

## BIOLOGICAL CONSIDERATIONS

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The striking success in the elimination of *Anopheles gambiae* from Brazil has focused attention on the feasibility of the total eradication of a population of a disease vector. On the completion of this project Soper and Wilson (1943) remarked that, "Many workers in the control of mosquito-borne disease have been more than reluctant to accept the idea that man has it in his power to eradicate any mosquito anywhere, no matter what the effort made. . . . The traditional ingrained philosophy that species eradication is impossible, that a species is something sacred and eternal in spite of the dodo, the passenger pigeon and the dinosaur to the contrary, and that when species disappear they do so in response to 'cosmic' or 'biological' rather than man-made factors, is most persistent." Despite this somewhat pessimistic view of the inertia to be overcome by the eradication concept, in the decade since this was written, a number of efforts have been made to extend the principle of total eradication to other situations, and the ERLAAS project for the eradication of *Anopheles labranchiae labranchiae* from Sardinia has been one such effort.

We might first consider the position of the biologist in this matter. The biologist does not insist that species are either "sacred" or "eternal." Quite the contrary, a primary tenet of evolutionary theory is that species are limited in the dimension of time as well as space. Thus Simpson (1944) has devoted a book, *Tempo and Mode in Evolution*, to a study of the life span of species in geological time. The causes of the decline and extinction of species are many and complex. In recent times, geologically speaking, the species which have been most subject to extermination or near extermination by the activities of man exhibit certain special characteristics. First, they may be economically useful to man (for food and clothing). Second, they may be conspicuously easy to find. Third,

they may have a low reproductive potential. Fourth, they may be in poor adjustment to their environment at the time that man is added as a burden to an already unfavorable situation. That is, they may have a very limited range of habits and lack the ability is added as a burden to an already unfavorable situation. That survival of a species may be endangered by any one, any combination or all of these situations. The passenger pigeon, the heath hen, the ivory-billed woodpecker, the bison in America, the wisent in Europe and the dodo have all become extinct or are in danger of extinction because they have suffered from some combination of these characteristics.

Let us examine the mosquitoes which interest us because of their unfavorable effect on human activity as transmitters of disease, in the light of these characteristics which may affect their survival.

While it is true that mosquitoes are subject to pressure from man and the diminution of their numbers or eradication would be favorable to man, there is no daily economic gain apparent to the lay human population, so that man as a species does not exert continuous pressure. When herds of bison roamed the Great Plains of the western United States, the economic gain to be derived by going on a bison hunt for meat, tallow and hides did not have to be explained in schools or at public meetings, nor was it necessary that the hunts be undertaken by some governmental agency for want of interest by the lay public. To say that the organization of people to similarly set about the hunting of a mosquito species would be less spontaneous is a gross understatement.

The conspicuousness of such unfortunate creatures as the passenger pigeon, the heath hen and the bison, as contrasted with the inconspicuous nature of the breeding and resting places of mosquitoes need hardly be commented upon. It is well to point out, however, that when species, or strains of species, of mosquitoes are what we speak of as "domestic" they are, relatively at least, more conspicuous because they are in close proximity to man. A domestic species, or a domestic strain of a species, thus approaches the condition of conspicuousness and thereby becomes vulnerable. The domestic strains of *Anopheles gambiae* and *Aedes aegypti* which invaded the Americas carried this fatal failing with them.

The reproductive potential of mosquitoes is high, not only because of the relatively large number of eggs laid, but also because



the time required to mature is short. This permits a rapid recovery of populations following unfavorable environmental conditions, whether these conditions be cold (winter), desiccation (dry season in the tropics) or attack by man. The low reproductive rate of mammals and birds is in extreme contrast.

From this we can appreciate how the biologist on the one hand entertains no mystic belief in the immortality of species in geological time, while on the other hand he may believe that a species in good adjustment with its environment is very likely to continue to survive so long as there is no major change in the environment nor a change in its habit pattern which brings it into major conflict with that environment through the sort of limiting factors we have so briefly outlined. It is unfortunate for man that the mosquitoes which interest him as disease vectors are, in the regions where they are vectors, for the most part well adjusted to their environment.

With the understanding that there is nothing immortal about species, we come to our main purpose which is to explore and contrast, from the biologists' point of view, the fundamental differences between the introduced populations of *Anopheles gambiae* and *Aedes aegypti* attacked in Brazil, and the indigenous population of *Anopheles l. labranchiae* in Sardinia; to gather the evidence indicating that the population of the latter mosquito is indeed indigenous in Sardinia; and to give some basis for an appreciation of how the outcome of the ERLAAS project in Sardinia may have thereby been affected. We will attempt to show how terminological confusion may have brought about the concept of a closer parallel between the situations in Brazil and Sardinia than the biological nature of the mosquito populations would justify.

