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The Signature of Environmental and Parasite Stresses on the Wings of *Apis mellifera*

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Abstract

The decline of pollinators, particularly *Apis mellifera*, seriously threatens global ecosystems and agricultural productivity. This study investigated the effects of environmental stress (low vegetation cover, high internal hive temperatures, high internal hive humidity, and high parasitism rate by the mite *Varroa destructor*) on the fluctuating asymmetry (FA) of honey bee wings in seven apiaries in Dom Joaquim, a reference city for wild honey production in the state of Minas Gerais, Brazil. We evaluated the FA of 18 wing venations of 30 bees from five beehive boxes in seven apiaries, totaling 1050 bees. Our results showed that fluctuating asymmetry (FA) in wing venation traits M4 ($P = 0.013$), M12 ($P = 0.014$), M13 ($P = 0.020$), M14 ($P = 0.014$), and M18 ($P < 0.001$) differed significantly among apiaries, likely reflecting variation in local environmental stressors. These differences suggest that site-specific conditions may differentially impact colony stress levels, influencing developmental stability in honey bees. We also noted that the apiaries with lower native forest cover (<50%) and lower floral diversity showed higher FA in the M17 venation. Furthermore, internal hive conditions, such as elevated temperature and mite infestation, were associated with increased FA in venation traits M2 and M18. In contrast, higher humidity levels were linked to increased FA in traits M4 and M17. In general, the results highlight that the FAs of the M2, M17, and M18 wing venations are associated with multiple stress factors, suggesting that the FAs of these venations are the most recommended for use. This research also emphasizes the importance of preserving native vegetation and managing hive conditions to maintain the health and stability of honey bee populations. These results also demonstrate the potential of FA as a bioindicator of environmental stress in *A. mellifera*, which may help improve beekeeping management practices.

Introduction

Most angiosperms depend on pollinators for their reproduction, which is as high as 94% in tropical ecosystems and 78% in temperate ecosystems (Greenleaf & Kremen, 2006; Ollerton et al., 2011; IPBES, 2016). In addition, 87 out of the 115 most important food crop species worldwide require pollinators or have their production improved with the help of pollinators (e.g., Klein et al., 2007; Giannini et al., 2015; Novais et al., 2016; Ellis et al., 2020). Animal pollination contributes an estimated $235 to $577 billion annually to agriculture (Potts et al., 2016). In Brazil, approximately 68% of the 53 major crops rely on pollinators, and the decline of these agents could result in a production loss of 16 to 59 million tons, translating to an economic impact of $5 to $15 billion per year (Novais et al., 2016). In Mexico,

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80% of cultivars depend to some degree on pollinators and represent 54% of the monetary value generated by agriculture, equivalent to $ 23 billion per year (Ashworth et al., 2009). In addition to impacts on food security and the economy, the loss of these pollinators and their services can have enormous consequences for maintaining biological diversity (IPBES, 2016; Christmann, 2019).

Despite the economic and ecological importance of pollinators, the decline of several pollinator groups has been recorded across the planet in recent years (e.g., Quesada et al., 2011; Vasiliev & Greenwood, 2020; Brunet & Fragoso, 2024; Feuerbacher, 2025). Among the group of pollinators, bees are the most dominant, although not the most diverse. The effectiveness of bees in pollination is due to their ability to carry a high number of pollen grains on their bodies and their dependence on floral resources (Patel et al., 2021). It is estimated that among the 100 main cultivated species that provide 90% of the world's food, 71% are pollinated by bees (Kluser et al., 2010).

Apis mellifera is the species that provides the most significant coverage in the pollination service, both in wild environments and in agricultural fields (e.g., Williams, 1994; Steffan-Dewenter & Tscharntke, 1999; Winfree, 2010; Willmer, 2011). This bee species is a generalist, making it easy to manage and transport. *Apis mellifera* hives can be moved during the flowering period to places where pollinators are scarce, to provide a pollination service in agricultural areas, and produce honey. This activity, known as migratory or mobile beekeeping, has been gaining popularity around the world (Cestaro et al., 2017).

Apis mellifera contributes approximately 80% of agricultural pollination services (Breeze et al., 2011) and is considered one of the most valuable pollinators for various monocultures (Papa et al., 2022). Thus, the decline in *A. mellifera* populations represents a serious threat that could trigger an unprecedented collapse in the ecosystem and global food security (Gallai et al., 2009; Marshman et al., 2019; Freitas et al., 2022). Moreover, despite its increasing adoption, migratory beekeeping has raised concerns due to its potential to increase the prevalence and spread of pathogens and parasites in honey bee colonies (Alger et al., 2018; Martínez-López et al., 2022). This risk becomes even more relevant given the current global decline in bee populations and the expected expansion of this practice, driven by climate change and the growing demand for crop pollination (Martínez-López et al., 2022).

The massive decline of *A. mellifera* populations has been documented in the United States and Europe (e.g., National Research Council, 2007; Potts et al., 2010). Following the first recorded decline in the United States in 2016, it was observed that the collapsed colonies exhibited well-defined characteristics, leading the phenomenon to be treated as a syndrome. Called Colony Collapse Disorder (CCD), a healthy bee colony faces a sudden and drastic loss of adult worker bees (Goulson et al., 2015; Lee et al., 2015; Flores et al., 2021; Banaji, 2022). Tragically, this loss ultimately leads to the decay of the colony, as even if the queen survives, the insufficiency of worker bees prevents the colony from sustaining itself.

This critical imbalance leads to the inevitable death of the hive, revealing the intrinsic fragility of this highly interdependent system (Williams et al., 2010). Multiple stressors have been identified as contributing to this syndrome. These range from a lack of floral resources caused by deforestation and land use changes, high levels of agents (parasites and pathogens) that affect bee health, climate change, pesticide exposure (Pires et al., 2016; Evans & Chen, 2021; Freitas et al., 2022), among others; all of which can interact synergistically.

Habitat loss and fragmentation result in a reduced availability of diverse floral resources, affecting the quantity and nutritional quality of the pollinators' diet (Gallai et al., 2009; Winfree, 2010). These nutritional changes directly interfere with bee development, immune system, and stress tolerance (Di Pasquale et al., 2013, 2016; Vaudo et al., 2015; Ghosh et al., 2020). In preserved environments, there is a greater diversity of pollen, providing bees with a balanced diet of proteins, fatty acids, and essential minerals (Herbert et al., 1980; Avni et al., 2014; Ghosh et al., 2020). In addition, floral diversity can influence the diversity of microorganisms involved in the fermentation processes that pollen undergoes within the cells of the hive, determining the nutrients available to the bees (Gilliam, 1979).

Another factor that generates stress in bees is temperature conditions. Colonies expend significant energy to keep the optimum internal temperature stable, venting warm air out of the nest on very hot days and accumulating metabolic heat on very cold days (Becher et al., 2009). In dry conditions, the workers collect and distribute water in the nest and perform ventilation behavior (Kronenberg & Heller, 1982; Jones et al., 2004). Thermal instability within the ideal range inside the hive causes stress during the development of bee pupae (Kleinhenz et al., 2003). Additionally, high temperatures in low-humidity conditions can cause *A. mellifera* to lose body water (Abou-Shaara et al., 2012). Humidity is another environmental variable contributing to stress in *A. mellifera* (Ellis et al., 2008; Li et al., 2019; Zakharov et al., 2020; Vaca-Sánchez et al., 2023). Although high humidity can result in a reduction in *Varroa* mite infestations, it can simultaneously increase the proliferation of pathogens in bees (Ellis et al., 2008).

Several factors, including a lack of food resources, climate change, and chemical contamination, among others, render *A. mellifera* bees more susceptible to parasites and pathogens, leading to colony losses (Freitas et al., 2022). In addition, high infestation of the *Varroa destructor* mite, considered the main parasite of *A. mellifera* (Nazzi & Le Conte, 2016; Noël et al., 2020; Reams & Rangel, 2022), can reduce fitness, alter weight, and affect the development and lifespan of this species (e.g., Bowen-Walker & Gunn, 2001;

Duay et al., 2003; Amdam et al.,2004; Garedew et al., 2004; Strauss et al., 2016; Gregorc & Sampson, 2019). *Varroa destructor* is the vector of several virus species, including Deformed Wing Virus (DWV), *Varroa destructor* Virus-1 (VDV-1), and Israeli Acute Paralysis Virus (IAPV) (Moore et al., 2011; Levin et al., 2016). These viruses can cause physiological changes and some alterations in the formation of the wing, colour, and size of the abdomen (Moore et al., 2011; Piou et al., 2022).

In general, the stress experienced by *A. mellifera* colonies can be detected before it causes the population to decline, allowing for mitigation. One of the most widely used indicators to assess the stress of organisms is fluctuating asymmetry (FA), a measure that assesses whether a disturbance, whether genetic or environmental, has generated an instability between the right and left sides of an organ or even the body of an individual during development (Palmer & Strobeck, 1986; Parsons, 1990; Knierim et al., 2007; Soares et al., 2024). The greatest and most significant incidence of phenotypic variability occurs when the stress is so severe that it can lead to harmful changes in the biological system (Parsons, 1990). If the same genome produces the expression of a bilateral character, then any asymmetry between the sides could be a consequence of environmental disturbance (Clarke & Oldroyd, 1996; Woods et al., 1998).

