

AN AGGREGATION PHEROMONE MODULATES LEKKING BEHAVIOR IN THE VECTOR MOSQUITO *Aedes aegypti* (DIPTERA: CULICIDAE)

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1 **ABSTRACT.** Males of *Aedes aegypti* mosquitoes formed swarms in the laboratory, triggered by the onset of the photophase or by the presence of odors from a rat (which is a potential host for females). The swarm attracted both males and females and increased mating activity. The number of copulas per mosquito was positively correlated with the number of mosquitoes in the swarm and with the duration of the swarm. Swarming and mating activity increased with the presence of a host for females. Young sexually immature males, less than 24 h old, flew but did not swarm nor copulate. Observations using an olfactometer showed that swarming males produced a volatile pheromone that stimulates the flying activity of females a distance. Females also produce a volatile attractant. The results suggest that males, and possibly also females, produce an aggregation pheromone that attracts males and females towards the swarm. The characteristics of the pheromone-mediated swarm may be described as a 3-dimensional lek. Our results suggest that the development of pheromone-based control systems and/or pheromone traps for the monitoring of vector populations is feasible, adding a new tool to combat of this vector of several human pathogens.

KEY WORDS Lek, mosquito, swarm, aggregation pheromone, sexual behavior

INTRODUCTION

Many culicid dipterans mate in swarms. These swarms are formed by tens to thousands of individuals of flying male insects, and Clements (1999) summarized their characteristics as follows:

1. The individual members of a swarm fly in loops or zigzagging, within a limited space, located in relation to some feature of the environment.
2. The swarms consist predominantly of males. When conspecific females enter a swarm, males approach and attempt to couple with them.
3. Swarming is limited to certain times of the day characteristic of each species, usually close to dusk and dawn.

The great majority of researchers consider mosquito swarms a means to facilitate mating (Downes 1969, Reisen and Aslamkhan 1976, Baker et al. 1980, Sullivan 1981, Bock et al. 1983, Yuval et al. 1993, Clements 1999). In this regard they are similar to leks, which have been described for a variety of animal species, including some dipterans (Shelly 1987; Sivinsky 1989; Whittier et al. 1992; Kelly and Dye 1997). Although very little experimental evidence exists regarding the mechanisms of swarm formation and attraction to it, visual markers in the landscape have been proposed as essential (Blickle 1959, Downes 1958, 1969, Yuval 2006). Visual markers may modulate the location of swarms, but as known visual marks are fixed in

space and time, they cannot explain the initiation of swarming. In addition, many type of swarms do not use visual markers (Clements 1999).

Other authors have suggested that pheromones could mediate swarm formation in some species of Diptera (see, for example, Edwards 1920; Savolainen and Syrjämäki 1971). The use of auditory signals to attract females may be discarded in the case of *Aedes aegypti*, and probably for all Culicidae, because females do not possess functional Johnston organs, as males do. These organs perceive sounds produced by the wing beat of females and stimulate males to copulate (Roth 1948). However, these auditory signals are not used for the formation and maintenance of swarms (Downes 1969), as males of *Aedes aegypti* are practically deaf to their own wing beat (Tischner and Schief 1955, Keppler 1958) as is the case for other Culicidae (Tischner 1953).

Although hard experimental evidence is lacking for the role of chemicals or pheromones in modulating sexual behavior in Culicidae, the involvement of chemicals in these behaviors, particularly in sexual recognition through contact pheromones, has been suggested for *Deinocerites cancer* (Downes 1966, Provost and Haeger 1967, Conner and Itagaki 1984), *Aedes albopictus* (Nijhout and Craig 1971), *Culiseta inornata* (Kliwer et al. 1966, Lang and Folster 1976), and some *Culex* species (Gjullin et al. 1967). **2** More recently, for *Anopheles gambiae* and *Aedes aegypti*, a role for cuticular hydrocarbons in courtship has been reported (Polerstock et al. 2002). More information about swarming and the underlying mechanisms might allow the design of alternative control methods for some of those

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culicids, which are important vectors of the disease agents that cause malaria, dengue, yellow fever, viral encephalitis, and other important tropical epidemic diseases.

