Sociobiology
An international journal on social insects

RESEARCH ARTICLE - BEES

The Importance of Fluctuating Asymmetry as an Indicator of Environmental Stress: Wing Morphological Responses of *Euglossa melanotricha* to an Altitudinal Gradient

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Article History

Edited by
Willian Moura Aguiar, UEFS, Brazil
Received 13 August 2024
Initial acceptance 25 August 2024
Final acceptance 14 November 2024
Publication date 30 January 2025

Keywords
Apidae, Bee ecology, Climate change, Extreme ecosystems, Geometric morphometric techniques, Serra do Cipó.

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Abstract

Abrupt abiotic changes in mountain ecosystems act as environmental filters, influencing the phenotypic expressions of numerous organisms. On the other hand, these responses along altitude gradients for native species are little understood. In this work, we examined the alterations in wing morphology, wing size, and vein fluctuating asymmetry (FA) of *Euglossa melanotricha* (Apidae: Euglossini) as a response to climatic variables across an altitudinal gradient in the rupestrian grassland (campo rupestre) of Serra do Cipó, Brazil. We collected bee specimens at seven altitudes (1. 800 m; 2. 900 m; 3. 1000 m; 4. 1100 m; 5. 1200 m; 6. 1300 m; 7. 1400 m), capturing 20 individuals of *E. melanotricha* at each elevation site. A significant difference was identified in *E. melanotricha* wing veins fluctuating asymmetry along the altitudinal gradient where the higher FA values were recorded in sites at higher altitudes. On the contrary, we found that wing area, wing length, and wing width presented higher values in sites at lower altitudes. Concerning wing morphology, the larger and wider wings were recorded in sites at lower altitudes compared to sites at higher altitudes, where wings were narrower and less elongated. The results of this study demonstrate that changes in climatic conditions along the altitudinal gradient significantly impact the physical characteristics of *E. melanotricha*. Furthermore, it can be concluded that both FA and geometric morphometrics are valuable tools for monitoring environmental stress in pollinators.

Introduction

Mountainous regions represent environmental gradients that can induce physiological stress in various groups of organisms. This stress arises from the harsh climatic conditions associated with changes in altitude, which involve a combination of multiple environmental variables such as temperature, precipitation, UV-B radiation, and wind speed, among others. Additionally, mountainous areas exhibit high geophysical heterogeneity variation, including topography, geomorphology, and slope aspects (Vitasse et al., 2021). Therefore, mountain regions represent an ideal natural laboratory for exploring the main environmental factors shaping the diversity, plant and animal distribution as well as the phenotypic variation associated with physiological and morphological adjustments of organisms in response to environmental changes (e.g., Fernandes et al., 2016; Vitasse et al., 2021).

Because in altitudinal gradients, air temperature decreases by approximately 1°C with every 100 m increase in altitude,

while precipitation and UV radiation increase with rising altitude, it is possible to expect a prominent physiological stress in some organisms such as insects inhabiting higher altitudes (Fernandes & Price, 1991). This physiological stress may be expressed as alterations in the size and shape of insect bodies, reflecting the influence of environmental factors as important stressors (Kendall et al., 2019; Skandalis et al., 2009). Thus, body size and wing morphology are some of the most notable traits of terrestrial and flying insects once correlated with their performance and fitness (i.e., survival, longevity, fecundity, and physiological and metabolic traits) (Chown & Gaston, 2010). Wing shape imposes evolutionary constraints directly linked to aerodynamics and flight efficiency for some insect taxa, including bees (Nunes et al., 2013). For example, under stressful conditions in harsh environments characterized by low temperatures and intense winds, bees experience different effects such as diminished resource-searching ability (Santos et al., 2020), alterations in body size as well as in the length of body appendages, variations in hair length and density, and increase of fluctuating asymmetry (FA) in the body, wings, and other morphological traits (Santos et al., 2020; Vaca-Sánchez et al., 2023). In this way, bees' wings have a complex venation system where hemolymph circulates from the thorax to the wings, which prevents heat loss in cold environments. Therefore, the survival and reproduction of both bees and plants they pollinate are affected in harsh environments such as mountaintops, having important implications for biotic interactions and plant communities, considering that bees pollinate more than 85% of angiosperms (Hoiss et al., 2012).

Bee morphology and body size indicate the impact of environmental filters and geographic and seasonal barriers (Kendall et al., 2019; Skandalis et al., 2009). On the other hand, wing shape is subject to more substantial evolutionary constraints and directly affects aerodynamics and flight efficiency (Nunes et al., 2013). Additionally, body size can exhibit considerable plasticity in response to environmental cues, conferring some advantages to bees. For instance, survival, fecundity, and mating success are associated with larger body size (Kingsolver & Huey, 2008). As holometabolous insects, bees do not undergo morphological changes during adulthood. Therefore, variations in body size can result from natural selection, climatic variations, and resource availability during the larval stage (Chole et al., 2019; Chown & Gaston, 2010). In contrast, wing shape variability responds to environmental factors, considering the costs and benefits of dispersion in anthropized landscapes such as forest fragments and agrosystems (Gérard et al., 2021).

Fluctuating Asymmetry (FA) is a statistical measure that quantifies the random variations in shape and size between two sides of a bilateral trait of organisms (Palmer & Strobeck, 1986). FA is considered a reliable indicator of developmental instability of an organism, reflecting their ability to buffer genetic or environmental perturbations and express a genetically determined phenotype (Palmer & Strobeck, 1986); were these perturbations frequently stem from environmental disturbances. Since both sides of morphological traits are determined by the same set of genes, the degree of FA reveals an individual inability to maintain homeostasis during development under stress conditions (Møller & Swaddle, 1997). Consequently, high FA levels can correlate with lower growth, fertility, and survival compared to more symmetric individuals (Cuevas-Reyes et al., 2018).

