near East and North Africa; a "Siberian" subregion, including northern and central Asia; and a "Manchurian" subregion, including eastern Asia. Several special studies of local faunas in the European and Mediterranean subregions have been published, notably by Wesenberg-Lund (1921) on Denmark, Seguy (1924) on North Africa, Kirkpatrick (1925) on Egypt, and Marshall (1938) on the British Islands.

The Ethiopian Region

The mosquitoes of the Ethiopian Region have been excellently monographed in the three volumes by Hopkins (1936, culicine larvae); Evans (1938, anophelines), and Edwards (1941, culicine adults). Edwards has included a detailed discussion of the zoogeographical relationships of the mosquito fauna. 358 species have been described of which 312 are endemic. Nearly all of the genera and subgenera occurring in Africa, however, are also found in the Oriental Region: there is only one purely African genus (*Eretmapodites*), and three endemic subgenera (*Theomyia* of *Theobaldia, Dunnius* and *Skusea* of *Aëdes*).

There are, however, important differences between the Oriental and Ethiopian faunas. Edwards points out that "several Indian genera and subgenera are unrepresented in Africa, and certain subgenera of *Aëdes* and *Culex* are developed to a very unequal extent in the two regions. In Africa *Aëdimorphus* is the dominant subgenus of *Aëdes;* in India it is *Finlaya*. In Africa again the subgenus *Neoculex* of *Culex* is very much better represented than in India. . . . The contrast between the African and European faunas is as marked as the resemblance between these of Africa and India. Not only are numerous tropical genera and subgenera absent from Europe and North Africa, but the representation of the subgenera of *Aëdes* is very different." Curiously, there are fewer species common to the Ethiopian and Oriental regions than to the Ethiopian and Palearctic: there being fifteen species in the first category and twenty in the second. The Ethiopian and Neotropical regions have little in common: the genera and subgenera that occur in both are either cosmopolitan or tropicopolitan, and the highly characteristic South American groups have no representatives in any of the Old World faunas.

Edwards remarks that from the point of view of the zoogeographer the two most striking features of the Ethiopian Region are its isolation and its homogeneity. While it is marked off sharply from the rest of the terrestrial world by deserts and seas, there are no well marked physical barriers within the area. Madagascar, to be sure, is sufficiently distinct from continental Africa so that some authors treat it as a separate major region. Its mosquito fauna is comparatively unknown: of the 41 recorded species, 29 occur also in continental Africa,

11 are endemic, and one is a common Oriental species (*Aëdes albopictus*).

The Oriental Region

This region, as defined by Wallace, includes all India and China south of the limits of the Palearctic Region, the Malay Peninsula, and the islands of the Indies as far east as the Philippines, Borneo, and Java. Celebes, the Moluccas, and the Lesser Sunda Islands, lying east of "Wallace's Line," are placed in the Australian Region by Wallace, but are considered to be part of the Oriental Region by some zoogeographers. The problem of the proper separation of these two regions has been discussed by Mayr (1944), who favors "Weber's Line," which would include Celebes and the Lesser Sunda Islands in the Oriental Region, but would include the Moluccas in the Australian Region. Mosquito distribution is more in accord with this separation (Christophers, 1933), but the differences between the Oriental and Australian mosquito faunas are in any event not profound.

There is no general monograph or catalogue of the mosquitoes of the Oriental Region. The fauna of India, Ceylon, and Burma has been admirably monographed by Christophers (1933, anophelines) and Barraud (1934, culicines), and Borel (1930) has described the mosquitoes of Cochin China. The anopheline faunas have been described in numerous papers, such as those by Gater (1935) for Malaya; Toumanoff (1936) for Indo-China; Swellengrebel and Rodenwaldt (1932) for the Dutch East Indies; and Russell and Baisas (1935) for the Philippines.

The total mosquito fauna of the Oriental Region must be large, though perhaps not as large as that of the Neotropical Region. There are 293 species known from India, Burma, and Ceylon, an area that would probably include something over half of the total Oriental fauna.

The Australian Region

The geographical separation of the Australian and Oriental regions was discussed above. Taylor (1934) has listed 203 species of mosquitoes from the region. While the known fauna is thus much smaller than that of the Ethiopian or Oriental regions, there is a higher proportion of endemic groups (11 genera or subgenera) than in any

other region except the Neotropical. The mosquito fauna thus does not reflect the striking isolation shown by the mammalian fauna, since South America has many more characteristic, curious, and presumably primitive mosquito groups than does Australia.

Endemic groups include the rather primitive anopheline genus *Bironella* (with two subgenera); three of the four subgenera of *Tripteroides* (the fourth subgenus being predominantly Oriental), a group related to the South American sabethines; the New Zealand genus *Opifex*, with a single species notable chiefly for its aberrant mating habits; and five subgenera of *Aëdes*.

The Nearctic Region

This region is most conveniently defined by considering it to include all of the North American continent north of the United States and Mexican frontier. Actually, typically northern forms extend considerably to the south in the highlands of Mexico, and it is customary to include these highlands in the area. In many groups of animals, the fauna of the tip of Florida is Antillean, so that for these animals this area might be considered as Neotropical, though it is more logical to treat tropical forms found in Florida as intrusions from the Neotropical Region. The subdivisions of the Nearctic Region have been discussed by Dice (1943).

The faunas of North America and Europe are very similar in all animal groups, so that some authors treat the two together as a Holarctic Region. Mosquitoes form no exception to this rule. Matheson (1944) lists 121 species for the Nearctic Region, so that the faunas are closely comparable even in size (125 Palearctic species according to Martini, 1930). The mosquitoes of the Nearctic Region are probably more completely described than those of any other region, since there are no large ill explored areas such as exist, for instance, in some of the Asiatic sections of the Palearctic. Differences between the two faunas are due entirely to the fact that some groups from the Neotropical Region have extended into the southern part of the Nearctic, and from the Ethiopian and Oriental regions into the southern part of the Palearctic.

No mosquito subgenus is limited entirely to the Nearctic, and only one (*Culicella* of *Theobaldia*) is entirely restricted to the Nearctic and Palearctic. The two areas are, however, characterized by the great development of the subgenus *Ochlerotatus* of *Aëdes*, and by four of

the *Theobaldia* subgenera. The development of *Ochlerotatus* in North America is particularly striking, no less than 50 of the 121 known mosquitoes of the area belonging to this group. These mosquitoes have apparently been the most successful of all groups in achieving adaptations to temperate climatic conditions, since they may abound even in the far north; they are typically ground-pool breeders with a well developed diapause period in the egg stage, which provides an efficient mechanism for hibernation.

The Nearctic mosquito fauna has been described in many taxonomic papers. The entire region was covered in the monograph by Howard, Dyar, and Knab, and synopses of all of the known species were given by Dyar (1928) and Matheson (1929, 1944). The earlier monographs of state faunas, like that of Smith (1904) for New Jersey, are largely of historical interest now, though Smith's has been republished by Headlee (1945) with some additional notes. The fauna of the southern United States has been covered by Carpenter, Middlekauff, and Chamberlain (1946) and in somewhat less detail by King, Bradley, and McNeel (1939). There are numerous lists of mosquito records for given states, and a few papers which cover state faunas in more detail, with keys and descriptions of the species. Examples are the papers by Rozeboom (1942b) on Oklahoma and by Dickinson (1944) on Wisconsin.

The Neotropical Region

This region is normally defined as including all of the continent of South America, North America south of the United States border (except for the Mexican highlands), and the islands of the Antilles. Wallace remarks that the Neotropical Region includes more peculiar genera and families of vertebrates than any other region. The mosquito fauna is also very distinctive. Lane (1939) lists 501 species in a catalogue of Neotropical mosquitoes. He places these in 47 generic or subgeneric groups (differing in some minor details from the subgeneric groups of Edwards, 1932); 27 of these groups are exclusively Neotropical, and 8 others are represented outside of the region only by a few species that have extended their range into the southern United States. In other words, 75 per cent of the Neotropical mosquito groups are indigenous—an extraordinary percentage when one considers the conservative basis of generic and subgeneric groupings in the family. Furthermore, the great majority of the species belong

to these characteristic groups—373 of the 501 species listed by Lane.

The Neotropical fauna includes mosquitoes that, on morphological grounds, are considered to be the most primitive members of the family (*Chagasia*, *Trichoprosopon*). It also includes some very bizarre forms, such as the large, bright metallic *Sabethes* with broad "paddles" or scales on their legs. In mosquitoes, as in other animals, there are large groups of South American species that are purely indigenous and that appear to owe their success to specialization for a life in a wide range of forest habitats. *Microculex* and *Wyeomyia* are good examples.

The outstanding biological characteristic of South America is the vast tropical forest: the conditions of life in this forest must be taken into account in any discussion of the characteristics of the animals of the continent. Among mosquitoes, for instance, there is a large predominance of diurnal mosquitoes. These are predominantly forest insects, and their breeding places are apt to be container habitats: bromeliads, bamboo, leaf bases, flowers, fallen leaves, and fallen flower bracts. Of the 47 subgeneric groups found in the region, no less than 25 breed exclusively in such habitats, including a majority of the characteristic groups. Only three of the indigenous phylogenetic stems show a great proliferation of species adapted to pond and pool habitats: the anopheline subgenus *Nyssorhynchus*, the culicine subgenus *Melanoconion*, and the genus *Psorophora*.

The standard reference work on South American mosquitoes is the book by Dyar (1928). Bonne and Bonne-Wepster (1925) have written a book-length study of the mosquitoes of Surinam, and a monograph of the important sabethine group of genera has been published by Lane and Cerqueira (1942). Of special studies on the anophelines, the papers by Komp (1942) on the Caribbean and Cova-García (1946) on Venezuela are noteworthy. Discussions of the zoogeography of the Neotropical sabethines have been published by Lane (1943) and of the anophelines by Lane (1944).

Summary on faunal regions

The data on the extent of the faunas of the different regions have been summarized in Table XIII, and the extent of the distribution of the different subgeneric groups in Table XIV. A few liberties have been taken in preparing these tables: particularly the presence of *Aëdes aegypti* and *Anopheles gambiae* in America has been ignored,

since both are obviously human introductions of otherwise completely alien groups; the assignment by Edwards of three Oriental species to the American genus *Haemagogus* has also been disregarded, as this is merely a temporary convenience pending a more complete knowledge of the affinities of these species.

TABLE XIII COMPARISON OF REGIONAL FAUNAS

REGION	NUMBER OF SUBGENERIC GROUPS (EDWARDS, 1932)	NUMBER OF SPECIES	AUTHORITY
Palearctic	20	125	Martini, 1930
Ethiopian	29	358	Edwards, 1941
Oriental	40	—	
Australian	38	203	Taylor, 1934
Nearctic	21	121	Matheson, 1944
Neotropical	40	501	Lane, 1939
World	88	1400	Edwards, 1932

It is obvious that mosquitoes are predominantly a tropical group, with the richest development of genera and species in the Oriental and Neotropical regions. These two faunas are probably closely comparable in size, but they differ in the greater isolation and conse-

TABLE XIV DISTRIBUTION OF MOSQUITO SUBGENERA BY FAUNAL REGIONS (BASED ON DATA FROM EDWARDS, 1932)

DISTRIBUTION	NUMBER OF SUBGENERIC GROUPS
Palearctic only	2
Ethiopian only	4
Oriental only	8
Australian only	11
Nearctic only	0
Neotropical only	23
New World regions	6
All Old World regions	3
Holarctic	1
Ethiopian and Oriental	3
Oriental and Australian	3
Ethiopian, Oriental, and Australian	7
Common to all regions	7
All regions except Africa and Australia	2
All regions except Neotropical	2
Other distributions	6
Total subgeneric groups	88

quently greater degree of endemicity in the Neotropical area. The Holarctic areas are comparatively poor both in genera and species, and they are notably poor in endemic groups: there are no wholly endemic Nearctic groups, and the two endemic Palearctic groups are both obscure subgenera of *Culex*, one known only from the Eastern Desert of Egypt, the other with a wider distribution in the Mediterranean and Near Eastern areas.

