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LEKKING BEHAVIOR OF *ANASTREPHA FRATERCULUS* (DIPTERA: TEPHRITIDAE)

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ABSTRACT

Anastrepha fraterculus (Wiedemann) (Diptera: Tephritidae) displays a lek mating system. Males form groups in which they simultaneously display signals (acoustical, visual, or chemical) to attract females with the purpose of mating. Females visit the lek and choose among signaling and courting males to mate. Scarce information is available in *A. fraterculus* about the main factors involved in female choice and the behavior of displaying males. This information could be important within the context of pest control programs with a sterile insect technique (SIT) component, because departures from normal sexual behavior caused by artificial rearing could affect males' performance in the field. In this study we assessed *A. fraterculus* male behavior within the leks and analyzed the importance of behavioral and morphological traits on their copulatory success. The existence of preferred places for lek formation was evaluated in field cages with trees inside and analyzed by dividing the trees in sectors according to a 3-dimensional system. Males were individually weighed, marked, and observed every 15 min. Morphometric and behavioral characteristics of successful and unsuccessful males were compared. Most successful males grouped in a region of the tree characterized by the highest light intensity in the first 2 h of the morning. Results showed that pheromone calling activity is positively associated with copulatory success. Copulations were more frequent for males calling inside the lek, indicating that pheromone calling activity and presence in the lek are key factors for copulatory success. A positive association between copulatory success and eye length was found; some characteristics of the face were also associated with copula duration and latency.

Key Words: *Anastrepha fraterculus*, field cages, lek, mating behavior, morphometric traits, sexual selection, South American fruit fly

RESUMEN

Anastrepha fraterculus (Wiedemann) (Diptera: Tephritidae) presenta un sistema de apareamiento tipo lek. Los machos forman grupos y, en forma conjunta, emiten señales (acústicas, visuales, o químicas) para atraer a las hembras con el propósito de aparearse. Las hembras visitan el lek y eligen entre los machos para copular. La información acerca de los principales factores involucrados en la elección de la hembra y de la influencia del comportamiento de los machos en los leks en esta elección es escasa para *A. fraterculus*. Esta información es importante en el contexto de programas de control que incluyen la Técnica del Insecto Estéril. En el presente estudio se evaluó el comportamiento sexual de machos de *A. fraterculus* dentro de los leks, y la asociación de su comportamiento y de rasgos morfométricos con el éxito copulatorio. El lugar preferido de agrupamiento de los machos fue evaluado en jaulas de campo con árboles en su interior y dividiendo el árbol en sectores de acuerdo a un sistema de tres dimensiones. Los machos fueron individualmente pesados, marcados y observados cada quince minutos. Luego de finalizado el ensayo se midieron los rasgos morfométricos. El mayor éxito correspondió a machos agrupados en una región del árbol caracterizada por tener la mayor intensidad de luz en las dos primeras horas de la mañana. Los resultados mostraron que la actividad de llamado con feromonas está asociada con el éxito copulatorio. Las cópulas fueron más frecuentes para machos que llamaron dentro del lek, indicando que la actividad de llamado con feromonas y la presencia dentro del lek son factores importantes en la obtención de la copula. Los análisis morfométricos revelaron una asociación positiva entre el éxito copulatorio y el largo del ojo, y que algunas características de la cara están asociadas además con la duración de la cópula y la latencia.

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Anastrepha fraterculus (Wiedemann) is a quarantine fruit fly present in South America. It is a polyphagous species that attacks about 80 fruit species from more than 15 different families, many of them of economic importance (Malavasi et al. 2000; Norrbom 2004). Efforts to develop the sterile insect technique (SIT) to integrate it in the control for *A. fraterculus* are in progress and include the development of mass-rearing (Vera et al. 2007), irradiation protocols (Allinghi et al. 2007b), and studies of the sexual compatibility among different populations (Petit-Marty et al. 2004a; Petit-Marty et al. 2004b;Vera et al. 2006).

