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RESEARCH ARTICLE - BEES

Morphometric Changes in Three Species of Euglossini (Hymenoptera: Apidae) in Response to Landscape Structure

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Abstract

Habitat fragmentation and expansion of agricultural activities are anthropic factors that can strongly impact biodiversity. Thus, sustainable practices of land use, such as agroforestry systems, are adopted with the proposal of improving environmental quality and restore ecological processes. In flying insects, habitat fragmentation may cause changes in the wing shape and size. Therefore, we hypothesized that landscape structure would affect wing size and shape in three species of Euglossini (*Eulaema atleticana* Nemésio, *Euglossa cordata* (Linnaeus) and *Euglossa ignita* Smith). The analysed specimens were collected in five areas, four forest areas with strong anthropic influence and an agroforestry system area. The results of the wing shape analysis have showed that the individuals of the three collected species within the agroforestry system diverge significantly (p<0.05) from those collected in the other areas. On the wings of *Eg. cordata* and *Eg. ignita*, differences in shape have occurred mainly in the medial region, which actively participates in the individual's flying ability**.** The wing size has showed meaningful difference only to the population of *Eg. ignita* (p=0,005). For *Eg. cordata* and *El. atleticana*, there was a significant correlation (r<0.05) between the morphometric data and the landscape metrics, which shows a close relationship between these species and the forest cover. The wing shape and size pursue an important function for the individual's ability in the environment, such as dispersion capacity and fertility rate, respectively. We concluded that the morphometric differences can reveal the existence of environmental stress for Euglossine bees. Therefore, the results contribute to establish morphometry variability as a tool for environmental quality monitoring.

Introduction

The progressive degradation of terrestrial ecosystems has brought several challenges to conservation proposals, especially because some organisms are sensitive to changes in the environment, such as habitat fragmentation. According to Bennett and Saunders (2010) such fragmentation is a threat to populations, for it may cause loss of genetic diversity through inbreeding, variations in microclimatic conditions and in food sources, which also affects birth and mortality rates,

and may lead them to extinction. Therefore, proposals such as Agroforestry Systems (SAFs) are presented as alternatives for the maintenance of environmental quality and sustainable use of forest ecosystems (Torralba et al., 2016).

SAFs consist of a land use technique in which agricultural species are grown among the native species of a forest ecosystem, exotic tree species or a mix of both. The main advantages of SAFs in an agricultural matrix, as compared to monocultures, is their more complex structure in terms of vegetation stratification, niche diversification and energy

flows. This practice ensures the maintenance of ecosystem services and benefits for biodiversity (Torralba et al., 2016); promotes the increase of carbon and biomass stocks in the soil (Cardinael et al., 2017) and may contain a similar diversity of plant species to those of secondary forests (Sistla et al., 2016).

Despite the benefits for the environment, the impacts of the SAFs for the biodiversity is a topic that needs further investigations, because studies indicate, for example, that this kind of land use impacts the soil microbiota (Wang et al., 2017), it is stressful for *Ceroglossus chilensis* (Eschscholtz) beetles (Benítez et al., 2008), and may also influence the richness and abundance of bees in the environment (Kennedy et al., 2013). Bees, in turn, are considered key organisms for the functioning of agroforestry systems (Matos et al., 2013), however, studies show that these insects are affected by soil use practices (Neves et al., 2012) and that one of the reasons for the decline of solitary bees, in the world, is the agriculture intensification (Kremen, 2002).

In the case of the Euglossini tribe, a bee group which includes species with primitive social as well as solitary behavior (Freiria et al., 2017), the evaluation of the effects of habitat fragmentation on the wing shape and size has showed interesting results (Silva et al., 2009). Bees of this tribe, also known as orchid bees are important pollinators and, therefore, they are considered important targets for native plant conservation policies (Brosi, 2009) and for analysis of the effects of fragmentation (Aguiar et al., 2015). Only in the Brazilian Atlantic Forest more than 60 species of Euglossini have been described, and some species are endemic to this biome (Nemésio, 2009).

