Acta Zoologica

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Acta Zoologica

ORIGINAL ARTICLE

Developmental Stability Across Seasons but Not Months: Wing Asymmetry Responses of an Orchid Bee to Short-Term Climate Variability

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Received: 31 December 2025 | **Revised:** 15 February 2026 | **Accepted:** 2 April 2026

Keywords: bioindicators | fluctuating asymmetry | geometric morphometrics | orchid bees | temporal variability | tropical dry forest

ABSTRACT

Fluctuating asymmetry (FA) and wing shape variation have been widely proposed as sensitive indicators of developmental instability and environmental stress in insects, yet their temporal dynamics remain poorly explored in tropical systems. In this study, we examined fine-scale temporal and environmental variation in wing FA and shape of the orchid bee *Euglossa imperialis* Cockerell, 1922 within a tropical dry forest of central Panama. Using geometric morphometrics, we quantified bilateral wing asymmetry and shape variation across monthly sampling periods spanning dry and rainy seasons, integrating high-resolution climatic data. Overall FA values were low and showed no significant differences between seasons, indicating high developmental stability across broad seasonal regimes. Similarly, wing shape exhibited extensive overlap between dry and rainy seasons, with no discrete seasonal differentiation. In contrast, both FA and wing shape varied significantly among months, revealing a gradual and continuous temporal signal. Minimum temperature and relative humidity emerged as the main environmental variables associated with FA, whereas precipitation showed no detectable effect. These results indicate that developmental instability and wing morphology in *E. imperialis* are primarily shaped by short-term climatic variability rather than by categorical seasonal contrasts. Our findings highlight the importance of fine temporal resolution when evaluating morphological responses to environmental conditions and suggest that FA and wing shape function as scale-dependent indicators of environmental variation. Integrating geometric morphometrics with high-resolution climate data provides a powerful framework for refining the use of orchid bees as bioindicators in tropical ecosystems.

1 | Introduction

The degradation of terrestrial ecosystems, including habitat fragmentation and climate change, poses major challenges for conservation, particularly in tropical regions where insects respond rapidly to environmental disturbances. Habitat fragmentation involves habitat loss, subdivision of remaining areas, and edge effects, all of which increase the risk of species extinction (Hunter 2002; Ribeiro et al. 2019; Thomas et al. 1998). In Panama, forest ecosystems have experienced substantial

degradation, especially in the central region of the country (Roubik et al. 2021).

Within this context, orchid bees of the tribe Euglossini have regularly been proposed as bioindicators of habitat quality and conservation status due to their ecological importance as pollinators and their sensitivity to environmental change (Añino et al. 2019; Gonçalves and Faria 2021). Changes in land use and habitat structure have been shown to affect Euglossini communities, influencing species richness, abundance, and functional traits across a range of Neotropical landscapes (Leão-Gomes and Vasconcelos 2023). However, the suitability of orchid bees as bioindicators has also been debated (Gonçalves and Faria 2021; Leão-Gomes and Vasconcelos 2023; Vega-Hidalgo et al. 2020). In particular, concerns have been raised regarding whether traditional community-level metrics adequately capture the effects of environmental stress and climatic variability on these bees, highlighting the need for complementary approaches that focus on organism-level responses.

The ecological and biological traits of these bees have led some authors, such as Reyes et al. (2009), to classify them as effective bioindicators of forest conservation. However, Añino et al. (2019) raised a critical debate regarding whether orchid bees truly meet the criteria required for this role. In particular, these authors questioned whether orchid bees are affected by climate change in a manner comparable to other bee groups, potentially weakening their predictive value as bioindicators in this context. This debate was further developed by Gonçalves and Faria (2021), who responded to Añino et al. (2019) by presenting arguments supporting the use of orchid bees as conservation bioindicators. Within this framework, our interest lies in evaluating whether alternative and complementary approaches exist to enhance the use of these organisms as bioindicators of conservation.

One promising avenue lies in the analysis of morphological traits that integrate developmental conditions experienced during larval stages. In holometabolous insects such as bees, adult morphology is fixed at emergence and therefore reflects the cumulative influence of environmental stressors acting during development (Mirth et al. 2014). Traits associated with flight, particularly wing size, shape, and symmetry, are of special interest because they are tightly linked to dispersal ability, foraging efficiency, and fitness, and are known to respond to environmental filters across gradients of land use, altitude, and climate (South and Arnqvist 2009; West-Eberhard 2005; Windig and Nylin 2000; Young and Badyaev 2006).

Fluctuating asymmetry (FA), defined as small, random deviations from perfect bilateral symmetry, has been widely used as an indicator of developmental instability and environmental stress (Palmer 1994; Van Valen 1962). Because both sides of a bilaterally symmetrical structure share the same genetic background, departures from symmetry are generally attributed to the inability of individuals to buffer genetic or environmental perturbations during development (Breuker et al. 2006; Debat et al. 2006). Higher FA has been documented in insects exposed to a wide range of stressors, including temperature extremes, nutritional limitation, chemical contamination and habitat disturbance, supporting its use as a sensitive proxy for environmental stress (Benítez et al. 2020, 2025; Maestri et al. 2015; Parsons 1992).