Sexual behavior of *A. fraterculus* has been analyzed for some Brazilian and Argentinean populations (Malavasi et al. 1983; Petit-Marty et al. 2004a). Mating occurs early in the morning, with most copulations taking place during the first 2 or 3 h after dawn, when males aggregate in groups (known as "leks") to which females are attracted (Malavasi et al. 1983). Copulation takes place preferably on the undersides of leaves, half-way up in the tree (Malavasi et al. 1983). Environmental conditions such as temperature, sunshine, and wind speed affect mating behavior (Malavasi et al. 1983; de Lima et al. 1994; Petit-Marty et al. 2004a).

Lek formation is common in the genus *Anastrepha* (Aluja et al. 2000). Aluja & Birke (1993) defined the *Anastrepha* lek as "an aggregation of at least 3 males (pheromone) calling simultaneously in a clearly defined area, usually from adjacent leaves of a single branch". The largest lek was observed in *A. suspensa*, containing 9 pheromone calling males (Sivinski 1989). For *A. fraterculus*, Malavasi et al. (1983) recorded leks of 5 males separated by less than 80 cm from one another, grouped in the area of the tree with the highest incidence of sunlight and generally establishing territories on the underside of leaves of host and non-host trees. The stimuli eliciting lek formation in *A. fraterculus* are unknown but may involve light characteristics as well as male signaling behavior, mainly through pheromone release (Malavasi et al. 1983).

Sexual selection plays an important role in the evolution of species with lek mating systems. Males often defend a small territory and exhibit a wide repertory of signals (acoustic, visual, and/or chemical) to attract females. In this mating system, females visit the sites where males aggregate and display their calling behavior, and choose a mating partner (Burk 1983). This, in turn, could lead to sexual selection, through female choice, on male morphological or behavioral traits.

In the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann), which is also a lekking species, male mating success may be associated, at least partially, to morphometric traits (Norry et al. 1999; Rodriguero et al. 2002a; Rodriguero et al. 2002b). In *A. fraterculus*, the relative importance of these traits has recently been studied (Sciurano et al. 2007), but the importance of attendance at leks for the male copulatory success remains unknown. Considering that a high proportion of the matings in this genus apparently occurs at the lek (Burk 1983; Hendrichs 1986; Aluja et al. 2000), strong selection in males for their ability to acquire a position within the lek (and perhaps even an intense competition for the female preferred positions) could be occurring. Descriptions of male-male agonistic interactions had been made for *Anastrepha* species (Hendrichs 1986; Sivinski & Burk 1989). In fact, Hendrichs (1986) found that *A. suspensa* males compete for leaves in the center of leks and that matings usually occur there. However, evidence that females choose males based on the location of the male may be difficult to reconcile with studies that suggest that individual qualities are important in mate choice (Kotiaho et al. 2001; Sciurano et al. 2007). Studies directed to assess the traits involved in female preference and male mating success should take into account the particular mating system of each species. Thus, any behavioral test must allow the males to form a lek, but also the females to choose among males inside and outside leks (Zapien et al. 1983).

The aims of our study were (1) to analyze the distribution of males inside the tree and determine regions of lek occurrence, (2) to evaluate the effect of morphological traits and pheromone calling behavior of the males on their lekking behavior and their copulatory success, and (3) to reveal potential advantages and requirements to join a lek.

Materials and Methods

Biological Material

A. fraterculus wild adults were obtained from infested guavas (*Psidium guajava*) collected at Horco Molle (Tucumán, Argentina). This population has been evaluated in terms of its mating compatibility with other populations (Petit-Marty et al. 2004a; Vera et al. 2006) and its sexual behavior (Petit-Marty et al. 2004a; Allinghi et al. 2007a). Upon emergence, flies were sorted by sex and fed with brown sugar and hydrolyzed corn protein (2:1), the regular diet used at Instituto de Genética, INTA Castelar, Argentina (Manso 1998). When tested, males were 20 d old, and females were 17 d old. All males were weighed and marked 1 day before testing. The mark consisted of a small piece of colored paper glued with a dot of acrylic paint on their notothorax. A letter (Microsoft Word Arial size 4) printed on the paper was used to individually recognize all the males. This technique has been used in sexual behavior studies in the medfly (McInnis et al. 2002; Vera et al. 2002; Vera et al. 2003).