Among bees, the shape and size of their bodies probably reflect the influence of environmental, geographic and seasonal barriers (Skandalis et al., 2009). While the wing shape presents greater evolutionary restrictions and is directly associated to the aerodynamics of this structure (Nunes et al., 2013), the body size has high levels of plasticity in response to the environment (Ghosh et al., 2013) and confers several advantages to the individual. The survival, fecundity, success in mating rates will be higher if this individual is bigger (Kingsolver & Huey, 2008).

Holometabolous insects, such as bees, in the adulthood do not undergo morphological changes, therefore the variance in the body size may be a response to the natural selection, climatic fluctuations and the availability of resource in the larval stage (Chown & Gaston, 2010), while the variance in wing shape is a response to environmental variability due to the costs and benefits involved in dispersion on fragmented landscapes (Hill et al., 1999).

Then, a promising tool in the impact analysis of environmental changes on populations is the use of morphometry, a technique which allows the closure of morphological data in a set of numerical and graphical data, with the purpose of expressing, testing hypothetical relationships and detecting patterns within the collected data

(Richtsmeier et al., 2002). It should be noted that compared to other techniques, such as molecular techniques, morphometry is an economical and effective technique, and, therefore, it has been widely used by biological sciences to support works in the areas of taxonomy and population analysis (Francoy et al., 2012; Nunes et al., 2013; Dellicour et al., 2017).

Therefore, in view of the fragmentation of the Atlantic Forest, one of the world's hotspots, due to its high biodiversity, which is endemic and endangered, and the expansion of agricultural practices in its territory, like SAFs, this study has aimed to test the hypothesis that the landscape structure affects wing shape and size of three species of Euglossini (*Eulaema atleticana* Nemésio, *Euglossa cordata* (Linnaeus) e *Euglossa ignita* Smith).

Materials and methods

Study area

The study was conducted within the limits of the Environmental Protection Area of Pratigi (APA Pratigi), located in the state of Bahia, Brazil, between the coordinates 13º55'48''S and 38º57'13''W. The region has an average annual temperature of 25 °C, regular and abundant rainfall and relative humidity around 80% to 90% (Nascimento et al., 2007). The Environmental Protection Area of Pratigi (APA Pratigi) is defined by law as an area of sustainable use and it aims to protect the remnants of the Brazilian Atlantic Forest and associated ecosystems, in which extractive and agricultural activities are allowed in a controlled manner. Thus, the landscape of the Environmental Protection Area of Pratigi can be described as intensely modified and fragmented, composed mainly of remnants of Atlantic Forest in different stages of regeneration, agroforestry systems, mangrove, *restinga* (coastal ecosystem with sandy soils and various vegetal communities) and pasture.

Sampling

The specimens were collected at five sampling points. The landscape location and characteristics of each point are shown in Table 1. The collections occurred between June 2014 to May 2015, through aromatic traps, according to the model proposed by Aguiar and Gaglianone, 2008. A cotton swab soaked with synthetic compounds (eucalyptol, methyl cinnamate, benzyl acetate, vanillin and methyl salicylate) was added into each trap in order to attract the males of Euglossini. Traps were set in the vegetation at 1.60m from the ground and remained in the place from 09:00AM to 3:00PM. At the end of this period, the bees were sacrificed with ethyl acetate. In the laboratory, the specimens were assembled and identified through the identification keys (Nemésio, 2009) and by comparison with the reference collection. Thereafter, all the specimens were deposited in the entomological collection of the Environmental Studies Laboratory of the Universidade Estadual de Feira de Santana (UEFS).

Table 1. Collection points, geographic coordinates and landscape composition around collection points, totalling an area of 5 km radius.

