MICROBIOLOGY AND MOSQUITO CONTROL[1]

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Many, varied and most inadequately understood are the relations between mosquitoes and micro-organisms, a term used here in its broadest sense to cover all organisms that cannot be studied without the aid of a compound microscope. At the threshold of life even this instrument is of limited use, but, although numerous viruses are transmitted to man by mosquitoes, we are as yet unaware of any virus harmful to mosquitoes and thus of potential value as a biological control agent. This is not to say that such a viral pathogen does not exist, for atypical polyhedral inclusions occur in other Nematocera including the Tendipedidae (Weiser, 1948) and Tipulidae (Rennie, 1923). Noninclusion virus disease has been recorded from the latter family (Xeros, 1954), and *Calliphora vomitoria* suffers from polyhedrosis (Bergold, 1943).

There are several records of rickettsiae from mosquitoes, perhaps the best known species being *Wolbachia pipientis,* an apparently harmless parasite of the ovaries and testes of *Culex pipiens* (Hertig, 1936). It would be premature to draw any conclusions about either group from the existing literature, which is, however, more informative as regards relations between mosquitoes and bacteria, algae, fungi, protozoa and higher forms.

It is not intended to survey this literature, a task that in any case is now being completed by Dr. Dale W. Jenkins at Fort Detrick, Maryland. Instead, it is proposed to touch upon several types of relationship ranging from those between free-living aquatic micro-organisms and culicid eggs and larvae, to purely parasitic ones, with the object of highlighting some fruitful areas for ecological research of direct benefit to mosquito control.

Today, we are faced with control failures due to resistance and the non-selectivity of modern insecticides. At a time when biological remedies for these problems are being considered (Laird, 1959d; AIBS conference, 1960), it is important to realize that such remedies are by no means confined to the fields of parasitism and macro-predation.

We still know remarkably little of the factors influencing the choice of oviposition sites by mosquitoes, or of the relationship between these factors and the total ecology of the larval habitat. Mosquito workers investigating a particular locality soon learn to associate the major types of larval habitats with the species most likely to be found there. They are hard put to it, though, to explain this evidence of preference in their writings. Much of their difficulty is due to a narrowness of outlook and perhaps of basic training as well. Although they may attempt to define a larval habitat in terms of its more easily measured chemical and physical qualities, they too often overlook the fact that it is the home of a wide range of organisms other than mosquitoes. It is seldom that the field collector pays attention to these beyond making general notes on plant cover and the presence of particularly prevalent fish or large invertebrates. Should a gleaming dytiscid swim into his dipper, he may perhaps pop it into his jar along with the larvae, note subsequently that it has destroyed some of them, and proceed to carry out simple feeding experiments in which an otherwise starved beetle is provided with mosquito victims that it quite understandably (favoured as it usually is by the

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congested quarters provided) captures and devours. A good deal of the literature on predation concerns work of this sort, which, of course, has its place in basic research. Few indeed, though, are quantitative studies of micro-limnology, or even qualitative reports including the listing of accurately identified animals and plants associated with developing mosquitoes. Better knowledge of these associations can lead to the development of much more precise criteria for the designation of larval habitats.

Again, a note on parasitism often has its origin in the finding of sickly larvae bearing a thick and obvious vestiture of bacteria, algae, fungi or protozoa, or combinations of these. Close host specificity is inferred without the more sweeping investigation that would have both disclosed the epibionts on other, frequently smaller and less conspicuous inhabitants of the water body concerned, and revealed the development of polysaprobic conditions. The latter at one and the same time render the habitat less and less tolerable to most mosquito larvae, and burden the debilitated survivors with passengers that flourish on many kinds of substrates in the presence of pollution (Laird, 1959b). Such organisms are in fact biological indicators of pollution, and well known as such to those concerned with sanitary aspects of water management. Although they are not parasites in the strict sense of the term at all, their presence may contribute towards the death of undernourished, infested animals.

The first points to be emphasized, then, are the danger of drawing erroneous conclusions from inadequate data, and the need for a keener sense of the importance of those concerned with mosquito control having a thorough grounding in freshwater biology.