Geometric morphometrics is an important tool for analyzing the impact of environmental variation on populations, allowing morphological data quantification into numerical and graphical formats to explore and validate hypothetical relationships between environmental factors and morphological variations in different organisms, including bees (Cuevas-Reyes et al., 2018; García-Jain et al., 2021). Notably, geometric morphometrics is both cost-effective and efficient in comparison with other techniques, such as molecular methods. Consequently, it has found widespread use in biological sciences, supporting studies in taxonomy, population analyses, and ecology (Aguilar-Peralta et al., 2020; Dellicour et al., 2017).

Euglossini bees are widely distributed in the Neotropics, occurring from sea level up to 2000 meters in elevation. The highest diversity of Euglossini is observed at low and medium elevations. This bee tribe comprises approximately 250 species grouped into five genera (e. g. *Euglossa*, *Eufriesea*, *Eulaema*, *Exaerete*, and *Aglae*) spanning from northern Mexico to northern Argentina. In lowland wet forests of this region, Euglossine bees can constitute up to ca. 25% of the local bee communities. Euglossini bees, also known as orchid bees, play a pivotal role as pollinating agents for a multitude of plant species, including the following families: *Araceae*, *Euphorbiaceae*, *Gesneriaceae*, *Solanaceae*, and especially *Orchidaceae*. Male Euglossini bees collect essential oils primarily produced by orchid flowers, making them exclusive pollinators of approximately 700 orchid species. These neotropical bees exhibit the ability to fly long distances within continuous forests. Despite their ecological significance, little is known about their wing symmetry and morphological responses to altitudinal gradients (but see Vaca et al., 2023).

Therefore, we utilized *Euglossa melanotricha* to assess the effects of altitudinal gradient as an environmental filter in Serra do Cipó, Brazil on wing morphology. We addressed the following questions: (i) Does the wing size and morphology of *E. melanothrica* change with altitude?; (ii) Does the expression of environmental stress levels in fluctuating asymmetry increase as altitude increases along the gradient?

Material and Methods

Study area

The study was conducted in the Serra do Cipó (19°10'-20' S, 43°30'-40' W), situated in the southern part of the Espinhaço mountain range in Brazil. The Serra do Cipó encompasses a vegetation complex composed of grassy habitats called rupestrian grassland (*Campo Rupestre* in

Portuguese). This vegetation is predominantly sclerophyllous and exhibits high levels of endemism (e.g. Fernandes, 2016). The climate is characterized by the presence of dry winters and rainy summers, with an average annual temperature of 21°C and an average annual precipitation of 1500 mm. Our study area included seven sites located along an altitudinal gradient ranging from 800 to 1400 m.a.s.l. and separated at intervals of 100 m of elevation (see Table 1). We determined three different altitude classes where the seven study sites were grouped: i. Lower altitude: Rio Cipó (800 m a.s.l.), Usina (900 m a.s.l.) and Serra Morena (1000 m a.s.l.); ii. Medium altitude: Cedro (1100 m a.s.l.) and Pedra do Elefante (1200 m a.s.l.) and iii) Highest altitude: Quadrante 16 (1300 m a.s.l.) and Alto Palácio (1400 m a.s.l.) (Fig 1). For further detail on the sites see Fernandes et al. (2016).

Study species

Euglossa melanotricha Moure, 1967, a member of the order Hymenoptera, family Apidae, and tribe Euglossini, is a medium-sized bee measuring 13 mm in body length and exhibiting metallic coloration (Fig 2). It is frequently encountered in open savanna habitats in Brazil and Bolivia.

Table 1. Summary of the results of each generalized linear mixed model testing the elevation effects over area, length, width wing, and FA levels of wing veins for *Eulaema melanotricha* individuals along an altitudinal gradient in Serra do Cipó, Brazil.

Response variable	Explanatory variable	*F*	p <
FA M1	Altitude	1.78	0.12
FA M2	Altitude	1.81	0.18
FA M3	Altitude	0.56	0.013*
FA M4	Altitude	0.68	0.68
FA M5	Altitude	0.79	0.59
FA M6	Altitude	0.74	0.96
FA M7	Altitude	12.2	0.001*
FA M8	Altitude	3.02	0.015*
FA M9	Altitude	8.47	0.042*
FA M10	Altitude	9.06	0.027*
FA M11	Altitude	6.81	0.045*
Wing area (Warea) (mm^2)	Altitude	2.28	0.001*
Wing length (Wlenght) (mm)	Altitude	14.02	0.002*
Wing width (Wwindth) (mm)	Altitude	17.68	0.001*

*Significant values.