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Test Procedure

Lekking behavior and male copulatory success was assessed in standard outdoor field cages (2 m tall, 3 m diameter) containing a rooted tangerine tree (nearly 1.7 m tall), at the experimental field in INTA Castelar during Apr 2002 (time of sunrise 7:20 AM approximately). Sixteen marked males were released into each cage. Observations took place from 7:30 AM to 12:00 PM, covering the period of sexual activity for Argentinean populations of this species (Petit-Marty et al. 2004a; Vera et al. 2006). One hour after the males were released, 8 virgin females were released into each field cage.

Two observers were assigned to each cage. Fifteen min after male release, the position and activity of each male inside the cage was recorded. This recording was repeated every 15 min. To determine the position of the males inside the tree, all trees were divided in 24 sectors in a 3 dimensional arrangement: (1) according to cardinal axes we defined four quadrants (NE, NW, SW, and SE representing the northeastern, northwestern, southwestern, and southeastern quadrants, respectively); (2) regarding tree height we defined 3 evenly-spaced sections (approximately 40 cm high) of the canopy (1 for the lowest third, 2 for the middle third, and 3 for the highest third); and (3) according to the depth of the leaves in the canopy we defined 2 sectors (P), for leaves situated approximately 5 cm from the periphery or edge of the canopy, and (C), for central or core leaves. Both height and depth were relative to each tree and defined previously by all observers to avoid bias. The activities of the males were classified as pheromone calling (hereafter referred to as calling, the presence of an everted anal pouch at the tip of the anus), mating, walking, interacting with other males (face to face encounters between males), and resting (motionless). Mating start time and copula duration were recorded for each couple observed. The test was performed simultaneously in 4 field cages and repeated on 2 different days.

At the end of the observation period, all males were recovered and stored in the freezer. A sample of 25 successful males (70% of all mated males) and 68 unsuccessful males (74% of all unmated males) were selected at random and measured for 8 morphometric traits, as follows: head width (HW), face width (FW), eye length (EL), thorax length (THL), wing length (WL), wing width (WW), femur length (FL), and tibia length (TIL) (Fig. 1). All measurements were made with a stereoscopic microscope, Ernst Leitz Wetzler, with a 12.5× ocular containing a micrometric scale. WL and WW were measured with a 1× objective; HW, EL, THL, FL and TIL with 4× and FW with an 8× objective (for a more detailed description see Sciurano et al. 2007). The data were standardized to have mean zero and unit variance in each replicate (Norry et al. 1999) in order to overcome any possible effect of the cage or the day.

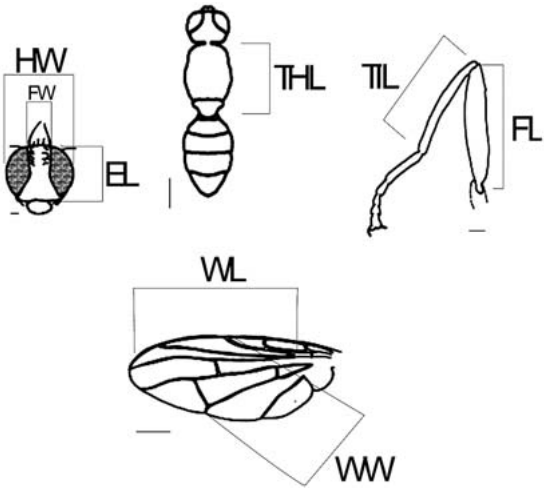


Fig. 1. Description of traits measured. HW, head width; FW, face width; EL, eye length; THL, thorax length; WL, wing length; WW, wing width; FL, femur length and TIL, tibia length. Bars = 1 mm.