It has been appreciated from the first that the populations of *Aedes aegypti* and *Anopheles gambiae* in the New World were introduced by human agency, in the case of *Aedes aegypti* probably several centuries ago at the time of the conquest of the New World, and in the case of *Anopheles gambiae* about a decade before the eradication effort against it was begun. This had led to discussion of the differences between introduced and indigenous mosquito populations, there being some awareness that mosquito populations which have been attributed to one species might have a limited range of habits in an environment into which they have been introduced, as in the case of *Aedes aegypti* and *Anopheles gambiae*

in the Americas, but more diverse habits in the region of their natural occurrence in Africa (Soper and Wilson, 1943 and Soper, 1948). It would appear, however, that the biological basis of these differences, which is of profound significance in evaluating the possibilities of success of eradication schemes elsewhere, has not been adequately brought to light. The importance of throwing light on this phase of the problem will be appreciated when it is recalled that the mystery of "anophelism without malaria," a classical problem in the epidemiology of malaria in Europe, was not understood until the *maculipennis* anophelines were sorted, by various ingenious means, into the several taxonomic categories comprising what had at first appeared to be a homogeneous population.

Even in the face of the meager knowledge we presently possess of the behavior and genetic composition of mosquito population units, it is now possible to demonstrate tangible biological differences between indigenous and introduced mosquito populations. In large measure the difficulty in comprehending the nature of the population units involved has been one of semantics. The taxonomist has applied a formal system of nomenclature to the naturally occurring mosquito populations in the laudable attempt to provide a set of file boxes into which the information on the morphology, physiology, behavior and genetics of these organisms may be sorted, stored and organized for most efficient use. The nomenclatorial system is necessarily somewhat complicated if it is to express in universal terms the complex and intricate relationships between populations and the place of individual organisms in a particular population. We must, therefore, at the outset define our terms, for certain of these which are in common usage have relatively strict meanings in technical taxonomy. The precision with which these definitions may be formed is unfortunately limited, since the exact meanings of these terms are, and will continue to be, points of controversy among taxonomists as the understanding of the nature of populations grows.

We must first concern ourselves with the biologists' definition of "species." The species concept does not envision a group of organisms as a naturally occurring genotypically pure line. For a working definition we can take the statement of Emerson (1938) quoted in Bates (1949), that species are, "genetically distinctive, reproductively isolated, natural populations." It is often an intri-



cate and difficult process to measure a population by these criteria, and for the most part biologists accept as a working approximation the thesis that populations with distinctive morphology, physiology and behavior are separate species, and name them as such without applying the genetical test. All too often, for practical reasons, morphological similarity alone is used as the basis for species definitions. The taxonomist working in a museum with a group of insects on pins or a collection of skins and skulls of mammals, is in no position to inquire into the behavior in nature of the organisms with which he is dealing, or their possible interfertility or intersterility. He merely sorts them into groups as best he can on the basis of distinctive morphology or coloration, and names these groups as species.

We must interest ourselves also in a second category recognized by the International Rules of Zoological Nomenclature since the mosquito population which concerns us most in Sardinia is often called by the taxonomist *Anopheles labranchiae labranchiae*—a trinomial involving the term "subspecies." This term has formal nomenclatorial status, and for it we may select the definition of Mayr (1942): "The subspecies, or geographic race, is a geographically localized subdivision of the species, which differs genetically and taxonomically from other subdivisions of the species." Where the ranges of the subspecies of a particular species are contiguous, the subspecies interbreed. The reason for dignifying these infraspecific variations exhibiting geographic integrity with the formal nomenclatorial status of "subspecies" has been expressed by Bates (1940): ". . . it is only through geographic separation that a single animal population can become divided into two or more populations sufficiently isolated from one another to permit divergent evolutionary trends to set in." At present the often, though not universally, accepted interpretation of the relationships within the *maculipennis* complex places the mosquito which interests us in Sardinia in the subspecies *labranchiae* of the species *Anopheles labranchiae* (Bates, 1940). The distribution of this subspecies aside from Sardinia includes the adjacent island of Corsica, central and southern Italy, Sicily, North Africa and southeastern Spain. The second subspecies of *Anopheles labranchiae*, in this view, is *atroparvus* which replaces subspecies *labranchiae* to the north and is widely distributed on the continent of Europe.

Finally, there are the trivial variations which exist throughout populations, without geographic correlation or evident survival value, which have no formal nomenclatorial status but are referred to by the nontechnical terms "variety," "strain," "form," "group," "breed," etc.

The International Rules of Zoological Nomenclature, since they seek to apply universal rules to cover the naming of all organisms in the animal kingdom, deal only with the main units of animal populations, i. e., categories of the magnitude of subspecies or larger. The terms "variety," "strain," "breed," etc., are colloquial and are not recognized under the formal code. Thus genetical variations within a species or subspecies which are not marked by morphological differences, but which may be of importance in disease transmission, go unrecognized insofar as designation by formal names is concerned. With this much understanding of the nature of the population units with which we are concerned, we may now consider the main problem of interest to us.

