Neotropical Entomology

ISSN: 1519-566X

journal homepage: www.scielo.br/ne

ECOLOGY, BEHAVIOR AND BIONOMICS

Body Size, Symmetry and Abundance of *Euxesta stigmatias* (Loew) and *Euxesta sororcula* (Wiedemann) (Diptera: Ulidiidae) in a Natural Reserve and in a Guava Orchard in Uberlândia, MG, Brazil

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Keywords
Fluctuating asymmetry, morphometry, population dynamics

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Edited by Fernando B Noll – UNESP

Received 16 May 2011 and accepted 11 July 2011

Abstract

The aim of this study was to comparatively characterize the monthly variation and the sex ratio of *Euxesta stigmatias* (Loew) and *Euxesta sororcula* (Wiedermann) populations from a natural reserve and from a guava orchard. It was also investigated whether density and the climate factors affected the individuals' size and fluctuating asymmetry (FA). In both environments, *E. sororcula* predominated over *E. stigmatias*. The number of individuals of *E. sororcula* collected in the ecological station was significantly lower than the number of individuals caught in the orchard. However, the number of captured individuals of *E. stigmatias* did not differ between environments. Significant sex ratio differences were detected between the collection sites. Female's frequency of *E. stigmatias* was significantly higher than male's frequency in the ecological station, but not in the orchard. An opposite pattern was encountered for *E. sororcula* population, showing a significant larger number of females only in the orchard. The species populations also differed in the degree of asymmetry presented. *Euxesta stigmatias* individuals showed no change on size or on FA along the captures and between environments. Moreover, *E. sororcula* tended to be more susceptible to climatic variations, considering the significant observed correlation between temperature and body size and the expressive body size and FA variations. This study illustrates the influence of the environment in some structural and dynamic parameters of the studied populations.

Introduction

Insect populations regulate their size over time in response to climatic factors and to variations in resource availability (Hannon & Ruth 1997, Andow 1991). However, environmental factors affect not only the population size, but also the dimensions or biomass of their component individuals (Lomônaco & Germanos 2001, Silva *et al* 2009).

Body size is a very important quantitative trait as it influences fitness, which describes the individual's ability to survive and reproduce (Schmidt-Nielsen 1984). Larger individuals tend to live longer and they are also often associated with higher fecundity and fertility (Wang *et al* 2009).

Environmental factors also affect the individual's capability to produce a phenotype with a perfect bilateral symmetry. If the expression of bilateralism is determined by the same genome, then the asymmetry between the sides is a consequence of modifications in the normal development programme, which may have genetic and/or environmental causes (Markow 1995). The population degree of asymmetry has been assessed by fluctuating asymmetry (FA), defined as small random deviations from perfect symmetry in organism traits that are bilaterally symmetric (Palmer & Strobeck 2003). Therefore, AF can be a good indicator of the individual developmental stability and fitness (Møller & Swadle 1997).

On the other hand, organisms also have the ability to resist against genetic disorders or environmental perturbations during development through buffering mechanisms programmed to produce a predetermined phenotype within the normal range of expression. This capacity is defined as developmental homeostasis (Lerner 1954), which may operate via canalization, a mechanism by which the variation of the phenotype is limited to one or a few forms under a set of genetic and environmental conditions. Moreover, the canalization also makes use of physiological or behavioral mechanisms to ensure developmental stability (Leung *et al* 2000). Thus, AF also provides a measure of how much a particular organism was able to buffer its development against stressful conditions. Because AF is inversely correlated with developmental homeostasis, it is often used to estimate the stability of development (Fuller & Houle 2002, Cárcamo *et al* 2008).

Body size and symmetry modulations in response to environmental factors can be produced by phenotypic plasticity (PF), which describes the ability to modulate the phenotypic expression without requiring genotypic changes (Scheiner 1993). Thus, PF can be considered an important adaptive tool for survival in unstable, heterogeneous or human disturbed environments (Gotthard & Nylin 1995).