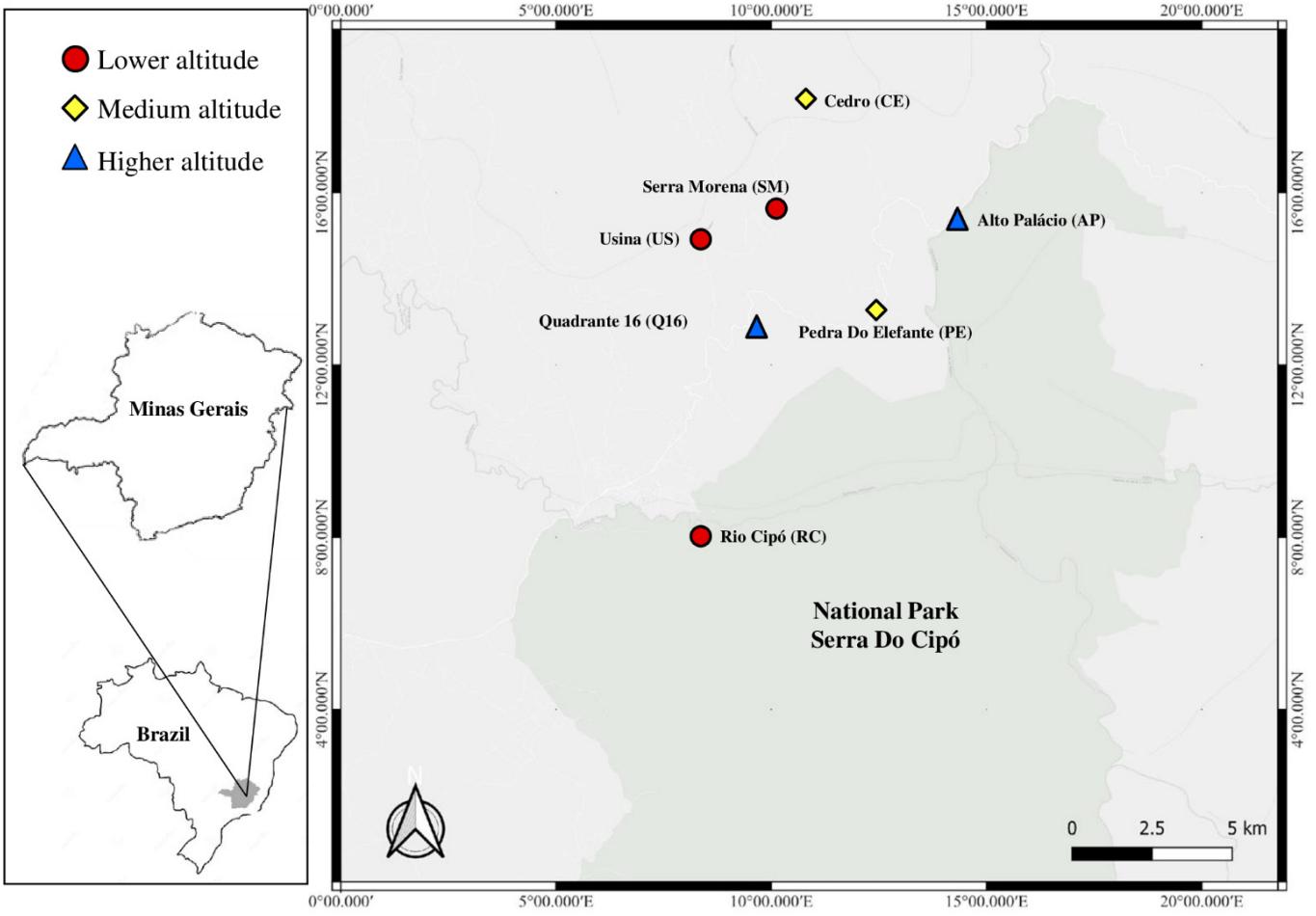

Fig 1. Map of *Eulaema melanotricha* collection sites with different elevations along the altitudinal gradient in the Sierra de Cipó, Brazil.

This species abounds in the entire Serra do Espinhaço mountain range, i.e., both Minas Gerais and Bahia states. Notably, *E. melanotricha* is an effective pollinator for orchid species and is abundant in tropical mountain ecosystems (e.g., Santos et al., 2020; Freitas et al., 2023). We specifically chose this species due to its widespread occurrence and remarkable phenotypic plasticity along altitudinal gradients.

Collection of Euglossa melanotricha

During the dry season (May-October) and the rainy season (November-April) of 2017, bees were collected in all altitudes using scent traps baited with fragrances such as benzyl acetate, beta-ionone, cineole, methyl cinnamate, methyl salicylate, eugenol, and vanillin. These traps were exposed for 48 hours, following the protocol outlined by Bezerra & and Martins (2001), as detailed in Santos et al. (2020). At each altitude, 20 individuals were randomly selected from the reference collection, which is deposited in the entomological collection of the Laboratory of Evolutionary Ecology and Biodiversity (LEEB) at the Universidade Federal de Minas Gerais, Brazil. In all collected bees, fore, and hind wings were dissected and separated using tweezers and mounted on blades for subsequent photography. After this, an M80 model stereomicroscope equipped with an IC80 HD built-in camera (configured at a magnification of 0.75 mm) was used for capturing wing photographs. Each digital image was edited using the stereomicroscope's software, with a 2 mm scale added.

