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MATING BEHAVIOR OF WILD *ANASTREPHA*
FRATERCULUS (DIPTERA: TEPHRITIDAE) ON A
CAGED HOST TREE

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ABSTRACT

The mating behavior of the South American fruit fly, *Anastrepha fraterculus* (Wiedemann) is described, following release of wild virgin flies onto a field-caged host tree (guava). Sexual encounters and attempted copulations occurred almost exclusively from 0700-0900 hours and were confined to leaf nodes or the bottom surface of leaves, where males stationed themselves, often forming leks, and appeared to emit a sex pheromone which was attractive to virgin females. There were no male visitations or sexual encounters on green guava fruit, even though females, after mating, frequently oviposited there.

RESUMEN

En este trabajo se describe el comportamiento sexual de la mosca de frutas suramericana, *Anastrepha fraterculus* (Wiedemann), en el árbol de guayaba, el cual fue cubierto por una tela para confinar las moscas virgenes. Los encuentros sexuales y tentativas de copulación ocurrieron casi exclusivamente entre 0700-0900 horas, principalmente en los peciolos en la face in-

ferior de las hojas donde los machos frecuentemente forman grupos (leks) que aparentemente emiten feromona sexual la cual atrae las hembras virgenes. Entretanto, machos y encuentros sexuales no fueron observados en las guayabas verdes aunque se hayan observado hembras haciendo oviposición despues de la cópula.

Anastrepha fraterculus (Wiedemann) a widely-distributed tephritid species whose range extends from the Rio Grande Valley in Texas to Argentina and Chile, is a serious pest of many tropical and subtropical fruits (Malavasi et al. 1980). The damage is caused by the larvae, which feed in the fleshy parts of fruit rendering it unfit for human consumption.

In several tephritids, visual, auditory, and chemical stimuli are important in sexual attraction and recognition. With respect to *Anastrepha* flies, Nation (1972) described for *A. suspensa* (Loew) the courtship behavior and evidence for a male-released sex pheromone that appears to be released during distention of the abdominal pleural region and anal pouch, accompanied by rapid wing fanning. It has been identified by Nation (1975) as a blend of 2 alcohols and 2 lactone esters. Male *A. suspensa* also produce distinct sounds through wing fanning which appear to be sexually stimulating to the females (Sharp and Webb 1977, Webb and Sharp 1976).

Studies of the mating behavior of tephritid flies under semi-natural or natural conditions have been reviewed by Fletcher (1977), Prokopy (1977, 1980), and Burke (1981). Recently, we described the distribution and activities of *A. fraterculus* flies on host and non-host trees in nature (Malavasi et al. 1983). In that study, we observed 26 encounters between males and females, 2 of which resulted in attempted copulation. Here, we present evidence suggesting the existence of a sex pheromone in *A. fraterculus* and report mating behavior of wild flies released onto a field-caged guava tree, a major host of this species.

MATERIALS AND METHODS

All of the flies used in all tests emerged from pupae obtained from infested guavas collected in Itaquera, 30 km away from São Paulo. Six hours after eclosion, the sexes were segregated and maintained in laboratory cages at 25°C and 14 h light:10 h darkness. The flies were provided with water and a mixture of 3 parts sucrose:1 part enzymatic yeast hydrolysate.

To gain information on whether *A. fraterculus* males release a sex pheromone, we used an olfactometer similar to that of Kobayashi et al. (1978). For each of the 21 replicates, 50 virgin females (30-40 days old) were placed in the large (30 x 30 x 30 cm) fly-holding chamber of the olfactometer. From 0800-1000 h, the females were assayed for response to the test cage containing either 10 males or no flies (control). The number of females attracted to each test cage after 2 hours was compared by the paired t statistical test.

All tests were conducted in a single field cage (3 m diam, 2.7 m tall) placed over a naturally-growing guava tree, *Psidium guajava* L. (1.2 m diam, 2.4 m tall), shaded by an overstory of *Tibouchina* trees. The guava tree had 740 leaves but no naturally growing fruit. Each test day 25 green, freshly-picked washed guava fruits (2-3 cm diam) were hung by wire from the guava tree. These green fruit were readily accepted as oviposition sites. We

also hung 4 yellow (ripe), freshly-picked guava fruits (3-4 cm diam), readily accepted as adult fly feeding sites. Flies (20-30 days old) were placed in a laboratory room with the natural photoperiod (0600-1900 h) at 23°C three days before release, and were released shortly after dusk (1800 h) into the cage. Release was accomplished by careful transfer of each fly from a laboratory cage to the bottom surface of a leaf, using a small piece of paper under the foretarsi as an aid. Thirty flies of each sex were released for each of the 5 replicates. The duration of each test was one day. All of the flies from a previous replicate were removed from the cage prior to the next replicate. Observations began at dawn (ca. 0600 h) and ended at dusk (ca. 1800 h). Our presence in the cage had no discernible effect on the flies.