In this study, we examined whether the numerous stress factors that can lead to massive bee mortality, such as low floral resource availability, climatic variations (temperature and humidity), and a high incidence of the mite *V. destructor*, can be identified in FA measurements on the wings of *A. mellifera*. The study was conducted in an area predominantly characterized by Atlantic Forest and remnants of Cerrado in Dom Joaquim, Minas Gerais, Brazil. Dom Joaquim is one of the 92 municipalities with the highest honey production (R$ 216,000.00) in the state of Minas Gerais (IBGE, 2023), and it has also shown increasing rates of land use conversion and significant climatic variability (MapBiomas, 2022). As such, this represents an ideal scenario for testing hypotheses related to the health of *A. mellifera* hives in the tropical region. The following hypotheses were tested: **i)** the environmental stress hypothesis, which predicts that bees from apiaries located in areas with less vegetation cover, therefore less availability of floral resources, and subject to higher temperatures or high humidity inside the hive, have greater fluctuating asymmetry in the wings; **ii)** the parasitism hypothesis, which predicts that bees in apiaries under high infestation of the *V. destructor* mites have greater fluctuating asymmetry, regardless of other stresses.

Materials and Methods

Study area

This study was conducted in the municipality of Dom Joaquim (between latitude 18º 58‘ S and longitude 43º 15’ W), Minas Gerais, Brazil. The study region has an Aw-type climate according to Köppen’s classification of climate types (1936), i.e., a tropical savanna climate with a dry season in winter, characterized by an average annual temperature of 21.5 °C, with dry, mild winters and rainy summers with high temperatures (Alvares et al., 2013). Dom Joaquim has 58% of its territory in the Atlantic Forest, 33% in agriculture, and less than 1% in Cerrado (https://brasil.mapbiomas.org/).

Seven apiaries were selected for the study, with a distance of at least 2 km between them, known as: 1. Água Limpa (18°58‘ 51.9’S 43°16‘ 08.7’W); 2. Britas (19°01’07.9‘S 43°17’12.6’W); 3. Chácara (18°56‘ 56.4’S 43°15‘ 28.1’W); 4. Grota (19°01’58.3‘S 43°15’19.0’W); 5. Fábrica (18°56’43.0‘S 43°23’34.3’W); 6. Palmital (18°57’15.6‘S 43°25’10.2’W); 7. Tribuna (19°02’32.8‘S 43°15’56.7’W). The apiaries were selected based on the beekeeper’s authorization, minimum distance, easy access to the apiary, and not using a complementary diet during the study period. The collection campaigns were carried out during the off-season (when floral resources are less available) in the region, between the end of November 2021 and March 2022. During the campaign period, average minimum temperatures ranged between 21 and 22 °C, while average maximum temperatures ranged between 22 and 23.5 °C. The minimum relative humidity (%) ranged from 70 to 80%, and the maximum was between 75 and 80% (Inmet, 2022).

Landscape analysis

To analyze the landscape, a buffer was created around the seven apiaries with a radius of 1 km. These circles, or masks of 314 hectares, were superimposed on the raster image of land use types and vegetation phytophysiognomies from Mapbiomas (2022), using a mapping of land use types and vegetation using LandSat 30 x 30m images (https://mapbiomas.org/)

Sampling floral resources around the apiaries

To determine the richness and composition of the floral resources available around the hives, flowering plant species were collected within a radius of 1 km around the apiary, i.e., in an area of 3,14 km^2 in each apiary, totaling 2,57 km^2 for the seven apiaries. The plant species collected in each apiary were identified in the field with a label containing the date of collection, the name of the apiary, flower color and habit (herbaceous, shrub or tree) and taken to the laboratory where they were herborized and the exsiccates sent for identification to the BHCB herbarium of the Botany Department of the Institute of Biological Sciences at UFMG, Brazil.

Sampling in the apiaries’ hives

Data and samples were collected from each apiary on two consecutive days without rain between 7:00 and 9:00 am. Before the collections began, a fumigator was lit to avoid accidents with the bees. Dry materials such as wood chips and foliage were used to burn in the fumigator and produce cold, white smoke. The use of smoke in beekeeping management is effective in controlling the defensive behavior of Africanised bees (Silva et al., 2012). When bees are exposed to smoke, they accumulate honey in their nectar vesicles for potential escape, thus becoming heavy with a distended abdomen and making them more challenging to sting (Gage et al., 2018). The use of smoke also masks the alarm pheromones released during stinging and the beekeeper's odor (Gage et al., 2018).

For this study, five hive boxes were randomly selected from each hive and numbered in sequential order, with labels applied to each. The smoke was applied around and in front of the hive to be opened, and then the hive was opened at a distance of 20 cm, allowing the smoke to reach a cold temperature. To measure the temperature and humidity inside the hive, a digital sensor connected to a thermo-hygrometer was inserted inside. The sensor was left inside until it reached stability, i.e., approximately five minutes.

To quantify *V. destructor* mite infestation, the central frame of the hive was selected. After verifying that the queen was absent from the frame, a plastic jar (diameter 10 cm, volume 471 mL) containing 300 mL of 70% ethyl alcohol was used to capture the bees that were near the frame. When the jar was passed close to the frame, the bees fell inside. A minimum of 300 worker bees were inserted into this jar at random. The same central frame of the hive was used to collect the worker bees to assess fluctuating asymmetry, according to Palmer & Strobeck (1986). During collection, a 50 mL Falcon tube containing 70% ethyl alcohol was positioned close to the frame, allowing the bees to fall into the tube. Around 50 bees fell into the falcon tube.

To quantify the mites found on the workers and determine the infestation rate in the hives, the first jar containing 300 bees immersed in alcohol was shaken vigorously to release the mites adhered to their bodies. The contents of the jar were then passed through a metal grid with an opening smaller than 0.5 mm, supported by a 500 ml glass beaker, used to separate the bees from the alcohol solution with mites. The number of bees found in the jar was then quantified. The alcohol solution with mites was placed over a funnel containing a white cloth, and the mites trapped in the white cloth were then more easily visualized and quantified. The following formula was used to assess the number of mites per 100 worker bees: number of mites/number of worker bees collected multiplied by 100 (Spivak & Reuter, 2001).

To assess fluctuating asymmetry and wing morphometry, we used jars containing 50 bees immersed in 70% alcohol. In each jar, 30 worker bees were selected at random and separated (30 x 5 hives x 7 apiaries = 1050 bees in total). Then, for each bee, we carefully removed the two pairs of wings, anterior and posterior, with fine-tipped entomological tweezers. These wings were placed on a smooth glass microscope slide (measuring 50 mm wide, 76 mm long, and about 1 mm thick), which had a frosted edge, allowing us to write the identification of the sample with a pencil. The following information was entered on each slide: locality name, hive box number, and sample number. The mounted wings of each individual were covered with a coverslip (size: 24 mm wide, 50 mm long, and approximately 0.13 - 0.16 mm thick) on the slides and viewed in a stereomicroscope with a 35 mm Apo 2.0xFWD objective and 0.63 zoom. The images observed under the stereomicroscope were photographed using an Axiocam 208 Colour camera. The software used to adjust the images captured by the camera on the computer was Zeiss Zen 3.4.

To measure the fluctuating asymmetry of the right and left wings of *A. mellifera*, the distances between 19 anatomical landmarks were identified on each forewing according to Smith et al. (1997) and Barour et al. (2016), totalling 18 venation measurements (M) (Fig 1).

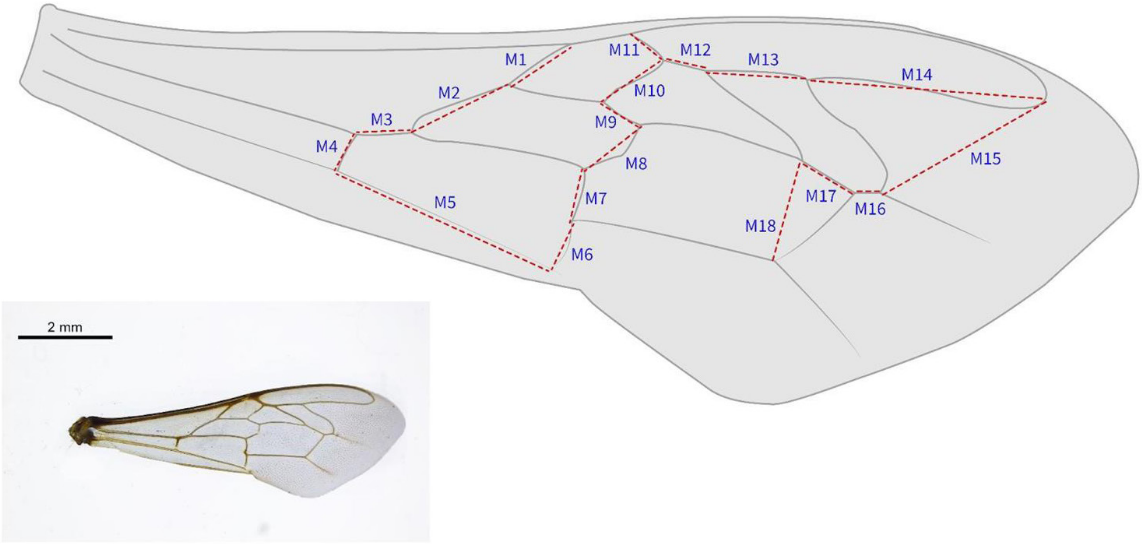

Fig 1. Illustration of the 18 venations (M1 to M18) of *Apis mellifera* wings from 19 anatomical landmarks used to assess fluctuating asymmetry.

The ImageJ programme was used to measure wing venation (see Cuevas-Reyes et al., 2018; Vaca-Sánchez et al., 2023). The calculation of PA per wing venation was based on the absolute difference of each of the distances between the veins on the right and left (|Di - Ei|), divided by the average of the distances (Di + Ei/2) (Palmer & Strobeck, 1986; Palmer, 1994; Graham et al., 2010). This PA formula accounts for the difference between right- and left-sided venation in relation to wing size (Cornelissen & Stiling, 2005; Vaca-Sánchez et al., 2023).