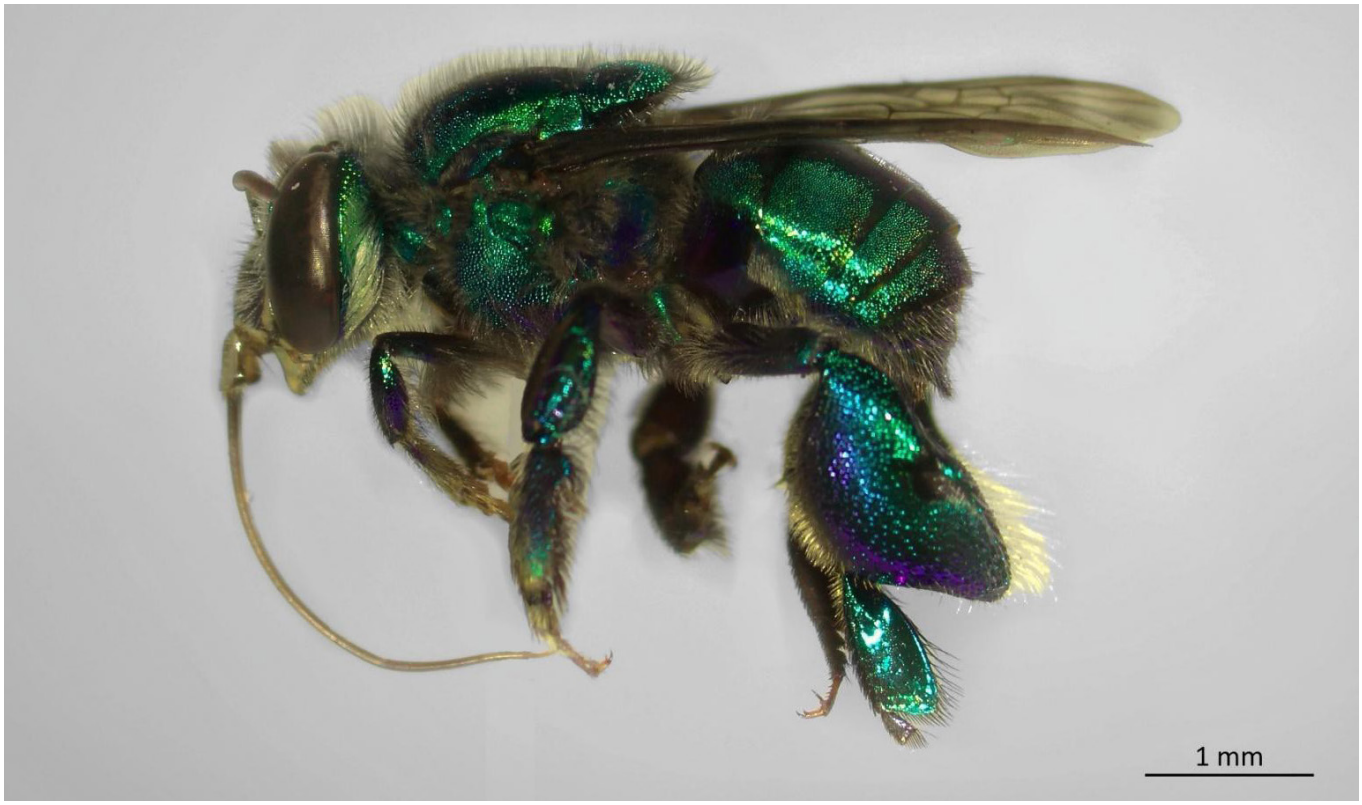

Fig 2. Side view photo of an *Euglossa melanotricha* Individual.

Climatic data

To assess the impact of climatic variables on wing size and fluctuating asymmetry of *E. melanotricha* along the altitudinal gradient in Serra do Cipó, climatic data for the study sites were obtained from the dataset provided by Mota et al. (2018). These data were collected using meteorological monitoring towers equipped with Onset HOBO® U30 data loggers, which recorded a range of climatic parameters as outlined in Fernandes et al. (2016). Seven stations were strategically placed at each study site, spanning altitudes between 800 and 1400 m a.s.l. The climatic parameters considered included air temperature (Atemperature), humidity percentage (H%), and wind speed (Wspeed). For our analyses, we calculated the mean value of each variable over the study period.

Fluctuating asymmetry and wing size

In each digital image of the wings obtained from the 20 *E. melanotricha* individuals at each altitude studied, wing length (W length), wing area (W area), wing width (W width), and the distances of 11 wing veins were quantified utilizing the ImageJ program (Fig 3).