Data Analysis

Location of calling males was used to determine if leks were actually forming, and if so, to describe the number of males inside each lek, the number of leks per tree, and whether leks were randomly distributed or followed some sort of spatial organization inside the tree. For those males that moved and called in different parts of the tree during the observation period, we considered their most frequent position in the analysis. In order to see whether it was possible to pool the data from the 8 replicates, differences among replicates for number of males in each position (height, depth, or cardinal position inside the tree) were analyzed with an ANOVA test. Nonsignificant differences were found ($P > 0.05$) and as a consequence, the analysis was performed by grouping data from all replicates. The observed frequencies of calling males found in each sector (NE, NW, SW, or SE; 1, 2, or 3; and C or P) were compared with the expected frequencies under random distribution by a Chi square test.

Sexual behavior of males was described considering (1) the time elapsed between the release of the males and the beginning of calling behavior (time to call), (2) the time spent calling, and (3) the time doing other activities. Time spent calling was calculated as the number of observations in which an individual male was observed pheromone calling divided by the number of observations on this male before attaining copulation (in

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the case of successful males) or by the total number of observations performed until the end of the observation period. Thus, if a male was pheromone calling all the time before mating, the time spent in calling behavior was one. The other activities registered such as walking, interacting with other males, or resting were grouped in a variable called 'other activities'. The sum of time spent calling and the time doing other activities equals one. For those males that mated, the time since female release until the achievement of copulation (referred to as 'latency') and copula duration were computed.

The time to call, the time spent calling (calling activity), and the body weight were compared between successful and unsuccessful males by means of a one-way analysis of variance (ANOVA). The association of time to call, calling activity, and weight with latency and copula duration of successful males were analyzed by means of Pearson product-moment correlation analysis. Statistical relationship between 8 morphological traits and copulatory success, copula duration, and latency was studied by step-wise multiple regression analysis.

Those males that exhibited calling behavior were classified according to whether they called inside a lek, always, sometimes, or never. The percentage of males that successfully mated was compared among the 3 categories by means of a Chi square test of heterogeneity. In addition, differences in time to call, time spent calling, body weight, and the 8 morphometric traits were evaluated among the same 3 categories (males that always, sometimes, or never called inside a lek). For the first 3 variables (time to call, time spent calling, and body weight), a one-way ANOVA was performed and for morphometric traits a MANOVA was used. In those cases in which significant differences were found, a Tukey test was performed. All statistical analyses were performed with Statistica for Windows (StatSoft, Inc. 2000) and STATISTIX 7 (Analytical Software 2000).

Results

Lekking Behavior and Mating Location

The distribution of the number of calling males on the different sections of the tree is shown in Fig. 2. Calling males tended to be grouped in the NE quadrant ($\chi^2 = 58.16$, $df = 3$, $P < 0.001$), in the peripheral leaves ($\chi^2 = 21.16$, $df = 1$, $P < 0.001$), and at a middle height of the tree canopy ($\chi^2 = 18.81$, $df = 2$, $P < 0.001$). Given that the preferred area to call was so small (one out of the 24 available, considering cardinal orientation, height of the tree, and depth in the canopy), only one group of calling males (i.e., lek) was evident in each tree. The largest lek in location NE2P contained 4 males. The position of the first male that called in

