

ciated with ovarian development and with the salivary glands." Undoubtedly, further investigation and more data are needed.

**SUMMARY.** The extract of the adult female *Culex tarsalis* has more antigenic components than does that of the adult male. The female extract formed 3 or more precipitate lines in the reaction against its homologous antiserum. Male extract reacting against its homologous antiserum produced only one or two lines. But when reacting against the anti-female serum, it elicited 2 or more lines. One explanation for the additional line or lines in this heterologous reaction is that possibly some antigens in the male mosquito are haptenes which are also parts of the complete antigens of the female. These haptenes react with the anti-female antibodies having sites complementary to those of the presumed haptenes and consequently precipitate out as extra lines. Pigeon antisera reacting with mosquito extracts formed very faint and diffuse precipitate lines.

### References

DOWNE, A. E. R. 1963. Mosquitoes: comparative serology of four species of *Aedes* (*Ochlerotatus*). *Science* 139:1286-1287.

FOX, I., KNIGHT, W. B., and BAYONA, I. G. 1963. Antigenic relationships among mosquitoes and sand flies demonstrated by agar-gel tests. *J. Allergy* 34:196-202.

METCALF, R. L. 1945. The physiology of the salivary glands of *Anopheles quadrimaculatus*. *J. Nat. Malar Soc.* 4:271-278.

SMITH, S., and SILVERMAN, P. H. 1966. Metamorphosis antigens of mosquitoes. *Mosq. News* 26:544-511.

WOLFE, H. R., and DILKS, E. 1949. Precipitin produced in chickens. IV. A comparison of the antibody responses of eight avian species. *J. Immunol.* 61:251-257.

ZAMAN, V., and CHELLAPPAH, W. T. 1963. Gel-diffusion studies with mosquito (Diptera: Culicidae) antigens. I. Antigenic analysis during metamorphosis. *Exper. Parasitol.* 13:108-112.

ZAMAN, V., and CHELLAPPAH, W. T. 1964. The agar gel-diffusion technique as a method of differentiating mosquito eggs. *Experientia* 20:429.

ZAMAN, V., and CHELLAPPAH, W. T. 1965. The agar gel-diffusion technique as a method of differentiating mosquito larvae. *Experientia* 21: 297-298.

## INTERACTIONS BETWEEN LARVAE OF *Aedes aegypti* (L.) AND *Culex pipiens* L. IN MIXED EXPERIMENTAL POPULATIONS<sup>1</sup>

T. MICHAEL PETERS,<sup>2</sup> BORIS I. CHEVONE<sup>3</sup> AND RICHARD A. CALLAHAN<sup>3</sup>

Department of Entomology, University of Massachusetts, Amherst, Massachusetts

Naturally occurring populations of mosquito larvae are forced to develop in the environment to which the female is attracted for oviposition. Similar attractants for different species frequently produce situations in which two species are forced to live together. Personal observations of single species populations in some situations and mixed populations in others

led the authors to perform an experiment to determine whether there are any mechanisms utilized by mosquito larvae to eliminate or reduce competition from other mosquitoes.

**METHODS AND MATERIALS.** Larvae used in the experiment were obtained from eggs of *Aedes aegypti* (L.) and *Culex pipiens* L. cultures maintained in our laboratory. The experiment was begun when a large number of *C. pipiens* egg rafts had produced larvae within a 1-hour period. At this time, *A. aegypti* larvae were vacuum hatched and were all within 1 hour of the

<sup>1</sup> This research was supported by Hatch Project No. 253—Revised.

<sup>2</sup> Project leader, Head of Department.

<sup>3</sup> Graduate Research Assistant.

same age as the *C. pipiens*. One hundred larvae of *A. aegypti* and *C. pipiens* in varying proportions were counted into 50 ml. of distilled water in each of 15 petri dishes (100 mm x 15 mm). The dishes were placed in a randomized block design within a growth chamber maintained at an average 81° F., w-5 78-83° F., and photoperiod of 14L:10D. After one day in which 50 mg. of dried brewer's yeast was supplied to each petri dish, the following feeding regime for each dish was followed; day 3, 25 mg. yeast; day 4, 50 mg. yeast; day 5, 100 mg. yeast; day 6, 150 mg. yeast; day 7, 50 mg. yeast; day 9, 100 mg. yeast; day 10, 50 mg. yeast; day 12, 50 mg. yeast. Water was changed prior to feeding. Using 100 as the number of individuals per replicate, we varied the proportion of *C. pipiens* to *A. aegypti* into treatments of: 0 to 100, 25 to 75, 50 to 50, 75 to 25, 100 to 0. Data were collected on the number of each species pupating within all replicates at 6-hour intervals starting with the appearance of the first pupa in any replicate.