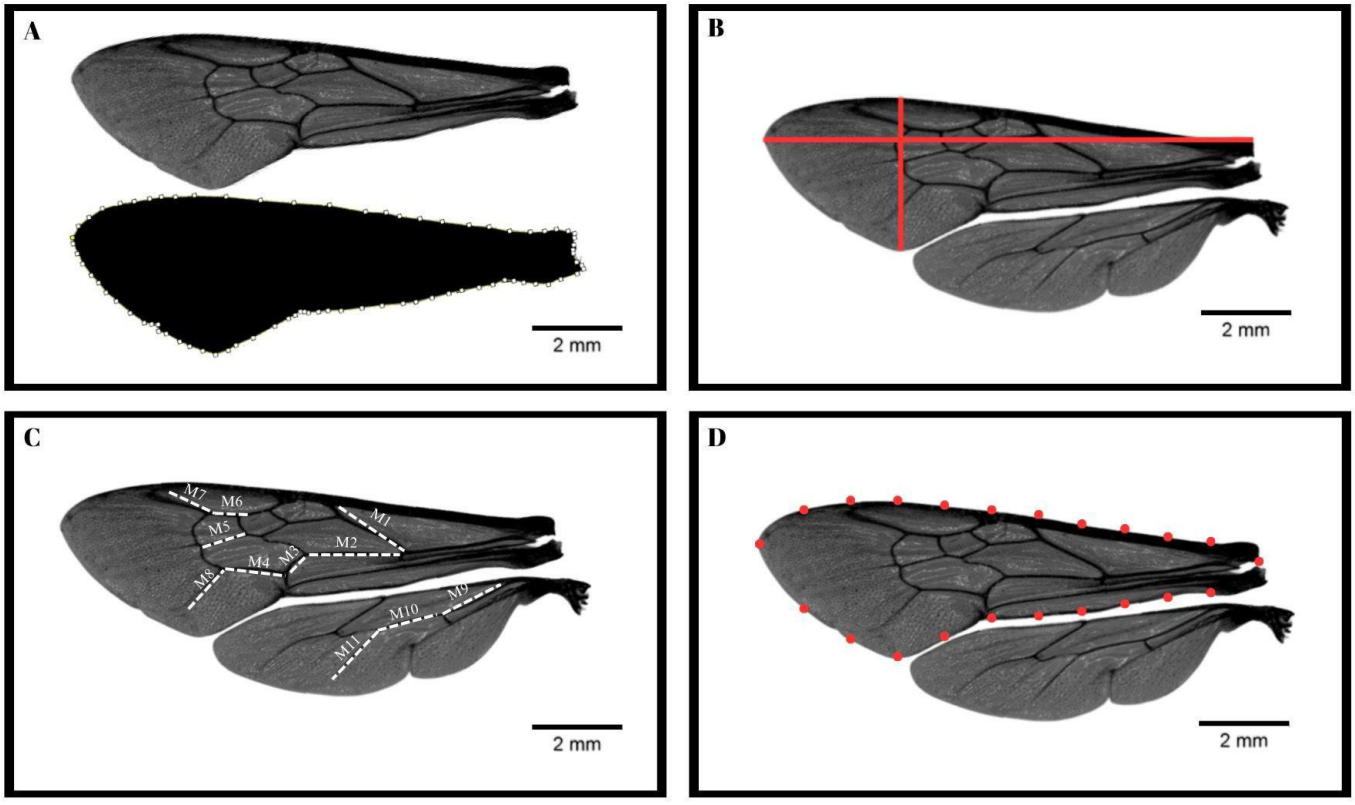

Fig 3. Wing measurements of the three wing traits size: (A) wing area, (B) wing length and width, (C) eleven wing veins of *E. melanotricha* for fluctuating asymmetry, and (D) morphometric measurements of wings of *E. melanotricha* showing the 22 morphological landmarks on the wings and two additional marks as size reference along the altitudinal gradient in Serra do Cipó, Brazil.

Additionally, FA was calculated from each digital image of the wings as the absolute value of the difference among the distances of the 11 wing veins on the right and left wings of *E. melanotricha* (| Ai - Bi |), divided by the mean distance (Ai + Bi/2) (following methods outlined in Cuevas-Reyes et al., 2018; Garcia-Jain et al., 2021).

Palmer and Strobeck (1986) distinguish between three types of asymmetries: fluctuating asymmetry (FA), directional asymmetry, and antisymmetry. FA quantifies the variance of random differences between the two sides of a bilateral trait, with a mean value of zero. Directional asymmetry refers to consistent biases in character within a species, favoring greater development on one side. In contrast, antisymmetry lacks symmetry and is characterized by a bimodal distribution of differences between the two sides, centered around a mean of zero (Palmer & Strobeck, 1992). We conducted a t-test and a Lilliefors normality test to confirm that our data specifically reflected FA. The average differences (Ai – Bi) did not significantly deviate from zero ($t = 5.2$; $p = 0.0001$), ruling out directional asymmetry. Similarly, the Lilliefors test indicated a normal distribution of differences (Ai – Bi) ($p > 0.05$), thereby discarding antisymmetry.

Geometric morphometric wing measurements

The differences in wing morphology among the *E. melanotricha* bees collected along the altitudinal gradient in Serra do Cipó were determined using geometric morphometric techniques. In each wing digital image used to assess FA, 22 anatomical marks (landmarks) were placed with two additional marks as a size reference (Klingenberg, 2011; Vaca-Sánchez et al., 2023). The landmarks represent wing shape and correspond to homologous loci that are unambiguous and repeatable marks across all wings (Cuevas-Reyes et al., 2018). We employed the TpsDig program to record the coordinates (x, y) of 22 anatomical landmarks in each wing image (Rohlf, 2015). To account for wing size effects, we conducted a Procrustes superimposition analysis using the MorphoJ program (Klingenberg, 2011). This analysis translates and rotates the landmark configurations into a common position, eliminating size differences (scaling) and providing centroid size and shape coordinates for the wings (Klingenberg, 2011). The average Procrustes shape coordinates, also known as the Procrustes mean, represent wing shape obtained by minimizing the sum of squared distances relative to other wing shapes within the sample. This shape serves as the reference for further analyses, including a Canonical Variation Analysis (CVA) (Cuevas-Reyes et al., 2018; García-Jain et al., 2021; Vaca-Sanchez et al., 2023).

Statistical analysis

The differences in wing area, length, width, and FA of *E. melanotricha* among sites with different altitudes were analyzed using general linear mixed models (GLMM). We carried out a separate model for each response variable.

The sites with different altitudes were considered fixed factors (explanatory variables), and the study site was considered a random factor. The response variables were the wing area, length, width, and FA of the nine veins.

To determine if the climatic variables (i.e., air temperature, humidity percentage, and wind speed) affected the wing area, length, width, and fluctuating asymmetry of bees along the altitudinal gradient, we performed a principal component analysis (PCA). Through this analysis, we obtained a PCA of the dispersion of points that showed if there exists an association of the wing area, length, width, and fluctuating asymmetry of individuals of *E. melanothricha* along the altitudinal gradient with the climatic variables (Stokes et al., 2012). Finally, to evaluate the morphological differences of wings of *E. melanotricha* individuals between different elevations along the altitudinal gradient of the Serra do Cipó, we performed a canonical variation analysis (CVA) using the MorphoJ program.

Results

We detected significant differences in FA levels in six out of the eleven wing veins analyzed (54.5 %) for *E. melanotricha* individuals along the altitudinal gradient in Serra do Cipó (Table 1). These veins showed higher levels of FA at higher altitude sites (Fig 4A, 4B, 4C, 4D, 4E, 4F, 4G). A contrary trend was observed for all attributes associated with wing size, where the area (Fig 4G), length (Fig 4H), and width of the wing (Fig 4I) decreased as altitude increased.