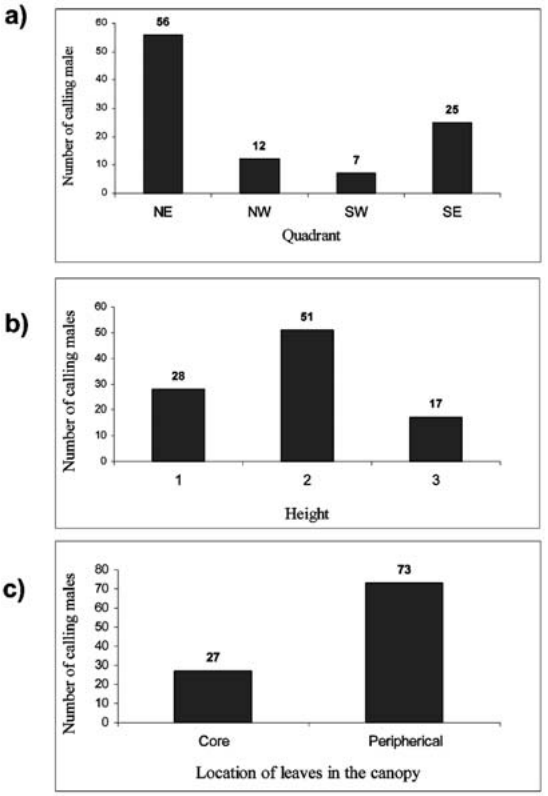


Fig. 2. Mean number of calling males observed in each part of the tree throughout the observation period: (a) number of males calling at each quadrant, (b) males calling in each height, and (c) males calling in peripheral and central leaves.

the tree determined the location of the rest of the pheromone calling males. In 58.88% of the cases, the first calling male was detected in the central third of the canopy (with the rest evenly distributed between the lowest and the highest thirds); in 70.6% of the cases the first calling male was located in the NE quadrant, with the remaining cases found in the SE quadrant. And finally, in all cases the first calling male was located in the P leaves.

Mating started at 9:30 AM and continued until 11:30 AM, reaching a maximum around 10:15 AM. Matings lasted from 15 min to 105 min; with a mean (± SE) of 67.30 ± 4.30 min. Mating couples had a spatial distribution similar to that of calling males (Fig. 2). They were found in the following areas: 90% in the P leaves, 72% in the NE quadrant, and 48% at height 2. In all, 35% of the couples were collected in the preferred area (periphery at an intermediate height of the NE quadrant). The percentage of males that mated was 28%. Considering that only half as many females as males were released per cage, this figure repre-

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sented 56% of the possible copulations. The mean number of copulations per cage was 3.9. Most of the copulations (90%) were observed on the underside of the leaves. Mean latency was 30 min and the last copulation was registered 150 min after releasing the females.

Lekking Behavior and Copulatory Success

The comparison of behavioral and morphological traits between successful and unsuccessful males indicated that successful males spent significantly more time calling than unsuccessful ones ($F = 3.97, P = 0.048$). The time to call was not different between the 2 classes of males ($F = 2.099, P = 0.151$) and 20% of unsuccessful males did not call at all. The mean body weight was 14.55 ± 0.17 mg. This variable did not differ statistically between successful and unsuccessful males (14.99 ± 0.35 mg and 14.30 ± 0.26 mg, respectively). Multiple stepwise regression analysis indicated that eye length (EL) was associated with copulatory success (Table 1).

Latency and copula duration were not associated with time to call, time spent calling, or body weight (Table 2). By contrast, the analysis of morphometric traits showed that latency was associated with face width (FW) and that copula duration was associated with FW and EL (Table 1).

We found that only 10% of the males called exclusively within the limits of the lek, while most of them (64%) never integrated to leks, and the rest (26%) entered the lek occasionally. Although males that called always inside the lek were less abundant, most of them (83.3%) copulated, while only 20.6% of the males that sometimes called inside the lek and 23.7% of the males that called always outside the lek mated. The differences among these classes were significant ($\chi^2 = 8.03$, $P = 0.018$). No differences in body weight or any of the morphometric traits were found among males that called only, sometimes, or never inside the leks (ANOVA for body weight: $F = 0.82, P = 0.441$; MANOVA for morphometric traits: $0.853 \geq P \geq 0.155$). However, significant differences were found in calling activity, both for the time spent calling (ANOVA: $F = 5.47, P = 0.009$, Table 3) and for the time required to start calling (ANOVA: $F = 6.35, P = 0.003$, Table 3). Males that never joined the lek spent less time calling than males that called alternatively outside or inside the lek (Tukey test: $P < 0.001$), while those that always called inside the lek showed intermediate (but non-significantly different) values for this variable. The same pattern was found when performing Tukey test for the time to call; males that never called inside the lek started their calling activity later than males that called alternatively inside or outside the lek ($P = 0.002$). The males that always called inside the leks showed intermediate values ($P > 0.05$). Interactions between males were not very frequent (only 8 observations) and therefore they were not analyzed.