Collection points	Geographic coordinates	Altitude	Landscape composition
Area 1	13°41'07.3" S / 39°05'18.8" W	71	Dense Ombrophilous Forest[1] (68,9%); restinga[2] (4%); mangroves (14,3%); pasture (1,2%).
Area 2	13°50'36.7" S / 39°17'21.5" W	230	Dense Ombrophilous Forest of Submontane[1] (60,5%); *Theobroma cacao* L., *Syzygium aromaticum* (L.) Merr. & L.M.Perry and *Bactris gasipaes* Kunth (33,7%); pasture (4,2%).
Area 3	13°54'50.4" S/ 39°27'23.9" W	470	Dense Ombrophilous Forest of Submontane[1] and Dense Ombrophilous Forest of Montane[1] (45,8%); *Theobroma cacao* L.(10,1%); *Hevea brasiliensis* Muell. Arg. and pasture (15,4%).
Area 4	13°53'51.8" S / 39°27'44.8" W	680	Dense Ombrophilous Forest of Montane[1] (43,4%); *Theobroma cacao* L. and agriculture (41,6%); pasture (13,4%).
Area-SAF	13°46'55.90"S 39°16'53.8" W	230	Dense Ombrophilous Forest[1] (50%); Musa paradisiaca L. (31,8%); *Theobroma cacao* L. and forest (11,9%); pasture (5,7%).

[1] Classification adopted by Instituto Brasileiro de Geografia e Estatística IBGE (2012) to characterize the kinds of landscape, which can occur throughout Atlantic Forest.
[2] Coastal ecosystem with sandy soils and diverse plant communities.

Morphometric analysis

In order to quantify the variance in the wing shape and size we adopted geometric morphometrics methods. For this purpose, 22 to 30 individuals of the three most abundant species in the study area, *El. atleticana, Eg. cordata* and *Eg. ignita*, were selected out from a total of 423 individuals (Table 2). Initially, the right anterior wing of each individual was removed and fixed on a microscope slide. After that, wings were photographed in a stereomicroscope with a coupled camera, integrated with the Leica Application Suite software.

The images of each wing were converted to the TPS format, through the tpsUtill 1.60 program (Rohlf, 2013). Afterwards, there was an insertion of landmarks and semilandmarks in the wing veins, through the tpsDig.2 program (version 2.18) (Rohlf, 2015). Around the marginal cell, on the wings of the *Eg. ignita* and *Eg. cordata* specimens, 13 landmarks and 13 semilandmarks were inserted, whereas on the wings of *El. atleticana*, 10 landmarks and 15 semilandmarks were inserted (Fig 1).

Data of semilandmarks were aligned afterwards, through the tpsRelw 1.49 program (Rohlf, 2010). The statistical analysis started with the Generalize Procrustes Analysis (GPA), which is characterized as a positional adjustment between all used wings. After the GPA, the wing shape of each individual was used to compare the variance of shape within and among populations (Adams et al., 2004).