This study was delineated to verify whether environmental factors may affect both individual's morphology and population's characteristics of two *Euxesta* species, *E*. *stigmata* (Loew) and *E*. *sororcula* (Wiedemann), inhabiting a natural reserve and a guava orchard in the municipality of Uberlândia, Minas Gerais, Brazil. We also investigated whether their abundance and the climatic factors influenced the size and the symmetry of individuals from these species.

Material and Methods

Studied organisms

The genus *Euxesta* is a complex of Neotropical endemic and abundant species, mainly distributed in Mexico, Peru, Puerto Rico, United States and the Virgin Islands (Steyskal 1968). Its dispersion across the Americas could be associated with the domestication and cultivation of corn (*Zea mays*) (Frias 1981). Nevertheless, both larvae and adults feed on a variety of other plants, including, potato, tomato, guava, sorghum, sugar cane, banana and orange (Capineira 2001). *Euxesta* has been considered a major pest in corn only when their larvae are associated with the primary pests *Helicoperva zea* (Boddie) and *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae) (Link *et al* 1984), but some would consider these insects as opportunistic and generalists on their feeding habits (Seal & Jansson 1993, Brunel & Rull 2010a).

Euxesta stigmata and *E. sororcula* are found in tropical and subtropical areas of the western hemisphere. In Brazil, *Euxesta* species have been encountered in high frequencies in corn fields in the states of São Paulo, Goiás, Paraná and Minas Gerais (Frizzas *et al* 2003). These pests require continuous insecticide application to control the adults, a process that may leave toxic residues in the food (Nuessly *et al* 2007). However, there is not a suitable alternative biological control strategy to be adopted in Brazil, because its natural enemies and field habits, especially between intercrops, are still unknown (Martos-Tupes 1982).

Sampling

Individuals were collected at the Panga Ecological Station (PES) and at the Água Limpa Farm guava orchard (ALF) in Uberlândia, Brazil, both belonging to the Universidade Federal de Uberlandia. The PES has 403.85 ha located between 19°09' - 19°11'S and 48°23' - 48°24'W, at 800 masl, presenting the typical Brazilian cerrado phytophysiognomics, including the semi-deciduous dry forest, the gallery forest and the dystrophic, mesotrophyc and grassland savannas (Cardoso *et al* 2009).

The ALF is approximately 27 km apart from the ESP (19°06 'S; 48 °20'W, 792 masl) and is located nearby soybean and corn fields and areas where banana, mango, guava, cherry, pineapple, orange and lemon are cultivated.

The region has hot and humid summers when there is a large water surplus, and cold and dry winters, when a high deficiency in soil water is common, especially during August and September (Nimer & Brandão 1989). The climatic data used in this work were obtained at the Laboratório de Climatologia, of the Instituto de Geografia, Universidade Federal de Uberlândia.

Insect collections were carried out from December of 2007 to August 2008, when a full guava orchard pruning had occurred. Eight plastic traps (constructed using PET bottles) were displayed in each area in order to capture the insects. These traps had three openings at their bases, having 300 ml of 10% molasses as attractant. They were set at 1.60 m high along the outside orchard guava corridors and at the boundaries of the different types of the reserve vegetation. The traps were left in the field for 15 days before the collection of the trapped insects. Then, the attractant was replaced for another 15 days of field exposure.

The captured insects were fixed in 70% ethanol and identified by Dr. Angelo Pires do Prado (Universidade de Campinas). All specimens were deposited in the "Coleção de Invertebrados do Laboratório de Ecologia Evolutiva da Universidade Federal de Uberlândia".

Morphometry

Five males and five females of each species were randomly caught from eight sorted samples that occurred between March 2008 and August 2009. These individuals had their left and right wings detached and mounted between slides and cover slips in order to be examined under a stereomicroscope (4.2 x). Fly wings are commonly used in morphological analysis because they allow measurements in a flat dimension, using their veins as fixed points (Klingenberg & McIntyre 1998). The dimensions of three wing segments (Fig 1) were taken using a digital caliper ruler. Each measurement was performed three times in order to estimate the measurements error.