Despite its importance as a vector of tropical diseases, very little is known about the swarming behavior of *Aedes aegypti*. Males do not form large swarms, as other Culicidae do, but are reported to mate near blood meal hosts. Males and females have overlapping daily flight activity (Lehane 1991, Clements 1999). However, small swarms of this species, in which mating occurs, have been observed near hosts (Goeldi 1905, McClelland 1959, Hartberg 1971, Gubler and Bhattacharya 1972). Similar swarms have been reported for other *Aedes* (*Stegomyia*) species, such as *Ae. pseudoscutellaris* (O'Connor, 1923) and *Ae. albopictus* (Gubler and Bhattacharya, 1972).

The present study focuses on swarming and mating behavior of *Aedes aegypti* in the laboratory, in order to elucidate the underlying mechanism regulating swarming and attraction to the swarms by this mosquito.

MATERIALS AND METHODS

Larvae were collected in flowerpots at the cemetery of Baruta, Estado Miranda, (Venezuela). Egg samples came from the División de Investigaciones, Dirección de Malariología, Ministerio de Sanidad y Asistencia Social, Maracay, and were referred to as strains Táchira F4 291099, Zulia F4 130400 and Rock IL 400. Starting from this mixture of field and laboratory-adapted material, we established a single laboratory colony at the University Simón Bolívar, Caracas. The mixture allowed for genetic variability of the breeding stock that in turn allowed better adaptation of the colony to the particular conditions at Universidad Simón Bolívar. Insects were separated by sex as pupae. Adults were allowed to emerge and were maintained, sexually segregated, in different rearing rooms at 25°C and 75% relative humidity. Photoperiod was 12L:12D without simulated dawn or dusk. The larvae were fed a mix of pulverized fish food and dry liver, and adults fed on sugar water. Adult females were allowed to feed twice a week on a captive white rat. The rat, placed in a metal cage, 20 × 15 × 15 cm wide, was placed inside the mosquito cage for 1 h.

Breeding chambers and observation chambers consisted of boxes with dimensions of 70 × 70 × 70 cm³ and 140 × 70 × 70 cm³, respectively, that had borders made of iron wire and were covered with a fine transparent cotton mesh, with sleeves for the introduction of the mosquitoes. The behavior of the adult insects was filmed, with the use of a videocamera (Sony Handycam) in an observation room, without the physical presence

of a human observer. All observations were performed during the photophase, because *Aedes aegypti* is known to be active diurnally (Lumsden 1957, Clements 1999). Preliminary observations by us detected no flight activity at night.

The following bioassays were performed.

Activity cycles

Adult insects were placed in an observation chamber; 24 h later their behavior was filmed, after the room used for observations was lighted. The groups observed were 1) 20 virgin males + 20 virgin females all 5 days old (3 replicate experiments), 2) 20 virgin females, all 5 days old (2 replicates), and 3) 20 virgin males, all 5 days old (2 replicates). Each 15 minutes, the number of insects flying was recorded during 3-min periods. The parameter measured was the weighted number of flying mosquitoes, defined as the number of insects flying multiplied by the time (in minutes) they were flying.

Swarming activity

Swarming activity for *Ae. aegypti* was defined operationally as the formation of a quasispherical cluster of flying insects about 30 cm in diameter, located in 1 of the lateral extremes on the cage where males flew in zigzag forming 3-dimensional loops. In order to measure swarming activity quantitatively, the insects were placed in an observation chamber as described above, and the groups studied (i.e., treatments) were 1) 20 adult females, 2) 20 adult males, 3) 20 adult females accompanied by 2 plastic 600-ml beakers, 1 containing 20 females, the other 20 males. The beakers were covered with small meshed cotton tissue and were placed in opposite ends of the observation chamber, alternating the location between replicate experiments to avoid biases in swarming behavior because of putative visual markers, 4) as in 3) but with 20 males, 5) 20 males mixed with 20 females. Six replicate experiments were performed for each treatment with the use of adult virgin mosquitoes 5–7 days after emergence.

From the filmed register of the 40 min immediately following the lighting of the room, we counted, for each minute, the number of individuals swarming, flying for more than 30 s (continuous flight) or approaching the beakers within ≤ 3 cm for more than 2 s. This was the period of maximal activity, as recorded with the previous bioassay. The overall duration of flight, for males and females segregated and mixed, was also determined. For males in any situation and for segregated females, the overall duration of flight was defined as the interval of time from the first continuous flight until the last one. For

females, in the mixed bioassay, because there were no continuous flights in this case due to the copulation activity, the overall duration of flight was defined as the time from the first copulation until the last one. We also considered the overall duration of swarming for males, with or without females, as the interval of time (min) they were swarming.

