# SEXUAL SELECTION ON MULTIVARIATE PHENOTYPES IN ANASTREPHA FRATERCULUS (DIPTERA: TEPHRITIDAE) FROM ARGENTINA

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# SEXUAL SELECTION ON MULTIVARIATE PHENOTYPES IN *ANASTREPHA FRATERCULUS* (DIPTERA: TEPHRITIDAE) FROM ARGENTINA

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## ABSTRACT

Despite the interest in applying environmentally friendly control methods such as sterile insect technique (SIT) against *Anastrepha fraterculus* (Wiedemann) (Diptera: Tephritidae), information about its biology, taxonomy, and behavior is still insufficient. To increase this information, the present study aims to evaluate the performance of wild flies under field cage conditions through the study of sexual competitiveness among males (sexual selection). A wild population from Horco Molle, Tucumán, Argentina was sampled. Mature virgin males and females were released into outdoor field cages to compete for mating. Morphometric analyses were applied to determine the relationship between the multivariate phenotype and copulatory success. Successful and unsuccessful males were measured for 8 traits: 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). Combinations of different multivariate statistical methods and graphical analyses were used to evaluate sexual selection on male phenotype. The results indicated that wing width and thorax length would be the most probable targets of sexual selection. They describe a nonlinear association between expected fitness and each of these 2 traits. This nonlinear relation suggests that observed selection could maintain the diversity related to body size.

Key Words: sexual selection, morphology, multivariate statistical techniques, sterile insect technique, morphometric analysis, *Anastrepha fraterculus*

## RESUMEN

A pesar del interés por la aplicación de métodos de control de bajo impacto ambiental sobre *Anastrepha fraterculus* (Diptera: Tephritidae), como la Técnica del Insecto Estéril (TIE), no existe aún información suficiente sobre su biología, taxonomía y comportamiento. Este trabajo tiene como objetivo evaluar el desempeño de moscas en jaulas de campo a través del estudio de la competitividad sexual entre machos (selección sexual). Para ello, se muestreó una población de Horco Molle, Tucumán (Argentina). En jaulas de campo se liberaron machos y hembras adultos vírgenes para evaluar la competición por el apareamiento. Se midieron ocho rasgos morfométricos en machos exitosos y no exitosos: ancho de la cabeza, ancho de la cara, largo del ojo, largo del tórax, largo del ala, ancho del ala, largo del fémur y largo de la tibia. Se realizaron análisis morfométricos para determinar la relación entre el fenotipo multivariado y el éxito copulatorio. Para evaluar la selección sexual sobre el fenotipo del macho se utilizaron diferentes combinaciones de métodos estadísticos multivariados y análisis gráficos. Los resultados demostraron que el ancho de ala y el largo de tórax serían los blancos más probables de selección sexual, y describen una asociación no lineal entre el éxito copulatorio y cada uno de estos dos rasgos. Dicha asociación sugiere que la selección observada mantendría la diversidad para el tamaño del cuerpo.

Translation provided by the authors.

The South American fruit fly *Anastrepha fraterculus* (Wiedemann) is an important fruit fly pest which, because of its wide host range, causes major economic losses to Argentina through direct effects reducing total production and indirect effects derived from restrictions to fruit and horticultural exports (Ortíz 1999). The species is abundant in the northwestern and northeastern regions of Argentina, which are characterized by hot and wet subtropical climates and separated from each other by an extremely arid region (Vergani 1956).

In 1994, Argentina embarked on an ambitious National Program for Control and Eradication of Fruit Flies (PROCEM-Argentina), whose strategy is based on integrated pest management. In this context, and with the objective of developing environmentally friendly approaches for their control (Manso & Basso 1999; Ovruski et al. 2003), one of the methods proposed is the sterile insect technique (SIT). Although it is being successfully applied against other tephritid species, most notably *Ceratitis capitata* (Wiedemann), it has not been yet developed for *A. fraterculus*. The SIT relies on reducing the reproductive potential of wild females by inducing egg sterility through the release of mass reared and sterilized males. Such reduction can be achieved only if the released sterile males are successful in mating and transferring sperm to wild females (Lux et al. 2002).

