AGAR GEL IMMUNODIFFUSION AND IMMUNOELECTROPHORESIS STUDIES WITH THE EXTRACTS OF MALE AND FEMALE ADULT *CULEX TARSALIS*[1]

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Antigenic relationships of various species of mosquitoes and antigenic changes during mosquito metamorphosis have been studied by the technique of the agar gel immuno-diffusion (Downe, 1963; Fox, *et al.*, 1963; Zawan, *et al.*, 1963, 1964, 1965; Smith and Silverman, 1966). This paper presents the results showing immunological differences of the two sexes of adult *Culex tarsalis*.

MATERIALS AND METHODS. Two-week-old adult male and female *Culex tarsalis* were obtained from a laboratory colony. They had been fed on apple and were subsequently starved for 24 hours before being homogenized in normal saline containing 0.01 percent merthiolate. The homogenates were centrifuged and the supernatants were kept frozen at —20° C. When needed, clear extracts were obtained by centrifuging the thawed supernatants.

Immune sera were prepared in 6 rabbits and 6 pigeons. Three animals of each kind in duplicate were injected respectively each time with a 2 ml extract of: (1) 100 females (2) 134 males and (3) a mixture of 50 females and 67 males. More males than females were used because of the difference in body weight; this averaged 1.55 mg for the female and 1.17 mg for the male. The extract was mixed with Freund's complete adjuvant at the ratio of 1:1 and injected into each animal in 3 injections at weekly intervals. The rabbits were injected subcutaneously in the neck region and the pigeons received a combination of intramuscular and subcutaneous injections in the breast. Immune sera were collected on the 10th day after the last injection. They were kept at —20° C. in small portions and used without pooling. The same concentrations of the 2 ml extracts were used as reacting antigens on agar slides.

One percent ion-agar was made up in normal saline for immunodiffusion. For reactions of pigeon antisera, agar in 8 percent saline was also used. In either case, the agar solution was buffered with sodium borate at pH 7.8 plus 0.01 percent sodium merthiolate. Approximately 8.5 ml. molten agar was poured on to a glass plate of 5.0 x 7.5 cm short of overflow. A plastic template 5 mm thick with holes of 2 mm in diameter set 10 mm apart was placed on the plate after the agar had solidified. Arranged in various neighboring positions, the antisera and mosquito extracts were transferred into the wells by fine pipets. The plates were kept in a moist chamber at 23° C., and the development of the precipitate lines was followed for 5 days. Rabbit antisera were used for absorption tests.

Electrophoresis of mosquito extracts was carried out in a normal saline-sodium barbital system at pH 8.2. Each agar slide (4 ml of merthiolated 1 percent Ion-agar in saline-barbital to a 2.5 x 7.5 cm microscope slide) was subjected to 5 milliamperes for 1.25 hours. Only rabbit antisera were used for immunodiffusion reactions after electrophoresis.

RESULTS. The results of immunodiffusion of the mosquito extracts against the rabbit and pigeon antisera are summarized in Table 1. Some of the precipitation reactions of rabbit antisera are shown in Figures 1 and 2. Pigeon antisera elicited fewer lines. These appeared only in 8 percent saline agar. They were also very faint and diffuse.

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TABLE 1.—Number of precipitate lines in agar gel resulting from immunodiffusion of *Culex tarsalis* extracts against antisera prepared in rabbits and pigeons. Number of lines of infrequent occurrences are in parenthesis.

C. tarsalis extracts	Rabbit antisera			Pigeon antisera		
	Anti-m	Anti-f	Anti-m+f	Anti-m	Anti-f	Anti-m+f
Male	1–(2)	2–3 (4)	2–3 (4)	1–(2)	2–(3)	2–(3)
Female	1–2	3–5 (6, 7)	3–5 (6, 7)	1–(2)	2–3	2–3
M+F	1–2	3–5 (6, 7)	3–5 (6, 7)	1–(2)	2–3	2–3

In the reactions of the male extracts against homologous antisera, usually one sharp line of precipitation was formed. This line was sometimes accompanied by a second faint one. However, when the reactions were carried out against either of the two heterologous antisera, two or more lines appeared. In the reactions of the female extracts against homologous antisera or against the antisera obtained from animals which had received injections of mixed male and female extracts, a greater number of precipitate lines were produced.

The reactivity of the mixed extract was about the same as with that of the female extract alone. Fewer lines developed in the reactions of all the three extracts against the anti-male antisera than against the anti-female or anti-male and female antisera.