Discussion

In the present study we analyze *A. fraterculus* male behavior and location during the hours of sexual activity and its relevance in their copulatory success. Males were found pheromone calling in a restricted part of the trees (on the northeastern quadrant, at an intermediate height, and in the peripheral leaves). This area represents one out of the 24 parts into which the tree canopy was divided, indicating that the grouping was very restricted and only a small portion of the tree is suitable or selected by the flies for calling and mating activity. This was considered as the area of lek formation. Given that most matings started before 11.00 AM, the preferred place to call corresponded to the one that received sunlight during the first hours of the day (the experiment was conducted in middle of autumn and the sunlight around 10.00 AM is concentrated in the NE and SE quadrants). This seems to imply that light determines, at least to some extent, the area of lek

Table 1. Association between copulatory success, copula duration, latency, and the 8 morphometric variables.

Dependent variables	Independent variables	Order	β'[1]	SD (β')	t (*df*)	*P*
Copulatory success	EL	1	0.43	0.18	2.36 (89)	0.020
	FW	2	-0.22	0.13	-1.68 (89)	0.097
	TIL	3	-0.19	0.16	-1.17 (89)	0.244
Copula duration	EL	1	0.50	0.18	2.79 (22)	0.007
	FW	2	-0.29	0.13	-2.24 (22)	0.028
	TIL	3	-0.19	0.16	-1.20 (22)	0.234
Latency	FW	1	0.59	0.21	2.78 (22)	0.011
	WL	2	-0.35	0.21	-1.65 (22)	0.113

[1]β', partial regression coefficient of the corresponding dependent variable on the independent variable; SD (β'), standard deviation of β'; *t* (*df*), Student's *t*-test (degrees of freedom). FW = face width; EL = eye length; WL = wing length; and TIL = tibia length.

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TABLE 2. RESULTS OF THE PEARSON PRODUCT-MOMENT CORRELATION ANALYSIS. CORRELATION COEFFICIENT *R*, AND THE ASSOCIATED *P*-VALUE IS PRESENTED FOR EACH PAIR OF VARIABLES.

	Weight	Calling activity	Time to call
Latency	$r = 0.06$, $P = 0.49$	$r = 0.04$, $P = 0.60$	$r = 0.20$, $P = 0.35$
Copula duration	$r = 0.14$, $P = 0.11$	$r = 0.15$, $P = 0.09$	$r = 0.22$, $P = 0.29$

formation. Probably flies look for the best place to display during the time of highest sexual activity (Petit-Marty et al. 2004a; Vera et al. 2006).

Factors such as light intensity, foliage density, and wind speed have been postulated to affect lek location in the medfly (Arita & Kaneshiro 1989; Hendrichs & Hendrichs 1990; Whittier et al. 1992). Malavasi et al. (1983), studying a Brazilian population of *A. fraterculus* that exhibits the same temporal pattern of sexual activity, also found that leks occurred on leaves receiving more sunlight in the morning. Given that tephritid males are usually attracted by pheromone emitting males (Kaspi & Yuval 1999), it could be proposed that in *A. fraterculus* the first active calling male positions himself in the best place of the tree (probably the one which receives more sunlight, NE2P in our case), and then new males arrive to join the calling male until the stimulus becomes sufficiently intense to attract females.