Bacterial associates of mosquito larvae include *Siderocapsa Treubii,* the bacilloid cells of which occur in mucoid masses surrounded by iron deposits. This microorganism was first described from underwater leaves in Indonesia and later reported from slides suspended in alkaline waters in the U.S.A. (Hardman and Henrici, 1939). It was first reported as a mosquito larval epibiont at Singapore, where it occurred on culicines and an anopheline, as well as on *Pistia* leaves and in surface scum (Laird, 1959b). The late Dr. Willis E. Snow (p.c., 1958), whose tragic death last year deprived the U.S.A. of the services of an able ecologist, found the same or a closely related organism in Alabama floodwater pools and on larval *Psorophora ciliata* and *P. howardii* developing there. Associated culicines gave rise to dwarfed adults, apparently through the crowding out of suitable larval food organisms by the iron bacteria, which rapidly became extremely abundant. However, normal-sized adults duly developed from the infested *Psorophora,* these predators capturing tiny immature amphibians as culicines became scarce.

A declining oxygen gradient resulting from burgeoning bacterial activity in freshly filled pools, triggers the hatching of the eggs of floodwater mosquitoes (Gjullin *et al.,* 1941; Laird, 1956; Horsfall, 1956). As larvae emerge they find a ready source of nourishment in some at least of the thriving bacteria which, as Dr. Snow indicated, are soon so numerous that there is no longer a hatching stimulus, any eggs remaining unhatched thus providing "some carryover for another generation if the initial brood does not survive." Surely the great importance of floodwater and snow-melt mosquitoes on this continent, makes the identification of the various bacteria concerned, and field studies of their bionomics, highly desirable?

Streptococcus margaritaceus, often common in polluted waters, may adhere to the body surfaces of larvae occurring there. More evident epibionts under such conditions, though, are *Sphaerotilus dichotomus* and the well-known sewage bacteria, *Sphaerotilus natans* and *Zoogloea ramigera.* Appearing as greyish-white "beards" to the unaided eye, these organisms, when noticed on mosquito larvae, will also be found if searched for on other insects, notably Tendipedidae and

Anisoptera, as well as on dead vegetation and submerged objects (Laird, 1959b). Such bacteria on larval *Culex pipiens fatigans* in Shanghai, were viewed as parasites by von Jettmar (1947), who asserted that his "streptobacilli" penetrated the cuticle of the host and destroyed the epithelial cells concerned with chitin production. Studies on *Anopheles maculipennis atroparvus* laboratory colonies in Italy (Laird, 1958) have since established that heavy growths of *Sphaerotilus* and another biological indicator of pollution, *Vorticella microstoma*, may so hamper infested insects that these, already greatly weakened by undernourishment due to the deterioration of their food supply with the development of polysaprobic conditions, die from exhaustion as pupation or hatching demand energy expenditure of which they have become incapable.

Such weakened larvae fall ready victims to invasion by aquatic fungi, a useful account of which has recently been published by Emerson (1958). Their strength may be inadequate for the shedding of the old larval skin at ecdysis, and further growth of the insects, trapped in prisons of their own making, leads to the appearance of the hoops and circlets described by Langeron (1928) and correctly interpreted by Gaud (p.c., 1953) as hairs of the new envelope viewed through the persisting former one. More bizarre effects are the development of twisted antennae and double sets of clypeal spines, and of bent siphons. Certain normally free-living protozoons invade wounded or moribund larvae, and sometimes even healthy ones. Ciliates of the genus *Tetrahymena* are prominent in this respect (Lamborn, 1921; Corliss, p.c. 1959). Of course, care must be exercised to distinguish between such instances of facultative parasitism and post-mortem invasion by simple scavengers, like the flagellates, *Trachelomonas hispida* and *Peranema trichophorum*.

Under mesosaprobic conditions, the pollution state characterizing most of the zone of oxidation, algal epibionts may be evident. These include species of *Characium*, flourishing on Cladocera and spreading to mosquito larvae and associated animals. Snow-melt pools are often mesosaprobic, due to the spring-time decay of organisms killed there at the freeze-up at the beginning of the preceding winter. Entomologists working in subarctic America (Jenkins, 1948; Jenkins and Knight, 1950, 1952) have noted the occurrence of green algae on the anal papillae of mosquito larvae. Similar observations were made during a visit to Ungava Bay, northern Quebec, in the early spring of 1959, and an algal epibiont then collected from the copepod, *Acanthocyclops vernalis*, and Tendipedidae (*Anatopynia alaskensis*), as well as from mosquito larvae (*Aëdes* spp. and *Mochlonyx* sp.), has been identified as *Chlorangium stentorium* (original data).