The mosquito subgenera show very wide distributions as compared with most animal genera. Only 48 are confined to a single region (23 of these to the Neotropical), and 7 have representatives in all of the major faunal regions of the world. This might be taken to represent exceptional powers of dispersal on the part of mosquitoes, but it probably rather reflects the conservative basis of mosquito classification. "Species groups" within the subgenera are apt to show a closer correlation with given geographical areas than do the subgenera themselves, and these "species groups" probably correspond more closely to the genera of other animals than do the broad subgenera.

A great deal of space is normally given in discussions of animal geography to speculation on the historical basis of contemporary distribution, in particular to concepts like "centers of origin" and "paths of dispersal." Christophers in various publications (particularly 1921) has made some stimulating observations on the distribution of the anophelines from this point of view. In general, however, our knowledge of mosquitoes is still too inadequate for the profitable discussion of such concepts; and conclusions based on the modern fauna alone can, in any case, never pass beyond the level of guesswork. This is shown by the completeness with which our concepts of mammal distribution are dependent on the fossil evidence, entirely lacking in a group like mosquitoes.

THE RANGE OF SPECIFIC POPULATIONS

A study of the precise geographical range of specific populations is of interest from several points of view. In the case of disease vectors, the distribution of particular species is an important element in the study of the geography of the disease. For the ecologist a study of the factors that appear to limit geographical spread may shed light on the ecological requirements of the species throughout its range. The student of evolution is very interested in geographical range because

of the importance of geographical isolation in speciation, because of the possible relation between the area occupied and the possible geological age of a species, and because the study of range may be of help not only in determining barriers to spread, but also in evaluating means of dispersal. In view of all of this, it is disappointing to find how little is known about the geography of the various individual mosquito species.

Such study is not simple for the various reasons set forth at the beginning of this chapter. England is probably the most thoroughly studied area on this globe from the point of view of field zoology; yet even for this area Marshall (1938) finds it advisable to include the following remarks, which apply with even greater force elsewhere: "Every 'locality record' is the result of a fortuitous meeting between a collector and his quarry. It follows, therefore, that a list of locality records of any common, widespread species of mosquito indicates the distribution, not of the species concerned, but of the entomologists who happen to have collected them."

An even greater handicap is the difficulty of defining specific populations. In 1920 a discussion of the distribution of *Anopheles maculipennis* would have centered on its Holarctic occurrence, its wide distribution through Europe and northern Asia and predominance in the northern and western parts of North America. This western American distribution would have been a noteworthy point, and Edwards (1921) in fact remarked that "in one or two cases (for example, *Anopheles maculipennis*) there seems to be definite evidence that the European fauna is more closely allied to that of western than that of eastern North America." In 1947 any discussion of the distribution of *Anopheles maculipennis* (by the present author at least) would involve a supraspecific group involving seven different populations in Europe and perhaps four populations in North America. The inclusion of the eastern North American *Anopheles quadrimaculatus* in this same series of populations would remove the basis of Edwards's remark. In the case of the *Anopheles maculipennis* group, at least, it seems probable that we have reached the stage at which we can recognize genetically isolated populations, and the study of the distribution of these populations thus attains general biological value. If and when it becomes possible to study genetic variation within a given mosquito population in correlation with geographical spread, as is now possible with *Drosophila* (Dobzhansky, 1947), this field of study will attain

even greater value. Our knowledge of mosquitoes even now, however, has reached a point where distributional studies are apt to form a valid basis for generalization.

The most widely distributed mosquitoes are probably the domestic species *Aëdes aegypti*, *Culex pipiens*, and *Culex fatigans*. *Aëdes aegypti* occurs throughout the tropical and subtropical zones of the world except "Japan, New Zealand and some of the smaller Pacific Islands" (Edwards, 1932). It presumably originated in Africa, since various closely related species are purely Ethiopian, and it has been spread by commerce. It is curiously domestic, breeding almost exclusively in artificial water containers, and over most of its range, at least, it has shown no tendency to invade natural breeding places. Its spread out of the tropical zone is generally considered to be limited by the 20° isotherm; the eggs are rapidly killed by freezing (Davis, 1932b), and this is probably the limiting factor. There is no direct evidence of physiological diversity in this world-wide population. Mathis (1934) found no differences among colonies from Greece, Cuba, Java, and West Africa kept under identical conditions.

The situation with the domestic *Culex* species is somewhat different, and was discussed in Chapter XVI. In general, *Culex pipiens* (and *molestus?*) inhabits the temperate areas of the world, and *Culex fatigans* the tropical and subtropical; in some places, however (for example, South Africa) the two apparently occur together. Since *Culex molestus* can only be distinguished from *pipiens* with certainty by biological procedures, the *pipiens* records from most parts of the world are open to question. A study of the relations among these species in the southern United States might yield interesting results.

The widest ranges among nondomestic mosquitoes are probably shown by the Holarctic *Aëdes*. Eleven species of the subgenus *Ochlerotatus*, one of *Aëdimorphus* (*vexans*), and one of *Aëdes* in the strict sense (*cinereus*) are found in the northern parts of both hemispheres, in most cases over extensive areas. *Aëdes vexans* probably has the widest range of all, listed by Edwards (1932) as "Palearctic, Nearctic and Oriental regions; Fiji, Samoa, New Caledonia." These wide ranging *Aëdes* have been the subject of little or no biological investigation, so that there is no basis for judging the possible homogeneity of the populations. The group to which they belong, however, is characterized by wide flight ranges of individual specimens.

At the other extreme, it is difficult to evaluate the significance of

very restricted ranges among mosquitoes because such ranges are apt to be characteristic of tropical islands and of high altitude species on tropical mountain ranges—two situations that have been very inadequately studied. The rate of endemicity among mosquito species on tropical islands—in the Antilles, for instance—seems to be very low, but that may be because material from the various islands has not been carefully studied and compared. "Relict species," ancient forms that have persisted in some small isolated area, have not been described. The most primitive surviving mosquito types (*Chagasia, Bironella, Trichoprosopon, Megarhinus*) all have species that range rather widely and that are locally common: *Bironella* species are largely confined to New Guinea, but that island might be called a subcontinent.

It is unfortunate that no special studies have been made of the mosquito faunas of high altitudes in the tropics. *Anopheles* and malaria are rare and sporadic in mountainous regions, hence a prime motivation for such studies has been lacking. The maximum altitude reached by various anophelines has, however, aroused a certain amount of attention, and records have been reviewed by Unti and Ramos (1942) and by Russell, West, and Manwell (1946).

The zonation of fauna and flora in tropical mountains is a striking phenomenon that has received considerable attention from field naturalists, and mosquito distribution, if and when it is studied in such regions, should be interpreted against this background. In Colombia four mountain zones are generally recognized: the tropical, from sea level to about 1,500 meters; the subtropical, from 1,500 to 2,800 meters; the temperate, from 2,800 to 3,400 meters; and the paramo, from 3,400 to snow line (about 4,600 meters). The limits of these zones are, of course, not precise even on a given slope, and show considerable variation in different mountain ranges. The vast majority of anopheline species are inhabitants of the tropical zone and most of them, indeed, are limited to comparatively level country by the habitats in which they breed. The few lowland species that extend their range very high into the mountains are apt to be adapted to running-water habitats, like *Anopheles pseudopunctipennis* and *A. argyritarsis* in tropical America. The first species has been found in many places at altitudes above 2,000 meters, and in Bolivia it has been recorded at 3,000 meters. For the most part, though, records of anophelines from above the tropical zone involve special species, probably with re-

stricted distributions, such as *A. hectoris* and *xelajuensis* in Mexico and Guatemala, *A. vargasi* in Venezuela, *A. garnhami* in Africa, and an undescribed species found by Dr. Ernesto Osorno at 3,000 meters in the Eastern Andes of Colombia.

Discontinuous distributions have not received much attention, probably because in most cases our information is inadequate for an evaluation of discontinuity. *Anopheles darlingi* presents a clear case of such a distribution: it is found over much of South America, including Venezuela and Colombia, but it has not been collected in Panama or Costa Rica, though reappearing in Guatemala and British Honduras (Komp, 1942). The isolated Central American population shows no structural peculiarities; behavior characters have not been compared. It has been suggested that *darlingi* in British Honduras may represent accidental human introduction, but this seems unlikely.

The possibility of spread of anophelines or other mosquitoes along north-south lines in the American continental area by human agency seems to me relatively unimportant, since the species appear to have reached relatively stable distributions along these lines through natural routes of dispersal. The occasional capture of *Anopheles albimanus* in southern Florida (Carpenter and others, 1946) probably represents, for instance, the extreme northern limit of the range of this species, where it either persists at a very low population level, or periodically is introduced by natural or artificial means, only to die out.

The danger of artificial spread of mosquitoes along lines of latitude, particularly between the African and South American continents is, however, very real, as was demonstrated by the appearance of *Anopheles gambiae* in Brazil (Soper and Wilson, 1943). The species was discovered in a very limited area near the city of Natal in 1930, where it must recently have been introduced. It spread very slowly, and apparently did not reach an ecologically favorable region until 1937, when it caused a serious outbreak of malaria in the town of Aracatí near the mouth of the Jaguaribe River, about three hundred kilometers in a straight line from the point of discovery at Natal. Once established in this favorable region it caused catastrophic epidemics of malaria, which led directly to the initiation of the justly famous campaign of eradication. Detailed ecological studies of the *gambiae* situation, of a mosquito species in a new environment, particularly from the point of view of association with the preexisting mosquito

populations, might have yielded very interesting results. Such studies were planned, but it was impossible to carry them through in detail because of the exigencies of the eradication campaign.

FOSSIL MOSQUITOES

Edwards (1923) wrote a review of the known fossil mosquitoes, and the material has been summarized by Edwards (1932, 1941), by Martini (1930), and by Marshall (1938). Two specimens that may represent species of *Culex* have been described from Wyoming and Colorado Eocene, and several Dixiinae are known from the Lower Oligocene Baltic Amber. The most numerous mosquito fossils, however, have come from the Middle Oligocene "Insect Bed" of the Isle of Wight. These include specimens quite surely assignable to *Culex*, *Aëdes*, and *Mansonia*, and perhaps also *Megarhinus* material. It thus seems quite certain that the main living mosquito stems were well differentiated in mid-Tertiary, so that they may have originated in early Tertiary or perhaps even earlier, since they form a rather primitive section of the Diptera, an order that probably arose in the Triassic. No fossil anophelines are known, but Edwards points out that this is not surprising in view of the very small number of mosquito fossils; on morphological grounds, the group is considered to form the more primitive existing mosquito stem.