In bees, wing fluctuating asymmetry has been shown to increase under stressful conditions associated with land-use change, altitudinal gradients and extreme environments, reflecting constraints on developmental stability imposed by harsh or variable conditions (C. P. Klingenberg 2019; Polak 2003; Vaca-Sánchez et al. 2025, 2023; Van Dongen 2006). These responses are particularly relevant in systems characterized by strong environmental heterogeneity, where subtle developmental effects may precede detectable changes in population size or species composition. Similar patterns linking increased FA to environmental stress have also been documented in other taxa, including flies and marine invertebrates, reinforcing the general applicability of FA as a bioindicator of anthropogenic and environmental disturbance (Benítez et al. 2025; Floate and Fox 2000; Moya et al. 2025). The methodological power of fluctuating asymmetry has been substantially better by the application of geometric morphometrics (Benítez et al. 2020; C. Klingenberg 2015). Unlike traditional morphometric approaches based on linear distances, geometric morphometrics captures shape as a multivariate property while preserving the spatial relationships among homologous landmarks (Adams et al. 2004; Rohlf and Marcus 1993). This framework allows for a more accurate and statistically robust quantification of subtle shape variation and bilateral asymmetry, improving the detection of environmentally induced developmental instability. The combined use of geometric morphometrics and FA has proven effective across diverse ecological contexts, including agroecosystems, polluted landscapes, altitudinal gradients and highly sensitive environments such as Antarctica (Moya et al. 2025).

Importantly, this integrative approach also enables the evaluation of fine-scale temporal variation, an aspect that remains underexplored in studies of pollinators. In systems such as tropical dry forests, where environmental conditions can fluctuate markedly over short temporal scales, developmental responses may be shaped more strongly by intra-annual variability than by broad seasonal contrasts. *Euglossa imperialis* Cockerell, 1922 is a medium-sized orchid bee widely distributed across Neotropical forests and recognized as an important pollinator of numerous orchid species (Roubik and Hanson 2004). In Panama and surrounding regions, it is among the most common and abundant Euglossini species across different landscape matrices (Roubik et al. 2021), making it particularly suitable for evaluating organism-level responses to environmental variability.

In this study, we use *Euglossa imperialis* as a model species to examine whether wing fluctuating asymmetry and shape variation reflect fine-scale temporal and environmental variability in a tropical dry forest of central Panama. By integrating high-resolution climatic data with geometric morphometric analyses of wing shape and asymmetry, we aim to assess the extent to which developmental instability is modulated by short-term environmental conditions. Through this approach, we seek to strengthen the use of orchid bees as bioindicators by incorporating organism-level developmental responses that complement traditional ecological metrics.

2 | Materials and Methods

2.1 | Study Area

The study was conducted in the vicinity of the Cerrozuela Hydric Reserve (8.3524, −80.4841), located within the Dry Arc region of Coclé, central Panama. This region is characterized by consistently high temperatures, typically exceeding 27°C, and by the lowest annual precipitation in the country. Average rainfall is below 400 mm during the dry season and reaches up to 1600 mm during the rainy season (Castrellón et al. 2021). These climatic conditions generate strong seasonal contrasts that are representative of tropical dry forest systems (Figure 1).

2.2 | Environmental Monitoring

Environmental monitoring was conducted at Cerrozuela between January and November 2023 using an automated meteorological station based on open-source hardware and software (Arduino). The station was equipped with sensors measuring air temperature, atmospheric pressure, rainfall and relative humidity, which operated continuously throughout the study period, providing high-resolution environmental data.

The monitoring system was built around an Arduino MKR1000 WiFi. These sensors have been previously tested and validated through direct comparison with a Vantage Pro-2 meteorological station (Davis) operated by the Atmospheric Physics Laboratory of the University of Panama. The use of the Arduino platform ensured a cost-effective, flexible and reliable alternative for long-term environmental data acquisition under field conditions.

2.3 | Specimen Collection, Storage and Identification

Active sampling was conducted using a 25-m linear zigzag transect following the methodology of Santos and Ramos (2016), using Rigar Balsam (Rigar S.A. Laboratory, Panama City, Panama) as the chemical attractant. This site was sampled during six periods, each consisting of two consecutive days, conducted in January, March, May, July, September and November 2023. Considering that the objective of the study was to analyse temporal variation in FA, exactly the same site was sampled each month under stable climatic conditions (no rainfall).

In Panama (especially on the Pacific slope, where the Dry Arc/Coclé region is located), the dry season typically extends from December to April (with December as a transition month and the driest period concentrated in January–March). In our study, the dry-season months were considered to extend from January to early May, based on the precipitation gradient observed during that year.