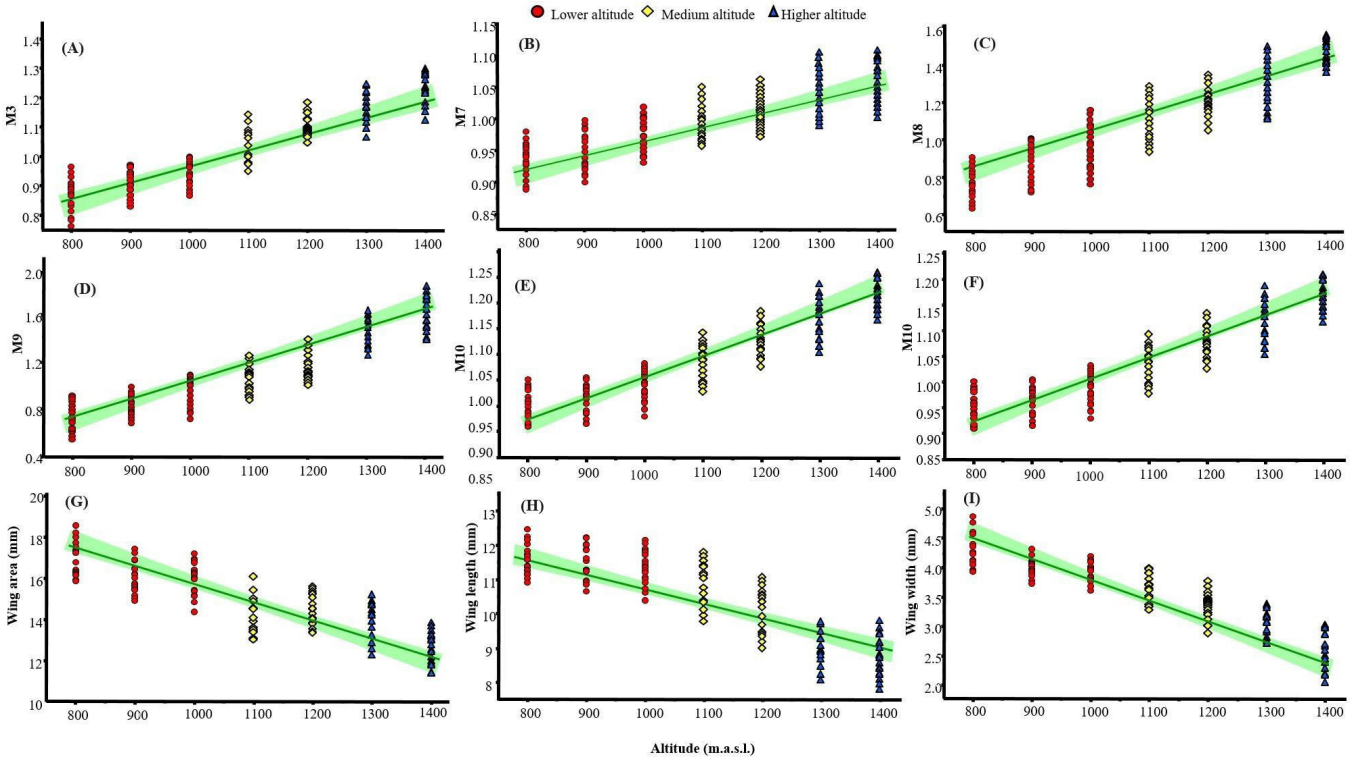

Fig 4. FA patterns of wing veins: (A) M3, (B) M7, (C) M8, (D) M9, (E) M10, (F) M11 and the three wing size traits analyzed: (G) wing area (Warea), (H) wing length (Wlength) and (I) wing width (W width) for *Eulaema melanotricha* individuals along an altitudinal gradient in Serra do Cipó, Brazil. Different study sites are represented along the altitudinal gradient by the following symbols: (1) red circles correspond to lower altitudes, (2) yellow diamonds represent the medium altitudes, and (3) blue triangles are the higher altitudes.

We also detected associations of FA levels of the wing veins and the three wing size traits of *E. melanotricha* individuals (i.e., area, length, width) with climatic variables along the altitudinal gradient (Fig 5A). We first observed that individuals of *E. melanotricha* analyzed along the altitudinal gradient of Serra do Cipó were separated into two different groups, where the PC1 axis explained 49.9 % and the PC2 axis explained 29.5 % of the total variance: (i) *E. melanotricha* individuals from higher altitudes (1200-1400 m a.s.l.) were grouped on the left side of the axis, where humidity percentage (H%) had a positive relationship with fluctuating asymmetry (FA), while wing area (Warea) had a negative relationship with H% and FA (Fig 5A); (ii) On the right side, individuals of medium (1000-1100 m a.s.l) and lower altitudes (900-800 m a.s.l) were grouped showing negative relationships between wing width (Wwidth) and wing length (Wlength) with air temperature (A temperature) and wind speed (Wspeed) (see Fig 5A).

Wing morphology of *E. melanotricha* individuals differed statistically among elevations along the altitudinal gradient according to canonical variation analysis that distinguished three principal groups: lower altitudes (800-1000 m a.s.l), medium altitudes (1100-1200 m a.s.l), and high altitudes (1300-1400 m a.s.l). CVA1 explained 75.12% of the total variance and CVA2 explained 17.51% (Axis 1 $\lambda = 0.0014$ df = 1010; 2 = 984.7; $p < 0.0001$, Axis 2 $\lambda = 0.7214$ df = 49,