It has been remarked that if mosquitoes or sand flies were as large as dogs, or even mice, we would know a great deal more about their classifications because they would be so much easier to observe, describe and manipulate. To illustrate the fundamental difference between an introduced and an indigenous mosquito it is also much easier to consider by analogy some species we know more about, such as *Canis familiaris*, the dog. A basic difference between dogs and any mosquito species we might choose to discuss is the fact that dogs have been domesticated and their breeding manipulated for several thousand years, while mosquitoes have, in the main, been subject only to the chance selection of nature.

Keeping this difference in mind, however, we can now appreciate that within the concept of a single species may be such diverse creatures as the dachshund and the great Dane, as well as both pointers and setters. These breeds or "strains" of the species *Canis familiaris* are strikingly different in both their appearance and characteristic behavior patterns, but as they are freely inter-fertile they are of only one species. Our understanding of a species tends to be colored by our special interest in that species. In the case of coon dogs we are interested primarily in the particular traits that enable the breed to rapidly and successfully track down raccoons. In the case of most anopheline mosquitoes, the aspect of



the behavior in which we have been most interested, and about which we know the most, are those characteristics which make a particular population either a successful or unsuccessful vector of malaria.

It is at once apparent that a Pekingese would be of no possible use in hunting foxes. If this breed (or strain) were turned loose it is doubtful if it could long survive by its own efforts. A mongrel dog of the same species would in all probability make out well enough under these circumstances. The Pekingese has been selectively bred for characteristics which enhance its value as a lap dog but which have little or no survival value in nature. There was a time when primitive *Canis familiaris* lived by its own efforts, and the mongrel of today retains many of these characteristics of survival value, untampered with by man.

In the case chosen as an illustration, the strains are marked by both morphological and behavioristic differences, i. e., the coon dog and the Pekingese look different and also act differently. But it is also possible for strains of a species to have the same morphological appearance, but act differently, i. e., two coon dogs may look alike but one will be a good hunter while another with the same training will not. Differences in behavior alone are of course more subtle, and often difficult to define.

Unlike the numerous clearly defined breeds of *Canis familiaris*, the populations of which mosquito species are comprised are still in the wild "mongrel" state. Exceptions to this general rule are colonies of these insects which have been bred in the laboratory for such purposes as experiments in the transmission of malaria, or for studies in resistance to certain toxicants. Such inbred laboratory standardized populations are usually referred to as "strains" of a particular species. A mosquito species as it occurs in nature must be considered as "mongrel" since there is free opportunity for interchange of all the genetic material of which it is comprised.

The work of Huff (1931) can be used to illustrate this point. This author was able to show that the ability of *Culex pipiens* to transmit infections of *Plasmodium cathemerium* to birds behaved as a simple recessive Mendelian character. By selective breeding he was able to produce one group of *Culex pipiens* which was relatively susceptible to *Plasmodium cathemerium* and another group refractory to infection with this parasite. It would appear that, had his

experiments been carried further, he would have been able to produce two strains of *Culex pipiens*, the one completely susceptible, the other completely refractory. (One strain would be a homozygous dominant for infectivity, the other a homozygous recessive which was refractory to infection.) Since this very important characteristic of this mosquito species appears, as these experiments would indicate, controlled by only one gene, there is no reason to suppose that any other morphological, physiological or behavioristic character of these strains would be concurrently affected. We would then have the anomalous situation in which we would say with equal accuracy either that, "*Culex pipiens* has been shown to be an effective transmitter of *Plasmodium cathemerium*" or "*Culex pipiens* has been shown to be incapable of transmitting *Plasmodium cathemerium*." Both statements would be correct as far as they went but neither would be the whole truth. The truth of the matter is that our nomenclatorial system is too coarse a tool to deal with this situation. To express the situation accurately we must resort to some such colloquial term as "strain" and say: "There is one strain of *Culex pipiens* which is an effective vector of *Plasmodium cathemerium* and another strain which is incapable of transmitting this parasite, but the whole unselected natural population of this species is composed of a mixture of both strains, and is in consequence intermediate in its ability to transmit."

It is possible for man to exercise selection of strains in the field, without conscious effort. We unfortunately have no information on the genetical composition of *Aedes aegypti* or *Anopheles gambiae*, either concerning the African populations, or those which invaded the New World. But we do know that in Brazil the populations of both these species exhibited the kind of behavior we associate with the term "domestic," while in Africa, in addition to domestic behavior, both also demonstrate what we call "non-domestic" or "sylvan" behavior. The consistency over a period of time with which the wide range of habits has been observed in Africa, and the more limited domestic habit found in the Americas, clearly indicates the heritable genetic basis of behavior in the strain imported into the New World.

Considering *Aedes aegypti* first, we know that in Africa it is very generally associated with man, breeding in a great variety of artificial receptacles close to dwellings. But, like the other members



of the subgenus *Stegomyia* to which it belongs, it also breeds in Africa in small accumulations of water in tree holes, coconut hulls and leaf axils. In Bwamba County, Uganda, Haddow (1945) found that while it was true that the species occurred in domestic receptacles, it was more often found breeding in tree holes, sometimes in virgin rain forest, and that in Bwamba it is essentially a sylvan and zoophilous species. Garnham, Harper and Highton (1946) working in Kenya found larvae a mile or more inside the forest.