The differences in shape, were analysed by using the multivariate statistical methods of Canonical Variable Analysis (CVA) and Procrustes Distance. The differences in shape were illustrated by deformation grids, generated by the *Thin-plate-spline* technique (Bookstein, 1989). These analyses were conducted in the MorphoJ program, version 1.06 (Klingenberg, 2008).
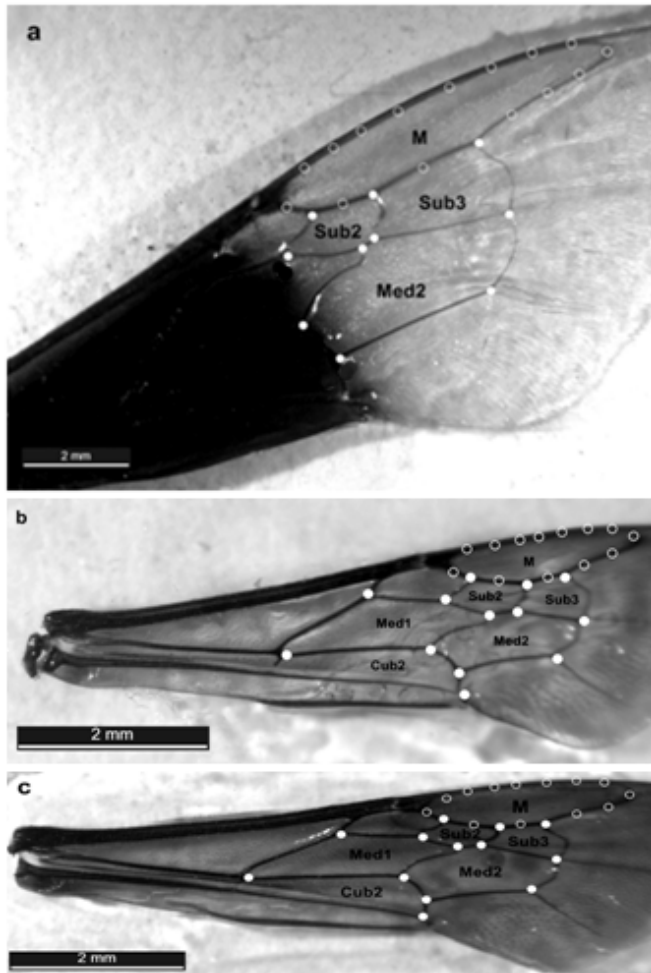
Furthermore, the Procrustes Distance was used for the Cluster Analysis by the *Unweighted Pair-Group Method with Arithmetic Mean* (UPGMA), thus, the average values of shape served as a basis for assessing the degree of similarity between the analysed groups. This analysis was conducted in the PAST program, version 2.17 (Hammer et al., 2001) and the results were represented in the mode of a dendrogram, produced from a bootstrap with 1000 repetitions.

We used the centroid size data, which is a geometric measure, defined from the square root of the sum of the square distance of each anatomical landmark, from the centre of the shape to evaluate the wing size (Monteiro & Reis, 1999). The centroid size values were extracted from the MorphoJ program and submitted to a Variance Analysis (ANOVA one way) in the PAST program.

The relationship of the variance in the wing shape and size with the characteristics of the landscape was conducted through the Mantel Test, a statistical method used for the correlation of two distance or similarity matrices, through the PAST program (Hammer et al., 2001).

Landscape classification

The landscape metrics were calculated for an area of 5 km radius around the five collection points through the V_LATE 2.0 extension of the Arcgis 10 program. This radius metric was estimated based on Euglossini flight capacity as presented by Pokorny et al. (2014). In turn, the landscape

Table 2. Number of individuals examined at each collection point.

| Species	Area 1	Area 2	Area 3	Area 4	Area-SAF
Eulaema atleticana	31	26	30	26	29
Euglossa cordata	30	28	29	28	26
Euglossa ignita	29	29	28	22	32

metrics (with emphasis on forest fragment cover area) were obtained for three indexes: 1) Total Area; 2) Total of core area, adopting 100m as an area subject to edge effect; 3) Average size of the fragments (McGarigal & Ene, 2013) (Table 3). Therefore, the soil use and occupation were surveyed around the five collection points through the use of data in shape file format produced by the Organization for Conservation of the Southern Lowlands of Bahia (OCT) (see Fig S1, Supplementary material - doi: 10.13102/sociobiology.v66i2.3779.s2234).

Table 3. Landscape metric for an area of 5 Km radius around the collection points.