An indispensable requirement for the application of the SIT against *A. fraterculus* is the evaluation of the sexual behavior of wild flies and identification of the factors that affect mate choice, in order to predict the expected performance of laboratory strains under field conditions. Mate choice may be the consequence of 2 different processes: (1) positive assortative mating and (2) sexual selection. The first implies that mating partners are phenotypically more similar to each other than expected by random mating (Falconer & MacKay 2001). Positive assortative mating will take place if mate choice preference differs between laboratory and wild females, determining that mass reared males mate preferentially with released females rather than with wild females. This process does not involve selection in natural conditions but may have negative consequences when SIT involves bisexual releases.

Sexual selection implies that certain individuals have advantage over other individuals of the same sex in exclusive relation to reproduction (Darwin 1871). Two different kinds of evolutionary processes can account for the evolution of sexually selected traits (Darwin 1871): (1) "intrasexual selection" or competition for mates between members of the same sex, usually males, and (2) "intersexual selection", which involves active choice of particular individuals of the opposite sex, usually female choice of mates.

Rodriguero et al. (2002a, b) demonstrated the occurrence of both selective processes operating on several morphological traits correlated with body size in *C. capitata*. Furthermore, body size is usually considered a quality index (Churchill-Stanland et al. 1986; Blay & Yuval 1997; Taylor & Yuval 1999; Kaspi et al. 2000). Because wings, legs, and head are apparently related to courtship behavior in *A. fraterculus* (unpublished results) and thorax length is an index of body size, we compared these traits in mating pairs and non-mated males to identify morphological character-

istics involved in the male copulatory success. To understand the process of sexual selection on male morphological traits, different multivariate statistical techniques were used to detect, quantify, and visualize multivariate selection in individuals from a wild population from Argentina.

## MATERIALS AND METHODS

### Field Cage Experiments

A wild population from Horco Molle, Tucumán (26°48' latitude South, 68°20' longitude West) was sampled in Feb 2001. Wild flies were obtained from infested *Psidium guajava* L. fruits taken from the tree and the ground. The fruits were sent to the Laboratorio de Insectos, Instituto de Genética "E. A. Favret" at INTA Castelar, where they were kept in plastic trays with a sand layer to allow pupation. Periodically, the sand was sieved to obtain pupae that were maintained under controlled conditions (25 ± 1°C, 80 ± 5% RH and a photoperiod of 12:12 (L:D) until adult emergence. Emerged adults were removed from flasks every 24 h and were separated by sex and kept under laboratory conditions (23-27°C, 50-70% RH and a photoperiod of 12:12 (L:D) until they reached the age of 20 ± 1 d.

Experiments were conducted under outdoor field cage conditions. Screened nylon cages (2.5 m high × 2.5 m diameter) were erected over rooted tangerine (*Citrus nobilis* L.) trees. Mature adults (250 males and 25 females) were released inside each cage. We choose this proportion for 2 reasons: (1) the number of calling males forming a lek in nature is usually much higher than the number of receptive females visiting a lek, and (2) a high number of males results in stronger selection on male mating success.

During observation periods, which ranged from 0800-1400 h, mating pairs were scored and gently removed from the cage by capturing them in a vial. Copulating males were designated "successful", while those males that failed to mate during the test were designated "unsuccessful". The whole experiment involved 8 replicates carried out on 12, 13, and 17 of Apr 2001. Although this design measures only mating success, the ability to mate may be considered a key component of sexual selection. In fact, previous studies (Petit-Marty et al. 2004a, 2004b) showed that sperm transfer is verified in virtually all copulations achieved under similar experimental conditions.