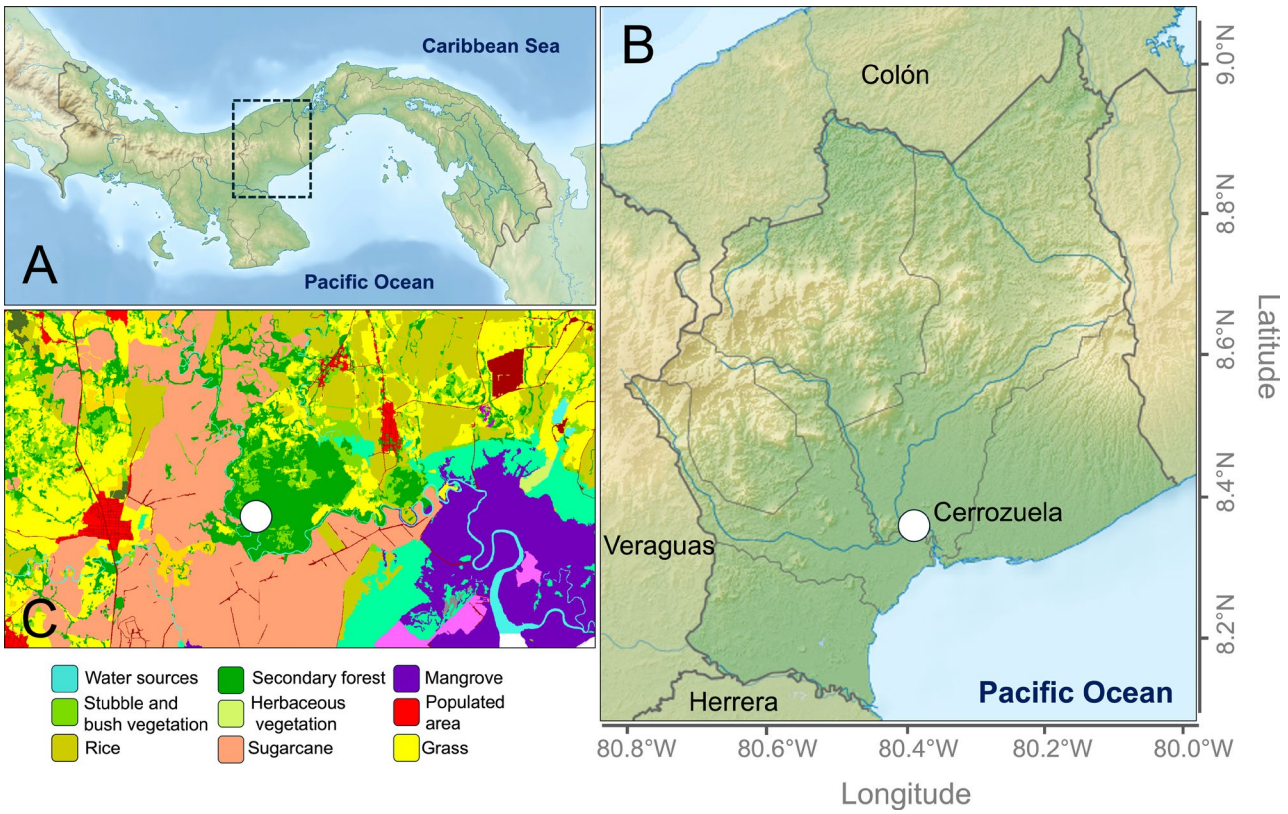

FIGURE 1 | Geographic location and land cover of the study area in Panama. (A) Map of Panama showing the location of the study region (dashed box) between the Caribbean Sea and the Pacific Ocean. (B) Detailed map of the central Pacific region indicating the sampling locality of Cerrozuela. (C) Land cover classification surrounding the study site, illustrating dominant vegetation types and land uses, including secondary forest, mangrove, agricultural areas (rice, sugarcane, grasslands), herbaceous and bush vegetation, water bodies and populated areas. The white circle indicates the exact sampling location. [Colour figure can be viewed at wileyonlinelibrary.com]

Captured bees were preserved in 70% ethanol and transported to the G.B. Fairchild Invertebrate Museum of the University of Panama (MIUP), where specimens were mounted on entomological pins and properly labelled. Species identification was performed using the taxonomic keys provided by Roubik and Hanson (2004). Because not all Euglossini species are equally frequent or abundant (Roubik et al. 2021), the focal species selected for this study was *Euglossa imperialis*, which is among the most common and widely distributed orchid bee species across different landscape matrices in the study region. For the analysis of fluctuating asymmetry (FA) in relation to environmental variation at Cerrozuela, a total of 120 individuals of *E. imperialis* were analysed, corresponding to 20 individuals per sampling month. All field collections were conducted under collecting permit ARB-046-2021, issued by the Ministry of Environment of Panama.

2.4 | Estimation and Analysis of Fluctuating Asymmetry (FA)

For the analysis of fluctuating asymmetry (FA), the forewings of *Euglossa imperialis* specimens were carefully removed. Wings were mounted on microscope slides and fixed with transparent adhesive tape, then photographed using a Leica stereomicroscope equipped with a digital camera. Digital images of both left and right wings were landmarked using 15 homologous landmarks per wing with TPSDig2 v2.31 (Figure 2) (Rohlf 2015).

Landmark data were organized in TPS format and imported into R, where they were structured as data frames and analysed using the package geomorph (Adams and Otárola-Castillo 2013). To minimize measurement error, all landmark configurations were digitized twice and averaged prior to analysis. Landmark datasets were subsequently subjected to a Generalized Procrustes Analysis (GPA), which removes the effects of size, rotation and translation, allowing shape differences to be attributed exclusively to variation in shape (Rohlf and Slice 1990). Fluctuating asymmetry was then estimated for each individual by quantifying differences between the left and right wings after Procrustes alignment. FA was calculated as the mean Euclidean distance between corresponding landmarks of the two sides, providing an individual-level measure of developmental instability.

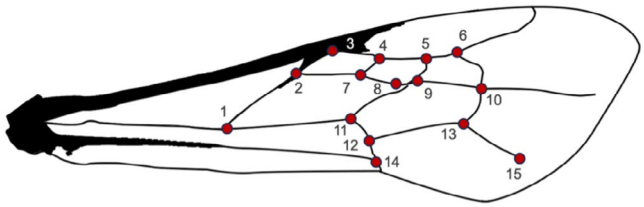

FIGURE 2 | Forewing configuration of *Euglossa imperialis*, showing the 15 landmarks used to characterize wing shape. [Colour figure can be viewed at wileyonlinelibrary.com]

Following FA protocols for geometric morphometrics (Benítez et al. 2020) estimation, statistical analyses were conducted to evaluate the effects of environmental and temporal factors. Generalized Additive Models for Location, Scale and Shape (GAMLSS) assuming a Gamma distribution were used to assess the influence of sampling conditions and environmental variables. Specifically, these models were applied to evaluate the effects of climatic variables, including maximum temperature, minimum temperature, mean temperature, temperature range, relative humidity and precipitation, on FA values in specimens collected at Cerrozuela (Table 1). All GAMLSS analyses were implemented in R using the package gamlss (Rigby and Stasinopoulos 2005). Temporal comparisons across sampling periods (months and years) also included Linear Discriminant Analysis (LDA) to evaluate the degree of separation among groups based on FA values. LDA was performed using the package MASS.