The absolute value of the difference between the right and left sides represents a good estimator of variance in FA between the wings, indicating whether there is no directional asymmetry (left or right side consistently larger, i.e. the differences between the left and right sides that are distributed around a mean is significantly greater or less than zero) or antisymmetry (no symmetry, where there is a bimodal distribution of the differences between two sides around a mean of zero) (Palmer & Strobeck, 1986; Palmer & Strobeck, 1992). To confirm that the measurements showed fluctuating asymmetry and thus rule out the possibility that the data represented directional asymmetry or antisymmetry, a Lilliefors normality test was performed on the absolute data, and a Student's t-test was used to compare the distances between the right and left sides.

Data analyses

To assess the similarity of flowering plant species found between the apiaries, a Jaccard similarity analysis was conducted, and a dendrogram was constructed using the same Similarity Index and paired group algorithm (UPGMA). All the variables analyzed were first subjected to the Lilliefors and Levine tests (Quinn & Keough, 2002) to verify that they conformed to a normal distribution pattern. Pearson's correlation analysis was carried out to assess the relationship between vegetation cover and flowering plant species. It was observed that the apiaries did not vary significantly in the percentage of native vegetation cover around them. Therefore, the data on the percentage of native vegetation cover were grouped into two groups (1. greater than 50% native vegetation cover; 2. less than 50% native vegetation cover). Based on this grouping, the FA of each wing venation (18 venations) was compared between hives in places with more than 50% and less than 50% native vegetation cover using the T-test when the FA data was parametric and the Mann-Whitney test when the data was non-parametric. To compare the FA of each wing venation between the apiaries, we used Analysis of Variance (ANOVA) when the data were parametric and the Kruskal-Wallis statistical test when the data were non-parametric. Tukey's test was used to compare the FA of each venation between apiaries. Canonical Correspondence Analyses (CCA) were carried out to assess the relationship between climatic variables and indoor parasitism on the FA of different venations (18), using the FA measurements of each venation in the main matrix and the characteristics of each apiary (temperature and humidity of the hive and the infestation rate of the *V. destructor* mite) in the secondary matrix. Simple linear regression was used to assess the effect of each environmental variable in the hive: temperature, humidity, and mite infestation on the FA of each venation.

Results

Surrounding vegetation cover

Within the buffers sampled, the following types of vegetation were found: 1. Forest (dense, open, and mixed ombrophilous forest and semi-deciduous seasonal forest, deciduous seasonal forest, and pioneer tree formation); 2. savannah (Cerrado); 3. rocky outcrop (campo rupestre). 4. pasture; 5. mosaic of uses (areas of agricultural use where it was not possible to distinguish between pasture and agriculture); 6. eucalyptus; 7. water (body of water: river and lakes) (Fig 2, Table 1S).

The native forest cover (Atlantic Forest) around the apiaries was over 37%, reaching a maximum of 71.49 % in the Tribuna apiary (Table 1S, Fig 2). The Palmital apiary also showed high forest cover (70.39%), followed by Fábrica (69.66%) and Britas (58.93%). It is observed that most of the apiaries studied (4) have more than 50% native vegetation cover. Native forest cover was less than 50% in only three areas: Grota (43.26%), Chácara (37.96%), and Água Limpa (37.88%). Pasture areas were present in all seven sites, with the highest percentages found in the Chácara (39.98%), Grota (36.39%), Água Limpa (35%), and Britas (29.41%) apiaries.

Land cover by a mosaic of uses accounted for around ¼ of the area around some apiaries: Água Limpa (26.49%), Tribuna (24.28%), Palmital (22.88%), Chácara (22.06%), and Grota (19.73%). Other types of vegetation cover, such as Cerrado, Rock Outcrop, and Eucalyptus, occurred in smaller proportions. Cerrado cover was found in two apiaries, Água Limpa (0.19%) and Grota (0.62%). Rocky outcrops were only found in the Palmital apiary (3.53%). Eucalyptus areas were observed in the Palmital (0.46%) and Fábrica (0.45%) apiaries, while the use of land with water, such as rivers and lakes, was minimal and only occurred in Água Limpa (0.43%).

The native vegetation cover and flowering species richness around the apiaries showed a high correlation ($r = 0.781$; $p = 0.022$). A total of 26 flowering plant species, belonging to 13 families, were found during the study period around the seven apiaries (Table 2S). The Asteraceae family stood out as having the highest number of flowering species at the time of the study (7 species: *Ageratum fastigiatum, Baccharis dracunculifolia, Baccharis trinervis, Chromolaena squalida, Cyrtocymura scorpioides, Sphagneticola trilobata, and Vernonanthura beyrichii*), followed by five species of Fabaceae (*Acacia mangium, Anadenanthera colubrina,*

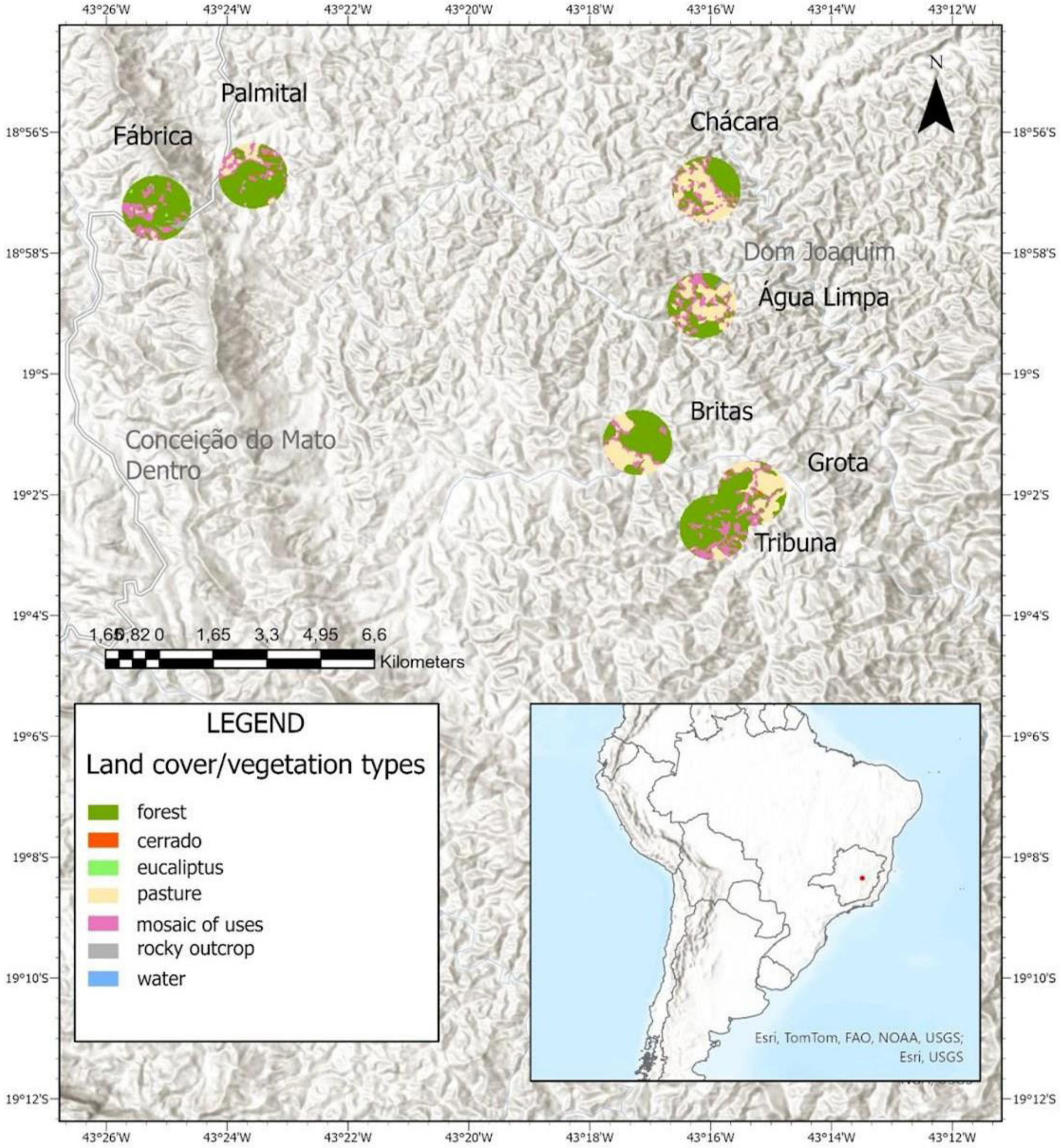

Fig 2. Map of land use and vegetation around the areas of the selected apiaries in Dom Joaquim, Minas Gerais (Fábrica, Palmital, Chácara, Água Limpa, Britas, Grota and Tribuna), Brazil.

Mimosa xanthocentra, Piptadenia adiantoides, and *Senna macranthera*). Three families each had two flowering species during the study: Convolvulaceae (*Distimake cissoides* and *Ipomoea saopaulista*), Lamiaceae (*Mesosphaerum sidifolium* and *Mesosphaerum suaveolens*), and Malpighiaceae (*Banisteriopsis malifolia* and *Stigmaphyllon lalandianum*). Most of the families found (8 families) had only one flowering species: Boraginaceae (*Varronia curassavica*), Cucurbitaceae (*Momordica charantia*), Euphorbiaceae (*Mabea fistulifera*), Malvaceae (*Hibiscus rosa-sinensis*), Melastomataceae (*Pterolepis glomerata*), Myrtaceae (*Eucalyptus saligna*), Solanaceae (*Solanum lycocarpum*), and Verbenaceae (*Duranta erecta*). Of the species identified, none were found to have a toxic effect on bees.

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The greatest similarity of flowering plant species was found between the Palmital and Fábrica apiaries (Jaccard Index (IJac) = 81.8%) and those with Tribuna and Britas (Fig 3). These four apiaries stand out for having more than 50 % native vegetation cover. The Grota and Chácara apiaries showed the greatest similarity to each other (IJac = 14%). The composition of species found in Água Limpa was less similar to other apiaries.