It is disappointing to find that very few Culicidae have been found among the amber insects. Brues (1933) made an ingenious attempt to determine the sort of collecting sample represented in the amber by comparison with a similar sort of sample from a contemporary New England forest. He obtained his sample by exposing sheets of "tanglefoot" on tree trunks, thus simulating the presumed conditions under which the amber fossils were formed. Among over 21,000 insect specimens caught by this means, there were only 26 mosquitoes. The scarcity of mosquitoes in the Baltic amber material thus surely reflects the habits of the insects, rather than any scarcity of mosquitoes in the amber forests.

CHAPTER XIX

TECHNIQUES IN MOSQUITO STUDY

> From a purely scientific point of view, I have always regarded the question, who first made a biological observation, as a matter of sublime indifference. It must never be forgotten that even with regard to biological observations, which can only rarely be committed to paper with the same convincing exactness as an anatomical structure, the exact apprehension of a given fact can only be acquired through repeated observation. It is further of the greatest significance that the biological observations are tested by different scientists and in different latitudes; only in that way can our suppositions and hypotheses be registered among real scientific facts. It must further be remembered that the study of Nature must always begin with the slightest possible literary ballast. He who has first crammed his head with all that has been written upon a subject will, at the moment of observation, when standing face to face with Nature, soon understand that his whole learning is only felt as a burden and restricts his power of observation. I for my own part have always been of the opinion that it is exactly the smallest equipment of human knowledge which gives the greatest peace in my studies, creates the scientific sovereignty over observations and thoughts and—as far as possible—moves the milestones of time nearer to the borders of eternity.—C. WESENBERG-LUND

IT WOULD seem advisable to include in this book some summary of the special methods that have been developed for investigating mosquito behavior. Such material should increase its usefulness as a reference work for students actually involved in mosquito investigations. It is assumed that any such investigator would have available taxonomic guides to the local mosquito fauna and one or more of the standard textbooks of malariology or parasitology. The taxonomic guides (which were cited for the various geographical regions in the previous chapter) generally include a section on the handling of preserved materials—on methods of pinning adults, making microscope

slides of larvae or male genitalia, and so forth—so that such information would seem to be superfluous here. Similarly, the malariological and parasitological textbooks generally contain sections on the techniques of microscopy involved in mosquito investigation, so that such information would also seem superfluous in the present connection. There remains the area of methods of field and laboratory handling of live mosquitoes, which is generally treated in a very inadequate fashion in such books.

FIELD STUDIES: LARVAE

Most mosquito larvae live at the water surface in areas covered with vegetation, and they are readily alarmed by water movement or by shadows. Their collection, then, involves skimming the water surface with a minimum of disturbance. Various types of dippers have been devised for this purpose, the most common perhaps being a round metal dipper of about 400 cc. capacity, with a small screened "window" on one side for draining off water, and with a hollow handle into which a cane, or long pole, can be inserted, so that the dipper can be used from the bank. Each field worker becomes used to his own type of apparatus, and is apt to find any other kind awkward. Thus we have never liked this conventional type of mosquito dipper, and instead have used shallow rectangular white enamel trays, photographic developing trays, usually the 16 by 21 cm. size. The straight sides of such a tray enable one to skim the water surface with maximum efficiency. There is no handle, and so the collector must usually roll up his trousers and get into the water, but this intimate contact with the breeding place also has its advantages. Either L. W. Hackett or Marshall Barber originated the remark that sun-loving anophelines cannot effectively be studied by shade-loving malariologists; and it is equally true that purely terrestrial entomologists work under a considerable handicap in studying aquatic mosquito larvae.

The collection of larvae from very shallow water, characteristic of some types of marsh and seepage, presents special difficulties. If the water is too shallow for the use of a tray or dipper, the best system seems to be "puddling," muddying the water and then patiently waiting with a pipette to collect the larvae that appear at the surface. Direct inspection and individual collection with a pipette is, in fact, the best way to get larvae in many situations. Various sorts of apparatus

can be devised for the collection of larvae from tree holes and other container habitats, but the basis of most such apparatus is a 100 cc. transfer pipette with a large rubber bulb, or else some system for siphoning out the water. Larvae from water containing plants, such as bromeliads, aroids, or Heliconia flowers, are best collected by dumping the entire water contents of the plant into a bucket or tray, and repeated washings of the plant will usually increase the larval collection considerably.

The nature of the records of field work, of course, depends on the purpose for which the larval collections are being made. Detailed suggestions on methods of keeping records are thus superfluous, and may even be misleading since a new approach may be productive of new and different results. For routine survey purposes some standard method of classifying breeding places may be used, schemes such as those outlined in Chapter XI. For research purposes, however, considerable thought should be given to the environmental factors to be recorded and measured, and the records of the field work probably deserve as much attention as the collecting itself, or more. Punch cards are very useful for the analysis of data of the sort collected during field surveys, but again care should be taken not to "freeze" any such record system too early in an investigation. A liberal and routine use of photographs of breeding places may yield more information on subsequent analysis than detailed descriptions.

One of the great needs in mosquito biology is for quantitative field studies; and by such studies, mosquito work may contribute greatly to the development of ecology, to the understanding of the environmental relationships of animals. Methods for such work must depend on the inventiveness of the investigator. It is a good idea to consult the ecological literature for suggestions on technique. Shelford (1929) has published a book dealing with ecological methods, with an extensive bibliography, and there is a good section on methods of collecting in the well known volume by Ward and Whipple (1918). Perhaps the best source of ideas is general reading of research articles published by marine and fresh-water biologists, since their problems and their methods of attack are bound to be of interest in relation to mosquito studies.

The commonest method of expressing density of mosquito larvae is in terms of individuals "per dip," a method that gives a rough idea of abundance, but that is bound to vary greatly with the type of dip-

per used and with the skill and experience of the operator. Bates (1941c) and Goodwin and Eyles (1942) have described methods whereby a given area of pond can be fenced off and the total larval population of the enclosed sample area collected. A measurement of density can also be obtained by placing a screened cage (one meter square is a convenient size) over different parts of a breeding place, collecting the adult mosquitoes that emerge every day.

An index of larval survival in a given breeding place can be obtained from the proportions of different larval stages in a given collection. The four larval instars of a particular mosquito species can be recognized readily, with a little practice, by noting the size and shape of the head, which is constant for a given instar. We find it most convenient to sort the larvae, with the aid of a reading glass, into four watch glasses; the watch glasses are then checked to be sure no larvae have been wrongly assigned, before the final count is made. The relative proportions of first- and fourth-stage larvae vary characteristically in different sorts of breeding places. Some data on Albanian habitats were published by Bates (1941c).

It is important, in making larval surveys, to find reliable methods of larval identification, since the breeding out of all material makes a study hopelessly complicated. With *Nyssorhynchus* larvae, at least, we find that identification is easier with live than with dead material, since the larvae will orient themselves right side up in a hollow ground slide so that characters can easily be checked with a compound microscope. The use of ice water for such larvae reduces movement to a minimum. Where several anopheline species are found in association, it is useful to raise numbers of first-stage larvae, so that the species composition of the adult and young larval populations can be compared.

A review of the material on larval ecology in this book makes it apparent that no rules can be made as to what chemical and physical factors of the larval environment should routinely be measured: the subject is still very much a field for active investigation. Chemical analyses of water by standard methods (the American Public Health Association publishes a convenient guide) is apt to be difficult in an ordinary entomological laboratory, but reagent manufacturers (such as Hellige and LaMotte) manufacture special kits for soil and water analyses by colorimetric methods that are relatively simple to use. If temperature records are kept, it should be remembered that tempera-

ture in the water two or three inches below the surface may be different from that of the surface film where the larvae live, and that temperatures at the moment of making a larval collection have little significance if they cannot be interpreted in terms of the daily temperature cycles.

FIELD STUDIES: ADULTS

The collection of adult mosquitoes may be carried out by catching individuals that come to bite, by "sweeping" with an insect net in foliage, by searching special resting places such as stables, or by the operation of traps of various sorts. The collection of mosquitoes with animal bait or by sweeping involves no special technical problems. Collection in resting places depends mostly on a knowledge of the habits of the mosquitoes, discussed in Chapter II. The use of stable traps and light traps, however, deserves some comment.

We have found stable traps to be the most useful general method of collecting adult nocturnal mosquitoes, whether with the object of getting the mosquito material, or with the object of collecting data on the abundance and habits of different species. Stable traps have several great advantages over light traps: the specimens may be taken alive and (with care in collecting) in good condition; mosquitoes are not mixed with a mass of other kinds of insects; and the traps are independent of power supply. This last factor has prevented any extensive testing of light traps in tropical regions, though battery operated traps could surely be devised. The advantages of light traps, of course, are that many traps can be operated economically, and that male as well as female mosquitoes are caught. I know of no parallel studies with the two types of trap in a given area, but the species composition of the fauna sampled by each type of trap would probably be different.

A stable trap of the type we use in Colombia is shown in Plate 14. There is no point in giving detailed specifications for the construction of such a trap, in so far as size and materials may vary depending on the circumstances under which the trap is to be used. A standard size and construction would be useful, since it would permit comparisons between studies in different areas, but this is an ideal not likely to be attained in the immediate future. Essentially the idea is to enclose an animal with materials strong enough to resist breakage by the animal, with a large enough extent of wire screening to permit

diffusion of attractant stimuli from the animal, and with an ingress baffle that allows mosquitoes to enter readily but inhibits their excape. The baffle design is perhaps the most important single factor in trap construction, and the type that we have found successful in Egypt and Colombia is illustrated in Figure 9. The important point is to have

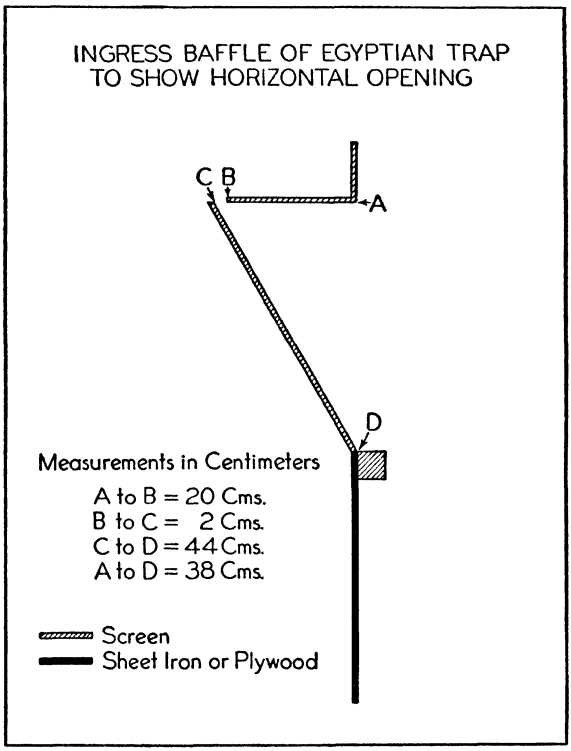


FIG. 9. CONSTRUCTION OF INGRESS BAFFLE WITH HORIZONTAL OPENING FOR "EGYPTIAN-TYPE" STABLE TRAP (FROM BATES, 1944*b*).

the opening in a horizontal plane, since mosquitoes enter readily by flying upward, but are not apt to escape by flying downward. A similar type of baffle can be used for making traps that fit on the windows of houses or stables.