Signals triggering swarming

Mosquitoes were exposed to stimuli that triggered swarming behavior in preliminary observations. The tests were as described above and the groups of virgin adults 5–7 days old used were: 1) 25 males, 2) 25 males + 25 females, all virgin adults of 5–7 days old. The swarming triggering stimuli tested were: 1) presence of a mammal host (at 2 p.m., when daily activity of mosquitoes was very low, a human observer entered the room and placed himself close to the observation chamber), and 2) initiation of the photo phase (the observations began at the onset of the light).

Effect of age on the copulation and swarming behavior

Twenty virgin adults of each sex were placed in observation chambers 12 h prior to dawn. The observations started immediately after the lighting of the room for a period of 1 h. The age of the males tested were 18 h and 4, 8, 12, and 22 d. Five replicate experiments were performed for each age category, except for 4 days of age, when 7 replicate experiments were performed. The females used in all the bioassays were 5–7 d old. We counted the number of males flying, swarming, or copulating for each minute during 1 h.

The parameters analyzed in this bioassay were age of males, maximum number of males flying, maximum number of males swarming, duration of a large swarm (we defined a large swarm as one in which the number of males forming the swarm ≥ 9), and copulation frequency.

Olfactometric bioassays

Guided by preliminary observations and by observations during experiments described in the previous section, and partially based on an olfactometer designed for *Lutzomyia longipalpis* sandflies by Morton and Ward (1989), we designed an olfactometer (Fig. 1) that allowed testing the effect on the behavior of the mosquitoes of various odors. The apparatus consisted of an electric air blower producing an air flow of 36 cm/s. The airflow passed through the odor source first, and then reached the cage containing

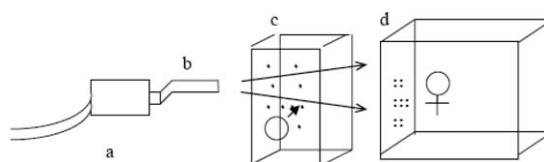


Fig. 1. The olfactometer indicating: (a) air pump, (b) plastic pipe (30 cm long and 0.5 cm diameter) + glass tube (15 cm long, 0.5 cm diameter); (c) source of odor stimulus (male mosquitoes); (d) cage with test (female) mosquitoes. Distance between (b) and (c) and between (c) and (d): 10 cm.

the test mosquitoes, which was identical to the one described above. The cages (Cage c in Fig. 1) served as containers for various different sources of odor described below. These cages were placed 10 cm from the air blower and 10 cm from the cage containing the mosquitoes to be tested.

In each bioassay we tested the response of 30 virgin females of 5–7 days of age. The test mosquitoes were placed in their respective cage the night before the bioassay, for acclimatization. The day of the bioassay, 20 min before the beginning of the photophase, one of the following stimuli (putative odor source) was presented:

1. Twenty-five virgin males 5–7 days of age (emitter males), which showed swarming behavior during the bioassay. The mosquitoes were located in a cage similar to the one described above, but smaller ($25 \times 25 \times 25 \text{ cm}^3$).
2. A white laboratory rat located in a metallic cage of approximately the size of the animal. The cage was placed, with the help of a tripod, just below the air current.
3. Both the rat and the male mosquitoes, in their respective cages, placed in succession in the path of the air flow.
4. A control (Control A), where an empty rat cage and an empty mosquito cage were placed in succession along the air stream.
5. Thirty virgin females (testing if females secrete a pheromone) of *Aedes aegypti* were placed in a small cage as described in 1.

In order discriminate between the influence of visual and olfactory stimuli, we did the following:

6. Twenty-five virgin emitter males as described in 1, but hidden behind a sheet of brown cardboard. The male assemblage was enclosed in a large cylindrical plastic bag with an input port for air flow and an exit port for the air through a glass tube. This tube blew the air into the cage with the test mosquitoes.
7. A control (Control B) consisting of all the apparatus described in 6, but without emitting mosquitoes in the cage.