Once per hour from 0600 to 1800 h, we took a census of the entire tree and cage wall and ceiling for ca. 15 min, recording the location and activity of each fly observed. During the interval between each hourly census, we charted the activities of randomly selected flies for up to 5 min each.

RESULTS

LABORATORY EVIDENCE FOR SEX PHEROMONE: In each test, a mean of 7.8 virgin females were attracted toward a test cage containing males while a mean of 2.5 virgin females were attracted toward an empty test cage ($t = 2.16$; $P < 0.05$).

FIELD CAGE ACTIVITIES:

Location of flies. During the entire day, more males and females, and considerably more mating pairs, were located on the guava tree than on the cage wall or ceiling (Table 1). Of those on the tree, the majority of non-copulating individuals were located on the bottom surface of leaves. Some females were on the top surface of leaves, green fruit (where ovipositions were frequent), and ripe fruit, with none on branches or leaf nodes. Some males were on ripe fruit and leaf nodes, with few or none on other structures. All males observed on leaf nodes were "calling". All mating pairs were on the bottom surface of leaves, although copulation may have been initiated elsewhere.

Location of encounters. The majority of male:female and male:male encounters, attempted copulations, and successful copulations occurred on leaf nodes (Table 2). A few male:mating-pair encounters occurred on the bottom surface of leaves. No female:female or female:mating-pair encounters were observed.

Time of copulation initiation. Copulation was initiated almost exclusively between 0700 and 0900 hours, with very little sexual activity before or after this period (Table 3).

Courtship behavior. All flies spent the night on the bottom surface of leaves or on the cage wall or ceiling. Male "calling" began when the temperature reached ca. 18°C and light intensity ca. 800 lux (i.e. at ca. 0700 h each day at summer), and ceased at ca. 1030 h. The predominant sites of calling coincided with the predominant sites of encounters: leaf nodes and the bottom surface of leaves. A calling male usually spent ca. 30 min at the same site before leaving. Calling males appeared to form leks consisting of 3-8 individuals, with each male located on a separate leaf node or leaf and

TABLE 1. CENSUS OF THE LOCATION OF MALES, FEMALES, AND MATING PAIRS OF *Anastrepha fraterculus* ON A FIELD-CAGED GUAVA TREE. COMBINED DATA OVER ALL CENSUS PERIODS FROM 0600 H (DAWN) TO 1800 H (DUSK).

Sex	Total no. observed on tree	% of total number at each location on tree						Total no. observed on cage wall and ceiling
		Leaves		Fruits		Leaf Nodes	Branches	
		Bottom surface	Top surface	Green	Ripe			
♂	500	71 (0) ^a	2 (0)	0 (0)	15 (0)	11 (93)	1 (100)	282 (11)
♀	428	74	4	11 (72) ^c	11	0	0	302
mating pairs	106	100 ^b	0	0	0	0	0	19

^a() % which were "calling."

^bFor site of copulation initiation, see Table 3.

^c% which were ovipositing.

TABLE 2. LOCATION AND OUTCOME OF OBSERVED ENCOUNTERS AMONG *Anastrepha fraterculus* ON A FIELD-CAGED GUAVA TREE. COMBINED DATA OVER ALL CENSUS PERIODS FROM 0600 H (DAWN) TO 1800 H (DUSK).

	Leaf nodes			Bottom surface of leaves			Other		
	♂:♀	♂:♂	♂:MP ^a	♂:♀	♂:♂	♂:MP	♂:♀	♂:♂	♂:MP
No. encounters	36	28	0	13	15	7	4	0	0
No. attempted copulations	31	28	0	6	9	5	3	0	0
No. successful copulations	31	—	0	3	—	—	3	—	—

^aMP = Mating pair

TABLE 3. TIME OF COPULATION INITIATION OF *A. fraterculus* ON A FIELD-CAGED GUAVA TREE.

Time	Leaf Nodes	Bottom surface of leaves	Branches	Cage wall	Total
0600-0700	1	0	0	0	1
0700-0800	16	2	1	2	21
0800-0900	6	3	3	1	13
0900-1000	1	1	0	0	2
1000-1800	0	0	0	0	0

all within ca. 50 cm of one another. Leks were particularly pronounced in the parts of the tree receiving the greatest sunlight. The same was true of lek formation on a non-host tree in nature (Malavasi et al. 1983).

