

A PROPOSED METHOD OF SEARCH FOR MICRO-ORGANISMS PATHOGENIC TO MOSQUITOES

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INTRODUCTION. Pathogens which parasitize both higher animals and insects are numerous and of many different kinds. A large number come from unknown sources and may be mutants occurring at widely-separated intervals of time and space. It might be possible to use pathogens for the control of mosquitoes if more effective pathogens could be discovered. Attempts have already been made to do this with known pathogens, but other unknown microscopic mosquito pathogens—bacteria, viruses and fungi—may very well have existed unrecognized and been thrown away with a dipper-full of wild-caught mosquito larvae.

Undiscovered microscopic or sub-microscopic pathogens of mosquitoes in existence (or which might come to exist at some future date through mutations) might be discovered by a randomized, purposeful search. On the theory that a systematic "shot-gun" method of search for moribund mosquito larvae might reveal such pathogens, the following protocol is suggested for such a search.

MATERIALS AND METHODS. 1. In a suitable rearing room maintain 10 Pyrex rearing pans fitted with adult-capturing cones of any material capable of withstanding repeated sterilization. An indeterminate number of any instar mosquito larvae of any species or any number of species will be introduced into the pans (along with their native water).

2. Alongside each of the rearing pans keep another similar pan which shall contain only the native water of its twin.

3. Each of the twin sets should have its own instruments for the transfer of larvae. The instruments should not be interchanged in order to avoid cross-contamination.

4. From time to time (but at least every 3 days) make a gross examination of the "colonies." Any dead or moribund larvae should be transferred to the "twin-pan."

5. Any adults which might hatch will be captured by the cones and might be assumed to be uninfected. (It is realized that there are pathogens such as *Thelohania legeri* and some viruses that are transmitted transovarially without patent or harmful effects in the adult females. Therefore, a representative sample of adult females may be checked for microsporidia if desired.)

6. At intervals, replenish the "colonies" from

native, wild-caught larvae taken from the same general locales. (Times of replenishment may be haphazard, so as not to interfere with any other work that may be in progress at the time, and may improve the time-space separation in the search for pathogens.)

7. On the same day of each month, perform the following procedures:

(a) Remove and destroy all living, apparently healthy, larval and adult mosquitoes, collect an aliquot of their rearing water to be pooled in covered jars and stored for a year at room temperature. Scrub the rearing pans and collection cones with a brush and strong detergent and sterilize in an oven or an autoclave.

(b) Remove the dead larvae from either pan of the set, place them in labeled vials along with an aliquot of the "twin-water," and store for a year at the temperature of the lower portion of a refrigerator, i.e., at a temperature of about 40° F.

(c) The old "twin-pans" will then become the rearing pans for new batches of larvae which will be caught from the same general locales.

(d) The newly-caught larvae should be accompanied by enough native water both to fill the newly-sterilized old rearing pans (which now has become the "twin-pan" in its set) and to replenish the partially-evaporated water in which the new "colony" is to be reared.

(e) Place the newly-sterilized cones over the new "colonies" and repeat all steps mentioned heretofore.

8. If, when performing step # 4 (above), any unusual mortality is noticed, the following procedure is suggested:

(a) Fill 10 additional sterilized rearing pans with freshly-collected natural water from the native locale and introduce fresh, wild-caught larvae from any source.

(b) Add an aliquot of the rearing water from the "mortality-producing" pan to these new colonies in an attempt to induce mortality in the new stock.

(c) Should any significant mortality be observed in these new colonies, at once notify the nearest college or university with a Department of Entomology and inform them of your findings so that appropriate laboratory follow-up action may be taken.

DISCUSSION. It may be argued that natural selection may render useless any pathogen which might be discovered. This is true—but the same methods by which they were discovered may also be used to discover other new pathogens. Further, even though through natural selection, mosquitoes may become more resistant to, or tolerant of, a pathogen, survivors may be more

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susceptible to control by other methods because of being parasitized.

It is realized that many agencies are not staffed or equipped to follow all the suggestions made above. Modifications can be made at every step

to conform to changing circumstances. As with any blind search, repeated failure can be expected—but someone, somewhere, sometime, may encounter valuable pathogens of mosquitoes by using these methods or modifications of them.

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Further information may be obtained from Dr. Robert W. Jones, III, Chief, Foreign Students Education Branch, Fogarty International Center, National Institutes of Health, Room B2CO5A, Building 31, Bethesda, Maryland 20014.