In absorption tests all the precipitable components of the anti-male sera, but not all those of the anti-female were absorbed by the reciprocal mosquito extract (Table 2). Evidently the female mosquito had more of the precipitating antigenic components than did the male. This conclusion is also supported by the results of immunoelectrophoresis shown in Figure 3.

TABLE 2.—Absorption tests on rabbit anti-mosquito sera.

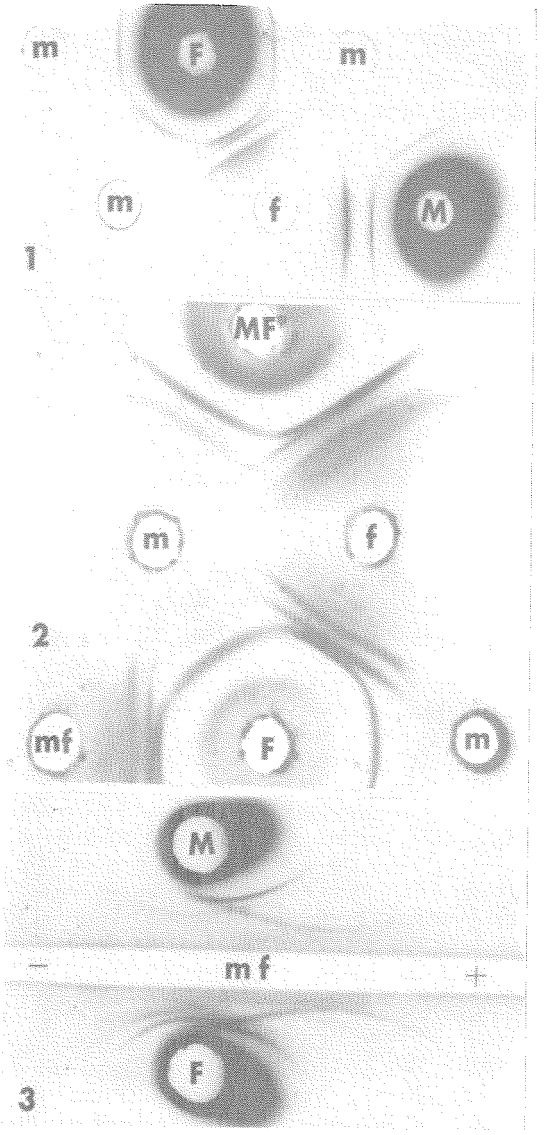
Rabbit antisera	Absorbing extracts	Testing extracts	Results
Anti-f	Male	Female	+
Anti-m	Female	Male	—
Anti-m+f	Male	Female	+
Anti-m+f	Female	Male	—

DISCUSSION. The lack of precipitation reaction of pigeon antiserum in our test system is in accord with the result of Wolfe and Dilks, 1949. They showed that the pigeon does not produce detectable precipitins against beef serum.

One explanation for the appearance of one or more extra precipitate lines in the heterologous reactions of the male mosquito extract is that certain male antigens may be haptenes, which are also parts of some of the complete antigens in the female. These haptenes react with the anti-female antibodies having sites complementary to those of the same haptenes and therefore precipitate out as extra lines. This hypothesis may also explain the lack of reactivity of the anti-male antisera in which no precipitable antibodies corresponding to the presumed haptenes are present.

The females were supposedly nulliparous or postparous because they had not been fed with blood, and those autogenous ones had already laid eggs before the end of the 2 weeks period when they were used. However, ovarian activities, the possible presence of some residual eggs or possibly other factors such as the salivary glands secretions of the female mosquito (Metcalf, 1945), may have contributed to the immunological difference of the 2 sexes of *C. tarsalis*.

The results of this study and the explanations disagree with those of Smith and Silverman, 1966. They found in *Anopheles* and *Aedes* a lack of reactivity from adult female antisera as compared to that of adult male antisera and attributed this difference to the "production of proteinaceous materials in the female asso-



FIGS. 1 and 2.—Immunodiffusion reactions of adult *C. tarsalis* extracts against rabbit antisera. Extracts: M-male, F-female, and M+F —a mixture of male and female. Corresponding antisera: m, f, and m+f.

FIG. 3.—The immunoelectrophoresis pattern of adult *C. tarsalis* extracts developed against rabbit anti-m+f antiserum.

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.

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INTERACTIONS BETWEEN LARVAE OF *AEDES AEGYPTI* (L.) AND *CULEX PIPIENS* L. IN MIXED EXPERIMENTAL POPULATIONS[1]

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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

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