Mesosaprobic waters often yield Cladocera bearing rotiferan epibionts, notably *Brachionus rubens* (Laird, 1956, 1959b). They, too, spread to larval mosquitoes, which may even form temporary anchorages for small Cladocera browsing among bacterial, algal and ciliate epibionts.

Ciliates, sessile Peritricha, are in fact the most immediately obvious epibionts indicative of the different levels of pollution. Epistylids and many vorticellids are mesosaprobes. Dichotomously branching colonies of up to 46 individual zooids of *Epistylis lacustris* grow upon a diversity of aquatic animals, including microcrustaceans and mosquito larvae. *Vorticella campanula* and *V. convallaria* have been recorded from *Lutzia* larvae under similar mesosaprobic conditions.

The commonest of all mosquito ciliates is undoubtedly *Vorticella microstoma*, a genuine sewage organism found throughout not only the mesosaprobic zone but the polysaprobic one as well—the latter being the zone of massive pollution and decomposition, in which reduction of the polluting substances proceeds vigorously. Heavily infested larvae appear to the naked eye as if clothed with a whitish fur, and often bear *Sphaerotilus* too.

Although the epibionts that have been mentioned show no special preference for mosquito larvae over other available substrates, and as has been indicated merely reflect environmental conditions, this is not to say that they may not be harmful on occasion. Larvae developing in mesosaprobic waters, although weighed down by masses of Peritricha, may still pupate and give rise to healthy adults (Laird, 1956). Should a polysaprobic situation develop, though, as in the case of intermittently manured water hyacinth ponds in the Oriental Region, the advent of unfavourable chemical and physical conditions, coinciding with the supplanting of preferred food organisms by bacteria and saprozoic protozoa including facultative parasites, combine to prejudice survival of the mosquitoes. Already undernourished larvae, hampered by increasingly heavy growths of polysaprobic epibionts, are hard put to it to hold their own in the competition for such suitable nourishment as remains. Successive moults leave them feebler, more weakly chitinized, and less fitted for survival (Laird, 1959b).

I have dwelt at length upon these aspects of microbiology, because in them lies the key to a more selective approach to some, at least, of our mosquito control problems. Knowledge of larval habitat ecology has made little progress since the localized naturalistic control efforts of the years between the World Wars—when Rice and Savage (1932) used cattle manure for weekly treatment of borrow pits in Assam and thereby completely prevented the breeding of *Anopheles minimus,* and Ramsay and Savage (1932) in India, Hargreaves (1933) in Uganda and Martial (1937) in Indo-China, to name only some of those concerned, achieved similar results with pollution of ponds by rotting vegetation. Williamson (1936, 1949) should be referred to for a full discussion of these matters which, in the face of the threatening resistance impasse, fully warrant critical reappraisal.

This account must not conclude without brief reference to some of the *bona fide* parasites of mosquitoes. An example of a long established parasite, completely adjusted to its host which does not *appear* to suffer harm from its presence, is the gregarine protozoon *Lankesteria culicis.* This organism has been found wherever searched for in *Stegomyia* populations and in certain other mosquitoes too. Its trophozoites occur in the larval gut, first within epithelial cells and then free in the lumen. At pupation the gregarines, now mature gametocytes, enter the Malpighian tubules and associate in pairs to form spherical gametocysts. Their individuality is soon lost, repeated nuclear divisions take place, and myriads of spores are finally formed. These are released upon rupture of the sporocysts in the Malpighian tubules or hind gut of the adult. Accounts of this well-known organism include those of Ray (1933) and Ganapati and Tate (1949). Emphasis was earlier placed on the fact that the host does not *appear* harmed. This is a reflection of the lack of appreciation of the importance of such parasites as population-regulating factors, a function not necessarily bound up with a high level of pathogenicity. It is questionable whether the Malpighian tubules can suffer massive damage without at least some harm being caused thereby, and as *Aedes aegypti,* one of the natural hosts for *Lankesteria culicis,* is being increasingly used as a laboratory test animal, it would be wise to ensure from the outset that colonies established for resistance testing and other purposes, are gregarine-free.