The richness of flowering species around the seven apiaries studied varied from seven to eleven species. In general, apiaries with more than 50 % cover had a higher richness of flowering species. The Fábrica apiary had the highest flowering species richness, with 11 species belonging to eight families: Asteraceae (three species), Fabaceae (two species), Cucurbitaceae (one species), Lamiaceae (one species), Malpighiaceae (one species), Solanaceae (one species) and Verbenaceae (one species) (Table 2S). In the Palmital and Brita apiaries, 10 flowering species were found each. In Palmital, the flowering species belong to eight families: Asteraceae (2 species), Fabaceae (two species), Cucurbitaceae (one species), Lamiaceae (one species), Malpighiaceae (one species), Malvaceae (one species), Solanaceae (one species), Verbenaceae (one species).

Nine flowering plant species belonging to six families were found in the Grota and Tribuna apiaries. Although the Grota apiary had less than 50% native vegetation cover, its richness was comparable to that of Tribuna, the apiary with the highest native vegetation cover among the apiaries. The families with the most flowering species found in Grota were not the same as in Tribuna, although these two apiaries are very close neighbors. In Grota, Fabaceae was the family with the most flowering species, followed by Convolvulaceae. In Tribuna, more flowering species from the Asteraceae family were found.

In the Água Limpa apiary, eight flowering plant species were found belonging to three families: Asteraceae (six species), Fabaceae (one species), and Solanaceae (one species). Finally, the apiary with the smallest sample of flowering species was Chácara, where seven plant species belonging to three families were recorded: Asteraceae (three species), Fabaceae (three species), and Lamiaceae (one species).

Characterization of the A. mellifera hives: temperature, humidity, and mite infestation found inside the hives.

In general, the hives in the seven apiaries studied had an internal temperature ranging from 28.9°C to 38.1°C (mean ± standard error = 33.99 ± 0.34°C, Table 3S). Humidity in the hives ranged from 34% to 78% (mean ± standard error = 53.31 ± 2.46%, Table 3S). The rates of *V. destructor* mite infestations per hive averaged 17.17% (± 1.77 %) and ranged from 3 to 42 per 100 bees. The apiary with the highest average internal temperature per hive was Palmital (36.96 ± 0.34 °C), followed by Chácara (35.0 ± 0.35 °C (Table 3S).

The apiary with the highest average internal humidity per hive was Britas (73 ± 2.24). The highest average *V. destructor* mite infestation rate per colony was observed

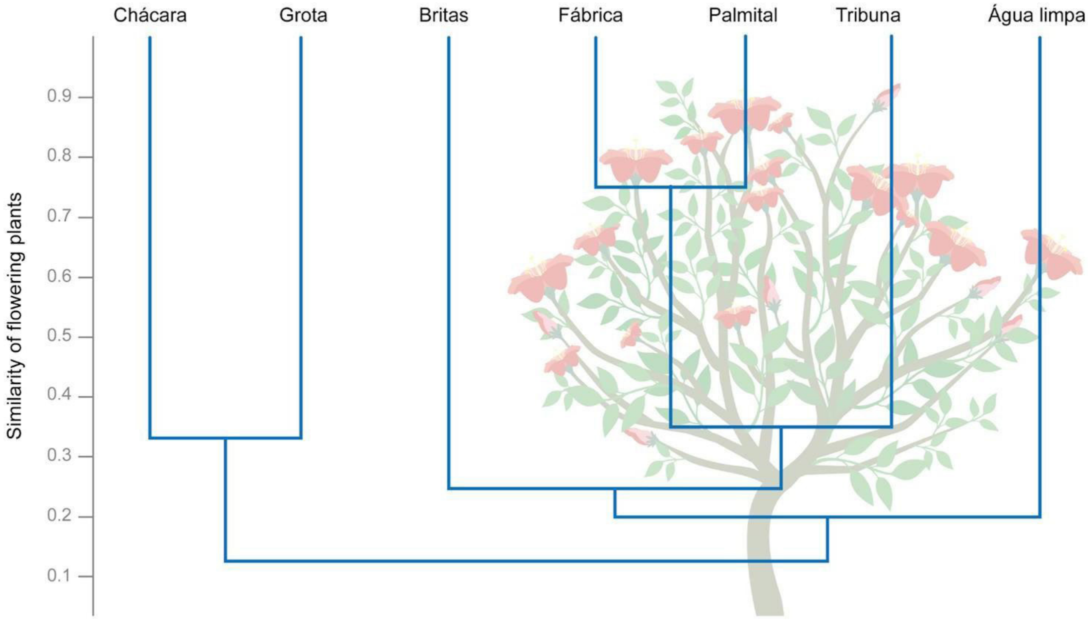

Fig 3. Jaccard similarity dendrogram (cohenetic correlation coefficient = 0.9345), paired group algorithm (UPGMA).

in Palmital (34 ± 3.21%), followed by Água Limpa (22 ± 3.40%), and Chácara (20.40 ± 3.59%). These values of mite infestation rate per hive are above the recommended values (greater than 12% mite infestation) for Brazil and could be harmful to *A. mellifera*. It was also noted that in the same apiary the hives showed a wide variation in temperature, internal humidity and mite infestation (Table 3S).

Fluctuating asymmetry of wing venation between apiaries

Fluctuating asymmetry of five of the 18 bee wing venations varied between the apiaries studied: M4 (P = 0.013), M12 (P = 0.014), M13 (P = 0.020), M14 (P = 0.014), M18 (P = <0.001) (Fig 4 A-E, Table 4S). There was no difference in the FA of the other 13 venations between the apiaries (Table 4S, p > 0.05).

FA of the M4 venation (FA M4) of the wings of *A. mellifera* was statistically lower in Água Limpa (Mean ± standard error = 0.003 ± 0.04 mm) than most of the apiaries evaluated: Brita (0.086 ± 0.006 mm), Chácara (0.068 ± 0.0038 mm), Fábrica (0.069 ± 0.021 mm), Grota (0.09 ± 0.02) and Tribuna (0.076 ± 0.0091 mm) (P = 0.013, Fig 4, Table 4S). The FA M4 values between Brita, Chácara, Fábrica, Grota, Palmital, and Tribuna did not differ from each other.

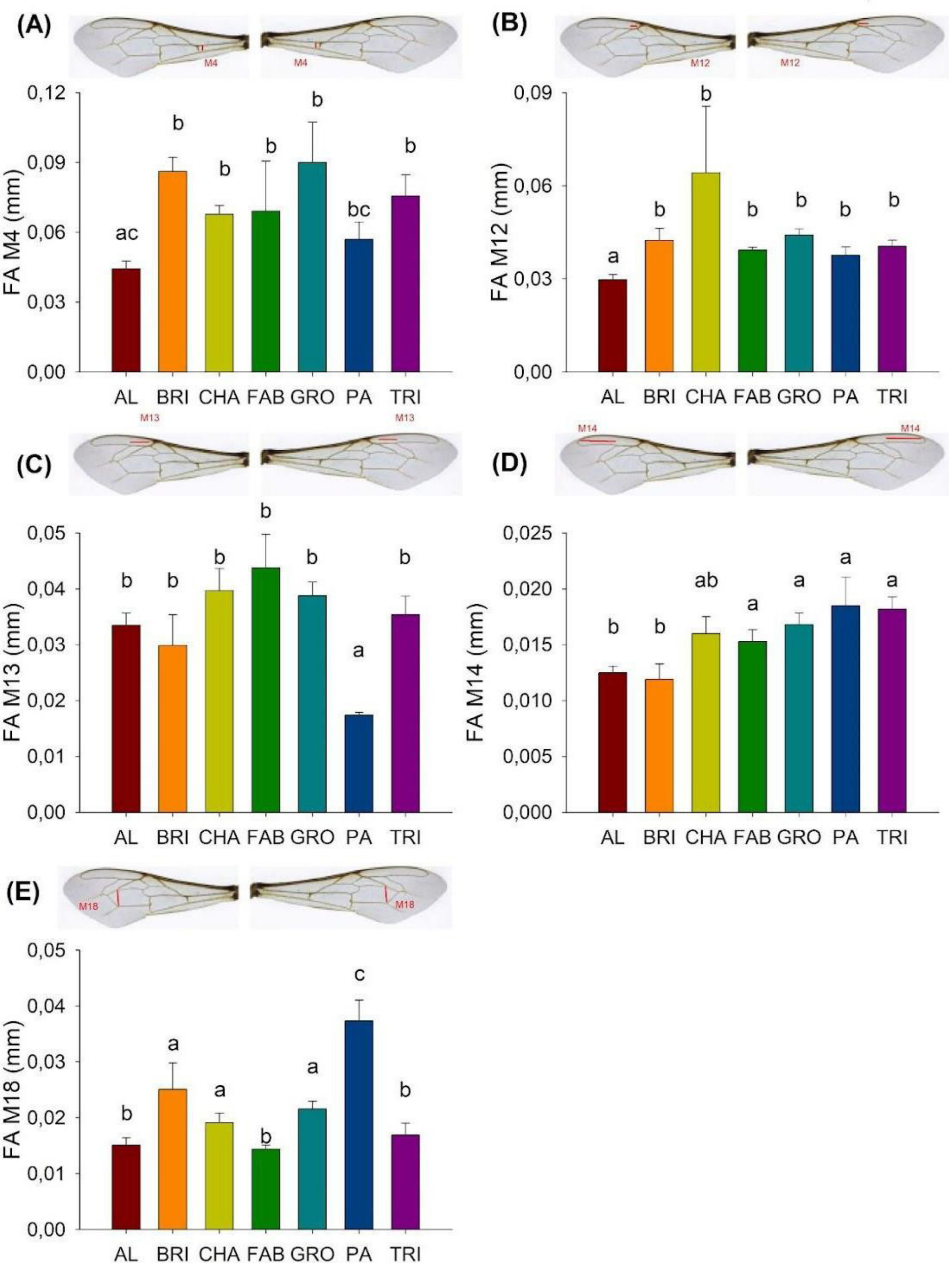

Fig 4. Mean values (± standard error) of fluctuating asymmetry (FA) of venations M4 (FA M4), M12 (FA M12), M13 (FA M13), M14 (FA M14), M18 (FA M18) found in the seven apiaries: Água Limpa (AL), Brita (BRI), Chácara (CH), Fábrica (FAB), Grota (GRO), Palmital (PAL), and Tribuna (TRI), located in São Joaquim, Minas Gerais, Brazil.