Collection points	Total Area of Forest (ha)	Mean Patch size (ha)	Total core Area (ha)
Area 1	5,367	536.7	4,481
Area 2	4,753	148.5	3,096
Area 3	3,420	142.5	1,807
Area 4	2,667	106.7	1,373
Area-SAF	3,928	178.5	2,208

Fig 1. Landmarks (points) and semilandmarks (circles) used for shape and size of the wing analysis of *Eulaema atleticana* (a), *Euglossa cordata* (b) and *Euglossa ignita* (c). The wing cells were named according to the classification adopted by Francoy *et al* (2012): Marginal (M), 2nd submarginal (Sub2), 3nd submarginal (Sub3), 1st medial (Med1), 2nd medial (Med2), 2nd Cubital (Cub2).
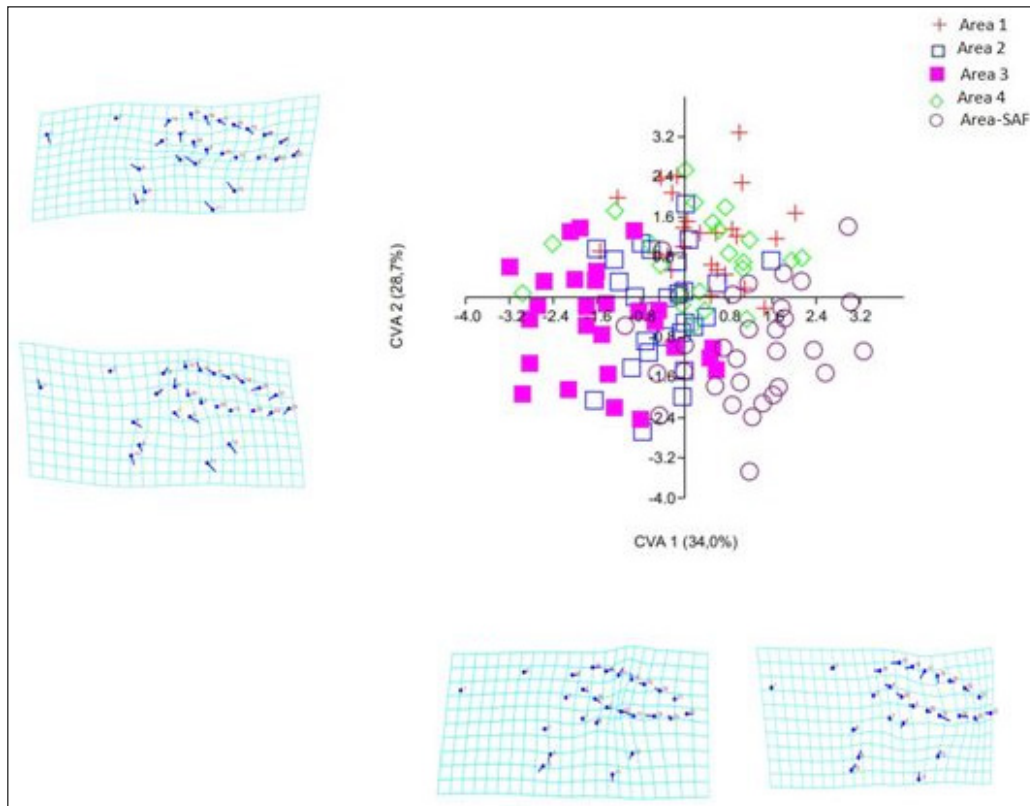
Fig 2. Scatter plot of the five populations of *Euglossa ignita* in relation to the canonical variables CVA 1 and CVA 2, arranged in a Cartesian plane and obtained from the wing shape. The deformation diagrams for the negative and positive sides of each axis are around the graph.

Results

The first three variables were necessary to explain more than 80% of the total variation in the Canonical Variable Analysis (CVA). The dispersion graph and the deformation grid have revealed important differences in the wing shape of populations of the three species, with emphasis on *Eg. ignita* species and the individuals collected within the SAFs.

The wing shape of the *Eg. ignita* populations (Fig 2) has diverged mainly between the following cells: 2nd medial, marginal and 3rd submarginal. For SAF's population, the deformation grid CVA 1 has demonstrated that there was a reduction of the 2nd medial and the central portion of the marginal region, accompanied by an increase of the 3rd submarginal.