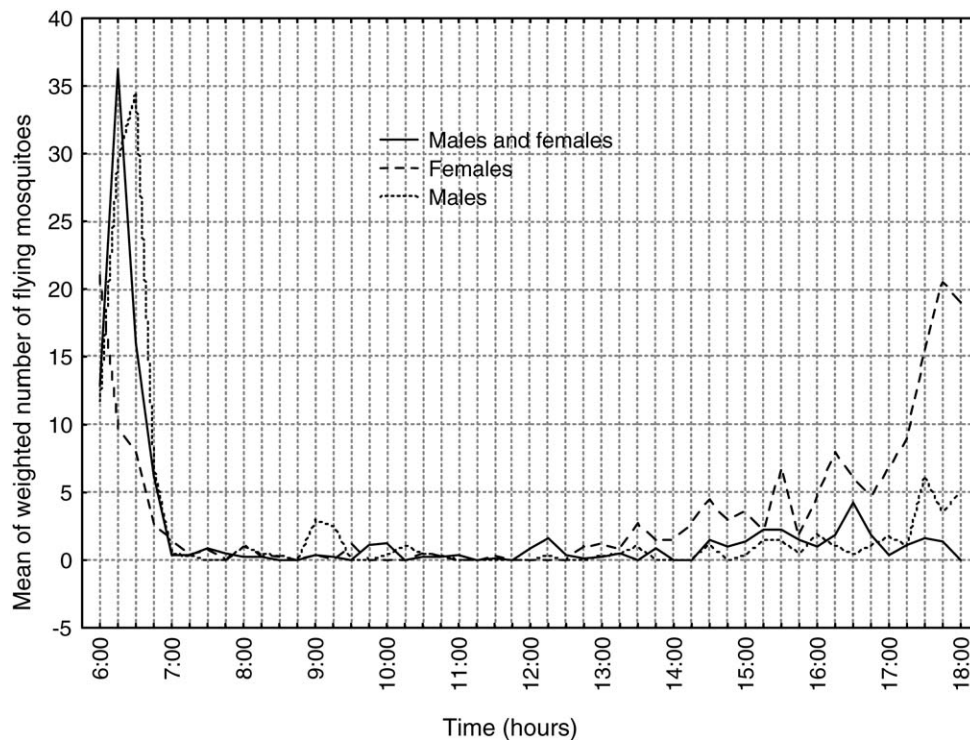


Fig. 2. Flight activity of *Aedes aegypti* during the photophase.

The behavior of the test mosquitoes was carefully videorecorded, as described previously. The number of insects performing each of following behaviors was counted during 10 min: flying, sitting, probing, copulating, and swarming. Each test was repeated 9 times with fresh batches of mosquitoes. Test mosquitoes were only females unless stated otherwise. For test 1, groups of females and groups of males were tested separately.

RESULTS

General observations

No mosquitoes were seen flying at the moment the light was turned on, or before it when red light was used to illuminate the cages during the scotophase. Figure 2 shows the flight activity, expressed as the weighted number of flying mosquitoes during photophase. Both sexes, either mixed or segregated, had maximum flight activity during the first hour after the onset of photophase. Both sexes, when segregated, increased their flight activity slightly at the end of photophase.

Swarming

Only males were seen forming swarms. We never observed segregated females swarming.

Females initiated flights at the beginning of photophase by flying very slowly for short distances; these flights progressively became longer and faster. Generally, less than 50% of segregated females were seen flying at the same time (Table 1). In contrast, for males at the beginning of the photophase, a few individuals started with faster and longer flights, after which progressively more males joined the flying groups. Thus, after a short period of time, more males than females were observed flying in our experimental settings (Table 1). The flight of males became progressively more coordinated and cohesive, until a swarm was formed for a certain period of time. In the bioassays, the maximum number of males flying occurred when there was a swarm present.

The number of flying insects of each sex did not seem to be affected by the presence of individuals of the opposite sex placed in small beakers inside the flight chamber (Table 1), despite the fact that we observed flight activity inside the beakers, especially for males.

The overall duration of flight for segregated females (Table 1) was not affected by the presence of males in a beaker. When males and females were mixed, the overall duration of flight for females was much shorter than when they were segregated, because females stopped flying and rested after copulation. The presence of

Table 1. Behaviors elicited by *Aedes aegypti* males and females at the beginning of the photophase.¹

Variables ²	Females	Females + both sexes in beakers	Females + males	Males	Males + both sexes in beakers	Males + Females
V ₁	29.1 ^a ± 9.29	31.62 ^{ab} ± 19.31	ND	78.63 ^c ± 12.97	59.0 ^{bc} ± 19.7	74.88 ^c ± 20.61
V ₂	31.5 ^a ± 7.92	32.33 ^a ± 7.2	13.17 ^b ± 5.12	31.5 ^{ac} ± 9.59	34.8 ^{ac} ± 5.88	38.0 ^c ± 4.9
V ₃	-	-	-	24.0 ^a ± 10.99	24.17 ^a ± 8.61	34.67 ^b ± 5.78
V ₄		13.17 ³ ± 7.0			0 ⁴ ± 0	
V ₅		3.16 ³ ± 2.71			0.4 ⁴ ± 0.55	

¹ Values are mean ± SD. For each variable, means followed by different letters indicate statistically significant differences ($p < 0.05$, Mann-Whitney test). ND = not determined. $N = 6$ replicates.