GAMLSS models extend generalized linear models by allowing not only the mean (μ) of a distribution to be modelled as a function of covariates, but also additional distributional parameters such as dispersion (σ), skewness (ν) and kurtosis (τ). Partial effects in GAMLSS represent the isolated contribution of a given covariate to a specific distributional parameter while holding other covariates constant. These effects facilitate interpretation of how individual environmental variables influence both the central tendency and variability of FA and are commonly visualized through effect plots to identify trends and nonlinear relationships (Rigby and Stasinopoulos 2005).

2.5 | Wing Morphometrics Analyses

To visualize patterns of wing shape variation and explore differences among sampling sites, a Principal Component Analysis

TABLE 1 | Monthly variation in climatic variables recorded at Cerrozuela Hydric Reserve (central Panama) during the sampling period.

Month	Tmin (°C)	TAVG (°C)	Tmax (°C)	Thermal range (°C)	Relative humidity (%)	Precipitation (mm)
January	22.05	26.63	31.81	9.76	88.88	97.01
March	22.90	26.73	32.24	9.34	79.48	36.30
May	23.30	26.92	32.81	9.51	89.18	309.61
July	22.21	26.78	33.01	10.80	91.78	330.44
September	22.14	26.08	33.86	11.72	92.08	351.77
November	21.93	25.16	30.47	8.54	89.68	502.65

Note: The table summarizes minimum temperature (Tmin), mean temperature (TAVG), maximum temperature (Tmax), thermal range, relative humidity, and cumulative precipitation for each sampling month. These environmental variables were used to evaluate the influence of fine-scale climatic conditions on fluctuating asymmetry in *Euglossa imperialis*.

(PCA) was conducted using the covariance matrix of individual shapes (Jolliffe 2002). PCA was used to represent the morphospace and provide a qualitative assessment of shape variation among sites. To further reduce dimensionality and maximize shape differentiation between groups, a Canonical Variate Analysis (CVA) was performed, generating new shape axes that emphasize among-site differences (Campbell and Atchley 1981). To statistically evaluate differences in wing shape among seasons and months, we performed Procrustes ANOVA using permutation procedures (10,000 iterations), as implemented in the geomorph package. This approach partitions shape variance after Generalized Procrustes Alignment and tests for group effects while accounting for the multivariate nature of shape data. Pairwise Mahalanobis distances among months were calculated from Procrustes-aligned wing shape coordinates in tangent space, and statistical significance was assessed using 10,000 permutations of group labels.

Statistical differences in wing shape among sites were assessed using permutation tests (10,000 iterations) based on Mahalanobis distances, which quantify the magnitude of shape divergence between group mean configurations. Geometric morphometric analyses were carried out using MorphoJ v1.07 (C. P. Klingenberg 2011) and the geomorph package in R v4.0.4 (Adams and Otárola-Castillo 2013).

3 | Results

3.1 | Fluctuating Asymmetry and Seasonal Patterns

Fluctuating asymmetry (FA), quantified as the mean Euclidean deviation between left and right wings after Procrustes superimposition, showed low absolute values across individuals, indicating subtle but consistent levels of developmental instability. Individual FA values ranged approximately between 0.205 and 0.222, with a central tendency around 0.21 in both seasonal groups.

A direct comparison between seasons revealed no statistically significant difference in FA between the Dry Season and the Rainy Season (one-way ANOVA, $F_{1,118} = 1.49$, $p = 0.224$). Boxplot distributions showed substantial overlap between seasons, although Dry Season individuals exhibited a slightly higher median FA compared to Rainy Season individuals (Figure 3A). This pattern suggests that, despite contrasting environmental conditions between seasons, overall levels of developmental instability remain broadly comparable at the seasonal scale.

3.2 | Environmental Drivers of Fluctuating Asymmetry

Generalized linear models assuming a Gamma distribution identified significant associations between FA and several climatic variables. Minimum temperature (Tmin) showed a positive relationship with FA (estimate = 0.0050, $p < 0.001$), whereas mean (TAVG) and maximum temperature (Tmax) exhibited negative effects (TAVG: estimate = −0.0110, $p < 0.001$; Tmax: estimate = −0.0032, $p = 0.016$). Relative humidity was also positively associated with FA (estimate = 0.00027, $p = 0.001$), while accumulated precipitation (Rain) showed no significant effect.

The ANOVA decomposition of the GLM confirmed relative humidity as the strongest environmental predictor of FA ($F = 14.56$, $p < 0.001$), whereas temperature variables and rainfall did not explain significant additional variance when considered independently. These results indicate that FA is more closely linked to atmospheric moisture conditions than to precipitation per se.