Table 4. Mantel Test used to compare the environmental data matrices and morphometric data (wing shape and size) of *Euglossa cordata* and *Eulaema atleticana*, with 5000 permutations.

| Correlation of matrixes	R	p
Euglossa cordata		
Shape x Altitude	0,04113	0,0991
Size x Altitude	0,02437	0,1984
Shape x Mean Patch Size	0,1121	0,0053*
Size x Mean Patch Size	0,1296	0,0085*
Shape x Total Core Area	0,07339	0,0104*
Size x Total Core Area	0,08407	0,0116*
Eulaema atleticana		
Shape x Altitude	0,1579	0,3019
Size x Altitude	0,0378	0,1442
Shape x Mean Patch Size	0,0804	0,0339*
Size x Mean Patch Size	0,1151	0,0164*
Shape x Total Core Area	0,0627	0,0285*
Size x Total Core Area	0,0885	0,0116*
Shape x Total Area of Forest	0,0375	0,0883
Size x Total Area of Forest	0,0542	0,0349*

*p< 0,05, significant

From the values of the distance of Procrustes (p<0,05), the differences of shape were meaningful for the three species. For *Eg. ignita* and *El. atleticana*, the highest values of dissimilarity were found among the population of Area 4 and of the SAF (p=0,01 and p=0,007, respectively). For *Eg. cordata*, were found among the population of the SAF and Area 3 (p=0,01). The results of the Cluster Analysis (Fig 3) have demonstrated the wing shape of the collected populations in the SAF differs significantly from the other places. These differences in the wing shape have put the SAFs population as an isolated group. The formed dendrogram for each species has showed the following cophenetic correlation: *El. atleticana* 83%, *Eg. cordata* 95% and *Eg. ignita* 86%.

Variations in the wing size, based on the centroid size, have been significant only for *Eg. ignita* (ANOVA, F=5,923 and p=0,00045) (Fig 4). We have verified that the largest variation interval has been identified for Area 4 and SAF populations, while the smallest variation interval has been observed for the Area 3. According to Tukey's Range Test, the Area 3 population has been the only one that has differed statistically from the Area 4 population (p=0.0248) and Area 5 (p=0.0005) indicating the individuals with the lowest values for wing size.