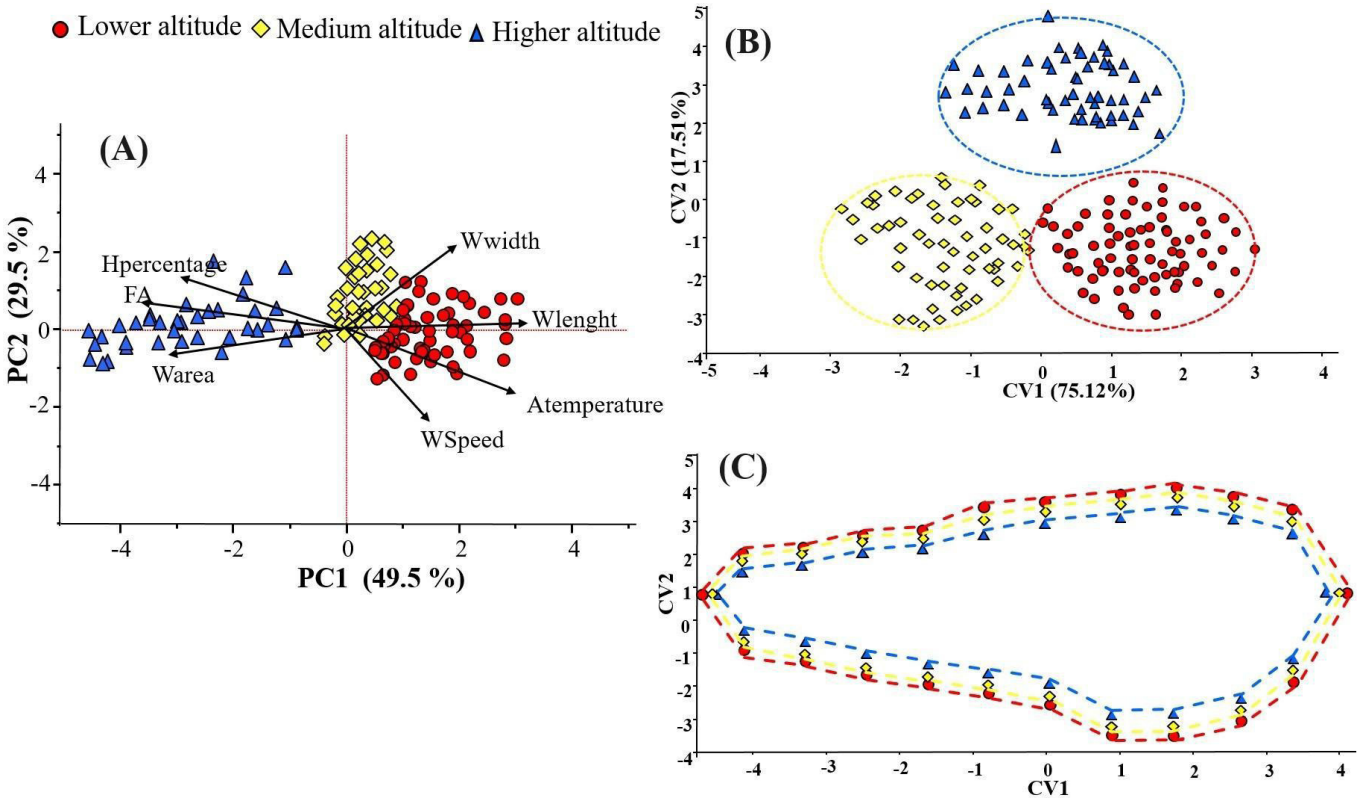

Fig 5. (A) Results of principal component analyses of fluctuating asymmetry of veins, wings size, width, length of *E. melanotricha,* and climatic variables along the altitudinal gradient. The Ws = wing size, Wl = wing length, Ww = wing width, FA = mean fluctuating asymmetry of the eleven veins, AT = air temperature, HP = humidity percentage, and WS = wind speed. The symbols of the study sites along the altitudinal gradient correspond as follows: lower altitudes are red circles, the yellow diamonds are the medium altitudes, and the higher altitudes are the blue triangles. Wing morphological variation of *E. melanotricha* among the sites along the altitudinal gradient: (B) Morphological difference and the wing shape of *E. melanotricha* showing the average coordinates of the configuration for wings along the altitudinal gradient. (C) Differences in wings shape morphology of *E. melanotricha* among the study sites along the altitudinal gradient of Sera do Cipó according to canonical variate analysis (CVA). The symbols of the study sites along the altitudinal gradient correspond as follows: lower altitudes are red circles, the yellow diamonds are the medium altitudes, and the higher altitudes are the blue triangles.

$p < 0.0001$) (Fig 5B). In addition, the wings of individuals from lower altitudes were longer and wider compared to those of individuals from medium, and higher altitudes, respectively, where their wing shapes were narrower and less elongated (Fig 5C).

Discussion

In flying insects, the shape and size of wings represent key functional traits associated with flight performance, dispersion ability, search for food, and even escape from predators. Therefore, these traits are subject to selection pressures based on the capacity for phenotypic responses or adjustments that allow them to persist in changing or harsh stressful environments (Dellicour et al., 2017). The results of our study indicated that there were significant differences in the wing vein asymmetry of *E. melanotricha* among the different elevations along the altitudinal gradient of Serra do Cipó. Specifically, changes in elevation turn out to be the main factor generating environmental stress in *E. melanotricha* individuals, increasing levels of fluctuating asymmetry in the wing veins as altitude increases. This results in an important environmental filter for wing morphological traits associated with bee performance (e.g., thermal performance, survival, longevity, fecundity, and physiological and metabolic traits), which in turn can affect their distribution and abundance across environmental gradients. Additionally, geometric morphometric analysis of the wing shape of *E. melanotricha* individuals exhibited substantial variation among the elevations analyzed in our study, where the wing morphology becomes less elongated and narrower as altitude increases. Body size is a fundamental trait that shapes multiple aspects of organismal physiology, ecology, and evolution of insects. Hence, a decrease in bee size may indicate limitations in its performance, growth, and reproduction. This could result in significant implications in the conservation biology of bees. This is particularly crucial given that wild bees constitute a highly diverse group of essential pollinators in natural and agricultural ecosystems (Freitas et al., 2022).

Our findings support the idea that altitude variation can influence these population-level changes. We observed a correlation between climatic variables, wing vein fluctuating asymmetry, and wing traits related to the size of *E. melanotricha* along the altitudinal gradient. This lines up with previous

studies that have reported morphological changes in the wings of mature bees resulting from stressors during their immature stages (Pinto et al., 2015; Vaca-Sánchez et al., 2023). For example, it has been documented that environmental stressors, such as lower temperature and humidity, influence phenotypic expression in various species of the Euglossini tribe, reducing wing size, increasing wing asymmetry, and changing wing morphology (Pinto et al., 2015; Vaca-Sánchez et al., 2023). At higher elevations, where temperatures are generally lower, the environment becomes more challenging for bees. This is because they need to attain higher temperatures to effectively carry out their foraging activities, which in turn affects flight performance and overall fitness *E. melanotricha*.