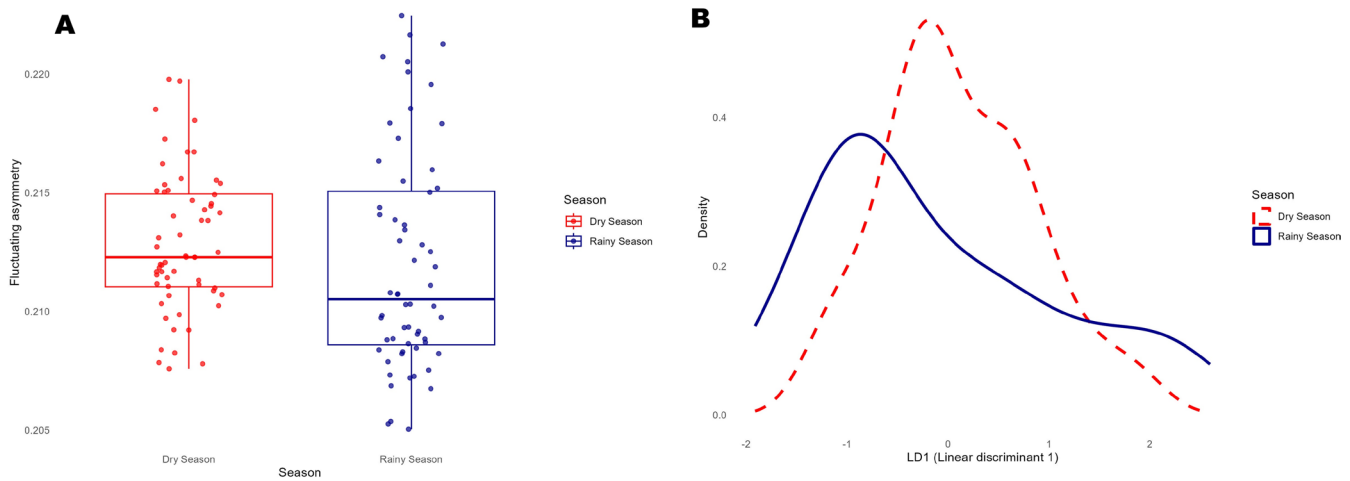

FIGURE 3 | Fluctuating asymmetry (FA) patterns in *Euglossa imperialis* across seasons. (A) Boxplots showing individual FA values for the Dry Season (red) and the Rainy Season (blue). Points represent individual specimens, boxes indicate interquartile ranges, horizontal lines represent medians and whiskers denote the range of variation. (B) Density distributions of the first linear discriminant axis (LD1) derived from Linear Discriminant Analysis (LDA) based on FA values. The Dry Season (red dashed line) and Rainy Season (blue solid line). Discriminant analysis based on FA further supported this result. Linear Discriminant Analysis (LDA) separating Dry and Rainy seasons produced overlapping density distributions along the first discriminant axis (LD1), indicating limited discriminatory power of FA alone to separate individuals by season (Figure 3B). While the Dry Season distribution tended to be centered toward slightly higher LD1 values and the Rainy Season distribution showed a broader spread toward negative values, both curves exhibited substantial overlap, consistent with the non-significant seasonal ANOVA. [Colour figure can be viewed at wileyonlinelibrary.com]

GAMLSS models further refined this interpretation. When month was included as a factor, July showed a significant negative effect on mean FA relative to January ($p < 0.001$), suggesting reduced developmental instability during mid-winter conditions. Additionally, FA variance (sigma) was significantly higher in September ($p = 0.019$), indicating increased heterogeneity in developmental stability during the transition toward the rainy season. Models excluding the month factor highlighted Tmin as a significant positive predictor of FA and suggested a tendency for mean temperature to reduce FA, reinforcing the role of thermal conditions in shaping developmental instability.

3.3 | Monthly Variation in Fluctuating Asymmetry

In contrast to the seasonal comparison, FA differed significantly among months (January to November; one-way ANOVA, $F_{5,114} = 3.89$, $p = 0.0027$). This result indicates that temporal variation at a finer scale captures differences in developmental instability that are not detectable when months are pooled into broader seasonal categories.

Month-based discriminant density plots revealed partial separation among months along LD1, with Dry Season months (January, March, and May) generally clustering toward intermediate LD1 values, whereas Rainy Season months showed greater dispersion. In particular, July exhibited a distinct distribution skewed toward lower LD1 values, while September and November showed broader and partially overlapping distributions. These patterns suggest that FA responds more strongly to month-specific environmental conditions than to the binary seasonal classification. These monthly patterns are consistent with the discriminant density structure observed among months (Figure 4).

3.4 | Wing Shape Variation Across Seasons and Months

Geometric morphometric analyses of wing shape revealed continuous but structured variation across individuals. The first three principal components accounted for a substantial proportion of total shape variance: 44.47% (PC1 = 24.45% and PC2 = 12.58%, PC3 = 9.83%), capturing the main axes of morphological variation in the dataset. Ordination of the shape space showed extensive

