MALE PHEROMONES OF *CULEX QUINQUEFASCIATUS*, *C. TARSALIS* AND *C. PIPIENS* THAT ATTRACT FEMALES OF THESE SPECIES

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Chemicals released by the females of many species of insects have been shown to attract males of the same species. Also males have been shown to produce chemicals that sexually excite females of the same species. Jacobson (1965), in a recent review, lists over 200 species which produce odors that promote mating.

Observations on the male behavior of *Opifex fuscus* by Kirk (1923), *Deinocerites cancer* Theobald by Haeger and Phinezee (1959) and *Culiseta inornata* Williston by Rees and Onishe (1951) suggest that sex attractants may be produced by the female of these species. Kliewer *et al.*, (1966) demonstrated that a sex attractant is produced in the female *C. inornata*.

The purpose of the present paper is to report the presence of male pheromones in *Culex pipiens quinquefasciatus* Say, *C. tarsalis* Coquillett, and *C. pipiens pipiens*.

MATERIALS AND METHODS. Tests for

pheromones in the three species were made in the laboratory in plywood olfactomoters measuring 34 in. long, 20 in. high, and 16 in. wide with a pyramid type of funnel extending horizontally 1 ft. beyond the end of the box section. The forward end and the bottom of the box section were covered with 18-mesh screen. The olfactometer rested on a 2 in. deep, plastic lined tray containing water to help raise the humidity. The top of the box was covered with a removable section of clear plastic, and a 10 in. x 10 in. plywood door section on one side provided an additional opening for introducing and removing insects.

Two 3 x 10 in. glass cylinders equipped with screen funnels at one end and a screen closure at the other were supported against openings 9 in. apart at the back end of the olfactometer. Air movement through the cylinders at the rate of about 30–40ft./min. was provided by a suction fan through a pipe connected to the end of the pyramid funnel section. The air was exhausted outside the building.

Mosquitoes used in the olfactometer tests were reared in the laboratory. Fifteen to 20 hr. after emergence, the adults were immobilized on a cold table at 1–8° C., and, if necessary, the males were separated from the females for tests in which unmated females would be used. The adults used for the mosquito extract tests and for the tests of untreated dead mosquitoes were killed on the second day after emergence by holding them at a temperature of —30° C. for about 30 min. The extracts were made by macerating the adults in benzene. This material was then centrifuged, and the liquid portion was vaporized to dryness.

Where live insects were used, the 1-ft. cube screen cages were separately enclosed. Each had a small slot at the top and air tube connections on one side. These air tubes extended for 40 in. from the cages to the olfactometer trap cylinders. The extracts of dead insects were placed in the cylinder in small petri dish half sections and the dead insects were tested in small flat screen boxes after a screen divider had been inserted near the middle. Similar empty dishes and screen containers containing glass beads were placed in the control cylinder. The cylinders were cleaned daily. The test materials were alternated in the two cylinders after each test.

Mosquitoes that entered the cylinders through the screen funnels during the night were discarded after counting and were replaced by others from the same population. Originally 250 were used; the number was reduced to 150 in later tests.

External light was excluded from the test room; lights placed low at the funnel end of the olfactometer provided about 300 lux (28 ft. candles) at the center of the olfactometer box from 6 a.m. to 10 p.m. A supplemental 7-w "dusk light" was operated on a 40-v current from 10 to 11 p.m. and from 5 to 6 a.m. Temperature during the tests ranged from 20–27° C. and relative humidity from 45–80 percent.

RESULTS. *Culex quinquefasciatus.* Information on pheromone attraction between the sexes of *C. quinquefasciatus* was first obtained with live adults. In a series of tests in which 250 female *C. quinquefasciatus* had a choice of entering a trap cylinder leading to an empty control cage or to a cage containing 1,400 males, the ratio of females entering was 1.9 to 1 in favor of the cylinder leading to the males. Females in a second series of tests had a choice of entering a trap cylinder leading either to a cage containing 1,400 males or to one containing 1,400 females. In this series, the ratio of females trapped in the cylinders was also 1.9 to 1 in favor of the cylinder leading to the male cage.

Dead male *C. quinquefasciatus* and benzene extracts of males placed in the glass cylinders at the opposite end from the trap entrance were also attractive to unmated females. The cylinders containing the dead males collected 4.2 times and the benzene extract of males 1.82 times the number taken in the empty control traps.

Tests were also made of females that

had been confined in cages with males for 6 days. These females were referred to as "mated," but no check was made to determine the percentage mating that had occurred. In these tests, the cylinder containing the dead males attracted twice as many females as the empty control cylinder.