Magoon (1935), who published the first detailed description of a stable trap, has emphasized the importance of making the trap demountable for easy portability. We have made most of our traps with

the sides held together by bolts and wing nuts, with the idea of thus increasing portability, but in actual practice we find that we never have occasion to dismount a trap except when it is to be used in a situation remote from a road, so that the parts must be carried for some distance by hand. Otherwise it is much simpler to load the trap, mounted, into the back of a truck. We use donkeys for bait. These animals are a convenient size, and readily learn to take their part in the investigation: jumping into the back of the truck for transportation to and from the trap, and rarely causing damage even to lightly constructed traps. Calves, sheep, and other medium sized animals can also readily be used as bait. Horses are also commonly used, but we have found them too large and too expensive.

Shannon (1939) has described a trap made of muslin or other netting material that can be used for diurnal captures from a horse. In general with diurnal mosquitoes we have found trapping methods less successful than direct captures from host animals: perhaps visual factors enter into host seeking to a larger extent with diurnal than with nocturnal mosquitoes.

A wide variety of insects (including many kinds of mosquitoes) are attracted to light, and various types of traps have been designed to catch such insects. The essential requirements are a light source, an ingress baffle, and some method of killing the trapped insects as quickly as possible. For mosquito collecting it is important to have some sort of a screening arrangement to prevent the entry of large moths and beetles. Mosquito studies and routine collections in the United States have been almost universally made with a standardized light trap called the "New Jersey Trap." This has been described in numerous papers (for example, Headlee, 1932) and detailed specifications can be obtained from the New Jersey Agricultural Experiment Station.

LARVAL CULTURE

The culture of larvae is a very important part of any mosquito investigation and one in which seemingly endless difficulties are often encountered. The literature on culture methods is considerable: apparently because each investigator, after surmounting his difficulties, has an irresistible urge to impart his discovery, without, however, checking back in any great detail on what has previously been published. The successful raising of mosquito larvae, like the culture of

bacteria, algae, fungi, ants, or guinea pigs, depends a great deal on an intangible factor of experience that cannot be communicated with printed words; so that while a review of the literature is helpful, indeed necessary as a source of suggestions, no technique is foolproof or guaranteed to work from the first trial.

Perhaps the most important point, and one that is usually not appreciated, is that the culture of each species of mosquito is a separate problem. Larvae from different kinds of habitats may require quite radically different handling in the laboratory, species breeding in ponds differing from species breeding in tree holes, and so forth. Species with apparently quite similar habits may also, however, require different handling; and species with widely varying habits may at times be found to respond well to the same type of culture medium. There is no rule of thumb. In a given laboratory, culture methods can generally be reduced to a few standardized types, but this is attained through a process of experience and a new kind of mosquito is always likely to present new problems. The material reviewed in Chapters VIII and IX of this book is perhaps sufficient indication of the inadequacy of our knowledge of larval requirements and of the empirical basis of most culture methods.

The problem in raising larvae, essentially, is to find a method of providing adequate food and at the same time maintain favorable physical and chemical conditions in the medium. This is especially true of anopheline larvae, which die rapidly in any medium in which a surface scum develops. In any given laboratory and with any given species of mosquito, it is generally necessary to run a series of comparative experiments before any technique can be standardized for mass breeding. It is particularly important to test the various types of water available: both in Albania and in Colombia we found that it was impossible to breed anopheline larvae in laboratory well water, presumably because of unfavorable materials present in ground water. In Albania the unfavorable effect was perhaps related to the high nitrate content, in Colombia to the high iron content. By a series of tests with water from a variety of possible local sources, the most satisfactory type can readily be selected for routine purposes. Rain water, stored in a cement tank, proved adequate for the anophelines raised in Albania, while in Colombia water from a near-by stream was found to be better than rain water for anophelines of the *Nyssorhynchus* group.

A wide variety of substances have been tested as food for mosquito larvae, but the most commonly used materials are probably commercial fish foods, yeasts, bread crumbs, and dog biscuit. Again, in any given situation, a series of tests should be made with different available food materials, once a satisfactory water source has been found. Dried prepared food of the sort used for aquarium fish may make excellent larval food (Martini, 1921; Woodhill, 1936) but it is apt to be expensive and hard to keep under tropical conditions. Of yeasts, both the baker's and brewer's type have been found satisfactory in some laboratories. Boyd, Cain, and Mulrennan (1935), in a technique that has been widely adopted for *Anopheles quadrimaculatus*, use fresh baker's yeast, keeping a constant supply available on slides arranged with corks so that they float just beneath the surface. In the tropics or in remote situations, dried yeasts will generally be the only type available. We have obtained excellent results with dried brewer's yeast, of the sort put up in tablet form by the vitamin manufacturers, in raising various kinds of larvae in Colombia: this yeast and rain water is a particularly good medium for *Haemagogus spegazzinii* and other tree-hole-breeding mosquitoes, and has proven adequate for a few anophelines, including *Anopheles pseudopunctipennis*. In general, however, we have found that yeasts do not make a satisfactory food for anophelines. The results will be excellent as long as the amount of yeast added to the water is at some ill defined "optimum," but pans may "go bad" over night and all of the larvae be found dead. With both the European and South American anophelines we have found less concentrated foods, bread crumbs or dog biscuit, to be more satisfactory.

There are endless varieties of both bread and dog biscuit, and again comparative tests must be made in any given situation. In Albania our "standard" food for all anophelines was bread bought in the local market, dried in the sun, ground in a coffee mill and applied daily to the surface of the larval pans with a salt shaker (Bates, 1941a). This type of food proved to be less satisfactory in Colombia, perhaps because of differences in the bread, or perhaps because of differences in the mosquitoes. In Colombia our "standard" food for anophelines is a brand of dog biscuit called "Ken-L-Meal." This was called to our attention by Dr. R. E. Heal, who found it to be the most satisfactory for mosquito larvae of a large series of brands that he tested. The

meal is ground fine with a mortar and pestle and applied daily to the water surface with a salt shaker.

Many kinds of infusions have been tried as larval culture media, and the standard hay infusion of the protozoologists is widely used. Boyd, Cain, and Mulrennan (1935) use alfalfa hay infusions that have "ripened" in the open for at least thirty days in raising *Anopheles quadrimaculatus*. A few anopheline species can be grown in such infusions, but they are most useful for rapid growing ground pool-breeding *Aëdes* and *Psorophora*, with yeast added as supplementary food. *Aëdes aegypti*, *Culex pipiens*, and similar species may be grown in very strong infusions of various sorts. The concoction and testing of such infusions can become a fascinating game.

For anopheline culture, in tests with a wide variety of species in Albania, Egypt, and Colombia, we have found a soil-water medium to be most satisfactory. A similar technique, called the "biphasic culture method," is successful with a wide variety of algae (Pringsheim, 1946), and it is notable that the culture of algae and of anophelines often presents similar problems and that the two frequently grow together very well. Bates (1941) has described the soil-water method in detail; it evolved from the sod method described by Shute (1936) and Vollmer (1936). The essential idea is to grow the larvae in dishes containing a layer of soil under the water; the soil seems to serve both as a source of food materials and as an adsorbing agent for deleterious substances. Again, tests must be made with a variety of soils in any given locality, though a "sandy-loam" type seems to be the most satisfactory. Food, such as bread crumbs or dog biscuit, must be added daily, and a proper balance of larval population is important—enough larvae to keep the surface swept clean, yet not so many as to produce overcrowding effects.

A comparative shallow layer of water over the soil often results in the most satisfactory survival and growth. It is interesting that L. E. Rozeboom finds that *Anopheles quadrimaculatus* can be raised with a minimum of effort in very shallow water over filter paper; perhaps the filter paper serves the same adsorbing function as the soil. We have not found the paper method to be very successful with South American anophelines, except for growing individual larvae in small vials.

There is a general conviction that anopheline larvae can best be

grown either in sunlight or with an artificial source of ultraviolet light. This was discussed in Chapter VIII.

LABORATORY MANIPULATION

Environmental control. Perhaps the most important single factor in laboratory studies of insect behavior is an adequate appreciation of the importance of temperature conditions. The rate of all metabolic processes in insects is directly dependent on the environmental temperature, and a description of any given process without a statement of the temperature at which the observations were made is quite meaningless. The development of parasites, the digestion of blood, the development of eggs, the rate of larval growth, the size and vigor of the resulting adults—all such things depend on environmental temperature. If observations and experiments are carried out at "room temperature," these temperature conditions can be measured and described, but the repetition of the experiments by other workers is made difficult because room temperature may be quite different in different laboratories. The control of temperature for many types of behavior observations is difficult and in many instances not necessary, but for any observations involving developmental rates it is very necessary, and any laboratory undertaking such studies should have adequate equipment for temperature control. Such equipment is expensive, but so are microscopes. Under field conditions, where a constant supply of electric current is not available, temperature control may present considerable difficulties, but if experimental work on the rates of processes is to be carried out, some method of achieving a reasonable amount of control must be worked out. Precise experiments can be left to the laboratories that do have an adequate source of electricity.

With electric power the maintenance of constant temperatures above room temperature is easy and relatively inexpensive. For larval experiments we have found large water baths in which the larval pans are floated to be very convenient. Such a water bath can be constructed very cheaply by a local carpenter: the bath proper can be made in the form of a large galvanized sheet-iron pan, enclosed in celotex or similar material for insulation, and heated by ordinary electric light bulbs enclosed in a wooden box under the iron pan. Temperature control can be achieved with either a mercury or bimetallic thermoregulator of the sort sold by various instrument com-

panies for temperature control in the usual small laboratory water baths. Homemade incubators for controlling air temperature can also be constructed by a good carpenter and thermostatic control achieved with the same sort of apparatus.

In the absence of electricity, constant temperatures are more difficult to achieve. In the tropics temperatures in a well insulated cellar are amazingly constant throughout the year, and in the temperate zone temperatures in such a cellar can be maintained at a uniform and relatively high temperature throughout the winter quite easily with kerosene-burning stoves (arranged so that fumes are conducted to the outside air by flues). An underground room provides, in any event, ideal conditions for many kinds of mosquitoes, especially those with nocturnal habits. Water baths similar to the electric ones can be constructed to be heated with kerosene lamps; and it is even possible to provide thermostatic control for such heating units by means of the damper system used on kerosene incubators, such as those used for hatching hens' eggs.

Constant temperatures at levels below maximum room temperature can be achieved by cabinets in which heating units are balanced against cooling coils. Ordinary electric refrigerators modified by the addition of heating coils for this purpose are manufactured by the Precision Scientific Company of Chicago, and probably by others. Anyone with a moderate knowledge of electrical circuits could probably readily arrange such a unit starting with a commercial electric refrigerator as a base.

Precise humidity control is more difficult to achieve than temperature control, and fortunately less necessary for most experimental work. The longevity of adult mosquitoes varies greatly under different humidity conditions, but for practical purposes roughly optimum conditions can usually be obtained with wet cloths, fans, and so forth. For experiments with different constant humidities it is usual to maintain humidities by means of different salt solutions or different dilutions of sulphuric acid. Tables showing the humidities given by such solutions have been published by various authors (for example, Shelford, 1929, p. 252). Considerable control over humidity has been achieved in modern air-conditioning units, and the installation of such units in at least one laboratory room would probably provide ideal conditions for experimental work.

It is important that a laboratory be adequately equipped for the

measurement of temperature, humidity, and light. Thermographs and thermohygrographs are rather expensive pieces of apparatus, but all work is greatly facilitated if several such instruments are available as a constant check on experimental environments. Humidity measurements are most often made with a sling psychrometer or some similar apparatus for checking on wet and dry bulb temperatures, but small hygrometers, operated by hair or paper and easily calibrated, are important in any mosquito work where conditions in small cages must be checked. Instruments for light measurement have become common as a result of their widespread use in photography, but it should be remembered that the type of light measurement used for photography is rather different from that needed in experimental work. "Illumination photometers," of which various sorts are manufactured by the Weston Electrical Instrument Corporation, are more useful for laboratory purposes.

