INSECTICIDE TESTS ON SEXED MOSQUITO LARVAE

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Insecticide tests with adult male and female insects have indicated that the male may be more variable and at times more susceptible than the female. This phenomenon has been documented by work on the American cockroach, *Periplaneta americana* (L.), (Heal and Menusan 1948, Forgash 1956), the house fly, *Musca domestica* L., (March and Metcalf 1949) and the mosquito, *Anopheles gambiae* Giles (Davidson 1958).

Mosquito larvicide tests are usually performed on unsexed larvae since for many species it is difficult to differentiate males and females at this stage of the life cycle. As a consequence, toxicity data on mosquito larvae consist of tests made on mixtures of the sexes in varying proportions. In a test of larvae at any given concentration of insecticide the sex ratio may vary considerably. Since individual replicates may also vary considerably in mortality rates it appeared worthwhile to examine the possibility that this may be due to a preponderance of male or female larvae in the replicate as the case may be be. The tests described in this paper were performed to determine if the susceptibility and variance factors of male mosquitoes are present in the larval stage.

METHODS AND MATERIALS. Certain species of mosquitoes may be sexed with relative ease in the 4th larval stadium by visual examination without magnification. Male gonads can be seen through the cuticle as two dark clumps located in the 5th and 6th abdominal segments when viewed from the dorsum. Fourth instar larvae of *Culiseta inornata* (Williston), *Culex boharti* Brookman & Reeves, and *Aedes nigromaculis* (Ludlow) were found to be especially suited for accurate sexing. In all tests, male and female larvae which had been reared under standard conditions from the same clutches of eggs were subjected simultaneously to the same insecticidal concentration. *C. inornata* and *C. boharti* were from laboratory colonies; *A. nigromaculis* was reared from eggs obtained by the method of Lewallen and Nicholson (1959).

Technical or purified samples of insecticides were prepared as 1.0 percent weight/volume solutions in acetone. Aliquots of the stock solution were diluted further with acetone to arrive at a series of concentrations which would give mortality figures within the approximate range of 15 to 95 percent.

Individual test units consisted of 100 ml. of distilled water in a 4-ounce paper cup containing 20 fourth instar sexed larvae which had been transferred from the rearing pans by means of a 1" x 3" piece of 16 mesh aluminum screen. Care was taken not to transfer excess water from the rearing pans to the cups. Controls were run with each batch of treated larvae; no mortality was observed in the controls during the test period of 24 hours.

Mortality data were obtained on three different types of insecticides. DDT was chosen as an organochlorine compound, parathion as an organophosphate, and Hercules AC 5727 as a carbamate.

One ml. of insecticidal solution was pipetted into each test unit, with each concentration run in duplicate. This procedure was followed on three separate occasions (three different batches of larvae) resulting in three replications of size 40 for each concentration, except with AC 5727 on *C. boharti* where only two batches of larvae could be tested before the colony was lost.

Treated and control larvae were held at 70° F±4° for 24 hours and received no food during this period. Percentage mortality was based on the criterion that larvae which responded normally when probed or disturbed by tapping the sides of the container were alive; all moribund or ab-

normal larvae were included in the counts with dead larvae.

Probit regression lines of mortality on log concentration were fitted by the maximum likelihood method (Finney 1952). Tests of heterogeneity, parallelism of lines (equal variance), and relative sensitivity of the sexes were applied to each set of replicates. The tests of relative sensitivity were performed for the pooled or individual replicates depending on the parallelism of the lines.

RESULTS. Table 1 indicates the cases in which heterogeneity and/or nonparallelism of the lines were significant at the .05 level. In all three cases of significant nonparallelism the component of variance due to replicates was significant at the .05 level and the component due to sex was nonsignificant. The relative sensitivity of the two sexes was not significantly different from one in any case. Hence, there was no evidence of a difference between the sexes in either variability of response or in sensitivity to the insecticides studied. This would indicate that when differences in mortality occur between replicates of insecticide tests involving these three species they are not due to a preponderance of one sex or the other. This may also be true of other species of mosquitoes.

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TABLE 1.—Cases of significant (.05 level) heterogeneity and nonparallelism of probit regression lines.

Species	DDT	Parathion	AC 5727
Aedes nigromaculis	Nonparallelism	.....	.....
Culex inornata	.....	Heterogeneity	Heterogeneity
Culex boharti	Nonparallelism	Heterogeneity	.....
	.....	Nonparallelism	.....

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