The FA of the M12 venation of the wings was lower in the Água Limpa apiary (mean ± standard error = 0.030 ± 0.0017 mm) than in all the other apiaries: Brita (0.042 ± 0.004), Chácara (0.0641 ± 0.022 mm), Fábrica (0.039 ± 0.00087 mm), Grota (0.044 ± 0.0020 mm), Palmital (0.037 ± 0.0027 mm) and Tribuna (0.041 ± 0.00197 mm) (P = 0.013, Fig 3, Table 4S). The largest M12 FA were observed in Brita, Chácara, Fábrica, Grota, and Tribuna, which did not differ from each other. The FA of the M13 wing venation was lower in Palmital (0.017 ± 0.00047 mm) than in the other apiaries studied: Água Limpa (0.034 ± 0.0022 mm), Brita (0.03 ± 0.006 mm), Chácara (0.04 ± 0.004 mm), Fábrica (0.044 ± 0.006 mm), Grota (0.039 ± 0.0024 mm) and Tribuna (0.035 ± 0.003 mm), which did not vary from one another.

The FAs of the M14 venation (FA M14) of the wings of bees from Água Limpa (0.013 ± 0.00056 mm) and Britas (0.012 ± 0.0014 mm) were smaller than those from Fábrica (0.015 ± 0.0011 mm), Grota (0.017 ± 0.0010 mm), Palmital (0.019 ± 0.0025 mm) and Tribuna (P = 0.014) (Fig 4, Table 4S). The FA M14 of the bees' wings did not differ between Água Limpa and Britas (P > 0.05). The FA M14 of the wings of bees from the Chacara (0.016 ± 0.0015 mm), Fábrica (0.015 ± 0.001 mm), Grota (0.017 ± 0.00103 mm), Palmital, and Tribuna (0.018 ± 0.0011 mm) apiaries did not differ (P > 0.05).

The FAs of venation 18 (FA M18) of the wings of Água Limpa (0.015 ± 0.001 mm), Fábrica (0.014 ± 0.0007 mm) and Tribuna (0.017 ± 0.002 mm) were smaller than Chácara (0.019 ± 0.0017 mm), Grota (0.022 ± 0.0014 mm) and Palmital (0.04 ± 0.01) (Fig 4, Table 4S, P = < 0.001). The FA M18 of Água Limpa, Fábrica, and Tribuna did not vary statistically from one another (P > 0.05). The FA M18 of Britas, Chácara, and Grotas did not differ from each other (P > 0.05). The FA M18 was the highest in Palmital among the apiaries (P = < 0.001).

Influence of vegetation cover on fluctuating asymmetry

Fluctuating asymmetry of the M17 venation of the wing of *A. mellifera* showed a significant difference between areas with low (37 to 43%) and high (59 to 71%) native vegetation cover (P = 0.016, Fig 5). The FA of the M17 venation in an area with low native vegetation cover (FC < 50%, mean FA ± standard error = 0.085 ± 0.0049) was 18% higher than in areas with high cover (FC > 50%, 0.072 ± 0.0027). FA of the other venations (M1 to M16, M18) did not differ statistically between areas of low and high native vegetation cover (P > 0.05).

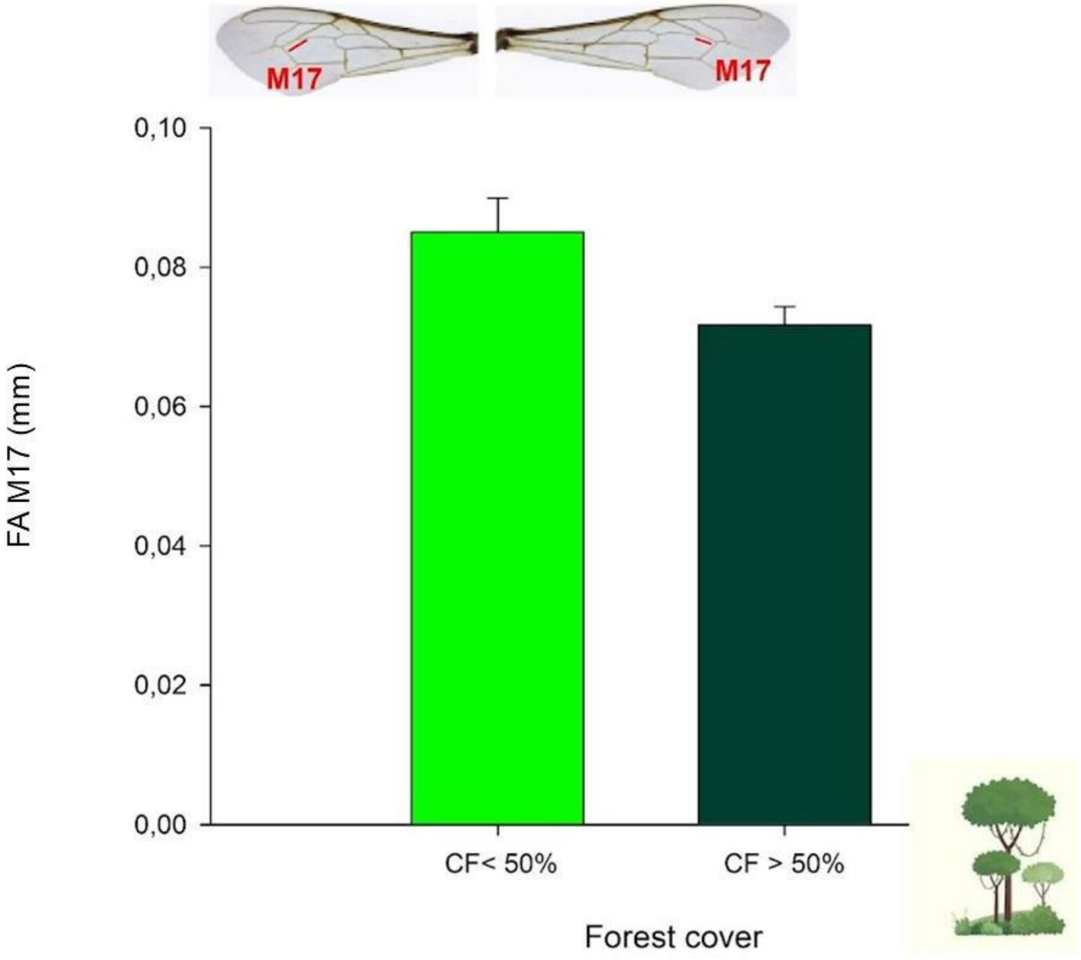

Fig 5. Effect of low (forest cover (FC) < 50%) and high (FC > 50%) native forest vegetation cover on the fluctuating asymmetry (FA) of the M17 venation.

Influence of temperature, humidity and mite infestation on fluctuating asymmetry

The Canonical Correspondence Analysis (CCA) ordination diagram shows the sites, FA and environmental variables (temperature, humidity and *V. destructor* infestation rate (Fig 6). The points for sites and FA have the same interpretation as in the CCA. They show the variation in FA measurements across sites. The environmental variables are represented by arrows (Fig 6). An arrow for an environmental variable indicates where there is a maximum change of that environmental variable along the diagram, and its length is proportional to the rate of change in that direction. Environmental variables with long arrows indicate that they are more correlated with the ordination axes than those with short arrows. The results of the CCA indicated that the FA of some venations is more associated with some stress factors (temperature, humidity, and mite infestation) inside the hive. The PC1 axis explained 61.87 % of the total variance, with the left side of the axis associated with the *V. destructor* infestation rate and the internal temperature of the hive, and the right side associated with the internal relative humidity of the hive (Fig 6). These results indicate that stress within the hive on FA levels in *A. mellifera*.

In the linear regression analysis, we found that hive conditions (internal temperature and humidity, and the rate of *V. destructor* infestation) influenced four venations (M2, M4, M17, and M18) of *A. mellifera* wings (Fig 7, Table 5S). We noted that the higher the internal temperature, the higher the FA of venation M2 (FA M2, $R^2 = 0.145$, $p < 0.024$) and M18 ($R^2 = 0.31$, $p < 0.001$). On the other hand, it was observed that the higher the internal relative humidity of the hive, the higher the FA of venations M4 ($R^2 = 0.212$, $p = 0.022$) and M17 ($R^2 = 0.239$, $p = 0.003$). The results also indicated that the higher the rate of *V. destructor* infestation found inside the hive, the higher the FA of venation M2 ($R^2 = 0.165$, $p = 0.015$), M18 ($R^2 = 0.16$, $p = 0.018$).