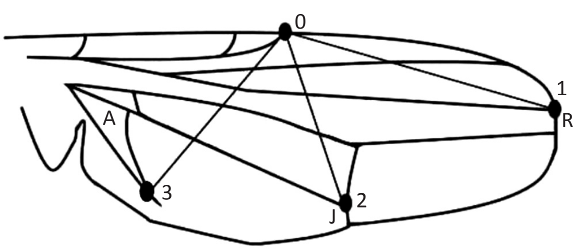

Fig 1 Morphometric measurements. The origin point of all segments (0-1. 0-2 and 0-3) corresponded to the interception of R_1 vein with the wing edge. Point 1 was established at the end of R_{4+5} vein at the wing apex. Point 2 corresponds to the junction of vein *m* to $M_3 + Cu_1$ and point 3 was marked at the apex of the Anal (A) cell projection.

Statistical analyses

Differences between the individual's frequency among species populations, areas, months and genders were verified using the Chi-square test (Zar 1984).

The morphometric measurements were simplified using the Principal Component Analysis (PCA) in order to obtain an index of size (Manly 1994) and its normal distribution was verified using the Lilliefors test. Differences in the index of size between species were investigated using a two samples *t* test. A three way ANOVA was performed to determine the effect of months, sex and collection areas in the size of individuals of each species (Zar 1984).

FA was calculated as the mean difference between the right (R) and the left (L) sides, i.e. $FA = [(\Sigma | (R - L) | /n]$ (Palmer & Strobeck 1986). A two-factor analysis of variance (ANOVA) was used to determine whether the between-sides variation was significantly larger than the measurement error (Woods *et al* 1998, Perfectti & Camacho 1999). According to Palmer & Strobeck (1986), it is necessary to distinguish FA from other kinds of asymmetry. A t-test was performed to verify whether the means of the signed right minus left distribution were not significantly different from zero, in order to discard the occurrence of directional asymmetry. Antisymmetry was tested by departures of the right-left frequency distribution from normality using Kolmogorov-Smirnov test. Size dependence of FA was tested for each sample by the regression of the unsigned absolute difference of the right minus left measurements on trait size. The normality of the data distribution was subsequently verified using the Lilliefors test. Then, the data were submitted to a two-way ANOVA in order to verify FA differences between areas and sexes (Zar 1984).

Correlations among individual's size, monthly frequency of occurrence, climatic factors (temperature, relative humidity and rainfall) and AF were made using the Person's correlation test (Zar 1984). All statistical procedures were performed using the computer software package Systat® for Windows®, version 10.2 (Systat 2000).

Results

Population data

Individuals of both species of *Euxesta* were captured only from March to July of 2008. In both environments, *E. sororcula* (n = 513) was predominant over *E. stigmatias* (n = 282) ($\chi^2 = 67.12$; $P < 0.001$; GL = 1). The number of individuals of *E. sororcula* collected in the PER (n = 157) was significantly lower than the number of individuals caught in the ALF (n = 356) ($\chi^2 = 38.59$; $P < 0.001$; GL = 1). However, the number of captured individuals of *E. stigmatias* did not differ between environments ($\chi^2 = 0.25$; $P < 0.005$; GL = 1) (n = 135 in ESP and n = 147 in ALF).

Euxesta sororcula was prevalent during the months of March (n = 44), April (n = 73) in the ESP, while *E. stigmatias* was more frequent in April (n = 54), May (n = 35) and June (n = 32), ($\chi^2 = 91.29$; $P < 0.001$; GL = 4) (Fig 2).