More spectacular in their effects are some of the microsporidian parasites. Garnham (1956) and Canning (1957) have described the infection of a laboratory colony of *Anopheles gambiae* with *Plistophora culicis,* from 50 to 100 percent of the adults being parasitized. However, the microsporidians were chiefly confined to the Malpighian tubules, and serious harm did not seem to result. *Plistophora collessi,* described from two Singapore culicines, is of rather more significance. This species causes a most effective sterilization. At the same time, the females, denied the possibility of laying eggs, seem able to lay spherical cysts each of which contains an average of four million spores. In view of so effective a means of seeding fresh larval habitats, it may seem surprising that the incidence of *Plistophora collessi* in nature is only of the order of 1 percent (Laird, 1959a). The explanation presumably lies in the fact that biological equilibrium between the parasite and its hosts was attained long ago, but once again, studies on the population-regulating significance of the association are highly desirable—this time, with a view to the possible value of the organism as a biological control agent for use in areas where susceptible vectors or pest mosquitoes are as yet free from infection.

Mermithid nematodes have been viewed by a number of authors (e.g. Welch, 1958) as promising material for biological control research. Jenkins and West (1954) reported infections of up to 100 percent of larvae in subarctic pools—although neighbouring pools were sometimes quite free from the parasites. Why this should be so we know no more of than the reasons underlying the erratic distribution and incidence of what are at present the most promising of all mosquito pathogens for biological control research, the fungal parasites of the genus *Coelomomyces* (Couch, 1945; Couch and Dodge, 1947; Laird, 1959c). The host usually dies without pupating, through destruction of the fat body and imaginal buds (Keilin, 1921; Iyengar, 1935), its body packed with sporangia. These may be plain or variously sculptured, and their presence in quantity makes infected larvae stand out from normal ones by a general rusty colouration and the chalky or tawny appearance of the anal papillae. After suitable conditioning the resting sporangia finally rupture, releasing flagellate zoospores.

An attempt to establish *Coelomomyces stegomyiae* in a previously uninfected mosquito population is currently proceeding in an isolated group of South Pacific atolls, the Tokelau Islands, under the sponsorship of the World Health Organization and with New Zealand government co-operation. Infective material derived from parasitized *Aedes albopictus* at Singapore was introduced into 761 larval habitats of *Aedes polynesiensis*, the primary vector of the filariasis parasite *Wuchereria bancrofti*, in the central and eastern South Pacific, in September, 1958. A return visit for assessment purposes is planned for April, 1960.

It is hoped that this pilot project will help pave the way for further introductions of mosquito pathogens, and specific predators too, from areas where they are endemic and in balance with host or prey populations to others in which they are not. It cannot be too strongly emphasized, though, that little of general value can be expected from such experiments until a great deal more has been learned concerning the distribution, host-parasite relationships and life histories of these mosquito enemies. Detailed and integrated studies on a global basis are called for if this approach to mosquito control is to become, as it must become, more than a blundering meddling with natural economy. Allied ecological research can lead towards a less direct form of biological control, too. By taking advantage of associations and cycles of freshwater organisms that are already worldwide, is it not possible to add specific environmental management techniques to the mosquito control armory? Both fields demand a global conception, an attitude of mind that is fortunately becoming rapidly more accepted in scientific fields today, thanks largely to modern advances in transportation and an increasing abundance of research funds. An interesting illustration of this was lately given by Friedmann (1960), who, in a detailed examination of the subject, commented that one of the advisers to a University of Michigan group contemplating establishing a field station for tropical zoology, suggested that they do so in the interior highlands of New Guinea rather than in relatively nearby Mexico!

New and exciting horizons for mosquito control will open up once field stations already in existence begin to devote attention, equal to that already accorded chemical insecticide research, to parasitology and ecology, under the guidance of a central planning group set up to prevent needless duplication of endeavor, arrange for rapid identifications, and integrate and make practical use of the data collected.

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