Discussion

Wing venation asymmetry fluctuations in *A. mellifera* proved to be an excellent indicator of environmental stress, be it inferred from variables related to the apiary's surroundings (native vegetation cover, richness of floral resources) or the internal conditions of the hive (temperature, humidity, *V. destructor* mite infestation). The results obtained contribute to knowledge about the impact of stressors on bees, primarily associated with climate change and habitat fragmentation,

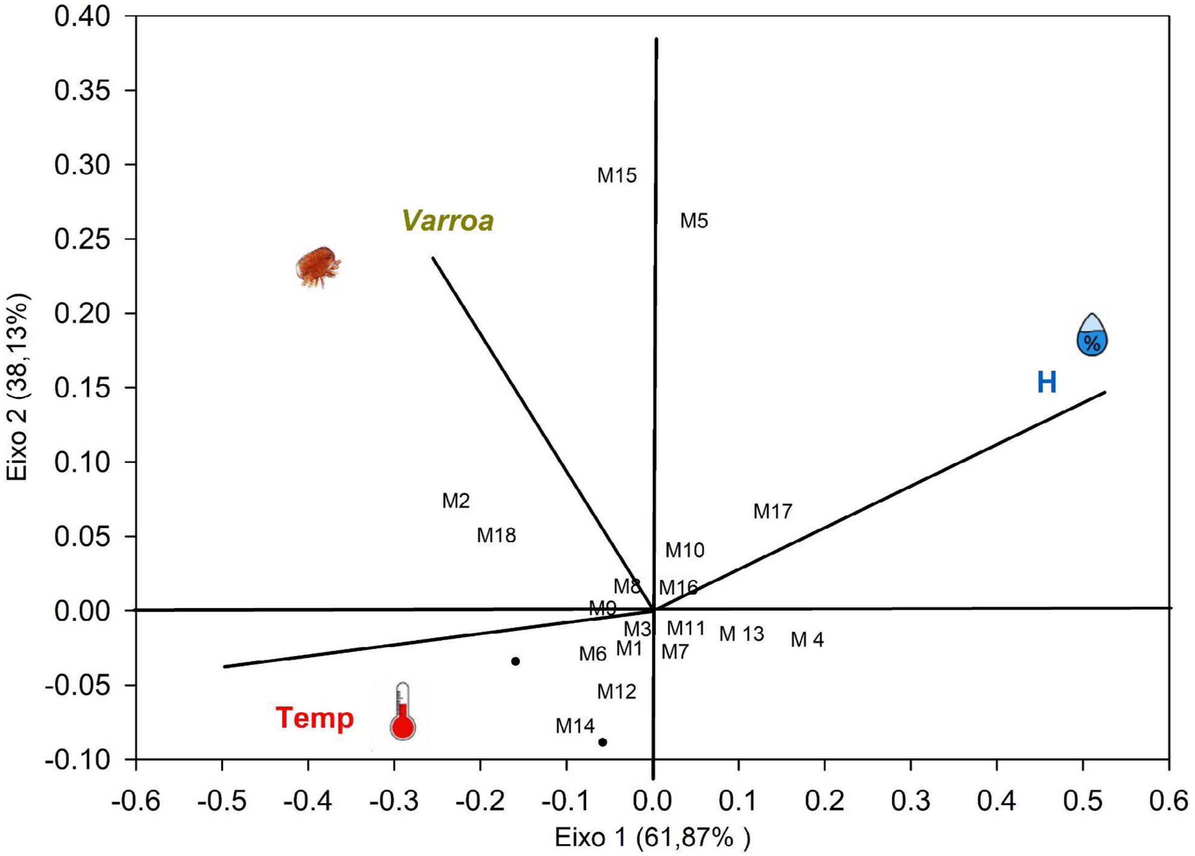

Fig 6. Canonical Correlation Analysis (CCA) with the main matrix showing the FA s of each venation (M1 to M18) found in each hive and the secondary matrix showing the variables of temperature (Temp, °C), humidity (H, %), and infestation rate of the *V. destructor* mite inside the hive. Axis 1 explained 61.87% of the variation, while Axis 2 explained 38.13%.

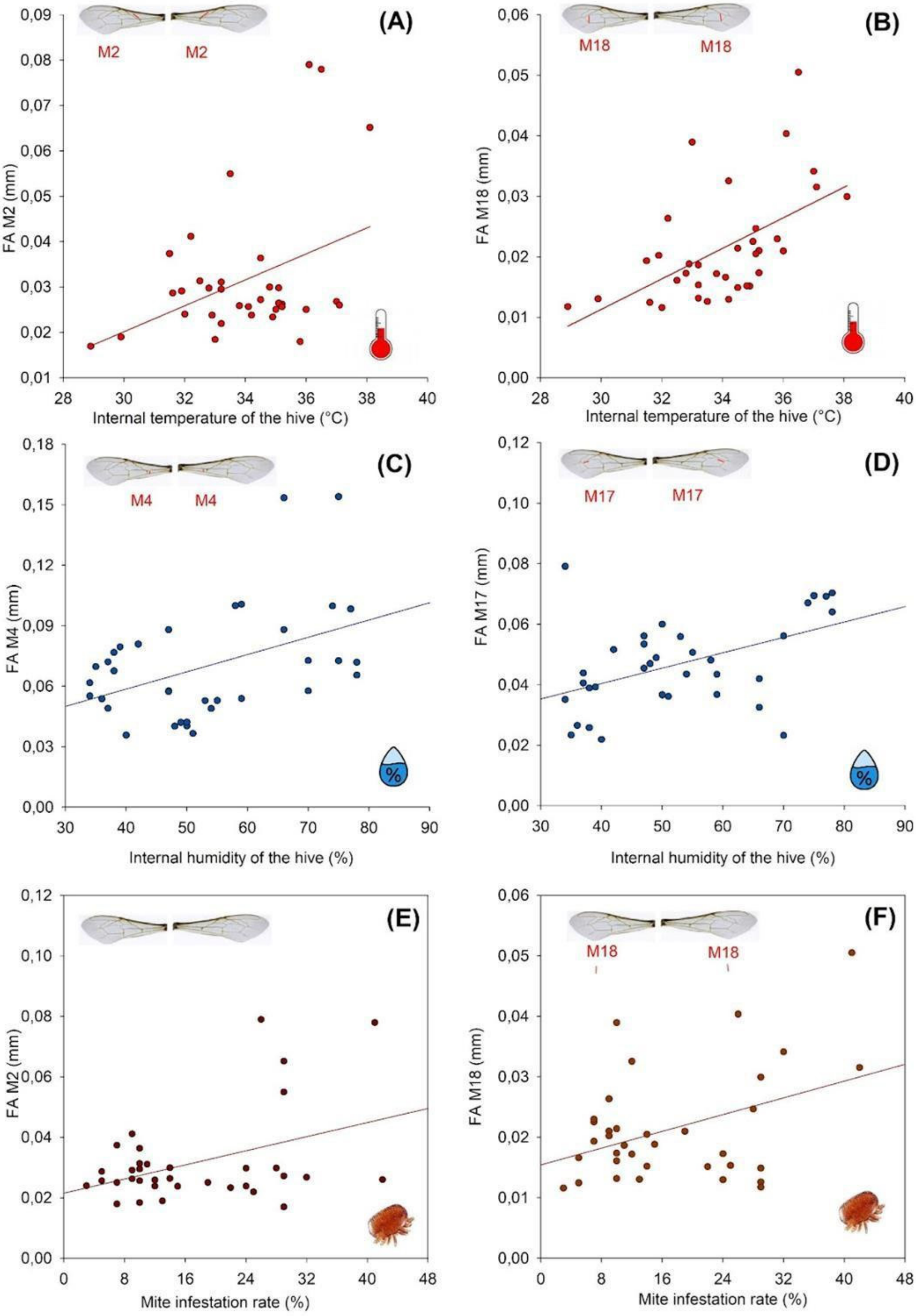

Fig 7. Relationship between the fluctuating asymmetry of venation M2 (FA M2), M4 (FA M4), M17 (FA M17), and M18 (FA M18), and the parameters of temperature (°C) and internal humidity (%) of the hives, and mite infestation rate (%).

two topics that are still underexplored in studies (see Havard et al., 2020). The work also indicates which *A. mellifera* wing venations may be more sensitive to environmental stress. In general, the impact of stress on FA in *A. mellifera* is centered on wing width and size (Nunes et al., 2015; Leonard et al., 2018).

In this study, we found that the FA of five wing venation traits (M4, M12, M13, M14, and M18) varied significantly among apiaries, suggesting that distinct local environmental factors to which the colonies are exposed may be influencing these morphological differences. The Água Limpa apiary exhibited the lowest FA values for most of the studied venation traits, standing out for its proximity to water bodies. This feature may be a key factor, as water availability is associated with the supply of essential micronutrients that support colony health, enhancing immune function, resistance to environmental stress, and bee longevity (Lau & Nieh, 2016; Eskov, 2018; Nearman et al., 2022). During periods of high temperatures, bees exhibit increased water demand (Eskov, 2018), and water also plays a crucial role in evaporative cooling within the hive (Kühnholz & Seeley, 1997). Therefore, the proximity to water sources may act as an alleviating factor against environmental stressors, as reflected by lower levels of morphological asymmetry. Additionally, we observed that four wing venation traits (M2, M4, M17, and M18) were particularly sensitive to one or more of the stressors evaluated in this study, including reduced vegetation cover, elevated hive temperature, humidity, and mite infestation.

The areas surrounding the apiary with low native vegetation cover (50%) had higher FA M17. This result shows that the reduction in vegetation cover is very important for the development and stress tolerance of bees, even generalists such as *Apis*, probably due to the reduced availability of a diet based on a greater diversity of floral resources (Gallai et al., 2009; Winfree, 2010; Ghosh et al., 2020). The results presented here also corroborate the studies conducted by Nunes et al. (2015) and Leonard et al. (2018), which found the impact of reduced vegetation cover on wing size and shape.