Euxesta sororcula peaked in April (n = 259) in ALF, but did not exceed 40 individuals ($\chi2 = 629.12$, $P < 0.001$; GL = 4) in the remaining months. *Euxesta stigmatias* was also predominant in April (n = 73), but this peak of occurrence was not far different from the number of individuals collected during the other months ($\chi^2 = 91.40$; $P < 0.001$; GL = 4) (Fig 3).

There was a significant difference on gender frequency between the sampling sites ($\chi^2 = 32.14$; $P < 0.001$; GL = 1). Males predominated in the PER while females in ALF

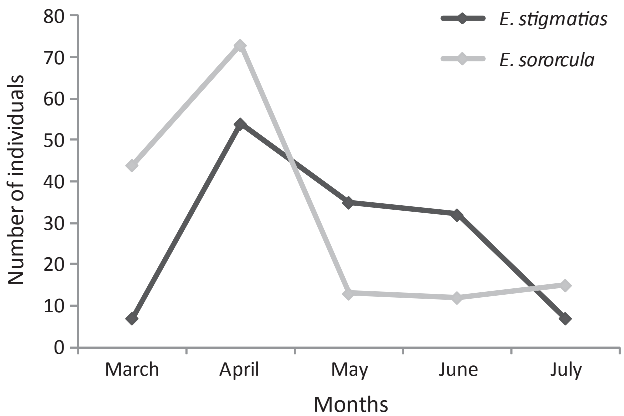

Fig 2 Monthly variation from *Euxesta stigmata* and *Euxesta sororcula* in the Estação Ecológica de Panga (PES) on collections made during the period from March to July 2008 using traps with molasses of sugarcane as attractive (no individual was captured in samples taken between December 2007 and February 2008).

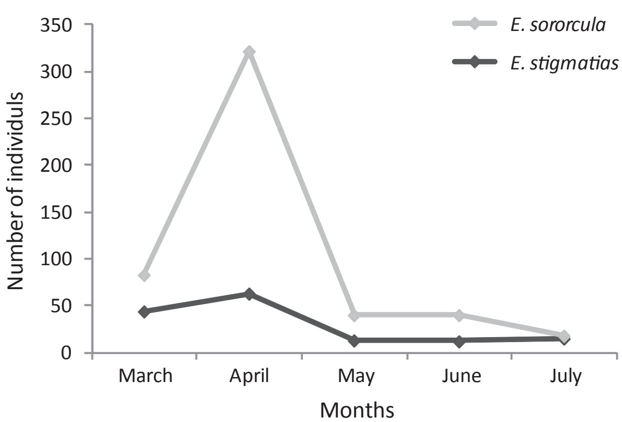

Fig 3 Monthly variation from *Euxesta stigmata* and *Euxesta sororcula* in the Fazenda Água Limpa (ALF) on collections made during the period from March to July 2008 using traps with molasses of sugarcane as attractive (no individual was captured in samples taken between December 2007 and February 2008).