Light control should not be forgotten in experimental work, and a variety of light sources and of filters of known wave-length transmission should be available. Variable resistances are necessary in any experimental work involving light control, and automatic time switches for light circuits are useful not only in experimental work, but for the routine maintenance of mosquito colonies of species that require special light conditions for mating.

Mosquito cages

Every worker develops his own pet designs for cages, as for other types of apparatus, and the dimensions, materials, and construction of such cages must vary according to the type of work contemplated. One of the most useful results of visits to different laboratories, however, is the collection of new ideas for cage or other apparatus design. We normally have available six sizes of cages which we refer to as "type A," "type B," and so forth, giving each individual cage a serial number. Thus if a record notes that the mosquitoes were in "Cage E-5," we automatically know the dimensions and construction of the cage.

Our smallest cages ("A" type) are used for field collections and for transfer or similar operations in the laboratory. They are made by covering a frame of welded brass wire with bobbinet, one end being left open as a sleeve. We have tried various sizes; 8 cm. square and 14 cm. long seems to be about the most convenient. If the cloth of one

side is dyed black with India ink, the enclosed mosquitoes can be observed much more readily. The "Barraud" cage, in which the netting is suspended from the brass frame (described in various publications including Russell, West, and Manwell, p. 253), seems to be an unnecessary complication, since we have never had trouble with mosquitoes becoming stuck in the corners of our field cages. We have also never found much use for collapsible field cages: these can be carried out to the field more conveniently folded up, but there is always the problem of finding space for them when they are assembled and full of mosquitoes.

The "B" and "C" type cages are used for laboratory manipulations; they are respectively 15 cm. square, 30 cm. long, and 25 cm. square, 40 cm. long; in both cases one end is made as a sleeve, and at least one side is glass, the others being screen or bobbinet. The "D" cages, the smallest size used for colonies, are 50 cm. on a side, and one lower quarter of the cage is enclosed with wide-meshed wire netting to serve as an animal compartment. The "E" cages are similar, but one meter high and 50 cm. square. This is about the smallest sized cage that can be used for mosquitoes in which the males have swarming habits. The letter "F" is used for room-sized cages—that is, any cage large enough so that the observer can enter the cage.

In any laboratory dedicated to experimental work with mosquitoes, it is convenient to have several such "room-sized" cages. Most of the cages of this type used in the Albanian and Colombian laboratories have been about two meters on a side and two meters high. A wide variety of mosquito species that fail to mate in smaller cages can be colonized in such rooms, but little additional advantage seems to accrue from further increase in size. Animals such as donkeys, calves, or man can be used as a source of blood meals in such cages, and the observer can check on the activities of the mosquitoes from the inside of the cage—a considerable advantage. In Albania we built a very large outdoor cage (Hackett and Bates, 1939), and Russell built a similar cage in India (Russell and Rao, 1942a), but it has never seemed to me that the expense of such cages was warranted by the results. One still cannot be sure that the reactions of the mosquito are "natural" because there is always the barrier of wire liable to be encountered on extended flights; and when the flight of a mosquito has been interrupted by this wire barrier its further activity may be definitely unnatural. Thus in Albania we found many mosquitoes on

the wire screens in the daytime, under definitely unfavorable environmental conditions, when there were many favorable resting places easily available that surely would have been utilized under completely "natural" conditions.

Miscellaneous apparatus

Adult mosquitoes are most readily handled with some type of suction catcher. The simplest type of catcher is a straight glass tube 30 cm. or so long, with an inside diameter of 10 to 15 mm., plugged at one end with a bit of smaller glass tube covered with bolting cloth, and with a long rubber tube reaching to the mouth. We find it better to make an angle at about the middle of such a tube, providing a slight barrier to the escape of the mosquitoes. If more than four or five mosquitoes are to be caught at a time, a Lewis-type catcher (Lewis, 1933) with a reservoir is convenient.

We find shell vials, 25 mm. in diameter and 50 mm. high, convenient for many kinds of laboratory operations. Individual ovipositions can be obtained from very large numbers of mosquitoes using such vials, each female being confined in a separate vial with a layer of moist cotton covered with filter paper over the bottom (Plate 16). The vials may be plugged with cotton wool or with cups of monel or aluminum screening. This technique was developed by Barber and Shannon for the study of the eggs of individual specimens of European *Anopheles maculipennis* (Hackett and Missiroli, 1935), and it has proven equally useful for obtaining routine ovipositions from thousands of South American *Nyssorhynchus*. The use of such vials enabled us to keep individual records on the behavior of very large numbers of *Haemagogus spegazzinii* in yellow-fever-transmission experiments (Bates and Roca, 1945) and proved also to be the most satisfactory method of maintaining these mosquitoes for long periods in the laboratory (Bates, 1947b).

There are many small useful tricks of mosquito manipulation that have been discovered by various workers and reported in a scattered literature. The section on technique in MacGregor's book (1927) is particularly full of suggestions, like his "egg harbour": enclosing mosquito eggs in a floating cork ring so that they do not become stranded on the side of the dish. He also describes various types of pipettes that are useful in larval manipulations. Methods of inducing mosquitoes to feed have been described by several workers (for example,

Burgess and Young, 1944; Knowlton and Rowe, 1935; Mattingly, 1946). Almost every biological paper contains some notes on technique, and a careful reading of the "methods" sections of such papers may be very rewarding. A general review of the literature on mosquito techniques would be a worth-while undertaking, but beyond the scope of this book.

LABORATORY COLONIZATION

The establishment of laboratory colonies of different species of mosquitoes is an almost indispensable tool in the study of mosquito behavior, since such colonies provide a continuous supply of relatively uniform material for experimental purposes. Some species of mosquitoes are very easily colonized, others require the development of special techniques, and some species, even though studied at length by many workers, have not been successfully established under laboratory conditions.

The first step in the colonization of a given species, of course, is to be sure that an adequate method has been found for raising the larvae: a step, as pointed out above, that may require considerable experimentation. When a method of handling the larvae has been worked out, large numbers of adults can be obtained from eggs laid by wild-caught females, though occasionally larvae collected from natural breeding places form the most convenient starting point.

The major difficulty in mosquito colonization is usually in inducing the adults to mate under cage conditions. There is no way of predicting mating behavior, since the most closely similar species may have very different mating habits. Consequently it is probably best always to make a first attempt with adults in small cages (such as the 50 cm. cube type); there is always the possibility that the species will mate readily under these conditions, and further experimentation is thus not necessary. Four or five days after the adults have been released in such a cage, a few females should be dissected to find out whether the spermathecae contain spermatozoa. The spermathecae are readily recognized as tiny, heavily chitinized spheres in the tip of the female abdomen—single in anophelines, three in most other mosquitoes. Wild females are almost invariably fertilized, and dissection of a few wild specimens for comparison with laboratory specimens will soon enable the observer to distinguish between full and empty spermathecae. Examination should be made with the high

dry objective of a compound microscope after dissection with a dissecting microscope. If the caged females are unfertilized, experiments with mating behavior must be undertaken; if they are fertilized, it remains only to induce them to feed, to keep them alive, and to induce them to oviposit—three problems that usually present few difficulties in the case of species that mate in small cages.

Only a small proportion of mosquito species have mating habits that allow fertilization to take place in a small cage. We find that if the first attempt to obtain fertilization in a small cage fails, it is most satisfactory to make the second trial in a room-sized cage. The observer can thus enter the cage and experiment with the mosquitoes under advantageous conditions. Some species that fail to mate in small cages will mate readily in large cages without any special modification of light or other environmental factors. With other species endless experimentation may be necessary. Failures may continue, but there is always the hope that at last one will find the right trick. Light is perhaps the most important factor, and tests should be made with different intensities, different colors, different positions to see whether swarm formation cannot be induced. Several species that we have tested (including *Anopheles multicolor* in Egypt and *A. pseudopunctipennis* in Colombia) form swarms most readily if there is a contrasting surface below the light. It is important also to choose a time for experimentation when the mosquitoes are active, and if the mosquitoes remain lethargic, to attempt by some means to stimulate them into random activity before attempting to induce swarm formation. With most anophelines, this means carrying out experiments during evening and night hours.

Other problems often arise after circumstances have been found under which a species will mate: the problems of getting the mosquitoes to feed, of keeping them alive, of obtaining eggs. In preliminary tests in a room-sized cage, when the observer must enter the cage for light experiments, it is easiest to depend on man as the source of blood. If the species only rarely feeds on man, it may be necessary to use a calf or donkey as food; and for routine maintenance some such animal is in any event most convenient. A bird (such as a chicken) may serve for a species that is reluctant to feed on a mammal.

For satisfactory longevity under cage conditions, some method of increasing the relative humidity in the cage may be necessary. Cages, particularly in the tropics, often become too warm, and some method

of lowering the temperature may be necessary: an effect that may be achieved by modifying the roof of the building or by the evaporation of water in or near the cage. Food (other than blood) may be important. Sugar solutions or fruits may be used, placed so as to be readily available to the mosquitoes. Sliced apples make an excellent supplementary food if they are available; or bags of raisins hung from the ceiling and periodically moistened. One must also be constantly on the alert against spiders becoming established in the cage: a very few spiders may have a remarkable effect on the longevity of adults in a mosquito colony.

Oviposition is generally not a problem, as most mosquitoes will readily lay eggs in a pan of plain water placed in the cage. With some species it may be necessary to have a fairly large pan, and it may be advisable to build an aquarium in the cage. Anophelines seem generally to oviposit better in pans with a black or dark background than in white enamel pans. Species of mosquitoes that breed in water rich in organic matter may require an infusion of some sort for oviposition in cages. Aëdine mosquitoes may require a moist surface to which the eggs can be stuck.

Only a few species of mosquitoes have been extensively maintained as laboratory colonies: *Culex molestus*, *C. pipiens*, *C. fatigans*, *Aëdes aegypti*, *A. albopictus*, *Anopheles atroparvus*, *A. quadrimaculatus*. Quite a few others have been maintained as colonies in particular laboratories, but in general only an insignificant proportion of the fauna has been tested for laboratory adaptation even in well studied areas. This is a very fruitful field for experimentation, and specific differences in behavior can perhaps be brought out more readily by colonization attempts than by any other method of study.

CHAPTER XX

THE STRATEGY OF MOSQUITO RESEARCH

> Because there are inherent advantages in emphasizing the distinction between strategy and tactics, I had intended to group my discussion around those poles: strategy as the art of deciding when and on what one will engage his strength, and tactics as the skill, economy, promptitude, and grace with which one utilizes his strength to attain the ends chosen by strategy.—ALAN GREGG

THIS book has been devoted to an attempt at summarizing what is known about the behavior of mosquitoes. One of the chief objects, as was stated in the introduction, has been to make this material more available to students of general biological theory. A summary of this sort in itself is bound to be incomplete, but it should at least serve as a key to the literature. The bibliography cited in the present review includes, certainly, less than a fifth of the papers that have been published on mosquito behavior alone, but an effort has been made to include references that in their turn contain bibliographies of the separate subjects. The vast accumulation of purely taxonomic papers has been given little attention, but these can be traced through the references in the regional monographs that have been cited. The morphology of mosquitoes has scarcely been mentioned, but since morphological studies have largely been carried out in connection with taxonomic work, the literature of the two fields is closely related. Internal anatomy, organ physiology, histology, embryology, studies of the sort that should receive considerable attention in any balanced summary of the biology of a group of organisms, have not been mentioned. But mosquitoes have been used very little for research in these fields, and it has generally been assumed that they do not differ strikingly from the other insects that have been studied from these points of view. Genetic studies with mosquitoes are completely lacking.

