ARTICLES

ATTRACTION AND OVIPOSITION STIMULATION OF GRAVID FEMALE MOSQUITOES BY BACTERIA ISOLATED FROM HAY INFUSIONS[1]

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Much research into the manner by which culicine and aedine mosquitoes find the proper sites for oviposition has indicated that one or more responses may be chemically regulated, Dethier *et al.* (1960). In 1928, Demina and Nikolskii reported that breeding areas with a high calcium content were preferred by ovipositing females of *Anopheles maculipennis* Meigen. Bates (1940), who tested several *Anopheles* species, obtained similar results with calcium, and Woodhill (1941), Manefield (1951), and Wallis (1954) reported that salinity and odor influenced the oviposition of *Aedes aegypti* (L.). However, Lund (1942) could not obtain any consistent response by *Anopheles quadrimaculatus* Say to calcium, mud, algae, salinity, phosphorus, ammonia, tannic acid, pH, ferric chloride, aluminum chloride, or sucrose. Thomson (1940), studying the oviposition of *Anopheles minimus* Theobald, stated that this species was visually attracted to shade when ovipositing. Bates (1940) also found that dark background colors were preferred by anophelines.

O'Gower (1963) used infusions of horse manure and dark surfaces and reported that in *Aedes aegypti* tactile and visual responses were the most important stimuli for oviposition. Gjullin *et al.* (1965) tested *A. aegypti* and *Culex pipiens quinquefasciatus* Say on grass infusions and log pond water and obtained an increased oviposition in these solutions over that in distilled water; they also found that furfural and methane stimulated oviposition, but that the oviposition rate in these compounds did not compare with grass infusions or log pond water. *Culex tarsalis* Coquillett did not respond significantly to those materials. Their evidence indicated that log pond water and grass infusions contained a chemical agent that influenced oviposition, but it did not definitely confirm an orientation response. Ikeshoji (1966) reported that he attracted *Culex pipiens fatigans* to various types of mosquito-breeding water and ether extracts of breeding water. He got no significant difference in their response to tap water and breeding water when the mosquitoes were antennectomized, therefore, he concluded that the breeding waters contained some volatile attractive substance.

No mention has been made in the literature as to the origin of the chemicals in mosquito breeding water and plant infusions which elicit responses known as attraction and oviposition stimulation. Our experiments demonstrate that bacteria are responsible for the production of these chemicals.

Materials and Methods. Two types of experiments were used to test responses of gravid females to hay infusion and bacteria. One series was conducted in a 213.4 x 25.4 cm cage covered with plastic screening and placed in a room with a window that provided natural light. The test material was placed in one end of the cage and a distilled water control in the opposite end; the females were permitted to visit either dish. The dishes used to hold the solutions tested on *A. aegypti* were lined with filter papers as this species lays on a moist substrate unlike *C. quinquefasciatus* which lays on the water sur-

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face. One hundred gravid females of *Aedes aegypti* or *C. quinquefasciatus* were placed in the end of the cage containing the distilled water. The number of eggs or egg rafts deposited in the two dishes in each species tested was recorded each morning until the females stopped laying. Three replicate tests were made for each of the two mosquito species.

The basic equipment used for the second series of tests were three olfactometers of the type described by Gouck and Schreck (1965). *C. quinquefasciatus* was the only mosquito species tested in this series of experiments. In the olfactometer the gravid mosquitos responded to the odor of the test solutions by flying upwind to glass traps inserted in the end of the device. Inverted wire screen funnels in the traps prevented the mosquitos from reentering the main chamber of the olfactometer. The females entering the two traps were counted each morning, and the number of egg rafts laid in the two solutions was recorded. The room containing the olfactometers was in complete darkness from 4:30 p.m. to 8:00 a.m. each day. In this experiment, three replicates were tested with distilled water and hay infusion and three with bacteria in distilled water and distilled water only.

The hay infusion was prepared by blending 10 grams of finely ground alfalfa hay in 200 milliliters of distilled water in a blender for 30 seconds. After the mixture was ground, the liquid was filtered through cloth and placed in an incubator for 24 hours. One part of the infused homogenate was added to 10 parts of distilled water and tested. The bacterial suspension was obtained by streaking nutrient agar plates with a bacterial inoculation loop of the infused homogenate and incubating it for 24 hours. Then bacteria were washed from the agar plates with swabs and distilled water and used without further dilution. The primary bacteria species in these hay infusions was *Aerobacter aerogenes*.

Results and Discussion. In the first test, the response of *Aedes aegypti* and *C. pipiens quinquefasciatus* to the hay infusion and to the bacteria demonstrated the presence of an arrestant and/or oviposition stimulant (Table 1). *A. aegypti* deposited 69 percent of their eggs on the paper in the beakers containing the hay infusion; *C. pipiens quinquefasciatus* females deposited 93 percent of their egg rafts on the infusion. The bacterial suspension stimulated 95 percent of the females of *C. pipiens quinquefasciatus* to deposit egg rafts.