### Morphometric Measurements

A sample of 70 successful and 135 unsuccessful males were selected at random after pooling the data from all replicates. These flies were measured for 8 morphometric traits (Fig. 1): head width (HW), face width (FW), eye length (EL), tho-
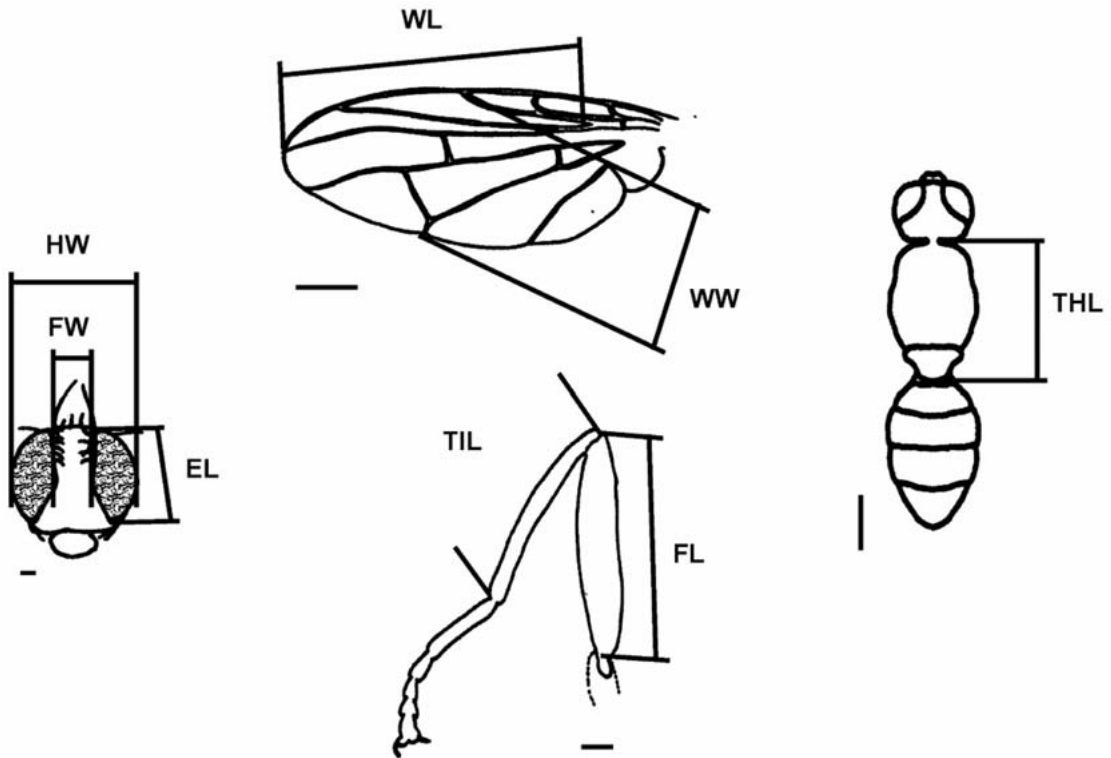
Fig 1. Description of measured traits: HW, head width; FW, face width; EL, eye length; THL, thorax length; WL, wing length; WW, wing width; FL, femur length and TIL, tibia length. Bar = 1 mm.

rax length (THL), wing length (WL), wing width (WW), femur length (FL), and tibia length (TIL).

Each fly was dissected on a Petri dish with a paraffin wax layer. Head traits were observed from the front and thorax length was scored from the dorsal view. In the case of bilateral limbs (wings and legs) the measurements were scored on the left side only. To measure HW, FW, EL, and THL, the body part was dissected and placed on a Petri dish with Bacto-agar (DIFCO laboratories, U.S.A.) 1% in $H_2O$. WL, WW, FL, and TIL were measured on a microscope slide ($24.4 \times 76.2$ mm) with a cover glass ($24 \times 40$ mm) sealed with synthetic balsam.

All measurements were made with a stereoscopic microscope, Leitz Wetzler, with a 12.5× ocular provided with a micrometric scale. WL and WW were measured at 1×, HW, EL, THL, FL and TIL at 4×, and FW at 8×.

Data Analyses

In the present work, the term 'fitness' was used to mean 'copulatory success'. Mated males (successful) were assigned an absolute fitness value of 1 ($W = 1$) while unmated males were assigned $W = 0$. Individual phenotypic values for each trait were standardized to have mean zero and unit variance according to the corresponding replicate (to the cage and to the day).

Pearson (1903) showed that multivariate statistics could be used to discriminate the direct and indirect effects of selection to determine which traits in a correlated ensemble are the targets of direct selection (Lande & Arnold 1983).

Multiple linear regression allows multiple traits to be considered simultaneously (Lande & Arnold 1983; Phillips & Arnold 1989; Brodie III et al. 1995). However, some difficulties exist in applying this analytical method to the data from selection studies (Schluter & Smith 1986; Mitchell-Olds & Shaw 1987; Wade & Kalisz 1990; Crespi 1990). To avoid some difficulties such as those derived from assumptions not fulfilled, different complementary statistical techniques were applied in the present study, as follows: (1) multiple logistic regression; (2) multiple stepwise regression; (3) principal components analyses (normalized varimax rotation) coupled with logistic regression; and (4) a nonparametric graphic technique known as 'cubic spline' (Schluter & Smith 1986; Schluter 1988).