Populations of this species have been introduced by human agency throughout almost all of the tropical, subtropical and portions of the temperate regions of the world. Such populations have been widespread in the New World, though eradication efforts in recent years have restricted their range in many places. The critical point to be noted in the New World is that, with only very rare exceptions, what has been called the "species," but what is in fact only a "strain," is always associated with human habitations, breeding in artificial containers, and nowhere can be considered sylvan in habit.

The *Aedes aegypti* of Kenya and Uganda are populations of the same species as that now occurring in the New World, as well as can be established by morphological comparisons, and this could be confirmed, no doubt, by the test of interfertility. But man has selected, without conscious effort, a strain of *aegypti* for importation to the New World. The strain was accidentally chosen for the characteristics of space preference and favored site for egg deposition, etc., which add up to what we call "domesticity." The individual *Aedes aegypti* that were accidentally brought by man from the Old World to the New, were in all probability those that were breeding in the water containers he carried with him. The portion of the *Aedes aegypti* population living and breeding in the African forest would not be subject to chance transportation overseas. This represents as careful a choice of strain as we exercise in selective breeding in the laboratory.

A similar situation may be observed in the case of the behavior of *Anopheles gambiae* in Africa, in comparison with the accounts of the population of this species which was imported to Brazil. In Brazil it was shown that the population of *Anopheles gambiae* was highly domestic, biting only indoors (Causey, Deane and Deane, 1943). "Even when captures were attempted with animal and

human bait right outside of houses teeming with *gambiae*, it was never possible to catch *gambiae* outdoors." (Soper and Wilson, 1943). While *Anopheles gambiae* in Africa is also considered in the main to be domestic, there are a number of reports of other behavior. De Meillon (1947) found "that when *gambiae* entered an area on the Transvaal highveld from which it is normally absent adults did not enter human habitations." At Freetown, Blacklock and Wilson (1941) report *gambiae* resting in the open on hedges and on young oil-palms. Symes found *gambiae* in an uninhabited area and considered "that it subsists mainly on animal blood until human settlement brings about a permanent increase in numbers" (quotation from Haddow, 1945, based on Symes, 1931). De Meillon has summarized the situation by pointing out that *gambiae* may (first) be almost entirely domestic, (second) may remain indoors at night only, or (third) may not enter human habitations at all.

Soper and Wilson (1943) conclude that the introduction of *Anopheles gambiae* into Brazil at Natal was probably effected by the transport of an adult or a few adults on fast mail-carrying French destroyers from Dakar. The population of *gambiae* in Brazil would thus be the progeny of one or a very few mosquitoes, with a limited genetic complement, selected for characteristics of domesticity by the circumstance of their boarding a ship in the harbor at Dakar.

It follows from this evidence that we would be in error if we concluded from the results obtained in eradicating strains of these two species in the Americas that a similar sort of effort would produce the same end result if applied against the whole genetic potential of these species in their native environment in Africa; just as we would be in error if we concluded from trials in the transmission of *Plasmodium cathemerium* with a laboratory selected strain of *Culex pipiens* refractory to this parasite, that the species *Culex pipiens* as a whole, in nature, was incapable of transmitting the infection. To put the situation accurately we can only make the qualified statement that strains of *Anopheles gambiae* and *Aedes aegypti*, characterized by domesticity, were eradicated—it was not the species *Anopheles gambiae* and *Aedes aegypti* with their full complement of genetic material enabling them to successfully weather periodic unfavorable conditions, whether natural or brought about by man, which were eliminated.



In Sardinia the ERLAAS project was concerned with a portion of a mosquito population which is widespread and successful in the western Mediterranean on both the continental shores and the islands encompassed by them. It is of importance to determine if the population of *Anopheles l. labranchiae* on Sardinia may properly be considered as indigenous there. If we could satisfy ourselves on this point we would have some basis for the belief that the population included the full scope of genetic material of a species or subspecies, from which we could expect some diversity in various of its characteristics. On the other hand, if we found evidence that *labranchiae* on Sardinia is a relatively recent introduction, since the advent of man, with the limited genetic material of a selected strain, we would be in some measure justified in making the analogy between the status of *labranchiae* in Sardinia and the condition and events illustrated by the introduction of domestic strains of *Anopheles gambiae* and *Aedes aegypti* into the Americas. It is also necessary to take some measure of just how domestic was the population of *labranchiae* on Sardinia.

The success of the original plan in the eradication of *labranchiae* in Sardinia depended, to a considerable extent, on whether it could survive on being denied the artificial shelters and the blood of man and his domestic animals (which is essentially what effective residual spraying over a period of years would have done).

This is a difficult point on which to gather direct evidence. The experience of Professor Missiroli and his associates in Latina has given rise to the hope that, in limited areas under intensive cultivation, where complete residual spraying of all human and domestic animal shelters is possible, the apparent absence of *labranchiae* larvae and adults may indicate eradication or near eradication. It should be noted, however, that this is a highly specialized situation. The bonification of the Pontine Marshes, with the radical change it brought about in the ecology of the area, must have seriously changed the native mammalian fauna. The pre-existing mosquito-native mammal relationship was modified some years before the advent of residual spraying, which then secondarily denied human and domestic mammal blood and artificial shelter to the *labranchiae* population.