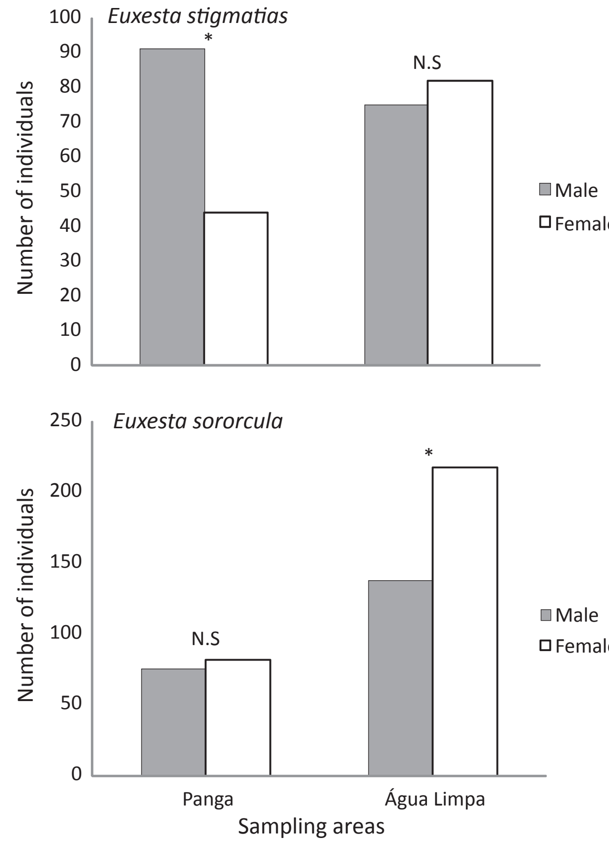

Fig 4 Number of males and females of *Euxesta stigmata* and *Euxesta sororcula* captured from March to July 2008 at the Estação Ecológica de Panga (PES) and the Fazenda Água Limpa (ALF) using traps of attraction with molasses from sugarcane as attractive (NS do not differ significantly. *statistically different at 0.1%).

(Fig 4). *Euxesta stigmatias* females were more frequent than males in PER ($\chi^2 = 16.36$; $P < 0.001$; GL = 1), while no significant difference was observed in ALF ($\chi^2 = 0.31$; $P > 0.05$; GL = 1). An opposite situation was registered for *E. sororcula*, that had higher number of females only in ALF ($\chi^2 = 17.97$; $P < 0.001$; GL = 1).

The correlations between the number of individuals monthly captured and climate factors were not significant (Table 1).

Body size

The PCA analysis revealed that all coefficients of the first main component were positive, which indicates that the multivariate index of size was adequately elaborated (Table 2). About 83.0% of the total variation in morphology corresponded to size variation (first main component), and the remaining 16.9% to shape distortion (the other main components). The indices of size presented normal distribution ($D_{max} = 0.053$; P = 0.739 for *E. stigmatias* and $D_{max} = 0.087$; P = 0.79 for *E. sororcula*). There were significant differences of size between species, with *E. sororcula* being larger (0.6 ± 0.82; n = 93) than *E. stigmatias* (-0.7 ± 0.65; n = 92) ($t_{0.05\ (2),\ 183} = -13.751$, $P < 0.001$).

The three-way ANOVA showed no significant

Table 1 Person's correlation between the number of individuals of *Euxesta stigmatias* and *Euxesta sororcula* collected per month and climatic factors (*n* values in paranthesis).

Variable	*E. stigmatias*	*E. sororcula*
Average temperature	r = 0.177; P = 0.183 (92)	r = 0.174; P = 0.180 (93)
Rainfall	r = 0.134; P = 0.141 (92)	r = 0.140; P = 0.146 (93)
Relative humidity	r = 0.212; P = 0.218 (92)	r = 0.317; P = 0.323 (93)

Table 2 First three principal components of the correlation matrix of three measures of wings from *Euxesta stigmatias* and *Euxesta sororcula*.

Variable	Principal components		
	1	2	3
Measure 1	0.961	0.195	-0.197
Measure 2	0.939	0.294	0.179
Measure 3	0.829	-0.559	0.026
Variance explained by components			
	2.491	0.437	0.071
Percentage of total variance explained (%)			
	83.041	14.582	2.376

differences in size of *E. stigmatias* between sexes (F = 0.179, P = 0.674), areas (F = 2.114, P = 0.150) and among months (F = 0.400, P = 0.808), nor the interaction between these factors were significant ($F_{area*sex}$ = 1.547 P = 0.218; $F_{area*month}$ = 0.964; P = 0.433; $F_{sex*month}$ = 0.694; P = 0.599 and F = $_{area*sex*month}$ 1.753; P = 0.148). The great size variability among samples may explain the lack of significance of the factors effects (Fig 4).