It may be advantageous, as a final chapter, to attempt to give this

material some perspective by means of a brief review in strategic terms. Mosquito research, historically, has almost entirely been carried out under the auspices of the medical sciences. It seems to me that these organisms also offer a rich field for exploitation from the point of view of some of the purely biological sciences, and that in any review of strategy both the medical and biological objectives must be considered.

The incentive for mosquito investigation has been largely practical, and it has been carried out or directed almost entirely by men with a primary interest in public health or medicine. The contact with the objectives of classical or academic biology has thus been tenuous. For the most part, mosquito research has been a function of public-health departments, or of special malaria or yellow-fever units, or of special institutes like the Tropeninstitut at Hamburg or the British Mosquito Control Institute at Hayling Island. Where research has been carried out under university auspices, it has generally formed part of the program of the departments of parasitology, tropical medicine, or public health of the medical school; and in the United States, at least, the medical and biological sections of a given university are usually rather widely separated, both by geography and tradition.

One can distinguish two rather fundamentally different objectives in this work: the collection of information of possible use in mosquito or disease control, and the collection of information for its own sake. By far the larger part of mosquito research has had, explicitly, the first sort of objective. Even the pure taxonomists are apt to quote the importance of species identification in practical work as justification for their studies of some obscure group of mosquitoes. The second objective has, however, not been unimportant by any means. The whole recent program of entomological investigation of yellow fever has been essentially in pursuit of information for its own sake, since the ideal control by means of vaccination was already at hand, and since there was in any event no hope of practical attack on mosquito cycles under forest conditions. Most of the regional studies of mosquito faunas, as distinguished from the purely anopheline surveys, would also belong in this class.

The collection of information of possible use in mosquito or disease control is in itself a very broad objective. After the initial discovery that arthropods could act as vectors of disease, medicine was faced with the large task of determining which diseases were transmitted by

which arthropods and under what circumstances. This task has still not been finished, as can be appreciated from the contents of the chapter on mosquitoes and viruses in the present book. The detection of the arthropod vectors has involved detailed studies under both laboratory and field conditions, since we have become increasingly aware that the phenomena of laboratory transmission mean little if they are not interpreted in terms of data collected under natural conditions, data concerning the epidemiology of the diseases and the ecology of the vectors.

The result of such studies is background information, against which the actual plan of control measures must be worked out. The most practical method of control may be through medical measures such as vaccination or chemotherapy, outside of the scope of mosquito strategy. If control is to be achieved through attack on the insect vector, however, it is obvious that further entomological study is necessary.

Three main types of control have been tested in the case of mosquito-born diseases: through insecticides, through engineering methods, and through biological methods. All involve further studies of the taxonomy, ecology, and physiology of the mosquitoes. In the case of insecticides there is a whole field of special research on toxicity, methods of application, methods of preparation, testing of new compounds. This is covered in an enormous literature that has not been mentioned in this book, since the material is completely foreign to the field of natural history in the sense used here, though such work has been the stimulus for a certain amount of investigation of insect physiology. In addition, however, the application of insecticides requires study of insect ecology for the primary decision as to which stage is to be attacked, whether larva or adult, and secondarily to determine the place and time of attack for achieving a maximum effect with a minimum expenditure of energy and funds. This has been the stimulus for much work in mosquito ecology.

Under engineering methods I would include all mechanical interference with the environment: drainage, mosquito-proofing, special tricks such as the construction of siphons for flushing streams. The more information there is available, the better such activities can be planned, but they have not served as the direct stimulus for the collection of much biological information on mosquitoes.

The possibility of biological control has served as the direct

stimulus for a great deal of investigation of mosquito ecology, since there is always the hope that control may be achieved through some slight modification of the environment if only the environmental relations are well enough understood. Many measures have been proposed, and a few, such as changing shade relations, or the introduction of predators (especially the American minnow *Gambusia*), have been tested on a fairly wide scale. The whole subject of biological, or "naturalistic," control has been reviewed at some length by Hackett and others (1938). The success of practical operations along these lines has not been very remarkable.

The above, I think, is a fair interpretation of the strategy of mosquito research within the framework of the medical sciences. The success of this work has been remarkable. To realize the accomplishment, we have only to compare our knowledge and control over the insect-born diseases in 1897 with our knowledge and control in 1947, a span of fifty years. No one considers the war on disease to be in any sense won, or even to have reached a point where any of the categories of scientists involved can afford to rest on their laurels. But I suspect that today, in any general overhaul of medical strategy, operations in the field of entomology, at least at the level of scientific research, are bound to receive scant attention. The great health problem among the insect-born diseases is still malaria, and here the crying need seems to be for concentration on strategic operations in what might be called the social field: in the organization and distribution of the knowledge and resources that are already available, in the education of the public and the training of operating medical and entomological personnel, in the development of methods of changing or improving the economy of malarious areas so that control operations of any sort may become feasible.

The entomological work that is most needed in this field of strategy is at the technological level. Mosquito control is still largely dependent on the application of insecticides, and while DDT is regarded by some as the answer to all prayers, there is surely still room for improvement and for additional information both concerning the insecticides themselves and the methods of application. But I would define "science" in the terms used by Conant (1947), who points out that "science emerges from the other progressive activities of man to the extent that new concepts arise from experiments and observations, and the new concepts in turn lead to further experiments and

observations." In the area of medical entomology, the development of new and fruitful conceptual schemes would seem to me to depend on a radical shift in point of view: a shift, in fact, from the strategy of medicine to the strategy of biology, since advances in the application of biology must necessarily depend on advances in biological theory.

I doubt whether anyone will question the statement that advance in general biological theory will contribute greatly to the understanding of the special problems of mosquitoes and disease. The converse is perhaps not so clear, whether mosquitoes themselves, as subjects of study, offer a fruitful field for investigation aimed at achieving a better understanding of broad biological relationships. I think they do, because of the tremendous advantage of the information accumulated in the course of the practical studies of medical entomology, because of the "law of the multiplication of the potential value of a subject of study," as outlined in the introductory chapter of this book. A more specific statement of the lines of investigation that appeal to me as particularly fruitful, both from the medical and biological points of view, may be a fitting way of closing this review.

The ultimate objective of the biological sciences is probably the explanation of living systems in universal terms. The physiologists, the cytologists, the biochemists, all of the people who are looking for uniformities, who are seeking to express living processes in terms of known chemical and physical phenomena, presumably have this objective or one of its outposts more or less clearly in mind. Mosquitoes as organisms offer no known advantages for this type of work, and the testing of general theory on our particular organisms will have to await results obtained from studies on the organisms that do seem most advantageous for this purpose.

There is, however, another general biological objective, perhaps subsidiary and surely related, yet perhaps to be attacked only by a different strategic approach. That is, the explanation of organic diversity, the description of the processes of organic evolution. It is in this field of strategy that mosquitoes may offer special advantages as subjects of study.

Goldschmidt (1940) has stressed the qualitative difference between macroevolution, the evolution of different groups and phyla, and microevolution, the evolution of specific populations. He has been much criticized for this stand by people who see only quantitative

differences between the two types of evolutionary product. Whether the distinction has any real basis or not, it has a certain convenience in orienting discussion, and if for the moment the phenomena of macroevolution are removed from consideration, we can perhaps more easily recognize the lines of attack that might be profitable in reaching a description of the essential phenomena of microevolution. Analysis of the former may well have to draw heavily on the evidence of paleontology, on experimental embryology, on types of investigation for which mosquitoes are in no way especially adapted.

On the other hand, the analysis of the phenomena of microevolution may well best be based on data concerning the dynamics of specific populations. Our lack of definite or extensive data in this area is surprising but very real. In one sense, at least, it is the field of ecology; but ecologists have become preoccupied with the description of total environmental situations, of habitats, a task that is almost insuperably complex and difficult. As a remedy, Elton has recommended attention to the simplest possible situations, such as the arctic tundra—but such situations are also in some ways very specialized, and perhaps not pertinent to some of the main problems. The more logical approach to my mind would be concentration on the environmental relationships of particular populations, moving slowly outward along the lines of increasingly complex relationships that would radiate from such populations.

In a study of this type, we would have first the problem of the description of the population on which interest is focused, then the problem of the description of the environmental situation in which the population occurs, in terms of relevance to this population. From these two general descriptions, we could attempt an analysis of the forces acting on the population in both qualitative and quantitative terms—that is, with regard to the nature of the forces, their amount, and the direction in which they act.

Explicitly, this would involve very detailed studies of the behavior of particular mosquito populations, or better, of groups of closely related populations, in physiological and ecological terms, both in isolation and in relation to various combinations of environmental factors. Such study is possible because a great many people have spent a great deal of time observing and experimenting with mosquitoes, providing a base from which more detailed and more precise undertakings can be launched. Such work, however, requires free-

dom from the pressures of practical considerations, and can only awkwardly be carried through in the framework of the administrative units of public health. It requires not so much the collaboration of epidemiologists as of academic biologists, and we should feel satisfied if we gain only an advance in theory.

The academic biologists are thoroughly aware of the importance of detailed study of population dynamics, of quantitative measurement of the forces that surely govern the process of organic divergence. If they can be interested in utilizing the rich material that lies available in mosquito studies, perhaps as a by-product of the investigations, they may produce contributions to that other area of scientific strategy, the attack on the problems of human disease.

APPENDIX

SYSTEMATIC LIST OF MOSQUITO SPECIES

THE various mosquito species and group names mentioned in the text are listed below according to the systematic order proposed by Edwards (1932). The author and year of publication of the various names are also cited, as a matter of general interest. The name of the author is placed in parenthesis in cases where the specific name was originally proposed with another generic term, in accordance with common zoological practice. Corresponding references have not been listed in the bibliography, but the sources of the names can easily be checked in any of the regional monographs. A brief notation of distribution has also been included for the various species and groups. This is meant merely to indicate the general area where the species or groups have been found, and preciseness has been sacrificed to brevity; the exact distributions, as far as known, can be found in the various regional monographs. The few synonyms that have been listed are terms that have been used in the literature published on mosquito biology since 1900; the synonymy of purely taxonomic interest has been ignored.

I have followed, somewhat doubtfully, the practice of making all mosquito names for categories higher than species correspond to the usage of Edwards. In some cases these names are clearly not in accord with present interpretations of the code of zoological nomenclature; in other cases proper usage is doubtful; and in a few cases generic concepts have been changed by subsequent revisers (for example, the American sabethine genera by Lane and Cerqueira). If I had made any generic changes, however, I would have been faced with the problem of evaluating each name and thus become involved in a field outside of my competence and outside of the scope of this book. The most satisfactory course thus seemed to be to use the classification of Edwards as a standard, just as one would adopt the practice of a particular dictionary as standard for usage in spelling and grammar. By this policy the names used in this book are not the "last word" in taxonomic practice, but they at least should be readily understood by anyone using the text, taxonomist or not. An arbitrary basis of nomenclature of this sort would be out of place in a taxonomic study; but nomenclatorial innovations would be out of place in the present book, and such innovations would be an inevitable consequence of a consistent

application of the provisions of the Zoological Code to the generic terms of the world mosquito fauna.