The Mantel Test (Table 4) has demonstrated that the elevation is not a determining factor for differences in wing shape and size of the species, even though it is a geographic characteristic which varies significantly within the study area. Although the value of p was significant, the value of R does not show that the shape and size of the wing are influenced by the landscape metrics. However, this data suggests that there is a trend of variation in the data, which may, in theory, be due to environmental variables. The wing shape and size of *Eg. cordata* and *El. Atleticana* have showed positive correlation with the Mean Fragment Size and the Total Core Area (p< 0,05), and the wing size of *El. atleticana* has showed correlation with the Total Area of forest.
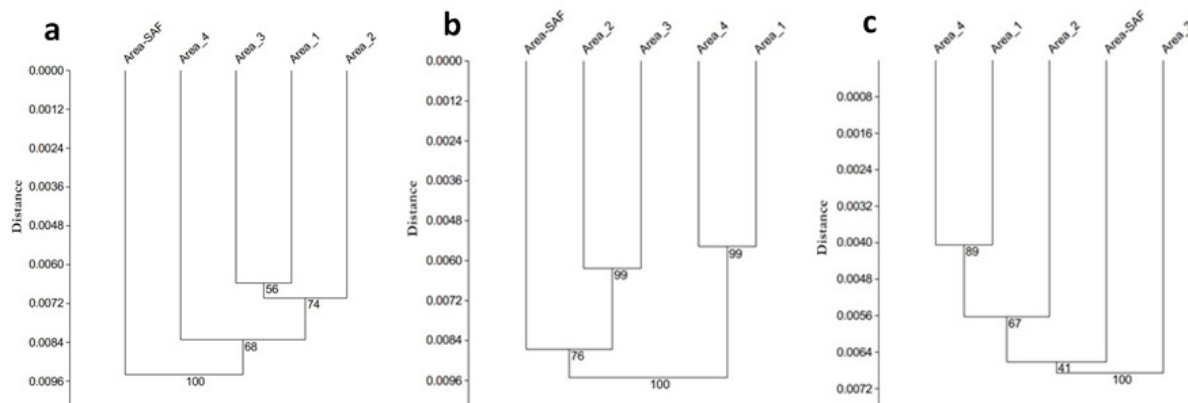
Fig 3. Dendrogram UPGMA obtained from the average values of the shape. (a) *Euglossa cordata*, with cophenetic correlation of 95%; (b) *Euglossa ignita* with cophenetic correlation of 86%, (c) *Eulaema atleticana,* cophenetic correlation of 83%.
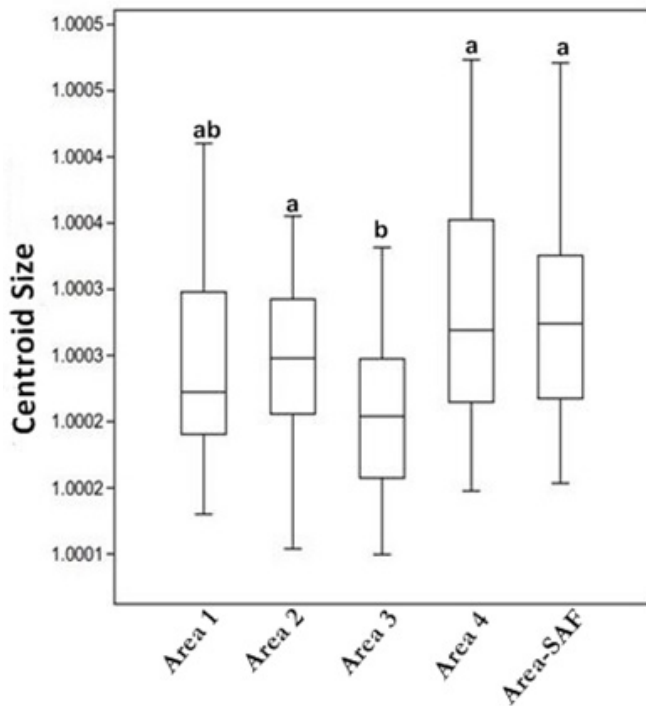
Fig 4. Box Plot and Tukey's Range Test for the centroid size of *Euglossa ignita*. The horizontal lines represent the medians; the rectangles represent the interquartile range (25-75%), while the vertical lines inform the intervals of variance. By Tukey's range test, the same letters do not differ statistically from each other.

Discussion

The morphometric analysis has demonstrated that the wing shape of the three species has varied significantly among the populations, especially those which were collected within the SAF. This result indicates that the landscape may be causing these changes in the populations, since we have found a correlation between the landscape metrics and the wing shape and size of two species: *Eg. cordata* and *El. atleticana*. Furthermore, the medial region, which is known to perform a key role in the flying capacity of insects (Johansson et al., 2009), it has undergone changes in *Eg. cordata* and *Eg. ignita* populations. Conditions which are found during foraging, may be a possible reason for the existence of these changes, because changes in wing morphology may be related to the costs and benefits that are involved in the dispersion pattern in fragmented landscapes (Hill, 1999). Landscapes that differ in extent of fragmentation provide different selection processes for dispersion, therefore, to deal with these differences, organisms have as alternatives the genetic adaptation or the phenotypic plasticity (Merckx & Van Dick, 2006).

Due to fragmentation, a new set of environmental conditions is generated, causing changes in ecosystem processes and occurrence of problems, such as edge effect. For Euglossini bees which are dependent on various forest resources, the creation of open areas or the introduction of agricultural activity, as in SAF, can cause a drop in the abundance of plants used as a source of resources, leading to foraging farther and farther. During foraging, these bees will be exposed to temperature variations and food scarcity, factors that are capable for influencing aspects of the phenotype and ecology of these insects (Whitman & Agrawal, 2009).