However, there were significant differences between sexes (F = 14.349, P < 0.001) and among months (F = 3.487, P <0.05) for *E. sororcula*, but not between areas (F = 0.825, P = 0.367). The interactions were significant for areas * months (F = 5.868, P < 0.001), but were not significant for the other interactions ($F_{sex*area}$ = 1.155, P = 0.286; $F_{sex*month}$ = 0.987; P = 0.420 and $F_{sex*month*area}$ = 1.521; P = 0.205). Accordingly to Tukey's multiple comparisons test, the individuals collected in May had significantly smaller size than those collected during the other months (Fig 5).

The correlation between the monthly obtained index of size for both species and the climatic variables were not statistically significant, except the positive correlation

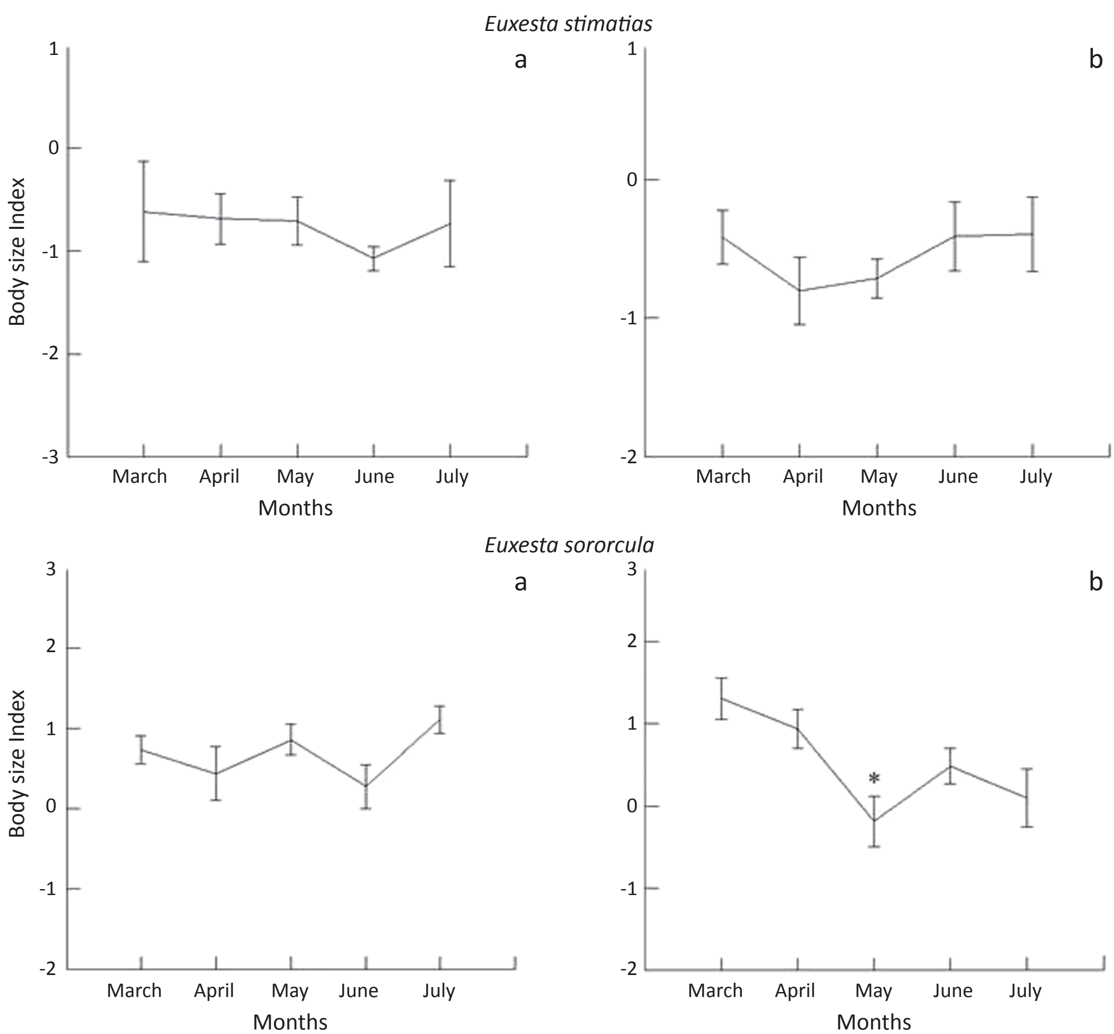

Fig 5 Multivariate size indexes from *Euxesta stigmata* and *Euxesta sororcula* in the Estação Ecológica de Panga – PES (a) and the Fazenda Água Limpa – ALF (b) during the month of collection. The index marked with an asterisk differ significantly (P < 0.05) from each other, according to the Tukey's multiple comparison test.

established between temperature and *E. sororcula* size (Table 3).

Table 3 Pearson correlation between the indices of size from *Euxesta stigmatias* and *Euxesta sororcula* and climatic factors (temperature. relative humidity. rainfall) (significant value in bold; *n* values in parenthesis).

	E. stigmatias	*E. sororcula*
Average temperature	r = 0.081; P = 0.898 (92)	r = 0.926; P = 0.027 (93)
Rainfall	r = -0.192; P = 0.759 (92)	r = 0.060; P = 0.924 (93)
Relative humidity	r = 0.057; P = 0.928 (92)	r = 0.437; P = 0.467 (93)

Fluctuating asymmetry

The FA error measurements were considered insignificant for both *E. stigmatias* (F = 6.600; P < 0.001 in ESP e F = 4.203; P < 0.001 in ALF) and *E. sororcula* (F = 5.676; P < 0.001 in ESP e F = 3,673; P < 0.001 in ALF). Antisymmetry and directional symmetry were discarded for all performed measurements. The arguments of Babbit *et al* (2006), explaining the non-obligation of normal adjustment for AF, were adopted.