Family CULICIDAE

Subfamily DIXINAE

(2 genera and 94 species listed by Edwards, 1932)

Subfamily CHAOBORINAE

(6 genera and 48 species listed by Edwards, 1932)

Subfamily CULICINAE

Tribe ANOPHELINI

Genus CHAGASIA Cruz, 1906	Neotropical
Three species listed by Edwards.	
Genus BIRONELLA Theobald, 1905	Australian
Subgenus *Bironella* Theobald, 1905	New Guinea
Subgenus *Brugella* Edwards, 1930	Ceram
Two species are listed in the first subgenus, one in the second.	
Genus ANOPHELES Meigen, 1818	Cosmopolitan
Subgenus *Stethomyia* Theobald, 1902	Neotropical
nimbus Theobald, 1902	S. America
Subgenus *Anopheles* Meigen, 1818	Cosmopolitan
Group A (*Anopheles*)	Cosmopolitan
a) *Anopheles* series	All regions except Ethiopian
algeriensis Theobald, 1903	Mediterranean and Western Europe
barberi Coquillett, 1903	Nearctic
barianensis James, 1911	India
Sometimes listed as variety of *plumbeus*.	
claviger (Meigen, 1804)	Palearctic
This species is frequently cited in the literature as *Anopheles bifurcatus* (Meigen, 1818; nec. Linnaeus, 1758).	
crucians Wiedemann, 1828	Nearctic
culiciformis Cogill, 1903	India
eiseni Coquillett, 1902	Neotropical
insulaeflorum (Swellengrebel & Swellengrebel, 1920)	India, East Indies, and New Guinea
(The *maculipennis* group)	
It is usually impossible to be sure, with references dated before about 1930, which specific population is covered by the term "*Anopheles*	

maculipennis." Where the distinction seemed important, an effort has been made in the text to specify whether a particular citation refers to *maculipennis* in the strict sense or in the broad sense. The following listing is adapted from Bates, 1940a, for the European forms and from Aitken, 1945, for the American forms, except that the American forms are made subspecies of the term "*occidentalis*" instead of the term "*maculipennis*."

maculipennis Meigen, 1818 Synonyms: *typicus* Hackett & Missiroli; *basilei* Falleroni	Europe
messeae Falleroni, 1926	Europe
melanoon melanoon Hackett, 1935	Italy
melanoon subalpinus Hackett & Lewis, 1935	Mediterranean
labranchiae labranchiae Falleroni, 1926 Synonyms: *pergusiae* Missiroli; *sicaulti* Roubaud.	Mediterranean
labranchiae atroparvus van Thiel, 1927 Synonyms: *fallax* Roubaud; *cambournaci* Roubaud & Treillard	Europe
sacharovi Favr, 1903 Synonym: *elutus* Edwards	Mediterranean, Asia Minor
occidentalis occidentalis Dyar & Knab, 1906	Canada and Pacific Coast of U.S.A.
occidentalis freeborni Aitken, 1939	Western U.S.A.
occidentalis aztecus Hoffman, 1935	Mexican highlands
marteri Senevet & Prunelle, 1927	Mediterranean
plumbeus Stephens, 1828 Synonym: *nigripes* Staeger	Europe
pseudopunctipennis Theobald, 1901	Western Nearctic and Neotropical
punctipennis (Say, 1823)	Nearctic
quadrimaculatus Say, 1824	Eastern U.S.A.
walkeri Theobald, 1901	Nearctic
b) *Myzorhynchus* series	Old World
barbirostris van der Wulp, 1884	Oriental
hyrcanus (Pallas, 1771)	Mediterranean and Oriental
hyrcanus hyrcanus (Pallas, 1771) Numerous varieties, of uncertain status, have been ascribed to the species "*hyrcanus*."	Asia Minor, Balkans
hyrcanus sinensis Wiedemann, 1828	China, Japan, Formosa, Malaya, India
c) *Lophoscelomyia* series Only two or three species.	Oriental

d) *Cyclolepteron* series	Neotropical
peryassui Dyar & Knab, 1908	S. America
Group B (*Arribalzagia*)	Neotropical
mediopunctatus Theobald, 1903	S. America
punctimacula Dyar & Knab, 1906	Mexico to Brazil
Group C (*Chrystia*)	Ethiopian
Only one species known.	
Subgenus *Nyssorhynchus* Blanchard, 1902	Neotropical
a) *Argyritarsis* series	
albitarsis Lynch Arribalzaga, 1878	S. America
argyritarsis Robineau-Desvoidy, 1827	Neotropical
darlingi Root, 1926	Neotropical
pessôai Galvão & Lane, 1937	S. America
b) *Albimanus* series	
albimanus Wiedemann, 1821	Caribbean
aquasalis Curry, 1932	Caribbean
benarrochi Gabaldon, Cova-García & Lopez, 1941	Venezuela, Colombia
noroestensis Galvão & Lane, 1937	Brazil
Perhaps a subspecies or synonym of *oswaldoi* Peryassú.	
rangeli Gabaldon, Cova-García & Lopez, 1940	Venezuela, Colombia
strodei Root, 1926	Neotropical
tarsimaculatus Goeldi, 1906	
This name is an absolute synonym of *albimanus;* it has been applied in the literature to several different populations of the *albimanus* series.	
triannulatus Neiva & Pinto, 1922	Neotropical
Subgenus *Kerteszia* Theobald, 1905	Neotropical
This group is very generally treated as a subgenus, though it was listed by Edwards (1932) as a "Group C" of *Nyssorhynchus.*	
boliviensis (Theobald, 1905)	Andean Region
Subgenus *Myzomyia* Blanchard, 1902	Old World
Group A (*Neomyzomyia*)	Australian, Oriental, Ethiopian
annulipes Walker, 1856	Australian
kochi Dönitz, 1901	Oriental
punctulatus Dönitz, 1901	Australia, New Guinea, Melanesia
The relationship of this and the following two species was discussed in Chapter XVI.	
farauti Laveran, 1902	Melanesia
koliensis Owen, 1945	Melanesia

Group B (*Myzomyia*)	Ethiopian, Oriental
aconitus Dönitz, 1902	Oriental
culicifacies Giles, 1901	India
funestus Giles, 1900	Ethiopian
minimus minimus Theobald, 1901	India, Malaya
minimus flavirostris (Ludlow, 1914)	Philippines
sergenti (Theobald, 1907)	North Africa
Group C (*Neocellia*)	Oriental, Ethiopian, Palearctic
annularis van der Wulp, 1884 Synonym: *fuliginosus* Giles, 1900.	Oriental
maculatus Theobald, 1901	Oriental
pulcherrimus Theobald, 1902	Near East, India
splendidus Koidzumi, 1920	India, China
stephensi Liston, 1901 var. *mysorensis* Sweet and Rao, 1937, of uncertain status.	Near East, India
superpictus Grassi, 1899	Mediterranean to India
Group D (*turkhudi*-group)	
multicolor Cambouliu, 1902	North Africa to India
Group E (*Pyretophorus*)	Ethiopian & Oriental
gambiae Giles, 1902	Ethiopian
ludlowi Theobald Many *ludlowi* references apply to *sundaicus* (Rodenwaldt, 1926), a widespread Oriental species.	Philippines, Formosa
melas Theobald, 1903	Ethiopian
subpictus Grassi, 1899 Synonym: *rossi* Giles	Oriental
vagus Dönitz, 1902	Oriental
Group F (*Cellia*)	Ethiopian, North Africa
pharoensis Theobald, 1901	Ethiopian, North Africa

Tribe MEGARHININI

Genus MEGARHINUS Robineau-Desvoidy, 1827 The genus is divided into three "groups" by Edwards (1932); these are often treated as subgenera by other authors (e.g., Lane, 1939).	Tropicopolitan
Group A (*Megarhinus*)	New World
Group B (*Ankylorhynchus*)	Neotropical
Group C (*Toxorhynchites*)	Old World

Tribe CULICINI

The *Sabethes* Group

The South American sabethine mosquitoes have recently been revised by Lane and Cerqueira (1942). They propose a number of generic changes, including the subordination of *Goeldia* to *Trichoprosopon* and *Sabethoides* to *Sabethes;* they separate a genus *Phoniomyia* from *Wyeomyia,* and recognize seven subgenera in *Wyeomyia.*

Genus TRICHOPROSOPON Theobald, 1901	Neotropical
compressum Lutz, 1905	S. America
digitatum Rondani, 1848	Neotropical
Genus GOELDIA Theobald, 1903	Neotropical
Subgenus *Goeldia* Theobald, 1903	Neotropical
frontosa (Theobald, 1903)	Guiana, Brazil
Subgenus *Ctenogoeldia* Edwards, 1930	Neotropical
Subgenus *Isogoeldia* Edwards, 1930	Neotropical
Genus TRIPTEROIDES Giles, 1904	Australian, Oriental
Edwards (1932) recognizes four subgenera, three restricted to the Australian region.	
Genus SABETHES Robineau-Desvoidy, 1827	Neotropical
belisarioi Neiva, 1908	S. America
cyaneus (Fabricius, 1805)	Neotropical
Genus SABETHOIDES Theobald, 1903	Neotropical
imperfectus Bonne-Wepster & Bonne, 1920	S. America
Genus WYEOMYIA Theobald, 1901	New World
Four subgenera listed by Edwards, 1932.	
Subgenus *Wyeomyia* Theobald, 1901	New World
bromeliarum Dyar & Knab, 1906	Costa Rica, Brazil
oblita (Theobald, 1907)	S. America
smithii (Coquillett, 1901)	Eastern U.S.A.
Subgenus *Dendromyia* Theobald, 1903	Neotropical
melanocephala Dyar & Knab, 1906	S. America
Genus LIMATUS Theobald, 1901	Neotropical
durhami Theobald, 1901	Neotropical
Genus TOPOMYIA Leicester, 1908	Oriental
Genus HARPAGOMYIA de Meijere, 1909	Oriental, Ethiopian

The *Uranotaenia* Group

Genus HODGESIA Theobald, 1904	Old World tropics
Genus ZEUGNOMYIA Leicester, 1908	Malaya
Genus URANOTAENIA Lynch Arribalzaga, 1891	Cosmopolitan

Group A (*Uranotaenia*)	Cosmopolitan
lowii Theobald, 1901	Southern U.S.A., Neotropical
sapphirina (Osten-Sacken, 1868)	Eastern U.S.A., Caribbean
Group B (*Pseudoficalbia*)	Old World tropics
obscura Edwards, 1915	Malaya