Possible changes in osmotic pressure of the solutions were tested by freezing point depression against a NaCl standard (accuracy 0.01° C.). Solutions were made with 2 mg. yeast/ml. distilled water and incubated up to 36 hours at 80° F. before testing. A Thomas freezing point thermometer, scale -5 to 1° C. in 0.01° C. (25 inch stem) was employed in a 16 x 100 mm test tube one-half filled with test solution. The entire bulb was immersed in the solution through a rubber stopper. The system was rotated in an ice-salt water solution at approximately -5° C.

**RESULTS AND DISCUSSION.** The percentages of each species surviving to pupation in the various treatments are presented in Table 1. These results show some interesting trends in interaction between species that could, but usually do not occur together. Analysis of *C. pipiens* mortality using the binomial population technique of Li (1957) is presented in Table 2. This shows a highly significant difference between treatments, and a significant difference in replicates within

TABLE 1.—Original population composition of *Culex pipiens* L. and *Aedes aegypti* (L.) and % of each species reaching pupation. 100 larvae per replicate.

Original population		% of each species reaching pupation	
<i>C. pipiens</i>	<i>A. aegypti</i>	<i>C. pipiens</i>	<i>A. aegypti</i>
100	0	66	..
100	0	83	..
100	0	81	..
75	25	55	82
75	25	55	86
75	25	60	100
50	50	26	90
50	50	46	90
50	50	32	90
25	75	24	96
25	75	24	100
25	75	32	95
0	100	..	100
0	100	..	97
0	100	..	93

treatments. The same type of analysis on mortality of *A. aegypti* shows no significant difference between treatments or between replicates within treatments (Table 3). Therefore, although the presence of *A. aegypti* significantly affects *C. pipiens* mortality at the population level and compositions tested, the presence of *C. pipiens* at this level has no statistically significant effect on *A. aegypti* mortality.

Figure 1 presents the regression lines and equations for *A. aegypti* and *C. pipiens* survival. It shows the quantitative effects that the percent of one species (abscissa) in the population has on survival of the other species (ordinate) in that population. Inconsistency of slope sign is due to the mathematical methods used to simplify computations. Regression analysis showed the relationship between percentage sur-

TABLE 2.—Analysis of variance of *Culex pipiens* L. in mixed populations.

Source	df	S.S.	M.S.	F <sub>(c)</sub>
Total	734	178.09	.....	.....
Treatment	3	24.40	8.13	17.79*
Rep: Trt.	8	3.66	0.457	2.20**
Error	723	150.03	0.208	.....

\* Significant at the 1% level.

\*\* Significant at the 5% level.

vival of one species and percentage of the other species present to be strictly linear.

This experiment demonstrates the classic and widely accepted definition of inter-specific competition in which both species are inhibited when in contact as compared to their biologies when isolated (Haskell,

1949). Although *A. aegypti* affects *C. pipiens* survival much more, *C. pipiens* does have an effect, though statistically insignificant, on *A. aegypti* survival as is seen in Figure 1.