The ANOVAs showed no significant differences in the FA of *E. stigmatias* between areas and between sexes. However, the significant interaction between the tested factors (sex * area) were detected in two of the three performed measurements (Table 4) for *E. sororcula*, indicating that the FA for females was higher in the orchard while for males it was higher in the Ecological Station. The results of the correlation´s test were not significant (Table 5).

Table 4 Two-way ANOVA between Gender * Location by multivariate index of size from *Euxesta stigmatias* and *Euxesta sororcula* (significant values in bold).

Measure	Source	*E. stigmatias*			*E. sororcula*		
		df	F	P	df	F	P
1	Location	1	2.058	0.155	1	0.204	0.653
	Gender	1	0.156	0.694	1	0.736	0.393
	Location*sex	1	0.156	0.694	1	0.557	0.458
	Error	88			89		
2	location	1	1.306	0.256	1	0.171	0.680
	Gender	1	0.269	0.605	1	0.587	0.446
	Location*sex	1	0.050	0.823	1	4.008	**0.048**
	Error	88			89		
3	Location	1	0.723	0.398	1	0.027	0.865
	Gender	1	1.450	0.323	1	1.801	0.183
	Location*sex	1	2.109	0.150	1	6.434	**0.013**
	Error	88			89		

Discussion

The absence of both species in the traps between December and February may indicate their low tolerance to high temperatures as pointed out by Martos-Tupes (1982) and Albuquerque *et al* (2002). The affirmation of Seal *et al* (1996), that *E. stigmatias* prefer bright environments with low temperatures, also supports this idea. Moreover, *Pseudodyscrasis scutellaris* (Wiedemann) and *Euxesta bilimeki* (Hendel) (Diptera: Ulidiidae) showed reduced activity when the temperature reached 30°C or at high and low temperature extremes (Brunel & Hull 2010a,b).

The peaks of occurrence of both species coincided with the harvest of corn and sorghum, confirming Frizzas *et al* (2003) statement that the population dynamics of these flies is strongly influenced by resource availability.