Our study revealed a distinct wing shape pattern across the altitudinal gradient of Serra do Cipó, where individuals of *E. melanotricha* exhibited different wing morphologies among lower (800-900 m a.s.l), medium (1000-1100 m a.s.l), and higher altitudes (1200-1400 m a.s.l). This fact can be explained by the high responsiveness of this bee taxon expressed in morphological adjustments to environmental filters of altitudinal gradients. Wind speeds at higher altitudes may constrain foraging capacity, leading to smaller wing sizes and increased asymmetry. Despite this limitation, *E. melanotricha* remains a highly dispersive bee in the mountain landscape. A possible explanation for these results is that at higher altitudes, the density of the air is reduced, which challenges the flight capacity of insects and favors the selection of reduced body sizes such as short and narrow wings, minimizing energy costs associated with flight and thermoregulation. Otherwise, future studies shall expand to evaluate the fine adaptations of insects to the changing climate at tropical mountaintops.

An additional factor explaining the changes in wing asymmetry, size, and morphology in *E. melanotricha* is the temporal and spatial variation in food quantity and quality along the altitudinal gradient (Hoiss et al., 2012). Some studies have indicated that alterations in body size distributions across an environmental gradient may indicate variations in resource availability, such as fluctuations in the abundance and composition of floral resources. It is conceivable that pollen limitation may influence the body size and wing morphology of bees (Chole et al., 2019). Our study species, *E. melanotricha*, is closely associated with specific plant groups, particularly orchids, where male bees are crucial in pollinating orchid flowers. In a study conducted by Santos et al. (2020) across the altitudinal gradient of Serra do Cipó, a decline in Euglossine abundance was reported with increasing elevation, correlating this trend with climatic variables (e.g., temperature, wind speed) and vegetation structure (see also Freitas et al., 2023). Notably, the rupestrian grassland vegetation at lower altitudes displays greater stratification and structural complexity, while habitats at higher elevations exhibit simpler vegetation (Fernandes et al., 2016; Mota et al., 2018). The observed shifts in vegetation structure along the altitudinal gradient will likely contribute to wing shape modifications and an increase in fluctuating asymmetry in wing veins among *E. melanotricha*, driven by reduced food availability. However, according to the 'Species Energy Theory' proposed by Maurer et al. (1992), the average body size within a community tends to decrease with increasing elevation. This phenomenon is attributed to the greater vulnerability of larger species to extinction in resource-scarce habitats than smaller species. Our findings align with the observation that smaller wing sizes in *E. melanotricha* are prevalent at higher elevations, specifically between 1200 and 1400 meters. This pattern suggests that individuals with larger body sizes face greater energy demands, resulting in smaller populations competing for the same resources as smaller individuals at higher altitudes (Kingsolver & Huey, 2008).

Conversely, Euglossine bees are notably influenced by wind and temperature availability. Higher elevations in mountainous ecosystems such as Serra do Cipó exhibit increased mean wind speeds and decreased temperatures (see Fernandes et al., 2016). Notably, larger body sizes thrive at mid to high elevations in certain regions. Our findings align with this pattern, as we observed reduced wing sizes at higher altitude sites and a correlation between wing size in *E. melanotricha* and temperature across the altitudinal gradient.

The results of our investigation showed that the phenotypic expression of *E. melanotricha* in the Serra do Cipó region is strongly influenced by changes in climatic factors along the altitudinal gradient. Given the correlation between bee decline and global warming and the knowledge that mountainous regions are more vulnerable to climate change, we highlight the crucial role that environmental factors play as stressors for bees. Furthermore, geometric morphometrics and fluctuating asymmetry (FA) represent effective methodologies for monitoring and assessing the impact of environmental stressors on pollinators, particularly in the challenging habitats of the Espinhaço mountain range in southeastern Brazil.

Author's Contributions

M. S. V-S: Formal analyses, investigation, writing - original draft, writing-review & editing

P. C-R: Conceptualization, formal analyses, investigation, writing-original draft, writing-review & editing

I. S. P-D.: Methodology, investigation, formal analyses, writing-original draft

I. M.: Methodology, investigation, writing-original draft, writing-review & editing.

Y. O.: Conceptualization, methodology, investigation, writing-original draft, writing-review & editing.

K. C.: Methodology, investigation, writing-original draft.

GWF: Conceptualization, investigation, resources, writing-original draft, writing-review & editing.

Acknowledgments

Thank the A. Almeida and T. Freitas for their methodological assistance at the beginning of the project.

The authors also acknowledge the financial support of the Brazilian National Council for Scientific and Technological Development (CNPq), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) and Coordination of Scientific Research (UMSNH) for supporting the research and providing grants. MSVS received a post-doctoral fellowship from UNAM. Thanks also to Programa de Pesquisas Ecológicas de Longa Duração (PELD) - Campo Rupestre Serra do Cipó (CRSC –CNPq/FAPEMIG), Reserva Vellozia, Cedro Têxtil, ICMBio-PARNA Serra do Cipó, Pousada Serra Morena, and Pousada Pouso do Elefante for logistical support.

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

Declaration of Competing of Interest

All authors declare that they have no conflict of interest. The work represents original research carried out by the authors. All authors agree with the contents of the manuscript and its submission to the journal. All authors have read, understood, and have complied as applicable with the statement on "Ethical responsibilities of Authors" as found in the Instructions for Authors.

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