² V₁ = Maximum number of mosquitoes flying (in percentage) at the beginning of the photophase; V₂ = overall duration of flight (minutes) at the beginning of the photophase; V₃ = overall duration of swarming (min) at the beginning of the photophase; V₄ = number of times mosquitoes were attracted to males enclosed in beakers; V₅ = number of times mosquitoes were attracted to females enclosed in beakers.

³ Wilcoxon test between V₄ and V₅ for females, $p < 0.05$.

⁴ Wilcoxon test between V₄ and V₅ for males, NS.

females did not affect the overall duration of the flight of males. It is interesting to note that the overall duration of flight for segregated males and females was the same under these conditions.

The overall duration of swarms (Table 1) was much longer when males were mixed with females than when they were segregated. However, the presence of females enclosed in a beaker did not affect swarm duration. Thus, the possibility of copulations rather than female presence positively reinforced male swarming.

Table 1 presents additional data on the experiments where females were flying in the presence of males and where females were enclosed in beakers. The results showed that females were attracted to males in the absence of visual clues, as the bezakers were opaque.

In the case of males flying in the presence of males and females enclosed in beakers, they always formed swarms and occasionally they flew to the beaker with females, but never to the beaker with males (Table 1). The presence or absence of a beaker containing either females or males, however, did not affect the site of the swarm.

Signals triggering swarming

Both the onset of photophase and the presence of a host (a human observer), triggered swarming

in males (Table 2). When we compared those stimuli, the response of males toward a potential host was much faster and more individuals responded.

Table 2 also shows the copulation activity of mixed male and female groups to the presence of a host or the onset of photophase. Copulation frequency in the presence of a host was much higher than after the onset of the photophase.

The effect of age on copulation and swarming behavior

Table 3 shows copulation frequency and swarming behavior in males of different ages. Table 4 shows a correlation analysis of data in Table 3. It is interesting to note that copulation frequency was positively and highly significantly correlated with the size and temporal stability of the swarm. The age of males, after 24 h of age, did not seem to affect swarming and copulation. The correlation between the maximum number of males flying and the maximum number of males swarming was practically 1. Analyzing each replicate experiment, we observe that when > 8 males were flying, all of them were flying in a swarm. Males less than 24 h of age, which are known to be sexually immature, did fly but did not swarm or copulate (although we observed some attempts to contact females).

Table 2. Behaviors elicited by *Aedes aegypti* males in response to the presence of a host or to the onset of the photophase.¹

Behaviors	Host			Onset of photophase			
	2 min	4 min	10 min	2 min	4 min	10 min	
No. of males swarming	23.0 ± 3.34	23.0 ± 3.61	23.57 ± 2.51	0.14 ± 0.38	10.57 ± 8.08	15.43 ± 7.11	$p(2, 4, \text{ and } 10 \text{ min}) < 0.01^*$
Copulation frequency ²	-	-	30.14 ± 11.55	-	-	6.43 ± 3.73	$p < 0.001^*$

¹ Host vs. photophase: Mann-Whitney test.

² Determined at 10 min.

Table 3. Flight activity, swarming, and copulation (mean \pm SD) by *Aedes aegypti* males of different ages.

Age (days)	Maximum number in swarm	Duration of the swarm (min) with over 9 males	Frequency of copulations	Maximum number flying
0.75	0	0	0	9.8 \pm 1.5
4	9.47 \pm 4.81	5.14 \pm 7.97	5.14 \pm 4.41	10.28 \pm 3.9
8	9.6 \pm 7.27	14.6 \pm 14.14	5.8 \pm 4.92	10.8 \pm 5.72
12	10.4 \pm 2.7	5.1 \pm 5.02	1.4 \pm 1.52	10.4 \pm 2.7
22	14.4 \pm 3.05	16.1 \pm 5.96	5.6 \pm 5.46	14.8 \pm 3.27