Differential survival is not the only criterion we might have looked for in this

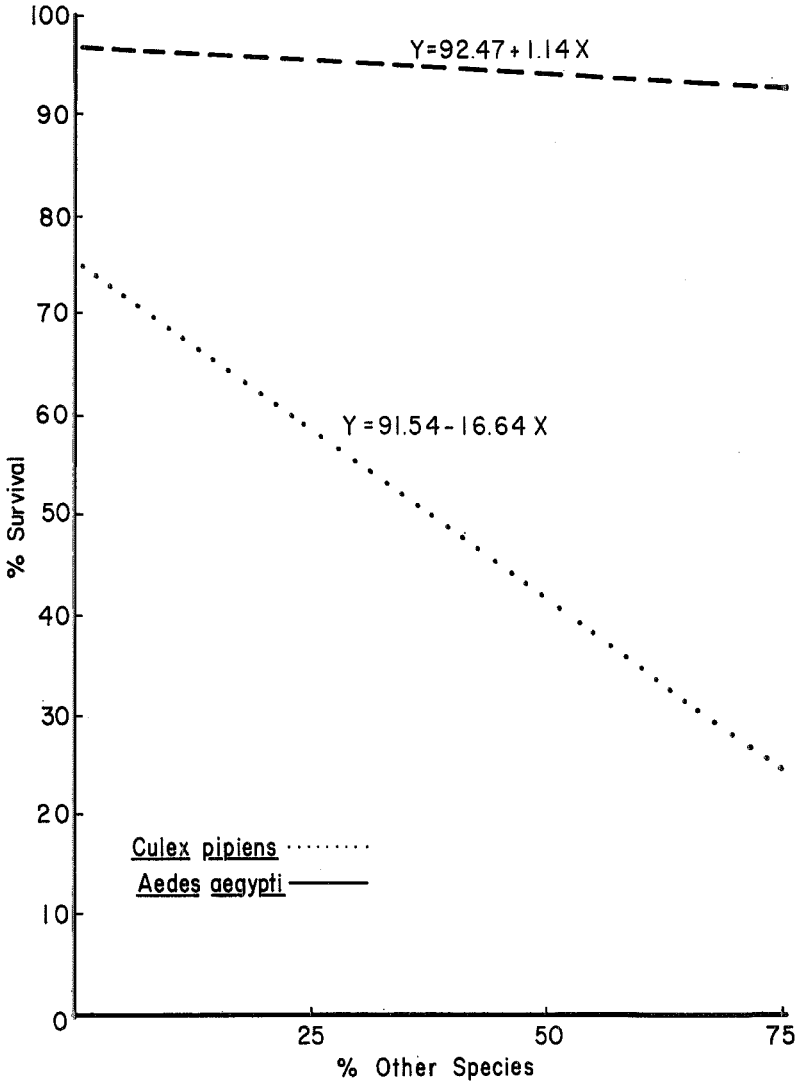


FIG. 1.—Larval survival as affected by the interaction between *Culex pipiens* L. and *Aedes aegypti* (L.) when reared at various population compositions.

TABLE 3.—Analysis of variance of *Aedes aegypti* (L.) in mixed populations.

Source	df	S.S.	M.S.	F <sub>(α)</sub>
Total	754	29.73	.....	.....
Treatment	3	0.54	0.180	0.031
Rep: Trt.	8	4.64	0.580	1.76
Error	743	24.55	0.033	.....

experiment. Others would be changes in the rate of development, ultimate size or weight/individual and a size or weight related characteristic, fecundity. The only criterion, other than survival, that we examined was rate of development as affected by population composition. Analysis of the data gathered at 6-hour intervals over the duration of the experiment showed no significant differences in developmental rates for either species regardless of the population composition. This may have resulted from the intervals selected by the authors since Christophers (1960) and Haddow *et al.* (1959) have shown that hourly observations are desirable in studying developmental rates in mosquito larvae.

Experiments in our laboratory subsequent to this experiment and to be reported in the future, indicate that the feeding regime provided food in great excess. This usually leads to pollution and increased osmotic pressure; factors that could differentially affect the survival of *A. aegypti* and *C. pipiens*. Osmotic pressure as determined by the freezing point depression technique showed less than 0.01 percent depression when compared to distilled water. This depression is well under the 1.5 percent sodium chloride equivalent reported as adversely affecting development (Wigglesworth, 1938). Another aspect of the pollution problem is that if pollution were a factor, *C. pipiens* would have shown proportionately greater mortality in the treatments with greater percentages of that species. Since it consumed food and used energy at a slower rate than the rapidly developing *A. aegypti*,

there would have been more food present in those treatments with a high proportion of *C. pipiens* and therefore a greater chance for pollution. However, Singh and Micks (1957) report that *C. pipiens* tend to occur more commonly in polluted water than *A. aegypti*.

*C. pipiens* is a relatively inactive larva rarely moving unless violently disturbed. *A. aegypti* is easily disturbed as a larva and aggregates in large groups probably due to a strong negative phototaxis. The general increase in mortality of *C. pipiens* as it becomes a smaller part of the population may be due to physical interference by the continually active *A. aegypti*. The interference seemingly does not bother *A. aegypti* at the population level tested. Another possibility is that *A. aegypti* produce metabolites which do not affect their own mortality at the population level tested, but do affect mortality of others, at least *C. pipiens*, when in the environment.

The adverse effect of *Aedes aegypti* (L.) larvae on those of *Culex pipiens* L. seems to be established, but the exact mechanism, whether physical or chemical, has not been demonstrated.

#### References

- CHRISTOPHERS, S. R. 1960. *Aedes aegypti* (L.). The yellow fever mosquito. Its life history, bionomics and structure. Cambridge Univ. Press, London 739 p.
- HADDOW, A. J., GILLETT, J. D., and CORBET, P. S. 1959. Laboratory observations on pupation and emergence in the mosquito *Aedes (Stegomyia) aegypti* (L.). Ann. Trop. Med. Parasitol. 53:123-131.
- HASKELL, E. F. 1949. A clarification of social science. Main Currents Mod. Thought 7:45-51.
- LI, J. C. R. 1957. Introduction to statistical inference. Edwards Brothers, Inc. Ann Arbor, Michigan. pp. 390-431.
- SINGH, K. R. P., and MICKS, D. W. 1957. The effects of surface tension on mosquito development. Mos. News 17:70-73.
- WIGGLESWORTH, V. B. 1938. The regulation of the osmotic pressure and chloride concentration in the hemolymph of mosquito larvae. J. Exp. Bio. 15:235-247.