The *Theobaldia-Mansonia* Group

Genus THEOBALDIA Neveu-Lemaire, 1902	Cosmopolitan except S. America
The generic term *Theobaldia* is preoccupied by *Theobaldia* Fischer, 1885, emendation of *Theobaldius* Nevill, 1878; accordingly the term *Culiseta* Felt, 1904 has been used by recent authors for the mosquito genus and subgenus (*see* Matheson, 1944, p. 220).	
Subgenus *Theobaldia* Neveu-Lemaire, 1902	Chiefly Holarctic
annulata (Schrank, 1776)	Palearctic
incidens (Thomson, 1868)	Nearctic
inornata (Williston, 1893)	Nearctic
subochrea Edwards, 1921	Palearctic
Subgenus *Allotheobaldia* Brolemann, 1919	Mediterranean, India, Africa
longiareolata (Macquart, 1838)	as for subgenus
Subgenus *Culicella* Felt	Holarctic
fumipennis (Stephens)	Europe
morsitans (Theobald, 1901)	Holarctic
Genus ORTHOPODOMYIA Theobald ,1904	New World, Palearctic and Oriental
Group A (*Orthopodomyia*)	Oriental and Neotropical
fascipes (Coquillett, 1905)	Costa Rica to Brazil
Group B (*Bancroftia*)	Neotropical, Holarctic
signifera (Coquillett, 1896)	Eastern U.S.A.
Genus FICALBIA Theobald, 1903	Ethiopian, Oriental, Australian
The species are placed in 4 subgenera by Edwards (1932).	
Genus MANSONIA Blanchard, 1901	Cosmopolitan
Strict interpretation of the Zoological Code requires that the generic and subgeneric term *Mansonia* be replaced by *Taeniorhynchus* Lynch Arribalzaga, 1891 (Edwards, 1941, p. 88).	
Subgenus *Mansonia* Blanchard, 1901	Neotropical
titillans (Walker, 1848)	Florida to Argentina
Subgenus *Rhynchotaenia* Brèthes, 1911	Neotropical
albicosta (Peryassú, 1908)	Brazil
chrysonotum Peryassú, 1922	Brazil

fasciolata (Lynch Arribalzaga, 1891)	Neotropical
juxtamansonia (Chagas, 1907)	Brazil
Subgenus *Coquillettidia* Dyar, 1905	All regions except Neotropical
fuscopennatus (Theobald, 1903)	Tropical Africa
microannulata (Theobald, 1911)	East Africa
perturbans (Walker, 1856)	Nearctic
richiardii (Ficalbi, 1889)	Palearctic
versicolor (Edwards, 1913)	Kenya, Uganda, Congo
Subgenus *Mansonioides* Theobald, 1907	Old World tropics
africana (Theobald, 1901)	Ethiopian
uniformis (Theobald, 1921)	Old World tropics
Genus AEDOMYIA Theobald, 1901	Tropicopolitan
The *Aëdes* Group	
Genus PSOROPHORA Robineau-Desvoidy, 1827	New World
Subgenus *Psorophora* Robineau-Desvoidy, 1827	New World
ciliata (Fabricius, 1794)	New World
Subgenus *Janthinosoma* Lynch Arribalzaga, 1891	New World
ferox (Humboldt, 1820)	New World
Synonyms: *posticatus* Wiedemann, *sayi* Theobald.	
Subgenus *Grabhamia* Theobald, 1903	New World
cingulata (Fabricius, 1805)	Neotropical
columbiae Dyar & Knab, 1906	Eastern U.S.A., Antilles
confinnis (Lynch Arribalzaga, 1891)	New World
discolor (Coquillett, 1903)	Nearctic
Genus OPIFEX Hutton, 1902	New Zealand
fuscus Hutton, 1902	New Zealand
Genus AEDES Meigen, 1818	Cosmopolitan
Subgenus *Mucidus* Theobald, 1901	Old World tropics
Subgenus *Ochlerotatus* Lynch Arribalzaga, 1891	Cosmopolitan
Group A	All regions except Palearactic
nigromaculis (Ludlow, 1907)	Nearctic
sollicitans (Walker, 1856)	Nearctic, Antilles
taeniorhynchus (Wiedemann, 1821)	New World
Group B	Holarctic
annulipes (Meigen, 1830)	Europe
cantator (Coquillett, 1903)	Nearctic
excrucians (Walker, 1856)	Holarctic
fitchii (Felt & Young, 1904)	Nearctic
flavescens (Müller, 1764)	Holarctic
maculatus (Meigen, 1804)	Europe

stimulans (Walker, 1848)	Nearctic
Group E	Holarctic
campestris Dyar & Knab, 1907 Synonym: *callithotrys* Dyar.	Nearctic
canadensis (Theobald, 1901)	Nearctic
caspius (Pallas, 1771)	Palearctic
dorsalis (Meigen, 1830)	Holarctic
mariae (Ed. & Et. Sergent, 1903)	Mediterranean
Group F	New World
infirmatus Dyar & Knab, 1906	Southern U.S.A.
nubilus (Theobald, 1903)	Neotropical
scapularis (Rondani, 1848)	Neotropical
serratus (Theobald, 1901)	Neotropical
Group G	Holarctic
cataphylla Dyar, 1916 Synonym: *prodotes* Dyar.	Rocky Mountains
communis (De Geer, 1776) Synonyms: *nemorosus* Meigen, *lazarensis* Felt & Young.	Holarctic
detritus (Haliday, 1833)	Palearctic
diantaeus Howard, Dyar & Knab, 1917	Holarctic
implacabilis (Walker, 1848) Synonym: *abserratus* Felt & Young.	Eastern U.S.A.
lateralis (Meigen, 1818)	Holarctic
pullatus (Coquillett, 1904)	Holarctic
punctor (Kirby, 1837)	Holarctic
Group H	Holarctic
rusticus (Rossi, 1790)	Europe
Subgenus *Finlaya* Theobald, 1930	Cosmopolitan
Group A	Austro-Malayan
kochi (Dönitz, 1901)	Papua, Solomons, Fiji
Group B	Neotropical, Oriental
terrens (Walker, 1856)	S. America
Group D	Oriental, Australian
japonicus (Theobald, 1901)	Japan, China
jugraensis (Leicester, 1908)	Malaya
Group G	New World, Palearctic, Oriental
atropalpus (Coquillett, 1902)	Eastern & Southern U.S.A.
fluviatilis (Lutz, 1904)	S. America
togoi (Theobald, 1907)	Japan, China, Siberia
varipalpus (Coquillett, 1902)	Western Nearctic
Group H	New World, Palearctic, Oriental

geniculatus (Olivier, 1791)	Palearctic
leucocelaenus Dyar & Shannon, 1924	S. America
triseriatus (Say, 1823)	Eastern U.S.A.
Subgenus *Howardina* Theobald, 1903	Neotropical
dominici Rangel & Romero, 1907	Northern S. America
fulvithorax (Lutz, 1904)	S. America
Subgenus *Pseudoskusea* Theobald, 1907	Australian
Group B	Australia
concolor Taylor, 1914	N.S. Wales, Tasmania
Subgenus *Stegomyia* Theobald, 1901	Old World tropics
Group A	Ethiopian
aegypti (Linnaeus, 1762) The distribution of this species, presumably spread by human agency, has been ignored in making zoogeographical generalizations. Of the numerous synonyms only three, *argenteus* (Poiret, 1787), *fasciatus* (Fabricius, 1805), and *calopus* (Meigen, 1818) have been commonly used in recent literature.	Tropicopolitan
africanus Theobald, 1901	Tropical Africa
apicoargenteus Theobald, 1910	Tropical Africa
luteocephalus Newstead, 1907	West Africa
metallicus Edwards, 1912	West Africa
simpsoni Theobald, 1905	Ethiopian
Group C	
albopictus (Skuse, 1895) Synonym: *scutellaris* of some authors.	Madagascar, Oriental, Australian
scutellaris (Walker, 1859) Synonym: *variegatus* of many authors.	East Indian and Pacific Islands
Group D	Ethiopian, Oriental, Spain
vittatus (Bigot, 1861)	as for group
Subgenus *Aedimorphus* Theobald, 1903	Ethiopian, Oriental, Australian, Holarctic
Group A	Ethiopian
apicoannulatus (Edwards, 1912)	Sierra Leone
Group C	Ethiopian, Oriental, Australian
tarsalis (Newstead, 1907)	Tropical Africa
Group D	Ethiopian
abnormalis (Theobald, 1910)	Gold Coast
Group G	Ethiopian, Oriental, Holarctic
durbanensis (Theobald, 1903)	Ethiopian

vexans Meigen, 1830	Palearctic, Nearctic, Oriental; Fiji, Samoa, New Caledonia
Subgenus *Diceromyia* Theobald, 1911	Ethiopian, Oriental
Group A	Ethiopian
taylori Edwards, 1936	Nigeria, Tanganyika
Subgenus *Aëdes* Meigen, 1818	Oriental, Australian, Holarctic
cinereus Meigen, 1818	Holarctic
Genus HAEMAGOGUS Williston, 1896	Neotropical
Group A (*Stegoconops*)	Neotropical
equinus Theobald, 1903	Neotropical
Group B (*Haemagogus*)	Neotropical
janthinomys Dyar, 1921	Trinidad
lucifer (Howard, Dyar & Knab, 1912)	Panama, Colombia
spegazzinii Brèthes, 1912 Synonym: *capricornii* of many authors, not Lutz.	S. America
splendens Williston, 1896	Northern S. America, St. Vincent
uriartei Shannon & del Ponte, 1928	Brazil, Argentina
Genus HEIZMANNIA Ludlow, 1904	Oriental
Genus ERETMAPODITES Theobald, 1901	Ethiopian
chrysogaster Graham, 1909	Tropical Africa
ferox Haddow, 1946	Uganda
Genus ARMIGERES Theobald, 1901	Oriental, Australian
Subgenus *Armigeres* Theobald, 1901	Oriental, Australian
kuchingensis Edwards, 1915	Oriental
obturbans (Walker, 1860)	India to Japan and Australia
Subgenus *Leicesteria* Theobald, 1904	Oriental
flavus (Leicester, 1908)	Oriental
The *Culex* Group	
Genus CULEX Linnaeus, 1758	Cosmopolitan
Subgenus *Lutzia* Theobald, 1903	Tropicopolitan
Group B	Old World tropics
fuscanus Wiedemann, 1821	Oriental
Subgenus *Neoculex* Dyar, 1905	Cosmopolitan
apicalis Adams, 1903	Holarctic
hortensis Ficalbi, 1889	Palearctic
Subgenus *Culex* Linnaeus, 1758	Cosmopolitan
Sitiens series	Old World
thalassius Theobald, 1902	Ethiopian
tritaeniorhynchus Giles, 1901	Oriental, N. Africa

vishnui Theobald, 1901	Oriental
Tarsalis series	New World
coronator Dyar & Knab, 1906	Neotropical
stigmatosoma Dyar, 1907	Western U.S.A. to Venezuela
tarsalis Coquillett, 1896	Western and Southern U.S.A.
Salinarius-apicinus series	New World
nigripalpus Theobald, 1901	Neotropical, Southern U.S.A.
restuans Theobald, 1901	U.S.A., Mexico
salinarius Coquillett, 1904	Eastern U.S.A.
territans Walker, 1856	U.S.A.
Pipiens series	Cosmopolitan
fatigans Wiedemann, 1828 (?) Also commonly called *quinquefasciatus* Say.	Tropical, subtropical
molestus Forskal, 1775 Synonym: *autogenicus* Roubaud	S. Europe, U.S.A.
pipiens Linnaeus, 1758	Holarctic, Argentina, S. Africa
"variety" *pallens* Coquillett, 1899.	
Subgenus *Melanoconion* Theobald, 1903	New World
erraticus Dyar & Knab, 1905	Southern U.S.A.
Subgenus *Microculex* Theobald, 1907	Neotropical
Subgenus *Carrollia* Lutz, 1905	Neotropical
metempsytus Dyar, 1921	Neotropical
urichii (Coquillett, 1906)	Northern S. America
Genus DEINOCERITES Theobald, 1901	New World

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