Olfactometric bioassays

When female mosquitoes were exposed to odors emanating from swarming males, we observed very fast flight with long displacements, which we will call here *flight* (the normal flight of females was not quantified here). When odors emanating from a rat were blown onto females in the cage, they aggregated in and around the area where the air flow entered the cage, standing on the lateral cotton tissue wall facing the air flow. This behavior was called *probing*. Quantitatively, the number of females flying in Bioassay 1, when the air flow passed through a swarm of males, was 10.9 ± 2.9 , and the number probing was 0.1 ± 0.3 . The equivalent numbers for the control situation was 4.0 ± 1.6 and 8.6 ± 2.7 ($p < 0.05$, Mann-Whitney test, $df = 8$). The average number of males swarming in the emitter cage during Bioassay 1 was 12 ± 3 .

Bioassays with males as test mosquitoes showed that males always formed swarms at the onset of the photophase, independent of odor stimuli. We found no correlation between the number of test males swarming and the number of emitter males swarming.

Other stimuli

We observe that test female mosquitoes responded with flight to the presence of odors from male mosquitoes, but not to odors from a rat

(Table 5). In the presence of odors from a rat, females flew upwind and concentrated sitting on the area of the tissue wall where the air stream hit the cage (probing in Table 5). If both odors were presented together, test female mosquitoes showed the characteristic flight; that is, the odor produced by males elicited behavior that overrode that produced by a rat.

When performing experiments where "emitter" male mosquitoes were visually hidden from test mosquitoes, similar results were obtained: the number of females flying when the air flow passed through a swarm of males was 16.11 ± 4.05 and the number probing was 2.78 ± 2.28 . The equivalent numbers for the control test were 7.22 ± 1.92 and 8.6 ± 2.7 ($p < 0.05$, Mann-Whitney test, $df = 8$). These results exclude the possibility that mosquitoes were responding to visual stimuli.

When females were used as emitter mosquitoes (Table 5), test female mosquitoes responded similarly as they did toward male emitters, suggesting that also females emit a volatile chemical inducer of behavior (i.e., a pheromone).

DISCUSSION

Aedes aegypti formed swarms in response to the onset of photophase or in response to host odors. These observations agree with those made under completely natural conditions by Lumsden

Table 4. Pearson correlation coefficients between the variables indicated in Table 8.¹

	Age (days)	Maximum number in swarm	Duration of large swarms (min)	Frequency of copulations	Maximum number flying
Age	1.000	—	—	—	—
Maximum number in swarm	0.398 ($p = 0.067$)	1.000	—	—	—
Duration of swarms with over 9 individuals	0.339 ($p = 0.122$)	0.827 ² ($p = 0.000$)	1.000	—	—
Frequency of copulations	- 0.009 ($p = 0.968$)	0.536 ² ($p = 0.01$)	0.693 ² ($p = 0.000$)	1.000	—
Maximum number flying	0.406 ($p = 0.61$)	0.978 ² ($p = 0.000$)	0.85 ² ($p = 0.000$)	0.547 ² ($p = 0.008$)	1.000

¹ $N = 22$. Data correspond to males older than 24 h.

² Significant correlation for $p < 0.05$.

Table 5. Maximum number of females flying (for at least 60 s) and probing (for at least 90 s), when stimulated with odors from different sources.¹

	Control A	Males	Males + rat	Rat	Females
Maximum number of females flying	4.78 ^a ± 1.99	10.89 ^b ± 2.89	9.11 ^b ± 3.69	1.78 ^c ± 2.34	8.67 ^b ± 2.0
Maximum number of females probing	9.11 ^a ± 5.04	0.11 ^b ± 0.33	5.89 ^{ad} ± 3.26	16.78 ^c ± 5.22	3.56 ^d ± 1.81

¹ Values are mean ± SD. For each variable, means followed by different letters indicate statistically significant differences ($p < 0.05$, Mann-Whitney test). $N = 9$ replicates.