Fiske et al. (1998) found that male copulatory success in lekking species is associated mainly with male displaying activity, aggression rate, and lek attendance. Our results suggest that calling activity and the way in which the males participate in the lek are key factors determining copulatory success (successful males called more than unsuccessful ones, and those who called exclusively inside the lek attained a higher proportion of the copulas). Although agonistic interaction between males of other *Anastrepha* species has been proposed as an important factor to maintain the position in the lek (Aluja et al. 1983; Hendrichs 1986; Sivinski & Burk 1989), aggression did not seem to be important in *A. fraterculus*, given that direct interactions between males were infrequent in our experiment. According to Shelly (2000), several lines of evidence suggest that male-male conflict seems to have low influence on copulatory success compared with the effect of intersexual selection in *C. capitata*. Whittier et al. (1994) found that male-male contests in *C. capitata* are infrequent and that male copulatory frequency is unrelated to fighting ability. This low frequency of fights between males was also found in our study, probably indicating the intrasexual selection mediated by agonistic interaction is unimportant for the copulatory success of *A. fraterculus* males, at least at the density we set inside our field cages. Nonetheless, the method employed in our study to characterize male behavior could have underestimated male-male interactions, because these phenomena usually last only a few s. A more focused observation of male-male interactions will surely help to understand the importance of male aggressive behavior on copulatory success.

TABLE 3. CALLING BEHAVIOR OF MALES THAT ALWAYS, SOMETIMES, AND NEVER CALLED IN THE AREA OF FORMATION OF LEK.

Lek participation (*n*)	Mean time in minutes to call ± SE	Mean calling activity ± SE
Always (12)	58.50 ± 31.18	0.44 ± 0.26
Sometimes (34)	34.44 ± 29.94	0.64 ± 0.25
Never (81)	63.02 ± 35.25	0.25 ± 0.23

There is some evidence suggesting that larger males have higher copulatory success in *A. fraterculus* (Sciurano et al. 2007), *Anastrepha suspensa* (Loew) (Burk & Webb 1983), and *C. capitata* (Churchill-Stanland et al. 1986; Blay & Yuval 1997; Taylor & Yuval 1999; Kaspi et al. 2000). Conversely, other studies showed that size has no effect on male copulatory success, at least for *C. capitata* (Whittier et al. 1994; Vera 1996; Norry et al. 1999; Shelly 2000). In the present study, body weight, thorax length, and wing width and length were used as indicators of males' size, and no relationship was found between these variables and copulatory success. Our males were collected from guavas, a highly nutritious host, and after emergence were fed *ad libitum* until the field cage trials. Thus, it is likely that the males used in our study cover only a small range of possible sizes, a range that perhaps was not large enough to include those sizes that diminish the copulatory success.

Although overall size was not associated with copulatory success, probably other morphological characteristics could be the target of female selection. Indeed, from the morphometric analysis, we found that eye length was positively associated with copulatory success. Several morphometric studies on *C. capitata* have shown the importance of this variable on male copulatory success (Vera 1996; Norry et al. 1999; Rodriguero et al. 2002a; Rodriguero et al. 2002b), and it was shown that this selection operated via female choice, probably during close male-female interactions (Norry et al. 1999). A more detailed analysis of the importance of the eye length on copulatory success

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in *A. fraterculus* will probably provide an important tool to evaluate male sexual performance.

We additionally analyzed 2 variables, latency and copula duration, that could be associated with the mating success of males. Latency was expected to be negatively associated with mating success because copulations in *A. fraterculus* are restricted to a narrow time window during the first 3 h after dawn (Malavasi et al. 1983; Petit-Marty et al. 2004a). Copula duration was expected to be positively associated with mating success because sperm transfer could be ineffective in very short copulations (nonetheless it has been shown by Taylor et al. 2001 that long-lasting copulas does not imply sperm transfer, so this variable should be analyzed with care). Our results suggest that morphological traits such as eye length and face width were associated with latency and copula duration, indicating that these traits could have some relationship with the physiological state of the males. Face width was negatively associated with fitness, because it was positively associated with latency and negatively associated with copula duration. On the contrary, eye length was positively associated with copula duration, and therefore -coupled with face width- could be the target of sexual selection. The high correlation between these 2 variables should be taken into account.