FA M17 is also an indicator of stress in conditions of high humidity, while the FAs of venations M2 and M18 were indicative of two stress factors inside the hive. FA M2 and FA M18 increased with an increase in temperature, humidity, and mite infestation inside the hive. These results indicate that internal beehive conditions can pose significant stress for the development and management of bees. High humidity inside the hive can reduce the survival of *A. mellifera*, especially when acting simultaneously with high temperatures (Li et al., 2019). In high temperature conditions, high humidity prevents bees from losing water, and consequently, their body temperature does not decrease enough to prevent damage (Li et al., 2019). In addition, high humidity can favour the incidence of pathogens such as the fungus *Ascosphaera apis* and lead to the weakening and death of *A. mellifera* worker bees (Flores et al., 1996). High temperatures (over 37 °C) can lead to a decrease in the viral load of viruses such as the deformed wing virus; however, they also favour bee mortality (Dalmon et al., 2019). Observations by Medina et al. (2018) indicate that bees reared under heat stress showed no change in body size, but showed greater FA in the shape of the forewings. Increased temperature can interfere with foraging and, in the long term, lead to oxidative damage in the individual (Finkel & Holbrook, 2000; Zhao et al., 2021).

Varroa destructor is considered the parasite that most leads to mortality in beekeeping (Gregorc & Sampson, 2019). The high infestation of *V. destructor* in bees not only leads to a loss of body weight but also affects their ability to fly and the foraging orientation required to collect floral resources for their development (Noel et al., 2020). The stress for bees due to this high infestation of *V. destructor* can be even greater when associated with the transmission of viruses through this parasite (Miranda & Fries, 2008) or even climate change (Le Conte et al., 2010). Here, we note that the high rate of infestation of the V. destructor mite in the hives of the studied apiaries is not a common occurrence in the country (Pinto et al., 2022). In general, the infestation of *V. destructor* observed in all Brazilian regions averages around 4% (Peixoto et al., 2021). The high incidence of *Varroa* observed in some of the hives studied suggests that they may be weakened, reinforcing the need to monitor and adopt good practices and management to maintain beekeeping health (Jack & Ellis, 2021).

Overall, our results demonstrate that the environmental conditions surrounding the apiaries directly influence the stress levels of A. mellifera colonies, which in turn strongly affect the symmetry of their wings. The loss of vegetation cover, increased temperature and humidity inside the hive, as well as mite infestation, were associated with increased FA in specific wing venation traits. Specifically, reduced vegetation cover was associated with increased FA in venation trait M17, while elevated temperatures and mite infestations were linked to higher FA in venations M2 and M18. Additionally, increased humidity levels within the hive correlated with greater FA in venations M4 and M17. These findings highlight that a combination of these stressors interferes with the symmetry of *A. mellife*ra wing venations, particularly affecting three specific traits (M2, M17, M18). This indicates that environmental and hive-related factors can measurably impact honey bee developmental stability. In this context, FA can serve as a valuable indicator for monitoring the health of hives. Furthermore, the study underscores the importance of effective thermoregulation and control of *V. destructor* for maintaining the health of *A. mellifera* colonies.

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Data availability

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

Conflict of interest declaration

All authors declare that they have no conflict of interest.

Authors' Contribution

BAS: Methodology, investigation, writing-original draft, writing-review & editing.
YO: Conceptualization, methodology, investigation, formal analysis, writing-original draft, writing-review & editing.
IM: Methodology, investigation, writing-original draft, writing-review & editing.
MPS: Methodology, investigation, writing-original draft, writing-review & editing.
BD: Investigation, writing-original draft, writing-review & editing.
WKS: Formal analysis.
FFG: Formal analysis.
MQ: Investigation, writing-original draft, writing-review & editing.
GWF: Conceptualization, writing-original draft, supervision, writing-review & editing, funding acquisition.

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Supplementary Material

Table 1S. Percentage of land use in areas surrounding the apiaries located in Dom Joaquim, Minas Gerais, Brazil. The following land uses were identified: 1. forest (Mata Atlântica, including dense, open, and semi-deciduous forest); 2. savannah (Cerrado); 3. rocky outcrop (campo rupestre); 4. pasture; 5. mosaic of uses (areas of agricultural use where it was not possible to distinguish between pasture and agriculture); 6. eucalyptus; 7. water (body of water: rivers and lakes).

Apiary	Land use (%)						
	Forest	Cerrado	Rock outcrop	Eucalyptus	Pasture	Mosaic of uses	Water (river and/or lake)
Água Limpa	37.88	0.19	-	-	35.00	26.49	0.43
Britas	58.93	-	-	-	29.41	11.66	-
Chácara	37.96	-	-		39.98	22.06	-
Fábrica	69.66			0.45	1.89	11.25	
Grota	43.26	0.62	-		36.39	19.73	-
Palmital	70.39	-	3.53	0.46	2.75	22.88	-
Tribuna	71.49	-	-	-	4.24	24.28	-

Table 2S. Flowering plant species recorded in the Água Limpa, Britas, Chácara, Fábrica, Grota, Palmital and Tribuna apiaries, located in the municipality of Dom Joaquim, Minas Gerais, Brazil.

Nº	Apiary	Family	Plant species
1	Água Limpa	Asteraceae	*Baccharis dracunculifolia*
2		Asteraceae	*Baccharis trinervis*
3		Asteraceae	*Chromolaena squalida*
4		Asteraceae	*Cyrtocymura scorpioides*
5		Asteraceae	*Sphagneticola trilobata*
6		Asteraceae	*Vernonanthura beyrichii*
7		Fabaceae	*Anadenanthera colubrina*
8		Solanaceae	*Solanum lycocarpum*
9	Britas	Asteraceae	*Ageratum fastigiatum*
10		Asteraceae	*Chromolaena squalida*
11		Asteraceae	*Cyrtocymura scorpioides*
12		Boraginaceae	*Varronia curassavica*
13		Asteraceae	*Ageratum fastigiatum*
14		Euphorbiaceae	*Mabea fistulifera*
15		Fabaceae	*Senna macranthera*
16		Malpighiaceae	*Banisteriopsis malifolia*
17		Malpighiaceae	*Stigmaphyllon lalandianum*
18		Melastomataceae	*Pterolepis glomerata*
19		Myrtaceae	*Eucalyptus saligna*
20	Chácara	Asteraceae	*Baccharis dracunculifolia*
21		Asteraceae	*Baccharis trinervis*
22		Asteraceae	*Chromolaena squalida*
23		Fabaceae	*Acacia mangium*
24		Fabaceae	*Piptadenia adiantoides*
25		Fabaceae	*Mimosa xanthocentra*
26		Lamiaceae	*Mesosphaerum suaveolens*
27	Fábrica	Asteraceae	*Ageratum fastigiatum*
28		Asteraceae	*Baccharis dracunculifolia*
29		Asteraceae	*Cyrtocymura scorpioides*
30		Cucurbitaceae	*Momordica charantia*
31		Fabaceae	*Anadenanthera colubrina*
32		Fabaceae	*Senna macranthera*
33		Lamiaceae	*Mesosphaerum sidifolium*
34		Malpighiaceae	*Banisteriopsis malifolia*
35		Myrtaceae	*Eucalyptus saligna*
36		Solanaceae	*Solanum lycocarpum*
37		Verbenaceae	*Lantana camara*
38	Grota	Asteraceae	*Baccharis trinervis*
39		Convolvulaceae	*Distimake cissoides*
40		Convolvulaceae	*Ipomoea saopaulista*
41		Fabaceae	*Acacia mangium*
42		Fabaceae	*Piptadenia adiantoides*
43		Fabaceae	*Senna macranthera*
44		Lamiaceae	*Mesosphaerum suaveolens*
45		Malvaceae	*Hibiscus rosa-sinensis*
46		Verbenaceae	*Duranta erecta*

Table 2S. Flowering plant species recorded in the Água Limpa, Britas, Chácara, Fábrica, Grota, Palmital and Tribuna apiaries, located in the municipality of Dom Joaquim, Minas Gerais, Brazil. (Continuation)

Nº	Apiary	Family	Plant species
47	Palmital	Asteraceae	*Ageratum fastigiatum*
48		Asteraceae	*Cyrtocymura scorpioides*
49		Cucurbitaceae	*Momordica charantia*
50		Fabaceae	*Anadenanthera colubrina*
51		Fabaceae	*Senna macranthera*
52		Lamiaceae	*Mesosphaerum sidifolium*
53		Malpighiaceae	*Banisteriopsis malifolia*
54		Malvaceae	*Hibiscus rosa-sinensis*
55		Solanaceae	*Solanum lycocarpum*
56		Verbenaceae	*Lantana camara*
57	Tribuna	Asteraceae	*Chromolaena squalida*
58		Asteraceae	*Cyrtocymura scorpioides*
59		Asteraceae	*Sphagneticola trilobata*
60		Convolvulaceae	*Distimake cissoides*
61		Convolvulaceae	*Ipomoea saopaulista*
62		Fabaceae	*Senna macranthera*
63		Lamiaceae	*Mesosphaerum suaveolens*
64		Solanaceae	*Solanum lycocarpum*
65		Verbenaceae	*Lantana camara*

Table 3S. Parameters (temperature, humidity and infestation rate of *Varroa destructor*) measured from each hive box in each apiary studied: 1. Água Limpa; 2. Britas;3. Chácara; 4. Fábrica; 5. Grota; 6.Palmital; 7. Tribuna, located in the municipality of Dom Joaquim, Minas Gerais, Brazil.