The test in the olfactometer (Table 2) demonstrated an orientation response to an attractant in the hay infusion and in the bacterial suspension. Of the gravid *C. pipiens quinquefasciatus* attracted to the traps, 66 percent favored the odors of hay infusion over distilled water, and 78 percent responded to the odor of the bacteria. Thus the bacteria itself contained an attractant. Most mosquitoes were attracted to these solutions during complete darkness so visual stimulation was not involved.

Table 1.—Selection of oviposition site by gravid females offered a choice between distilled water and hay infusion or bacteria in distilled water (100 females/test; percentage of total number of females ovipositing).

Species	% Eggs or rafts laid in distilled water	% Eggs or rafts laid in hay infusion	% Eggs or rafts laid in bacteria plus distilled water
Aedes aegypti	33	67	..
Aedes aegypti	34	66	..
Aedes aegypti	27	73	..
Culex quinquefasciatus	8	92	..
Culex quinquefasciatus	1	99	..
Culex quinquefasciatus	11	89	..
Culex quinquefasciatus	5	..	95

TABLE 2.—Attraction of gravid females of *Culex quinquefasciatus* to distilled water, hay infusion, and bacterial odors in an olfactometer (200 females/test; percentage of total number of individuals trapped).

Percent attracted to—		
Distilled water	Hay infusion	Bacteria in distilled water
33	67	..
36	64	..
32	68	..
23	..	77
24	..	76
19	..	82

The attraction of *C. pipiens quinquefasciatus* to bacteria in the olfactometer test illustrates the method by which this species is able to find the proper larval breeding site. Gravid floodwater mosquitos are no doubt attracted to their breeding places by odors from the chemicals of fermentation deposited on the plant litter and soil soon after the rain pools have dried and it also seems logical that tree hole species may be guided to their breeding sites by the odors from their peculiar larval habitats. These species were not tested as we do not have them in our insectary. *A. aegypti* were not attracted to the odors of hay infusion in the olfactometer though they were stimulated to oviposit in these solutions (Table 1). The bacteria, isolated from the hay infusion, were not tested on this mosquito.

Bacteria have also been shown to be important for mosquito growth by Atkin and Bacot (1917), Rozeboom (1935), Buddington (1941), and Hazard *et al.* (in press) and to trigger the mechanisms causing egg hatch in some *Aedes* and *Psorophora* species by Atkin and Bacot (1917), Thomas (1943), Gjullin *et al.* (1941), and others. This is of interest as apparently the eggs hatched because of a reduction in oxygen during the logarithmic division of the bacteria at a time when a food supply is available for the newly hatched larvae. It would appear, then, that bacteria are closly associated with mosquito development and also influence the behavior of certain mosquito species, especially that behavior associated with attraction and ovipositioning.

SUMMARY. Bacteria isolated from hay infusion produce chemicals which stimulate gravid *Aedes aegypti* (L.) and *Culex pipiens quinquefasciatus* Say to oviposit. Olfactory attraction by these compounds was also demonstrated by tests comparing hay infusions with suspensions of the bacteria in distilled water in an olfactometer.

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COMPARATIVE OVIPOSITION SELECTION PREFERENCE BY *AEDES DORSALIS* AND *AEDES NIGROMACULIS* FOR THREE INORGANIC SALTS IN THE LABORATORY [1]

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INTRODUCTION

It has been suggested that differences in larval sites of different species of mosquitoes are the result of indiscriminate laying of eggs and selective pressures exerted in the larval stage by specific combinations of environmental conditions. It is presently accepted by most workers that ovipositing mosquitoes do not lay their eggs indiscriminately and that restriction of larvae of a particular species to a given habitat is largely a result of selection preference by the ovipositing females.

Aedes nigromaculis (Ludlow) is much more restricted in its larval habitats in northern Utah than is *Aedes dorsalis* (Meigen). A strong correlation exists between the presence or absence of *A. nigromaculis* and *A. dorsalis* in a given habitat and the salinity of the soils on which the females of these two species oviposit (Petersen and Rees, 1966). In an attempt to determine just how inorganic salts affect the distribution of these two mosquito species, oviposition selection preference studies were conducted in the laboratory with *A. dorsalis* and *A. nigromaculis* females to determine their preferences for variations in inorganic salts and to determine the effects of inorganic salts upon the maturation and hatching of the eggs.

MATERIALS AND METHODS. Since *A. dorsalis* and *A. nigromaculis* resist colonization, biting females were collected in the field and transported to the laboratory. Approximately 150 to 160 engorged females were transferred to 18″ x 8″ x 8″ cages and held in an insectary for 3 days.

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