Frías (1978, 1981) also described some life-cycle and fertility differences of populations of *Euxesta annonae* (Fabricius) and *Euxesta eluta* (Loew) (Diptera: Ulidiidae) co-occurring in a corn field, influenced both by temperature and humidity. Moreover, he found that the water content of the corn kernels, which varies accordingly to their maturity state, also influences the reproductive dynamics of these species.

Populations of *E. stigmatias* and *E. sororcula* differed in their population dynamics, although they peaked simultaneously in both areas. *Euxesta sororcula* was

Table 5 Spearman's correlation between the multivariate index of size and fluctuating asymmetry values from *Euxesta stigmatias* and *Euxesta sororcula* in the two sampling areas: Fazenda Água Limpa (ALF) and Estação Ecológica de Panga (PES).

Measure	*E. stigmatias* (*n* = 92)		*E. sororcula* (*n* = 93)	
	ALF	PES	ALF	PES
1	r_s = -0.114 (P > 0.20)	r_s = -0.038 (P > 0.50)	r_s = -0.163 (P > 0.20)	r_s = 0.134 (P > 0.20)
2	r_s = -0.157 (P > 0.20)	r_s = -0.178 (P > 0.20)	r_s = -0.103 (P > 0.20)	r_s = 0.106 (P > 0.20)
3	r_s = 0.171 (P > 0.20)	r_s = 0.109 (P > 0.20)	r_s = -0.019 (P > 0.50)	r_s = 0.023 (P > 0.50)

more abundant and prevalent than *E*. *stigmatias* and occurred with a frequency 70.8% higher than *E*. *stigmatias*. On the other hand, *E*. *stigmatias* could be using a wider variety of native resources in the ecological reserve (Oliveira & Gibbs 2000), and may have been able to maintain high frequencies of occurrence for a longer period than *E*. *sorocula*. The higher abundance of *E*. *sororcula* females in the orchard and of *E*. *stigmatias* females in the ecological station reaffirms the distinction between these species and indicates their refuge habitat preferences.

Body size also differed between individuals of both species. Although *E*. *stigmatias* was smaller than *E*. *sororcula*, no changes in size along the monthly collections and no size distinction between males and females were detected. On the other hand, *E*. *sororcula* presented monthly size variation, possibly influenced by temperature.

The larger size of males of *E*. *sororcula* as compared to female is probably associated with larger male's mating success, a pattern commonly observed in other flies (Baldwin & Bryant 1981). In fact, the male courtship behavior in *E*. *sororcula* was described and included the males's pursuit and apprehension of the female (Martos-Tupes 1982).

FA in *E*. *sororcula* was influenced by both sex and area of collection, contrary to FA in *E stigmatias* which remained invariable. The failure of *E*. *sororcula* in maintaining body symmetry makes this species a promising bioindicator of environmental disturbances (Leung *et al* 2000, Hasson & Rössler 2002). Organisms signaling stress caused by habitat modification are important to avoid irreversible environmental impacts (Silva *et al* 2009).

There is a still incipient literature database on Ulidiidae (Tephritoidea) (Brunel & Rull 2010ab), and the studied groups have restricted information regarding to its economic significance (Diaz-Fleischer & Aluja 2000) and distribution in natural, refuge areas (Aluja 1999).

In conclusion, it is possible to affirm that *E*. *stigmatias* and *E*. *sororcula* populations from ESP and the ALF have different patterns of variation, showing different responses to climatic factors. Individuals of these populations are also distinctly affected by climatic conditions and by the inhabited environment. *Euxesta sororcula* has greater potential than *E*. *stigmatias* to damage commercial guava plantations, and is a promising bioindicator of environmental stress. This study also shows that natural reserves may be a suitable refuge place for these species.

Acknowledgments

We thank CAPES for the scholarship granted to K.C.F.L. Souto, Dr Ângelo Pires do Prado for the flies identification and FAPEMIG for the publishing support.

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