Multiple logistic regression is a more natural model for studying the relationship between a dichotomous fitness measure and various phenotypic explanatory variables than is multiple lin-

ear regression. It does not rely on assumptions of normality for the predictor variables or the errors, and it allows the selection effect to vary nonlinearly (Janzen & Stern 1998). Among different multiple regression methods, stepwise regression analysis was used to find the smallest set of predictor variables that still yields an adequate prediction (Sokal & Rohlf 1979).

Principal component analysis (PCA) of morphological variables was used to eliminate bias due to the correlation among traits. A normalized VARIMAX rotation was applied to extract the first 3 principal components from the standardized phenotypic covariance matrix, because this method insures that components are orthogonal and simplifies interpretation by minimizing the number of variables that have high loadings on each principal component (Norry & Vilardi 1996; Norry et al. 1999; Rodriguero et al. 2002a, 2002b). The logistic regression of relative copulatory success on the rotated principal components estimates the regression of fitness on each of these composite variables ($\beta'$). Because copulatory success is a dichotomic variable (W = 1 or 0), the significance of $\beta'$ was tested by Chi-square test ($\chi^2$) (Schluter 1988).

Finally, to reveal the precise shape of the regression of fitness on the multivariate phenotype, we adopted Schluter's (1988) nonparametric visualizing technique known as 'cubic spline'. This nonparametric method is not restricted *a priori* to a particular model of selection and estimates complex functions with multiple peaks and valleys allowing a complete descriptive model of selection pressures on individuals (Schluter 1988; Brodie III et al. 1995), although a shortcoming is the higher sampling variation compared with parametric regression surfaces (Schluter 1988; Schluter & Nychtka 1994).

All statistical analyses were made with the software STATISTICA 6.0 (Statistica Statsoft 1998) and STATISTIX 7 Trial Version (Analytical Software 2000).

## RESULTS

For most traits, successful males had on average higher values than unsuccessful ones, but differences were only significant for wing width ($P$ = 0.003) (Table 1). The statistical relationship between fitness and phenotypic characters was evaluated by 3 multivariate methods. First, a multiple logistic regression revealed 3 variables as possible targets of sexual selection: thorax length ($\beta'$ = -0.59 ± 0.24; $P$ = 0.014), wing width ($\beta'$ = 0.94 ± 0.32; $P$ = 0.003), and femur length ($\beta'$ = -1.11 ± 0.50; $P$ = 0.025). Second, the possible correlation among traits was removed by means of a step-wise multiple regression analysis. This analysis identified wing width ($\beta'$ = 0.32 ± 0.08; $P$ = 0.003) and thorax length ($\beta'$ = -0.24 ± 0.08; $P$ =

TABLE 1. MEAN VALUES (MM) (± SE) OF MORPHOMETRIC TRAITS MEASURED IN SUCCESSFUL AND UNSUCCESSFUL SOUTH AMERICAN FRUIT FLY MALES FROM FIELD-CAGE MATING TESTS.

| | Males | |
|---|---|---|
| Trait[1] | Unsuccessful ($n$ = 135)[2] | Successful ($n$ = 70) |
| HW | 7.34 ± 0.44 | 7.41 ± 0.46 |
| FW | 4.60 ± 0.34 | 4.65 ± 0.34 |
| EL | 5.42 ± 0.36 | 5.45 ± 0.36 |
| THL | 2.44 ± 0.19 | 2.41 ± 0.18 |
| WL | 4.80 ± 0.26 | 4.83 ± 0.42 |
| WW | 2.40 ± 0.15[3] | 2.48 ± 0.29[3] |
| FL | 6.85 ± 0.40 | 6.85 ± 0.45 |
| TIL | 6.18 ± 0.39 | 6.22 ± 0.47 |

[1]Abbreviations of morphometric traits for this and subsequent tables are given in Fig. 1.

[2]$n$, number of individuals.

[3]Significant differences between successful and unsuccessful males ($P$ = 0.003).

0.002) as possible targets of sexual selection. Both characters were significantly correlated with fitness but with opposite signs. Third, the principal component analysis of morphological characters showed that although PC1 explains most of phenotypic variance, it is not associated with selective differences. Only PC2 and PC3 are significantly related with fitness. PC2 is mainly determined by wing width while PC3 is determined by thorax length (Table 2). Together, the first 3 principal components accounted for nearly 88% of the total variance in traits. The 3 analyses were con-