"Calling" in *A. fraterculus* was observed to be similar to that described for *A. suspensa* by Nation (1972). During this behavior the abdominal pleural region and anal pouch are distended, with both wings at right angles to the body axis and the coastal edges oriented upward. Periodically, the wings are vibrated rapidly (fanned) for ca. 5 sec.

The male was the resident in all 36 observed intersexual encounters. In most of these, the male was in the process of calling when the female arrived. Typically, the arrival of a female in flight occurred in 2 stages. First, the female landed within ca. 15 cm of the male, and then walked toward the stationary male, approaching frontally. The male and female continued to face each other at a distance of ca. 2 cm, and remained so for ca. 3 sec. Then the male leaped over the head of the female and onto her abdomen. The copulating pair immediately proceeded to walk to the bottom surface of a leaf. Of 29 observed mating pairs, 7 remained in copula for less than 1 h, 7 for 1-2 h, 12 for 2-3 h, and 3 for more than 3 h. As illustrated by the data in Table 2, calling males encountered and attempted copulation with other males just as frequently as with females, suggesting little or no ability of males to discriminate between the sexes prior to contact.

DISCUSSION

Although female attraction toward males may have been partly on the basis of male visual or auditory stimuli, we believe male-released sex pheromone during the process of "calling" was the principal stimulus.

This study shows that copulation in *A. fraterculus* on a caged host (guava) tree was initiated almost exclusively on leaf nodes on the bottom surface of leaves. It was the female which invariably flew toward a somewhat stationary male, rather than the male pursuing a female. Our evidence strongly suggests that stationary males on leaf nodes emit a sex pheromone attractive to virgin females. Leaf nodes and the bottom surface of leaves are the major sites of calling by male *A. fraterculus* on uncaged trees in nature (Malavasi et al. 1983). As in other non-temperate tephritids (e.g. Nation 1972, Feron 1962) the principal strategy employed by an *A. suspensa* male to attract a female appeared to be a release of sex pheromone coupled

with possible sound produced by wing vibration. The fact that calling and mating initiation were confined almost exclusively to the hours of 0700-0900 may be important in pre-mating reproductive isolation between different *Anastrepha* species simultaneously utilizing the same plant. For example, *Anastrepha ludens* (Loew) and *A. suspensa* are known to mate in late afternoon or early evening (Baker et al. 1944, Perdomo et al. 1975).

No inter or intrasexual encounters among *A. fraterculus* were observed on green fruit, even though already-mated females were frequently observed there attempting oviposition. This is in strong contrast to the situation in *Rhagoletis* flies, where males often attempt to force-mate already mated, ovipositing females on fruit (Prokopy 1980). The total absence of *A. fraterculus* males from green fruit suggests that the oviposition deterring pheromone deposited by the female on fruit after egg-laying (Prokopy et al. 1982) has no arrestant effect on the males.

This study, together with that of Malavasi et al. (1983), provides behavioral-ecological evidence useful in elucidating patterns of divergence between *A. fraterculus* and other *Anastrepha* species.

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SYSTEMATICS OF ASIOPSOCIDAE (PSOCOPTERA)
INCLUDING *PRNOTIOPSOCUS AMAZONICUS*
N. GEN. N. SP.

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ABSTRACT

Pronotiopsocus n. gen. is described with *P. amazonicus* n. sp. as the type species. The new genus is placed in family Asiopsocidae together with *Asiopsocus* Günther and *Notiopsocus* Banks. The 3 genera are diagnosed. Their probable plesiomorphous characters are derived by outgroup comparison with superfamily Caecilioidea. These characters are taken to represent a hypothetical common ancestor of the Asiopsocid genera, which is used to reconstruct the phylogeny of these genera. *Pronotiopsocus* represents one line of the phylogeny with *Notiopsocus* and *Asiopsocus* representing a sister lineage. Geographical distributions suggest great antiquity with a Pangaeian origin of the family. The furcation of the lineage bearing *Notiopsocus* and *Asiopsocus* may have coincided with the initial breakup of Pangaea. Absence of *Notiopsocus* from India, Madagascar, and Australia may be due to insufficient sampling of these regions or to the timing of origin and spread of the genus relative to the breakup of Gondwanaland.

RESUMEN

Pronotiopsocus gen. n. se describe con el tipo *P. amazonicus* sp. n. El nuevo género se coloca en la familia Asiopsocidae, junto con *Asiopsocus* Günther y *Notiopsocus* Banks. Los 3 géneros se diagnostican. Sus caracteres plesiomorfos probables se derivan por comparación con la superfamilia Caecilioidea. Estos caracteres se toman como representantes de un antepasado común hipotético de los géneros asiopsócidos, empleado para la reconstrucción de la filogenia de éstos géneros. *Pronotiopsocus* representa una línea de la filogenia con *Notiopsocus* y *Asiopsocus* representando una línea-