Apiary	Colony	Internal temperature (°C)	Internal humidity (%)	Infestation rate of *Varroa destructor* (%)
Água Limpa	1	32.80	50.00	24.00
	2	28.90	48.00	29.00
	3	34.50	49.00	29.00
	4	32.90	47.00	15.00
	5	29.90	50.00	13.00
	Average	31.80	48.80	22.00
Britas	1	33.00	70.00	10.00
	2	35.20	74.00	9.00
	3	35.10	78.00	14.00
	4	34.20	77.00	12.00
	5	31.60	66.00	5.00
	Average	33.82	73.00	10.00
Chácara	1	35.10	34.00	28.00
	2	36.00	47.00	19.00
	3	34.90	38.00	22.00
	4	33.80	37.00	12.00
	5	35.20	39.00	10.00
	Average	35.00	39.00	18.20

Table 3S. Parameters (temperature, humidity and infestation rate of *Varroa destructor*) measured from each hive box in each apiary studied: 1. Água Limpa; 2. Britas;3. Chácara; 4. Fábrica; 5. Grota; 6.Palmital; 7. Tribuna, located in the municipality of Dom Joaquim, Minas Gerais, Brazil. (Continuation)

Apiary	Colony	Internal temperature (°C)	Internal humidity (%)	Infestation rate of *Varroa destructor* (%)
Fábrica	1	33.50	75.00	29.00
	2	34.80	51.00	14.00
	3	33.20	53.00	25.00
	4	32.50	55.00	10.00
	5	34.20	54.00	24.00
	Average	33.64	57.60	20.40
Grota	1	34.50	78.00	10.00
	2	35.00	70.00	7.00
	3	32.20	75.00	9.00
	4	31.50	66.00	7.00
	5	33.20	59.00	11.00
	Average	33.28	69.60	8.80
Palmital	1	37.00	38.00	32.00
	2	36.50	37.00	41.00
	3	38.10	40.00	29.00
	4	37.10	36.00	42.00
	5	36.10	35.00	26.00
	Average	36.96	37.20	34.00
Tribuna	1	32.00	58.00	3.00
	2	35.80	59.00	7.00
	3	34.10	42.00	5.00
	4	33.20	34.00	10.00
	5	31.90	47.00	9.00
	Average	33.40	48.00	6.80

Table 4S. Mean (± standard error) of the fluctuating asymmetry of the wing venations of *Apis mellifera* from each apiary studied: 1. Água Limpa; 2. Britas; 3. Chácara; 4. Fábrica; 5. Grota; 6. Palmital; 7. Tribuna, located in the municipality of Dom Joaquim, Minas Gerais, Brazil. The wing venations highlighted in bold are those where fluctuating asymmetry (FA) showed *p* values less than 0.05, indicating statistical significance.

FA	Apiary								Statistical analysis
	Água Limpa	Britas	Chácara	Fábrica	Grota	Palmital	Tribuna	P	
FA M1	0.04 ± 0.01	0.06 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	P = 0.61	Kruskal-Wallis One Way Analysis of Variance on Ranks
FA M2	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.06 ± 0.01	0.03 ± 0.01	P = 0.07	Kruskal-Wallis One Way Analysis of Variance on Ranks
FA M3	0.07 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.08 ± 0.01	P = 0.60	One Way Analysis of Variance
FA M4	0.04 ± 0.01	0.09 ± 0.01	0.07 ± 0.01	0.07 ± 0.02	0.09 ± 0.02	0.06 ± 0.01	0.08 ± 0.01	P = 0.01	Kruskal-Wallis One Way Analysis of Variance on Ranks
FA M5	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	P = 0.62	Kruskal-Wallis One Way Analysis of Variance on Ranks
FA M6	0.05 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.05 ± 0 .01	P = 0.60	Kruskal-Wallis

Table 4S. Mean (± standard error) of the fluctuating asymmetry of the wing venations of *Apis mellifera* from each apiary studied: 1. Água Limpa; 2. Britas; 3. Chácara; 4. Fábrica; 5. Grota; 6. Palmital; 7. Tribuna, located in the municipality of Dom Joaquim, Minas Gerais, Brazil. The wing venations highlighted in bold are those where fluctuating asymmetry (FA) showed *p* values less than 0.05, indicating statistical significance. (Continuation)

FA	**Apiary**								**Statistical analysis**
	Água Limpa	**Britas**	**Chácara**	**Fábrica**	**Grota**	**Palmital**	**Tribuna**	**P**	
FA M7	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.01 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	P = 0.13	Kruskal-Wallis One Way Analysis of Variance on Ranks
FA M8	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	P = 0.71	One Way Analysis of Variance
FA M9	0.08 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.08 ± 0.01	0.10 ± 0.01	0.08 ± 0.01	P = 0.24	One Way Analysis of Variance
FA M1 0	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	P = 0.22	One Way Analysis of Variance
FA M11	0.06 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	P = 0.10	One Way Analysis of Variance
FA M12	0.03 ± 0.01	0.04 ± 0.01	0.06 ± 0.02	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	P = 0.01	One Way Analysis of Variance
FA M13	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.02 ± 0.01	0.04 ± 0.01	P = 0.02	Kruskal-Wallis One Way Analysis of Variance on Ranks
FA M14	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	P = 0.01	One Way Analysis of Variance
FA M15	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.03 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	P = 0.70	Kruskal-Wallis One Way Analysis of Variance on Ranks
FA M16	0.08 ± 0.01	0.07 ± 0.01	0.09 ± 0.01	0.08 ± 0.01	0.09 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	P = 0.09	Kruskal-Wallis One Way Analysis of Variance on Ranks
FA M17	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.03 ± 0.01	0.05 ± 0.01	P = 0.10	Kruskal-Wallis One Way Analysis of Variance on Ranks
FA M18	0.02 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.04 ± 0.01	0.02 ± 0.01	P < 0.01	One Way Analysis of Variance

Table 5S. Effect of hive conditions on conditions of the hive (temperature, humidity and *Varroa destructor* infestation) on fluctuating asymmetry (FA) of the 18 wing venations (M1 - M18) of *Apis mellifera*. The linear regression results that were significant are in bold (P < 0.05).

	R	**P**	**R2**	**P**	**Equation**
Temperature X FA M1	0.16	0.35	-	-	-
Temperature X FA M2	0.38	0.02	0.15	0.02	FA M2 = -0.0656 + (0.00286* Internal temperature)
Temperature X FA M3	-0.18	0.24	-	-	-
Temperature X FAM4	-0.18	0.29	-	-	-
Temperature X FA M5	0.07	0.69	-	-	-
Temperature X FA M6	-0.25	0.15	-	-	-
Temperature X FA M7	-0.18	0.30	-	-	-
Temperature X FA M8	-0.08	0.60	-	-	-
Temperature X FA M9	-0.07	0.69	-	-	-
Temperature X FA M10	-0.16	0.36	-	-	-
Temperature X FA M11	0.23	0.19	-	-	-
Temperature X FA M12	0.12	0.50	-	-	-
Temperature X FA M13	-0.41	0.01	-	-	-
Temperature X FA M14	0.45	0.07	-	-	-
Temperature X FA M15	-0.09	0.59	-	-	-
Temperature X FA M16	-0.23	0.18	-	-	-
Temperature X FA M17	-0.15	0.38	-	-	-
Temperature X FA M18	0.55	0.01	0.30	<0.001	FA M18 = -0.0645 + (0.00253* Temperature)

Table 5S. Effect of hive conditions on conditions of the hive (temperature, humidity and *Varroa destructor* infestation) on fluctuating asymmetry (FA) of the 18 wing venations (M1 - M18) of *Apis mellifera*. (Continuation)

	R	P	R2	P	Equation
Humidity X FA M1	0.078	0.66	-	-	-
Humidity X FA M2	-0.16	0.35	-	-	-
Humidity X FA M3	0.014	0.94	-	-	-
Humidity X FA M4	0.44	0.01	0.20	0.01	FA M4 = 0.0242 + (0.000858* Humidity)
Humidity X FA M5	0.29	0.10	-	-	-
Humidity X FA M6	-0.04	0.83	-	-	-
Humidity X FA M7	0.20	0.20	-	-	-
Humidity X FA M8	0.13	0.45	-	-	-
Humidity X FA M9	-0.15	0.40	-	-	-
Humidity X FA M10	0.28	0.10	-	-	-
Humidity X FA M11	0.28	0.11	-	-	-
Humidity X FA M12	-0.12	0.50	-	-	-
Humidity X FA M13	0.25	0.15	-		
Humidity X FA M14	-0.25	0.16	-	-	-
Humidity X FA M15	0.15	0.40	-	-	-
Humidity X FA M16	0.079	0.65	-	-	-
Humidity X FA M17	0.49	0.01	0.24	0.003	FA M17 = 0.0200 + (0.000508* Humidity)
Humidity X FA M18	-0.11	0.52	-	-	-
Varroa destructor infestation X FA M1	-0.05	0.76	-	-	-
Varroa destructor infestation X FA M2	0.41	0.02	0.17	0.02	FA M2 = 0,0215 + (0,000583* *Varroa destructor* infestation)
Varroa destructor infestation X FA M3	0.01	0.99	-	-	-
Varroa destructor infestation X FA M4	-0.31	0.06	-	-	-
Varroa destructor infestation X FA M5	0.21	0.21	-	-	-
Varroa destructor infestation X FA M6	-0.13	0.43	-	-	-
Varroa destructor infestation X FA M7	-0.20	0.25	-	-	-
Varroa destructor infestation X FA M8	0.01	0.96	-	-	-
Varroa destructor infestation X FA M9	0.24	0.17	-	-	-
Varroa destructor infestation X FA M10	-0.05	0.77	-	-	-
Varroa destructor infestation X FA M11	-0.10	0.56	-	-	-
Varroa destructor infestation X FA M12	0.03	0.85	-	-	-
Varroa destructor infestation X FA M13	-0.25	0.15	-	-	-
Varroa destructor infestation X FA M14	0.15	0.40	-	-	-
Varroa destructor infestation X FA M15	0.25	0.14	-	-	-
Varroa destructor infestation X FA M16	-0.01	0.95	-	-	-
Varroa destructor infestation X FA M17	-0.15	0.38	-	-	-
Varroa destructor infestation X FA M18	0.40	0.02	0.16	0.02	FA M18 = 0.0154 + (0.000346* *Varroa destructor* infestation)