TABLE 2. PRINCIPAL COMPONENTS (PC) ANALYSES COUPLED WITH LOGISTIC REGRESSION ANALYSES.[1]

| Trait | PC1 | PC2 | PC3 |
|---|---|---|---|
| HW | <u>0.75</u> | 0.50 | 0.32 |
| FW | <u>0.81</u> | 0.44 | 0.13 |
| EL | <u>0.70</u> | 0.48 | 0.42 |
| THL | 0.30 | 0.25 | <u>0.87</u> |
| WL | <u>0.81</u> | 0.01 | 0.46 |
| WW | 0.24 | <u>0.91</u> | 0.23 |
| FL | <u>0.70</u> | 0.32 | 0.52 |
| TIL | 0.64 | 0.33 | 0.54 |
| Eigenvalues | 5.92 | 0.66 | 0.46 |
| % total variance | 74.03 | 8.26 | 5.76 |
| Cumulative % | 74.03 | 82.30 | <u>88.05</u> |
| $\beta'$ | 0.13 | 0.57 | -0.31 |
| $\chi^2$ | 0.71 | 8.80 | 4.29 |
| $P$ | 0.399 | <u>0.003</u> | <u>0.038</u> |

[1]Traits with the highest factor loadings on each PC, cumulative variance percentage and where $P$-value < 0.05, are underlined.

sistent with each other indicating that direct selection favored males with broader wings and shorter thorax.

A 2-dimensional graphical representation of selection surfaces by means of the nonparametric method of cubic spline showed a nonlinear relationship between copulatory success and each of the characters that are probable targets of sexual selection (Fig. 2). Fig. 2a suggests that selection on wing width acts by favoring extreme phenotypes, while Fig. 2b shows a principal and a local maximum in the case of thorax length. Furthermore, a 3-dimensional selection surface plot (Fig. 3) allows visualization of selection on wing width and thorax length simultaneously by considering the loading of these traits on the major axes of the selection surface. This graph showed one absolute and 2 local maxima consistent with Fig. 2. The absolute maximum corresponds to males with the widest wings and large (but not largest) thorax. One local maximum is associated with shortest thorax and medium width wings and the other corresponds to medium-sized thorax and narrowest wings. The nonlinear relationship between ex-
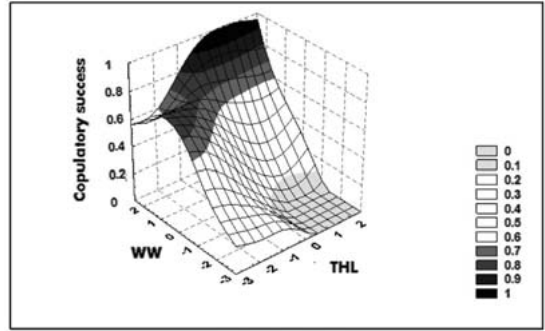


Fig. 3. 3-dimensional visualizations of selection. Copulatory success of male fruit fly *A. fraterculus* as a function of wing width and thorax length, simultaneously.

pected fitness and the probable targets of sexual selection suggests that observed selection could favor the maintenance of body-size diversity.

## DISCUSSION

Despite increased interest in applying environmentally-friendly control methods, such as SIT, against *A. fraterculus*, information about its biology, taxonomy, and behavior is still insufficient. Interdisciplinary work is necessary to develop mass-reared strains that mate successfully with wild populations. Some of the key subjects for research involve nutrition to optimize mass rearing methods in the laboratory (Manso 1999; Jaldo et al. 2001), the development of genetic sexing strains, population genetic studies to determine affinity and gene flow among different populations and the routes of colonization (Alberti et al. 1999), studies of sexual behavior, mating systems, and compatibility between wild and mass-reared flies (Petit-Marty et al. 2004a, 2004b), and morphometric studies to determine characters related to fitness (Russo et al. 2002).