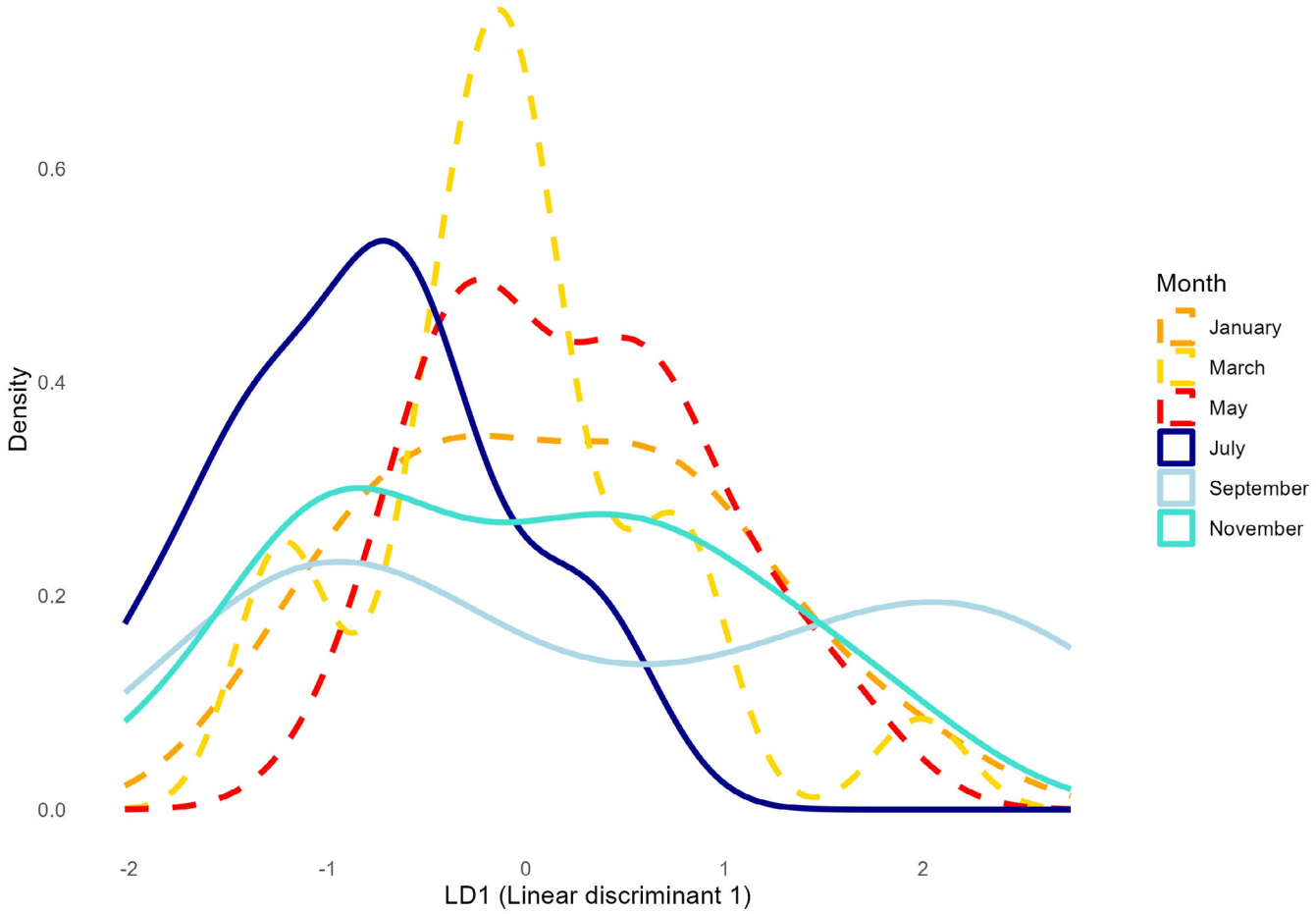

FIGURE 4 | Discriminant density of fluctuating asymmetry (FA) by month in *Euglossa imperialis*. Kernel density distributions along the first linear discriminant axis (LD1) from a Linear Discriminant Analysis (LDA) are shown for each sampling month. Dashed lines indicate dry-season months (January, March, May) and solid lines indicate rainy-season months (July, September, November). [Colour figure can be viewed at wileyonlinelibrary.com]

overlap between individuals sampled during the Dry and Rainy seasons, indicating that wing shape variation is not sharply partitioned by broad seasonal categories (Figure 5A). Individuals from both seasons were broadly distributed across the morphospace, with no clear separation along the primary axes of shape variation. In contrast, grouping individuals by month revealed a more nuanced temporal pattern. Although convex hulls corresponding to individual months partially overlapped, shifts in their position and dispersion were evident. Dry-season months (January, March and May) tended to occupy intermediate to positive values along PC1, whereas rainy-season months (July, September and November) showed greater dispersion across both PC1 and PC2. Canonical Variate Analysis (CVA) further emphasized these temporal differences by maximizing among-month shape variation (Figure 5B). While overlap among groups remained, several months exhibited partial separation along the first canonical axis, indicating subtle but consistent differences in wing shape associated with specific sampling periods. In particular, some rainy-season months showed displacement relative to dry-season months, suggesting that month-specific environmental conditions contribute to detectable shape differentiation when variation is explicitly maximized.

Procrustes ANOVA confirmed the absence of a significant seasonal effect on wing shape. Comparisons between Dry and