In other places, where the vector of malaria has been a species with habits somewhat similar to those of *labranchiae*, as in the

case of *A. quadrimaculatus* in Arkansas and *A. pseudopunctipennis* in Morelos, Mexico (Gahan and Payne, 1947), residual spraying of human and domestic animal shelters has been reflected in a significant reduction of adults in the area (of the order of 50% to 85%) but no approach to eradication or even good control of larvae. In Panama, where the vector is *A. albimanus*, a species which invades artificial structures sheltering man and domestic animals only during the active feeding period at night, no significant modification of the mosquito population of the area outside the houses was observed, except for the brief period of two or three weeks following each spraying, although excellent malaria control was obtained (Trapido, 1946).

To deal with the problem of whether *labranchiae*, or for that matter any other mosquito requiring a blood meal for oviposition, can survive in the absence of man and domestic animals, we might look into the history of the relationship. Because the studies of anopheline mosquitoes have been motivated by the interest in the group as vectors of disease, the tendency has been to work most on those aspects of the biology of these insects related to disease transmission, in this case, malaria. A fresh view of the anopheline-human relationship, quite aside from the malaria relationship involved, would undoubtedly prove profitable.

Setting aside malaria for the moment, how could we determine if the species *A. labranchiae* could survive in the absence of man and his dependent domestic animals? Obviously by removing all traces of the structures built by man and by removing man himself and his domestic animals—then observing if the mosquito survives. This is not feasible. Another way of going about the same thing would be to establish if *labranchiae* existed in Sardinia before the advent of man, and before the development of agriculture and animal husbandry as instruments of increasing and concentrating human populations. This line of investigation quickly leads us back into prehistory and a certain amount of speculation, although such information as may be deduced from prehistorical evidence can be as accurate or more accurate than a good many written records.

Prehistory is the era before the appearance of written records, and until recently it has provided us only with a relative chronology based on stratigraphy. Now, however, we are able more and more,



through the application of the methods of geochronology, to translate the evidence provided by prehistory into an absolute time scale. Geochronology may be defined as "the science of dating in terms of years those periods of the past to which the human historical calendar does not apply. It covers human prehistory as well as the whole of the geological past." (Zeuner, 1951).

What we are now specifically interested in establishing is whether or not *labbranchiae* existed in Sardinia before the advent of man. The record with regard to man seems fairly clear. The means of establishing the early presence of man in an area is usually that of searching for the artifacts of relatively imperishable stone or pottery he left behind. One of the prime criteria for determining that an anthropoid type was a man is the test of whether or not he made and used tools. The presence of stones which were modified into tools of one sort or another is accepted evidence of the presence of man. Archeologists also accept the premise that the mode of manufacturing these tools underwent a kind of evolution just as did man himself. Tools made by chipping stones are most primitive, while those showing grinding or polishing are more advanced. Cultures characterized by the presence of chipped stone tools are spoken of as being paleolithic, those of polished stone as neolithic. The working of metal came later, first copper or bronze and then iron, and the cultures characterized by these materials are termed the bronze or the iron age. The evolution of man through these steps to his present state occurred at different times in different places, and in some cases intermediate steps may be missing, as in the case of the South Seas where the arrival of Europeans brought the iron age to a stone age culture without the intervening bronze age. The absolute dating of these ages must be worked out separately for each locality.

It would appear that there is no record of the finding of paleolithic artifacts in Sardinia. Childe (1925), in referring to Sardinia, states, "In paleolithic times the island was uninhabited." In his maps showing the distribution of human cultures in Europe, the first culture indicated for Sardinia is in the second half of the third millennium B. C. The *nuraghi*, which so characteristically dot the Sardinian landscape, Childe attributes to the late bronze age which would be of the order of 1000 B. C. Zeuner (1951), who has gathered and evaluated evidence related to geochronology

from a great number of sources, presents a table (his figure 30), based on European data, which places the beginning of the neolithic age at about 3500 B. C., the beginning of the bronze age from about 2000 to 1500 B. C. and the beginning of the iron age at about 600 B. C.

The account of prehistoric man in Sardinia by Kendrick (1929) is also of interest here. "No trace of paleolithic man is recorded in Sardinia, and the earliest evidence of human occupation dates from the time when metal was first coming into use in the Western Mediterranean. There are, it is true, a few coastal stations in the south of the island that have yielded purely lithic industries, but in the greater number of the simple cave-dwellings wherein the earliest civilization is to be recognized, there are abundant copper implements together with the usual stone and obsidian tools. Indeed, it was probably the natural richness of the island in copper and lead that was responsible for the first settlements therein. The first civilization is, in a sense, the only prehistoric civilization of the island, for it continued without any remarkable alteration not only until the advent of the Greeks and the Carthaginians, but even until the coming of the Romans." Thus we see that Kendrick would date the first human culture on Sardinia as being of the bronze age.