In *A. suspensa* (Burk & Webb 1983) and *C. capitata* (Churchill-Stanland et al. 1986; Orozco & López 1993) male size has been shown to be related with mating success. However, this variable itself may not necessarily be the direct target of sexual selection, because such an association could be the result of selection on one or many traits correlated with it (Norry et al. 1995). In *C. capitata* some size-related traits have been detected as targets of sexual selection (Norry et al. 1999; Rodriguero et al. 2002a; Rodriguero et al. 2002b). In this species, the combination of stepwise regression plus principal component analysis, coupled with regression analysis, indicated that eye and thorax length were positively correlated with copulatory success, while face width was negatively correlated, suggesting that those males with largest eyes and thorax, and smallest
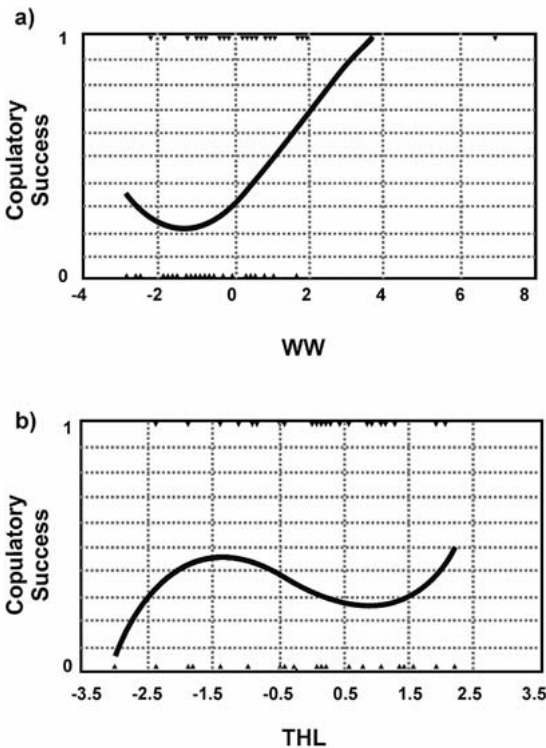


Fig. 2. 2-dimensional graphical representation of selection surfaces by means of nonparametric method of cubic splines. Copulatory success of male fruit fly *A. fraterculus* as a function of **a)** wing width ($y = -0.006x^3 + 0.036x^2 + 0.135x + 0.313$) and **b)** thorax length ($y = 0.032x^3 + 0.024x^2 - 0.126x + 0.324$). ▲ expected values — observed values.

faces have the highest copulatory success (Rodriguero et al. 2002a; Rodriguero et al. 2002b). Norry et al. (1999) revealed that copulatory success was also partially determined by intersexual selection on morphology, probably on size or shape of the head capsule. These results suggested that in *C. capitata* discrimination among potential mates on the basis of the male head morphology probably takes place when the female approaches the wing-fanning and head rocking male face to face (Calcagno et al. 1999; Norry et al. 1999; Rodriguero et al. 2002b). In contrast, there are other studies showing no relationship between male size and copulatory success (Whittier et al. 1994; Whittier & Kaneshiro 1995; Vera et al. 1996).

Our results suggest that thorax length, wing width, and femur length are possible targets of sexual selection in *A. fraterculus*. However, when the effect of correlation among traits is removed (stepwise regression and PCA), only thorax length and wing width are significantly correlated with copulatory success. Thus, the selection on femur length would be indirect, due to the correlation of this trait with other size-related traits. According to principal component analysis the PC1, an indicator or overall size, in the present case is not significantly associated with mating success. Our results indicate that wing width and thorax length are selected in different direction. All this evidence leads to the conclusion that under these experimental field-cage conditions sexual selection operates on body shape rather than body size level.

Rodriguero et al. (2002a, b) suggested the occurrence of correlational selection on multivariate phenotype as a by-product of directional selection acting on face width and thorax length separately and in the opposite direction in *C. capitata*. The selection surface analyzed in the present study described a nonlinear association between expected fitness and the 2 traits (wing width and thorax length) identified as targets of sexual selection, considered both separately or simultaneously. That nonlinear relation suggests that the observed selection could maintain diversity related to body size or shape. The different multivariate statistical methods and visual analyses yielded consistent results suggesting that sexual selection would affect multivariate phenotype in *A. fraterculus*.

Under natural conditions the adult male:female ratio should be near 1. However, the population of pheromone calling and signaling males is usually several times higher than the number of receptive virgin females that approach the calling males. In the present work, the proportion of males to females released inside each cage (10:1) is probably higher than that in the wild and might increase selective pressure on characters related to mating success. Our experimental design might lead to an overestimation of sexual selection. However, in natural conditions with huge population numbers very slight selective differences will have significant effects that would be impossible to detect under experimental conditions. In our experiment we did not evaluate the whole process of sexual selection because we did not measure post-zygotic components. However, mating success may be considered as the final result of many pre-zygotic components of sexual selection, such us male ability to integrate into leks, initiate pheromone calling, and display successfully the whole sequence of courtship activities that lead to female acceptance. In previous work (Petit-Marty et al. 2004b), we observed that virtually all matings achieved under similar experimental conditions were fertile. This means that an advantage in the ability to mate would be strongly selected under natural conditions.

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