(1957), McClelland (1959), and Hartberg (1971), who reported the occurrence of small swarms or microswarms of this species over or around vertebrate hosts, with peaks of flight activity during the hours immediately after dawn and before dusk. Copulations may occur without swarm formation (Downes 1969). We showed that swarms enhance mating opportunities and increase the frequency of copulations. Only males initiated swarming, and most copulation occurred mainly during swarming. After a critical swarm size is achieved, all *Ae. aegypti* males that are flying in the surroundings incorporate themselves into the swarm. These observations suggest that the swarming behavior of *Ae. aegypti* is similar in many aspects to that reported for *Anopheles*, *Culex*, *Psorophora*, *Ochlerotatus*, and other *Aedes*. However, swarming in *Ae. aegypti* differs from swarming of other Culicidae in the following:

1. Swarms of *Ae. aegypti* and other mosquitoes in the subgenera *Stegomyia* are strongly dependent on the presence of a vertebrate host. This contrasts with what happens in *Culex*, *Anopheles*, *Psorophora*, and others, which use stationary visual markers for organizing swarming behavior. Some of these species, such as *Culex pipiens quinquefasciatus*, form swarms over humans (Peloquin and Olson 1985). Some reports describe the migration of swarms formed originally over a visual marker to above the heads of a human subjects passing nearby (Clements 1999). Others describe how males of the swarming *Ochlerotatus cantans*, disturbed by a person walking through a resting site in late afternoon, formed short-lived swarms called "provoked swarms" (Nielsen and Greve 1950).
2. *Ae. aegypti* is a diurnal species, and in the laboratory males swarmed in response to the onset of light in the morning, despite the fact that the photoperiod transitions were abrupt. In contrast, males of some species of *Anopheles* and *Culex* in the laboratory only respond with swarming when the changes in luminosity at dawn or at sunset are gradual (Nielsen and Haeger 1960, Yuval and Bouskila 1993). This might be due to a faster adaptation to

laboratory conditions for domesticated *Ae. aegypti*, or it might reflect more profound differences.

3. The microswarms reported for *Aedes aegypti* and other *Stegomyia* are usually composed of few individuals (Goeldi 1905, McClelland 1959, Hartberg 1971, Gubler and Bhattacharya 1972). This contrasts with the large size of swarms, composed of hundreds or thousands of individuals, reported for other diptera (Cambournac and Hill 1940, Belkin et al. 1951, Williams and Patterson 1969, Peloquin and Olson 1985, Yuval et al. 1992). Yet Downes (1969) consider "it is also proper to refer to a single male as swarming if his behavior resembles that of individuals in larger swarms."

Grouping behavior in mosquitoes may fulfill different needs (Sullivan 1981, Sivinski 1984) and might show different forms. Our assays with *Ae. aegypti* in the laboratory might have revealed only one type of swarm, and other types might be described in the field. Swarm formation in *Ae. aegypti* could be considered a facultative behavior, as it occurs only under certain conditions and mating occurs also without swarming (Downes 1969, Sullivan 1981, Yuval and Bouskila 1993).

To our knowledge we provide here the first quantitative experimental report showing that swarms (at least those formed in the laboratory) enhance reproductive behavior for *Ae. aegypti*. Our experimental evidence included:

- The overall duration of swarming was positively affected by the presence of females.
- The size and/or the duration of the swarm were strongly correlated to the frequency of copulations observed in the swarm. Although we observed, strictly speaking, only pairing and did not know if this was followed by insemination, an increase in pairing activity is likely to imply also an increase in inseminating copulations.
- The presence of a potential host for females is a strong stimulus for swarming in males. This suggests that swarming is related to the reproductive necessities of the females. In this context, swarm makers may have evolved to replace the host as a congregation site, thereby characterizing *Ae. aegypti*'s swarming behavior.

ior as an undifferentiated stage of evolutionary development.

- Sexually immature males (i.e., under 24 h of age) never swarm, although they do fly.
- The maximum daily flight activity of males and females coincide.
- Females respond with a characteristic agitated flight to the presence of swarming males and also of flying females.
- There is a close correlation between the number of females induced to fly and the number of males swarming.

The mechanisms by which mosquitoes are attracted toward a swarm are not known, but our results can only be explained if we assume that the existence of an odor emitted by swarming males attracts both males and females to the swarm. This odor has to be secreted by the mosquitoes and thus can be considered as an aggregation pheromone. Females respond to the pheromone with their characteristic agitated flight, which is known to serve as attraction stimuli to males flying in the swarm, inducing them to try to copulate with them while flying. For the initiation of a *Ae. aegypti* swarm, several signals might be involved (Hartberg 1971), including presence of host odors (kairomones). Our results suggest that females also secrete a volatile attractant. We have no evidence of its function or to suggest that this is the same compound that is secreted by males. Further studies will be required to determine the chemical nature and function of these attractants.