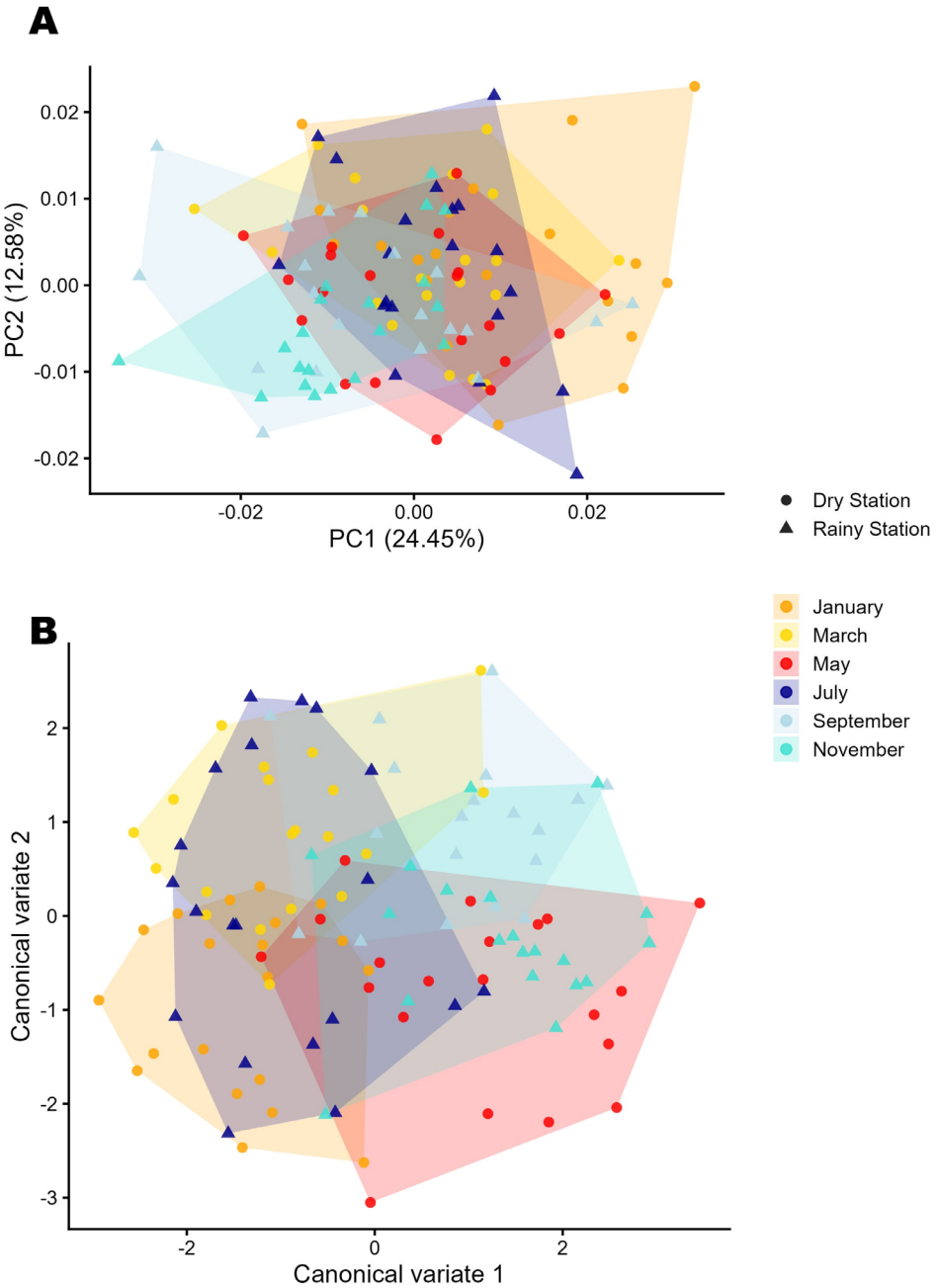

FIGURE 5 | Wing shape variation across seasons and months in *Euglossa imperialis*. (A) Principal Component Analysis (PCA) of wing shape showing overlap between Dry and Rainy seasons, with convex hulls illustrating month-level variation. (B) Canonical Variate Analysis (CVA) maximizing shape differences among months, revealing partial separation of specific sampling periods despite overall overlap. [Colour figure can be viewed at wileyonlinelibrary.com]

Rainy seasons revealed no detectable differences in overall shape ($F = 0.019$, $p = 0.885$), with season accounting for less than 0.01% of the total shape variance. When centroid size was included to account for potential allometric effects, neither size ($p = 0.363$) nor season ($p = 0.225$) significantly influenced wing shape, indicating that seasonal variation in morphology is independent of size-related effects.

Pairwise Mahalanobis distances indicated that shape differentiation was primarily structured along the seasonal transition (Table 2). Months corresponding to the rainy period, particularly November, showed significant differences from several dry-season months, including January and March. Likewise, September differed significantly from January, reinforcing the contrast between early dry and late rainy conditions. In contrast, most comparisons within the same season were not significant, suggesting relative shape stability within dry months and within rainy months. Overall, these results support a seasonal signal in wing shape variation rather than random month-to-month fluctuations.

4 | Discussion

This study provides evidence that fluctuating asymmetry and wing shape variation in *Euglossa imperialis* are structured by fine-scale temporal and environmental variability rather than by broad seasonal contrasts. Overall, FA values were low and remarkably consistent across individuals, and no significant differences were detected between the dry and rainy seasons. Similarly, geometric morphometric analyses revealed extensive overlap in wing shape between seasons, indicating that seasonal regime alone does not drive discrete morphological differentiation. In contrast, both FA and wing shape exhibited significant month-to-month variation, highlighting a gradual and continuous temporal signal. These results suggest that developmental instability and wing morphology in *E. imperialis* respond primarily to short-term climatic conditions and transitional periods rather than to binary seasonal classifications, emphasizing the importance of fine temporal resolution when evaluating environmental effects on insect morphology.