In addition to attempting to date the appearance of man in Sardinia and relate it to the time of arrival of the mosquito, we are also interested in when man took on the way of life which characterizes him now in most parts of the world, i. e., the living in concentrated groups made possible by his discovery of agriculture and the domestication of wild mammals and fowl providing a continuous food source which could be stored between growing seasons in the case of grains, or which could be butchered as needed in the case of domestic animals. A further requirement for the agglomeration of human population is an adequate and continuous supply of water. In temperate regions, with an annual cold period, there would also be need for cover extensive enough to shelter the entire group, i. e., either extensive natural caves, or shelters of wood, hides, stone or earth, made by man himself. All of the basic necessities for the beginning of the social existence which characterizes man today, favor in equal measure what we speak of as "domestic" mosquitoes. The clustering of man himself, together with the animals he domesticated, provided an abundant and continuously



available source of blood meals requisite to the maturation of eggs in anopheline mosquitoes; man's location near a dependable source of water established in advance that the mosquito maturing eggs after a blood meal would find a place to deposit them close at hand. Finally, the shelter required by man for protection from the adverse winter season served in equal measure as a resting place for the hibernation and diurnal retreat of adult mosquitoes.

Thus if we can date the time that man took up agriculture and animal husbandry we will have the date of the earliest time at which the man-domestic animal-mosquito relationship can have been established; for prior to this, even if an early man and the mosquito co-existed in the same area, man lived in no larger than family groups, and must of necessity have moved about in a ceaseless search for food. Zeuner (1951, figure 30) applying the methods of geochronology to the data on European man has placed the nature of the economy on a dated time scale. The paleolithic is characterized by hunters, fishermen and food collectors, while it is not until the neolithic (3500 B.C.) that the food producers (agriculturists) are found.

We may now consider the age of the mosquito population. The palaeontological evidence with regard to the *Culicidae* has been discussed by Edwards (1923), who states: "Since we have reason for believing that the order *Diptera* arose not later than the Triassic period, and since the *Culicidae* are certainly one of the more primitive families of the order, it is highly probable that members of this family existed during the Jurassic period, before the age of mammals; the fact that many *Culex* at the present day attack lizards and frogs, suggests that even the blood-sucking habit may have been developed at this early period." Zeuner (1951) in his figure 83 dates the Triassic at 152 to 182 million years ago, and the Jurassic at 127 to 152 million years ago. Fossil mosquitoes of a modern genus (*Culex*) are known from the Eocene (approximately 50 million years ago), and Edwards says further that, "In the Oligocene rocks of the Isle of Wight and Germany remains of *Culicidae* are numerous, but the species hardly differ from those of the present day; all the three subfamilies are represented, as well as the genera *Dixa*, *Chaoborus*, *Mochlonyx*, *Culex*, *Aedes*, and perhaps *Theobaldia*, *Mansonia* and *Megarhinus*." (The Oligocene dates back about 30 million years.) With regard to the anophelines

which are of particular interest to us we have the additional statement by Edwards (1923), "Although no fossil *Anopheles* has yet been found, there can be no doubt from its morphology that it is an old genus, most probably older than any culicine form; its nonoccurrence in the fossil state can be accounted for by supposing that it has always been, as it is now, less abundant than the *Culicinae*." It has also been pointed out that the scarcity of fossil mosquitoes in general is a reflection of their unsuitability for preservation as fossils rather than any lack of them in remote geological time.

In attempting to piece together the picture of the relationship of the anopheline mosquito *A. labranchiae* and *Homo sapiens* as an agriculturist and herdsman in Sardinia, we have thus far only established the fact that mosquitoes similar to if not identical to modern species existed millions of years ago, while the record of man in Sardinia is relatively brief, i. e., extending back only about 5,500 years from the present. It will be recognized that the information with regard to man is quite specific, while that for *labranchiae* is rather general, since there are no records of fossil anophelines. How then can we tie down the status of *labranchiae* in more specific terms? Recent work on the life of species in terms of geological time can help us on this point. Zeuner (1951) points out that some species have persisted without apparent alteration for as much as 30 million years, while others have evolved during periods of only half to one million years. The following generalizations are made by Zeuner based on his own work and that of others.

- (1) "There appears to be a fastest rate of evolution of species of the animal kingdom under natural conditions, namely about 500,000 years per species-step."
- (2) "In evolution the number of generations appear to be less significant than absolute time." That is to say, the fact that a mosquito may have several generations a year, while man may have only several in a century, does not necessarily mean that the mosquito will evolve more rapidly than man.
- (3) "Every species passes through an episode of rapid evolution but may become stabilized thereafter and persist unaltered for a long time."



Our knowledge of the great antiquity of the *Culicidae* would lead us to the conclusion that the group passed through its episode of rapid evolution many millions of years ago, and is now relatively stable. But, in any event, we could reasonably expect *labranchiae* to be not less than 500,000 years old.