Defining the area of lek formation allowed us to analyze where each male called during the hours of sexual activity. We found that a minority of the males called exclusively inside the lek, and most of them attended the lek only occasionally or never. But, from those that remained in the lek, 83% copulated, and only one quarter of the remaining calling males achieved copulation. This result showed the importance of calling inside the leks, and the fact that presence in the lek could result from strong selective pressures to keep these "preferred sites". We could not find any morphological difference among males from these 3 classes. So it seems that overall size and morphometric traits are not requirements to be a part of the lek. Conversely, differences were observed in their calling activity. Males which called only occasionally inside the lek spent more time in calling activity than the rest, and started to call earlier than the others. Thus, another advantage of staying in the lek is that less effort on calling is needed; this is an advantage under the assumption that pheromone calling is an energetically "expensive" activity (Yuval et al. 2007), although joining a lek may also increase the risk of predation (Hendrichs & Hendrichs 1998).

Based on our results, we can propose that *A. fraterculus* males exhibit 3 strategies related to pheromone calling behavior: (1) males that call always in the lek invest a moderate effort in calling and have a high probability of mating (83% in our case); (2) males that call outside the leks invest a moderate effort in calling with the advantage of lacking competitors (even though there were 63% of the males outside the lek, they located in the 23 remaining sectors of the tree) but with the disadvantage of lower probability to copulate (24% in our case) -perhaps these males were less motivated to mate or some undetected trait or interactions impeded them from entering the lek and thus acted as "satellite" males; and (3) males that call inside and outside the lek invest a lot on calling and have a low probability of achieving a copula (21% in our case). Probably these males try to occupy empty sites inside the leks and alternate this behavior with a male satellite strategy. Perhaps they would achieve a copula (as well as the successful males) if females continued arriving at the lek (in our experimental design only 8 females were released, and no more females were added after the males started to copulate). Insightful results would come from studies that register the number of unsuccessful mating attempts made by each type of calling male, to assess if males that call outside the leks attract less females or if they are being rejected more by attracted females.

In summary, the present study showed that, although *A. fraterculus* was considered a lekking species as many other of the same genus, the lek is formed not only by males that remain in it throughout the period of sexual activity, but also by males that call alternatively inside and outside the lek. Also, we found that the area of lek formation was the same in all the trees that we used, indicating that factors other than intrinsic properties of the trees are determining where the males should group to call. In all trees the area of lek formation was the one that received more sunlight during the morning, strongly indicating a major role of this variable. However, as Aluja et al. (1993) noted, the lekking systems in *Anastrepha* are very dynamic and highly influenced by male density, so the present results should be confirmed in nature, where males are allowed to join or leave a tree if it is already occupied by other calling males.

We conclude that the copulatory success of males is associated with morphometric and behavioral traits. Males with longer eyes, spending more time calling, and that call inside the lek, have higher chances of copulating than males with shorter eyes, spending less time calling, and calling outside the lek. Interestingly, those males that call alternatively inside and outside the lek start to call earlier and spend more time calling, and yet obtained less copulas than those inside the lek, and about the same amount as those who call always from outside. This suggests some hierarchical order in the behavioral traits: calling is important, but the males should call from a specific location of the tree. Also, it seems that females are evaluating some morphometric trait of the males, but the distribution of this trait is independent of male behavior (males that always,

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never or sometimes called inside the lek do not show differences in morphometric traits). Morphometric traits (in this case EL) could be used by females only after coming in close contact with males. It seems that *A. fraterculus* follows the pattern found for *Anastrepha striata* Schiner (Aluja et al. 1993) in that females use a chemical cue (e.g., calling pheromones) to locate males at long distances, and visual, acoustic and/or contact cues (e.g., courtship behavior, morphometric traits) to evaluate males at short distances.

We are still lacking sufficient information on male copulatory success in *A. fraterculus*. This information is vital within the context of SIT implementation, which has been proposed as a component of an area-wide integrated management of this pest (Guillén & Rodríguez 2007). The results of the present study shed light on several traits of *A. fraterculus* males that seems to be associated to their copulatory success. In fact, based on our results, morphometric traits such as eye length and face width should be considered in quality control tests rather than weight or overall size. Furthermore, studies or quality control tests directed to assess alterations of behavioral patterns due to mass rearing, irradiation (which is commonly used to sterilize the males) and handling should include not only calling behavior, but also lekking attendance, as we found that this seems to be a keystone on the way to a successful sterile male.

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