Statistically significant differences in attraction existed between test materials and controls in this series of tests (Table 1) except for one in which live males were tested against dead males. The dead males were not attractive to the live males. Also, attraction of females to males increased significantly with age in some of these tests and in subsequent tests with other species.

Culex tarsalis. The females of *Culex tarsalis* were also attracted to dead males in olfactometer tests. Attraction of 250 dead males placed in one cylinder was 1.2 times greater than the attraction of the empty trap cylinder. In tests with 1,000 and 1,500 dead males in the test cylinder, the attraction was 1.7 and 1.4 times greater, respectively, than that of the empty control cylinder. Males were attractive to females at significant levels when 1,000 and 1,500 males were used but not when 250 were used.

Benzene extracts of male *C. tarsalis* (not shown in tables) were about as attractive as dead males in the olfactometer tests. In 5-day duplicate tests with the extracts from 1,000 males, the cylinder containing the extract collected 1.5 times as many females as the empty control cylinder.

Culex pipiens. The female to male attraction of *Culex pipiens* was similar to that of the other two species. In tests with 3,000 dead males, the ratio of virgin females entering the trap cylinders was 1 to 1.6 in favor of the males, and the results were statistically significantly at the 1 percent level. The ratio was 1 to 1.9 in favor of the male baited cylinder when 1,500 dead males were used, and these results were significant at the 5 percent level. Mated females were also attracted to dead males but not at significant levels. The females used for the "mated" female tests had been caged with males for 4 days and were assumed to have mated, but they were not checked to determine whether mating had occurred.

Males of *Culex pipiens* were not attracted by dead females in another series of tests. The males preferred the empty control cylinder by a ratio of 1.4 to 1. Benzene extracts of females were also unattractive.

Tests of non-specific attraction. The females of *Culex pipiens, C. quinquefasciatus,* and *C. tarsalis* were attracted to the males of the other species. Female *C. pipiens* showed a low 1 to 1.2 ratio of attraction in favor of male *C. quinquefasciatus,* and females of *C. quinquefasciatus* showed a high ratio of 3 to 1 in favor of a trap containing male *C. tarsalis.* These results and those of other combinations are shown in Table 3. In all tests except that with *C. pipiens* females and *C. quinquefasciatus* males, the attraction was significant at the 1 percent or 5 percent levels.

DISCUSSION AND CONCLUSIONS. Earlier failures to show the presence of any attractants in the three *Culex* species caused us to make olfactometer tests overnight to avoid any cyclic or other effects that might cause the insects to be less responsive. The traps were baited from 4 p.m. until 8 a.m., but females did not begin to enter them until after 10 p.m. when the lights were turned off. No seasonal differences in the attraction of the females for the males was seen from July 1965, when the attraction was first noted, to August 1966.

Of the three species studied, *C. quinquefasciatus* showed the highest attraction of females to males. Populations of female *C. quinquefasciatus* and *C. pipiens* were not attracted to other female populations of their respective species. Groups of dead male *C. quinquefasciatus* were also unattractive to populations of males of this species. Although this attraction of females to the pheromone produced by the males of these species suggests that it is sex-related, no attempt has yet been made

TABLE 1.—Attraction of *Culex quinquefasciatus* in olfactometer tests as determined by the numbers of mosquitoes collected in overnight tests extending over 5–6 days (replicated 2–4 times).

No. and State of Insect (and age in days)	No. of Days Tested	No.[a] Attracted to indicated material (in parentheses)		Level of Significance[b]	
		Control Material	Test Material	Of Attraction	Of Age
		Females			
250 virgin ♀ ♀ (1–6)	6	124 (Empty container)	250 (1,400 live ♂ ♂)	5%	1%
250 virgin ♀ ♀ (1–6)	6	178 (1,400 live ♀ ♀)	342 (1,400 live ♂ ♂)	1%	1%
150 virgin ♀ ♀ (2–5)	5	159 (Empty container)	229 (Extract of 1,500 ♂ ♂)	1%	NS
250 virgin ♀ ♀ (2–5)	5	113 (Empty container)	476 (1,500 dead ♂ ♂)	5%	NS
150 mated ♀ ♀ [c] (6–11)	6	133 (Empty container)	273 (1,000 dead ♂ ♂)	1%	NS
		Males			
250 males (3–8)	6	64 (Empty container)	60 (1,500 dead ♂ ♂)		

[a] Includes all replicates.
[b] Within the test.
[c] Females had been caged with males for 6 days before test.

TABLE 2.—Attraction of female *Culex tarsalis* and female *C. pipiens* to males and of female *C. pipiens* to females as determined by the numbers of females collected in over-night olfactometer tests extending 4–7 days (replicated twice).