With this much background we may now examine in more detail the relationship of *labranchiae* to man and to the native fauna in Sardinia, and evaluate the two propositions: the one, that this mosquito is dependent on man for its existence; the other, that the species has in the past, and can at the present, get along very well in the absence of man.

1. An examination of a map of Europe and the Mediterranean basin during Pleistocene times (i. e., within the past one million years) shows that at the time of the greatest extension of land, Sardinia and Corsica were connected with northwestern Italy (figure 14 in MacCurdy, 1924). There would have been at that time no water obstacle to the free passage of *labranchiae* from the mainland to these land masses. From what we know about the probable antiquity of the anopheline species in the area, it is also likely that *labranchiae* was on the scene at that time, and quite capable of inhabiting this peninsula, as well as what was then the Italian isthmus connecting Europe to North Africa, where the species also occurs today. There is abundant evidence that during the interglacial epochs Europe had a much milder climate than at present, as proved by the fossil remains there of such mammals characteristic of warm regions as the hippopotamus, rhinoceros, hyena, etc.
2. Kendrick (1929) in discussing the archaeological reconstruction of the prehistory of man in Sardinian states: "The first habitations were perhaps cave dwellings, but it was not long before stone-built huts were erected. Many villages of such huts are known, one of the best examples being that of Gonessa in the southwest of the island. The little houses themselves were mostly round, and about 20 feet in diameter; inside they were furnished with benches, stalls for animals, and an altar near the entrance. In a strategic position near the huts were dwellings of grander character, perhaps the residences of chief-

tains, but fortified so that they might serve as watch-tower, refuge and defense for the whole village in times of danger. These are the *nuraghi*, double-storeyed towers in the shape of a truncated cone and built of large stones laid in regular horizontal courses sometimes with the aid of a clay mortar."

We are struck at once by this evidence that the first human habitations on the island were caves, with stone-built huts being erected later. To place the horse in proper relation to the cart we can only conclude that at the time of the geologically recent introduction of man to the island, it was he who invaded the habitation of the mosquitoes, rather than the converse. As man prospered through his exploitation of agriculture and animal husbandry, he increased and undertook the construction of stone huts to provide shelter for his greater numbers. This activity provided additional facilities for mosquitoes, and no doubt the mosquitoes increased also.

3. If we hesitate to accept the thesis that *labranchiae* was a resident of Sardinia at the time that island was connected with the European mainland, or that man invaded the mosquito habitat rather than the converse, we might next look into the possibility of the introduction of *labranchiae* onto this land mass by natural agency before the advent of man. The path of the seasonal flight of migratory birds follows an ancient pattern. In the present instance we are interested in waterfowl which feed in what is also a favored *labranchiae* habitat—fresh-water swamps with surface vegetation. Corsica and Sardinia are along the flyway of a number of species of waterfowl which winter in North Africa and summer in Europe. Dr. Alexander Wetmore, Secretary of the Smithsonian Institution, has kindly provided a partial list of species of ducks migrant between Europe and northern Africa which have also been recorded from Sardinia. This list excludes several species of rare, casual or accidental occurrence.

*Tadorna tadorna* (Linnaeus). Shelduck

*Anas platyrhynchos platyrhynchos* Linnaeus. Mallard

*Anas strepera* Linnaeus. Gadwall.

*Anas acuta acuta* Linnaeus. Eurasian Pintail

*Anas querquedula* Linnaeus. Garganey

*Anas crecca crecca* Linnaeus. Teal



*Spatula clypeata* (Linnaeus). Shoveler  
*Mareca penelope* (Linnaeus). Wigeon  
*Netta rufina* (Pallas). Red-crested Pochard  
*Nyroca ferina ferina* (Linnaeus). Common Pochard  
*Nyroca fuligula* (Linnaeus). Tufted Duck  
*Mergus albellus* Linnaeus. Smew

That such waterfowl are capable of transporting the eggs of mosquitoes adhering to their feet, legs and feathers is strongly supported by the unpublished observations of Dr. Herbert C. Clark and Lawrence H. Dunn who, while examining ducks shot by members of a hunting club at the La Jagua swamps in Panama for ectoparasites, also found the eggs of several species of mosquitoes, which hatched out in the laboratory. While anopheline eggs are not known to withstand prolonged desiccation, they might be expected to remain viable for the few hours flight from either Corsica or North Africa to Sardinia.

In the light of this information we might digress a moment to suggest that migrating waterfowl may reintroduce *labranchiae* into Sardinia, either from North Africa to the south, or Corsica to the north. The quarantine of ships, boats and aircraft, no matter how thorough or effective, will not block this means of reintroduction.

4. Evidence of the north or southward extensions of the ranges of mosquitoes within the separate hemispheres is lacking. The impression is strong that these ranges are well established, and have been determined by natural agencies before the advent of man, though of course they are frequently locally modified by man's alteration of the hydrology. A case illustrating this point very clearly is that of the status of *Anopheles albimanus* in Florida. The species is the common and abundant malaria vector in Cuba, and on a number of occasions specimens have been taken in the Florida Keys only a few miles to the north and in southern Florida itself. On several such occasions alarm has been expressed over the possibility that the species might become established as a malaria vector in Florida, but, whether control measures were taken or not, the species remains a rare curiosity, apparently being at the natural limit of its range to the north, with man serving neither to extend nor restrict its range.