Environmental variables showed a limited influence on fluctuating asymmetry in *Euglossa imperialis*, with minimum temperature emerging as the only consistently associated factor. The positive relationship between Tmin and FA suggests that lower thermal thresholds may play a role in shaping developmental stability, potentially through mechanisms linked to insect development and physiological regulation. In insects, minimum temperature can be particularly relevant for processes such as developmental timing, metabolic efficiency and dormancy or diapause-related responses, which have been reported for Hymenoptera in the Neotropical region (Cambra et al. 2021). The absence of strong effects of other climatic variables may also reflect the relatively low environmental variability characterizing the study area, where mean temperatures remain stable throughout the year and precipitation levels are comparatively low relative to other regions of Panama.

In addition to minimum temperature, relative humidity emerged as a significant predictor of fluctuating asymmetry, yet its ecological implications warrant further consideration. Atmospheric moisture can influence insect developmental stability through multiple mechanisms, including its effects on larval microclimatic conditions, metabolic regulation and water balance. In tropical systems, relative humidity may modulate evaporative stress and resource quality, potentially altering the precision of morphogenetic processes during wing development. Higher humidity levels could increase developmental variability by affecting thermoregulation and physiological homeostasis, particularly in environments characterized by fluctuating thermal regimes. Thus, the positive association between relative humidity and FA observed in this study suggests that atmospheric moisture may act as a subtle but biologically meaningful driver of developmental instability at fine temporal scales.

Notably, although no significant differences in FA were detected between dry and rainy seasons, greater variability in FA was observed during the rainy period. This pattern is consistent with previous findings in other euglossine bees, such as *Euglossa pleosticta*, where seasonality has been shown to influence the magnitude and variability of FA without necessarily producing clear seasonal shifts in mean values (Silva et al. 2009). Similar interpretations have been proposed in recent studies on insect wing fluctuating asymmetry, which suggest that FA may respond more strongly to short-term environmental heterogeneity and developmental noise than to broad categorical gradients such as season or habitat type (Benítez et al. 2014, 2020, 2015; De Coster et al. 2013; C. Klingenberg 2015; Van Dongen 2006). Together, these results reinforce the view that FA in euglossine bees reflects subtle, context-dependent developmental responses rather than a straightforward indicator of large-scale environmental stress, highlighting both the sensitivity and the limitations of FA as a bioindicator in relatively stable tropical systems.

TABLE 2 | Pairwise Mahalanobis distances among months calculated from Procrustes shape coordinates.

Months	January	March	May	July	September
March	1.5581				
May	1.901*	2.0304*			
July	1.3413	1.0815	1.6994*		
September	1.9592*	1.4175	1.4376	1.441	
November	2.1296*	1.993*	1.6906	1.886*	1.4516*

Note: Asterisks denote significant differences based on 10,000 permutation tests ($p < 0.05$).

In contrast to systems exposed to strong anthropogenic or physicochemical stress, the patterns observed in *Euglossa imperialis* indicate a limited expression of developmental instability (C. P. Klingenberg 2003). Studies conducted in pesticide-exposed agroecosystems and in Antarctic coastal environments affected by human footprint have reported clear increases in fluctuating asymmetry and pronounced shape divergence associated with chronic environmental stress (Benítez et al. 2025; Ivanković Tatalović et al. 2020; Moya et al. 2025). In those contexts, FA acts as a robust indicator of developmental disruption driven by cumulative disturbance (Van Dongen 2006). By comparison, the consistently low FA values and the absence of discrete seasonal differentiation in *E. imperialis* suggest effective developmental buffering under relatively stable climatic conditions, with FA reflecting subtle, short-term environmental variability rather than persistent stress. Similarly, while geometric morphometric analyses in impacted systems often reveal clear categorical segregation in wing shape, the extensive overlap observed here indicates that wing morphology in *E. imperialis* varies along a continuous temporal gradient, emphasizing the context-dependent and scale-sensitive nature of FA and shape-based indicators.

Overall, fluctuating asymmetry exhibited subtle but consistent variation across individuals, with no clear separation between Dry and Rainy seasons. In contrast, significant differences among months and strong associations with temperature and humidity indicate that FA responds primarily to short-term environmental variability rather than to broad seasonal contrasts. These results suggest that developmental instability in this system is modulated by fine-scale climatic conditions, particularly thermal and moisture regimes. This scale-dependent response provides an appropriate framework for interpreting the subsequent patterns of wing shape variation revealed by geometric morphometric analyses.

In conclusion, our results indicate that both fluctuating asymmetry and wing shape in Euglossa imperialis are characterized by high morphological stability across broad seasonal regimes, coupled with subtle but consistent responses to short-term environmental variation. While neither FA nor wing shape exhibited discrete differentiation between dry and rainy seasons, month-level analyses revealed fine-scale temporal patterns associated with thermal and moisture conditions. These findings suggest that developmental instability and wing morphology in this species are shaped by transient climatic fluctuations rather than by categorical seasonal contrasts. Consequently, FA and geometric morphometric traits should be interpreted as sensitive, scale-dependent indicators of environmental variation, whose ecological signal becomes evident primarily when temporal resolution is sufficiently fine. This integrative approach highlights the potential of combining FA and shape analyses to better understand organismal responses to environmental heterogeneity in tropical ecosystems, while also underscoring the importance of cautious interpretation when applying these metrics as bioindicators of conservation status.

Acknowledgements

We mostly thank Vicerrectoría de Investigación y Postgrado, Universidad de Panamá for all the help providing funding for this research. Additionally, we acknowledge the support provided by ANID/BASAL FB210018 through the Cape Horn International Center for Global Change Studies and Biocultural Conservation (CHIC).

Funding

This work was supported by Agencia Nacional de Investigación y Desarrollo (FB210018).

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data supporting the findings of this study can be obtained upon request from the authors.

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