150 Females per Test[a]		No.[b] Attracted to Indicated Material (in Parentheses)		Level of Significance	
Age in Days	Days Tested	Control Material	Test Material	Of Attraction	Of Age[c]
Culex tarsalis					
2–8	7	146 (Empty container)	168 (250 dead ♂)	NS	5%
2–6	5	79 " "	138 (1,000 dead ♂)	1%	NS
2–8	7	214 " "	302 (1,500 dead ♂)	5%	NS
Culex pipiens					
2–9	8	322 " "	512 (3,000 dead ♂)	1%	1%
2–7	6	177 " "	332 (1,500 dead ♂)	1%	5%
6–9[d]	4	59 " "	109 (1,000 dead ♂)	NS	NS
2–6	5	186 " "	132 (1,000 dead ♂)	NS	NS
2–6	5	184 " "	176 (extract 1,000 ♀)	NS	NS

[a] All virgins except those indicated under [d].
[b] Includes all replicates.
[c] Within the test.
[d] Females had been caged with males for 4 days before the test.

TABLE 3.—The attraction of the females of three species of *Culex* to the males of other species. Figures show the number of females collected in over-night tests extending over periods of 4 to 5 days that were replicated from 4 to 6 times.

150 Virgin Females per Test		No.[a] Attracted to Indicated Material (in Parentheses)		Level of Significance	
Age in Days	Days Tested	Control Material	Test Material	Of Attraction	Of Age[b]
C. quinquefasciatus					
2–5	4	204 (Empty container)	432 (dead *C. pipiens* ♂)	5%	5%
2–6	5	94 " "	285 (dead *C. tarsalis* ♂)	1%	1%
C. pipiens					
2–6	5	296 " "	381 (dead *C. tarsalis* ♂)	5%	NS
2–5	4	331 " "	451 (dead *C. quinquefasciatus* ♂)	NS	NS
C. tarsalis					
2–6	4	202 " "	404 (dead *C. quinquefasciatus* ♂)	5%	NS
2–5	4	224 " "	368 (dead *C. pipiens* ♂)	5%	NS

[a] Includes all replicates.
[b] Within the test.

to provide supplemental information on mating behavior or mating.

More than 250 male *C. tarsalis* were necessary to attract any significant numbers of females in these tests. In nature, such numbers might occur in mating swarms or where large numbers of newly emerged males may be resting. One may also speculate that small amounts of the pheromone may have an effect when the males and females were nearer each other than they were in our tests. The attraction of females of each of the three species to males of their own species and to males of the other two species suggests that all males in this group may have chemicals that release similar or closely related odors.

The proportion of females attracted to

males or male extracts differed significantly at the 5 percent or 1 percent level from the numbers attracted to the controls in all but three of the test series. The three low significance levels had several causes. In one test, the number of male *C. tarsalis* (250) was apparently too low; in the second with *C. pipiens,* the females were not virgin; in the third, virgin female *C. pipiens* showed only a low degree of interest in male *C. quinquefasciatus.*

The females of the three *Culex* species showed an increased attraction to the males as they grew older. All females used in the olfactometers were from the same population and were the same age on each succeeding day. The increased attraction of the females to the males occurred when the females were from 2 to the maximum of 7 days old.

Benzene was used in the limited number of extract tests made of males. However, *n*-hexane and chloroform were also effective for this purpose.

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MANATEES AS A NATURALISTIC BIOLOGICAL MOSQUITO CONTROL METHOD

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John Esquemeling, a Dutch adventurer who accompanied the English buccaneer Sir Henry Morgan on the sacking of Old Panama and who later published memoirs in his book "The Buccaneers of America" in 1678, included a full page discourse about the manatee. The sea travelers of Christopher Columbus days found that the flesh of the manatee was very good to eat and whenever the opportunity arose, they welcomed a change from their hard biscuit and salt pork diet. One of the areas the sea travelers frequented for manatee provisions was a place called Boca del Dragon which we know today as one of the inland harbors of Bocas del Toro, the northwest province of the Republic of Panama on the Caribbean Sea. According to Esquemeling, the Spaniards called them "manetines" while the Dutch called them "sea-cows" because they had a head, nose and teeth similar to that of a cow. Their flesh was the color of a land cow but the taste was like that of pork. The flesh contained a large amount of fat which the buccaneers melted and retained in earthen pots for use as grease. The manatees were reported from 20 to 24 feet in length.

In 1961, nearly 300 years later, the Division of Sanitation, Health Bureau, Canal Zone Government, became interested in

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