Such introductions as have been spectacularly successful (from the point of view of the mosquitoes) have been made by man himself along lines of equal latitude from one continent to another (*Aedes aegypti* and *Anopheles gambiae*), natural agencies before the advent of man having failed to bridge the vast oceanic gap to a suitable habitat of like latitude on another continent.

5. One is struck at once in Sardinia by the fact that the conspicuous native mammalian fauna of the island includes wild boar, moufflon and rabbits, whose domestic counterparts, pigs, sheep and rabbits were, by all accounts, before the advent of ERLAAS, hosts of *labranchiae*. While *labranchiae* is considered a very domestic species, Hackett (1937) reports that "in southern Italy and Sardinia, where *labranchiae* prevails, from 10 to 20 per cent live on human blood." We see then that the bulk of these mosquitoes feed on other animals and the implication is clear that the species could get along very well without man, and indeed with only the wild counterparts of man's aggregations of domestic animals.
6. It is very much more difficult to search natural resting places of mosquitoes than the conveniently accessible structures built by man to shelter himself and his domestic animals, but Dr. T. H. G. Aitken has accumulated a number of records of *labranchiae* resting in such places during the summer and hibernating in winter. It has been suggested that *labranchiae* is never found more than five kilometers from some structure made by man, and reasoned from this that the species is completely dependent on man. But if circles five kilometers in radius were drawn about every such structure below the 1000 meter level on Sardinia it is likely that little, if any, part of the island below this level would not be covered. This observation, therefore, can only be taken as a statement of the evident fact that man-made structures are widely dispersed on most of the island. A more useful method of judging how closely *labranchiae* may be bound to man would be that of reviewing positives in relation to centers of human population. Dr. Aitken has had this done, with interesting results. Of all *labranchiae* positives found during 1950 only 8.2 per cent were less than one kilometer from a



village center, while 33.3 per cent were more than five kilometers distant. The comparable figures given by him for 1949 are 8.6 per cent and 21.5 per cent (ERLAAS memoranda from Dr. Aitken, dated January 15, 1951 and January 27, 1951). It might be explained that the small proportion of positives near villages is a result of attrition following the residual spraying of dwellings, but this in no way diminishes the significance of the fact that a substantial number of such positives as were found, were more than five kilometers distant from these centers of population, and in several cases were as much as 11 to 12 kilometers distant.

The great weight of all this evidence indicates that *labranchiae* antedated man, the agriculturist and herdsman, on Sardinia by a vast period of time, maintaining itself on the native mammal population. It is unlikely that it would have abandoned this relationship in its entirety during a period so brief in the geological life-span of a species as five or six thousand years.

This conclusion is of considerable significance in the eradication effort on Sardinia. *Anopheles labranchiae* as a long-time native resident of Sardinia may be expected to have the full genetic complement of that species. It is not a selected strain of the species, accidentally chosen for its domestic characteristics, and is therefore not bound to man for its survival. It may be expected to demonstrate the resilience of species in their native environment to adverse conditions, whether natural or man-made. On being denied access to man and his domestic animals it might be expected to survive through the portion of the population which maintains its original relationship with the native mammal fauna and original natural resting places. The experience of ERLAAS during the 1949 and 1950 seasons would appear to demonstrate that *Anopheles labranchiae* has by this means fulfilled the biological potential of an indigenous population with a high reproductive rate and a good adjustment with its environment. In just what way or ways *labranchiae* expressed this biological potential in avoiding complete destruction, we unfortunately cannot be sure.

It has recently been suggested by Soper (1951) that if indeed the ERLAAS program has been effective in eliminating the portion of the *labranchiae* population on Sardinia bound to man, then:

"Are the few remaining *labbranchiae* in Sardinia direct descendants of the *labbranchiae* of thousands of years before adaptation to life with man occurred and, therefore, incapable of building up the heavy infestation responsible for the previous highly malarious state of the island?" To expect such an outcome, we would have to assume that the behavior characteristics which we sum up with the term "domesticity" are controlled by a very few genes, and that the ERLAAS program had completely eliminated the portion of the *labbranchiae* population with these genes which bind it to man's activities, leaving a residual population homozygous for the characteristics which relate it only to the native fauna, and completely dissociate it from man. That the selective action on the *labbranchiae* population has been so drastic and complete (as, for example, in the converse case of the selection of a very few domestic individuals of the species *Anopheles gambiae* and *Aedes aegypti* for introduction into the New World) is unlikely, since, despite the continuous attrition produced by the residual spraying program in Sardinia, some of the positives of the last year of the ERLAAS operation were found in domestic situations. We may also recall that since *labbranchiae* invaded the habitat of man when he settled on the island in prehistoric times, as would appear from the evidence assembled earlier in this paper, the residual population of *labbranchiae* will retain sufficient plasticity to do so again.



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iii

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