According to these authors, phenotypic plasticity guarantees to individuals an ecological niche amplitude and occupation of new spaces. In anthropogenic environments, such as areas with forest remnants, the percentages of incident light, humidity, temperature and wind speed are modified (Bennett & Sauders, 2010). In accordance with Reed et al. (2011), the effects of these changes are commonly observed in species with brief life cycles and with high population and fertility rates, because they allow the appearance of morphological changes in a short period of time. In these circumstances, the Insecta class is widely used in analyses of phenotypic plasticity (Moczek, 2010).

The interaction between environmental conditions (temperature, humidity, geographic location, altitude) and wing shape was identified in some groups of insects, namely: Diptera (Prudhomme et al., 2016), Lepidoptera (Bai et al., 2015), Hymenoptera (Silva et al., 2009), demonstrating that the wing shape is an indicator of stress during the development of these organisms. However, at the landscape level, investigations about the effects of landscape changes on wing shape are still scarce. Silva et al. (2009) reported that specimens of *Euglossa pleosticta* (Dressler), which were collected in border and interior environments of the forest fragment, showed important differences in the wing symmetry of the individuals, which suggests that changes in wing shape were due to instability in larval development, as a result of climatic and anthropic factors operating over the environmental conditions within the border as well as in the core area of the fragment. Outomuro et al. (2013) identified *Trithemis* dragonflies collected in open areas had wings wider than those collected in forest areas, demonstrating that wider wings were a favourable condition in open areas.

Beyond the shape, the wing size of insects may also indicate the environmental conditions to which the individuals were exposed. Thus, the occurrence of individuals with larger wings within the SAF suggests they were able to forage over larger ranges, using the agricultural areas, or returned to the SAFs with a greater supply of resources. Similar results were found by Merckx and Van Dick (2006) for butterflies of the *Pararge aegeria* (Linnaeus) species. In assessing the larval development of this species in three landscapes (agricultural landscape, forest landscape and landscape with forest fragments), authors notice that larvae that developed in agricultural landscape had more thoracic mass and higher wing loading than individuals developed in the forest landscape.

In insects, the wing size can be used as a proxy for the size body (Nijhout & Callier, 2015). For bees, the body size represents a greater ability in the environment, for it endow large individuals with high foraging capacity and

mating success (Kingsolver & Huey, 2008). Euglossini bees are benefited by the body size, as long as the males collect aromatic compounds in the plants, which are possibly used for the attraction of females, and consequently, they help in the dispersion of pollen. Euglossini females find resin in the plants for building of the nests, as well as, nectar and pollen which are inserted into the nest for feeding the offspring. For this cause, Euglossini bees tend to occupy larger fragments to guarantee the necessary resources for feeding and nesting (Brosi, 2009). Therefore, fragmentation and other changes in the landscape are problems that can impact strongly the populations of these bees. These impacts can reflect on the body size, which may indicate the decline of resources in the environment (Renauld et al., 2016).

Some researches (Benjamin et al., 2014; Renauld et al., 2016) have pointed out that fragmentation and proliferation of agricultural areas affect the bee's abundance regarding body size. Thus, the persistence in a particular environment will depend on the dispersion potential of the species, therefore, bees with greater mobility will be less prone to the effects of habitat loss (Jauker et al., 2013). Considering that smaller habitat patches may have fewer resources, only those individuals with greater foraging capacity will be able to use the dispersed resources in the landscape.

Bees are pollinators of great importance in tropical areas, however, evaluations about the impact of the simplification of ecosystems on these taxa are still scarce. Our study has demonstrated that the variations in the shape and size of Euglossini bees are good indicators of environmental variance and the SAFs have made a significant influence on the morphological characteristics of these bees. Thus, to consider the speed with which natural environments are converted into agricultural areas, the response of these pollinators, to these changes, becomes a fertile field for future researches and proposals for conservation of bees.

Acknowledgments

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