The importance of pheromones in swarm formation has been reported very scarcely and has not been reported previously for any culicid. Authors such as Sivinski and Petersson (1997) doubted that pheromones are useful in swarming behavior because of the difficulties in determining the source of the signal inside the swarm. This, however, was related to attraction of the sexes, which among *Aedes aegypti* is achieved apparently by sound. The aggregation pheromone that might be used by this species seems to stimulate attraction toward the swarm, but does not seem to act for courtship or mate selection, where other cues may be involved.

Pooling our knowledge of swarming mosquitoes, we might suggest that *Ae. aegypti* swarms function as follows: Stimulated males initiate a small swarm and secrete an aggregation pheromone, which attracts more conspecific males to the swarm. After a critical swarm size has been achieved, conspecific females are attracted to the swarm. These females might also secrete the same or a different aggregation pheromone. Inside the swarm, females attract males using sound and/or visual stimuli. Females select fast-flying and rapid-responding males by flying fast inside the swarm, forcing males to

follow and to copulate in flight. Behaviors displayed during swarming could favor mate selection based on fast flying, body size, sound, pheromones, or a combination of several criteria (Jaffe 2002), including assortative mating. Situations similar to the one described here have been reported for leks of Tephritidae and Phlebotominae (Prokopy and Hendrichs 1979, Webb et al. 1983, Ward and Morton 1991), where a pheromone attracts individuals to a lek and later other mate-selection processes take place.

Regarding the question of whether mosquito swarms can be considered a lek, our results showed that mosquito swarms actually function as a lek in the sense defined by Höglund and Alatalo (1995), who proposed that the term *lek* should be used more flexibly than the strict definition by Bradbury (1981) allows. A lek could thus be any aggregation that fulfills the following criteria: Males have to be more aggregated than a random spatial distribution would allow, and fertilization should be the prime motive for females to visit the swarm. We showed here that both criteria are fulfilled for *Aedes aegypti*.

Even if we use the stricter definition of lek by Bradbury (1981), swarms of *Ae. aegypti* can be considered as complying with the required criteria:

1. Males do not show parental care
2. Leks formed by males are temporarily restricted to a "mating arena," in which most mating occurs. In the case of mosquitoes, the restriction occurs in a "volume" of space.
3. The male territories do not contain resources vital to females, or, according to a later modification (Bradbury 1985), there is no male-regulated access to resources that might appear in the territory. In the case of *Aedes aegypti* and some other Culicidae, swarms are formed near a host used by females for their blood meal. Some authors do not consider this criterion a requirement for the definition of a lek (Alexander 1975).
4. Females have the opportunity to freely select a mate in the arena.

Some authors consider that for aerial swarming of mosquitoes and other insects, in opposition to substrate-based aggregations, this criterion is not fulfilled because they feel it is unlikely that courtship and mate selection might occur while flying and thus do not consider swarms to be leks (Bradbury 1985, Shelly and Whittier 1997). However, we observed clear aggression between males for access to a flying female. It is conceivable, therefore, that the fastest-flying male or the strongest male might have greater odds in copulating successfully. Other authors propose that the position of an insect within a swarm might influence its sexual opportunities (Thorn-

hill 1980, Sivinski and Petersson 1997). So, in species where male size is positively correlated with reproductive success, larger individuals probably occupy certain parts of the swarm that are safer or preferred by females. For example, a central position may be more attractive because it might be sheltered from predators. A peripheral position, however, could provide better access to females approaching the aggregation (Hamilton 1971). Taking into account that mosquitoes are extremely good fliers, males might display sophisticated courtships and male–male aggression behavior, which might be as or even more efficient than classical territorial aggression behavior that has been described for leks working on a surface. For example, females can approach a mosquito swarm from any direction, so that selection should favor those fast-flying males that detect the female first. This strategy will favor circular flight, trying to cover all directions, which seems to be what happens in the swarms described here.

Our observations suggest then that mosquito swarms are undistinguishable from leks and suggest that sophisticated inter- and intrasexual behavior is still to be discovered. Knowledge of reproductive behavior of economically important mosquitoes might be very relevant to control, especially in the design of vector control programs involving the sterile male technique, or the release of genetically modified vectors aimed at spreading genes to natural populations, as these are based on models of population dynamics and genetics of vector populations in which the frequency of polyandry may play a critical role. Thus, we believe that furthering our understanding of the chemical, ecological, and behavioral aspects of mosquito's reproduction will allow us to establish successful control programs of these insects eventually.

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