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Wing morphometrics reveals the migration patterns of Africanized honey bees in Northeast Brazil

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Abstract

Climatic differences can directly affect the population structure of organisms. The Northeastern Brazilian covers an area of about 1.5 million square kilometers, in which the semi-arid part corresponds to approximately 60%. It is probably the most vulnerable region to climatic variations in Brazil. Here, we investigated the variability of Africanized honey bees in different localities from Northeast Brazil during the dry season and the influence of drought periods in morphological variation among populations. Analyses were carried out with data collected by traditional and geometric morphometrics of bees sampled during the dry season and showed a subtle morphological variation in agreement to the climatic pattern. Furthermore, once we added samples collected during the rainy season, we observed a change in its pattern, with a very different result from the same population sampled during drought periods. The geometric morphometrics results emphasized that samples collected during the rainy season in semi-arid areas would be more similar to bees from humid coastal areas. These results probably reflect the probable dispersion pattern of these bees between humid coastal and semi-arid areas.

Introduction

Since its introduction to Brazil, in 1956 (Gonçalves, 1974), Africanized bees have occupied almost the entire American continent, from northern Argentina to the USA (Rinderer et al., 1993). They can be easily identified based on morphometric features, like the pattern of wing venation (Francoy et al., 2008) and are well adapted to the local environment, especially to Northeast Brazil, a region with very similar features to the original African environment where these bees were originally sampled. Northeast Brazilian covers an area of about 1.5 million square kilometers. Approximately 60 percent of its total area presents semi-arid conditions (Silva, 2004) corresponding to 11 percent of the area of Brazil (Marengo et al., 2017). This extensive area is the most vulnerable region in Brazil regarding climatic variations where the periodicity and duration of the droughts usually are high (Marengo et al., 2017; Silva, 2004). Previous studies have shown that the presence of Africanized honey bee colonies in semi-arid is high during the rainy season, but these bees become scarce in the dry season (Freitas et al., 2007). It suggests that Africanized honey bees could migrate from semi-arid areas to regions where environmental conditions are more favorable during drought periods (Freitas et al., 2007). In areas constantly subjected to disturbance, bees must continually adapt to these changes, otherwise absconding can occur towards more favorable areas, a fact that is even more exacerbated in Africanized honey bees (Freitas et al., 2007).

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These climatic differences between nearby localities can directly affect the population structure of organisms established in these regions. Diniz-Filho et al. (2000) observed that the variations among local populations of African subspecies of *Apis mellifera* L. were correlated with their distribution in different climates. Diniz-Filho and Malaspina (1995) observed spatial variation in populations of Africanized honey bees distributed throughout Brazil. Hence, here we evaluate the variability of Africanized honey bee populations within Northeast Brazilian region, including semi-arid, during the rainy season and dry season comparing traditional and geometric morphometrics of wings. Our main expectation is that the populations' variability to be related to the differences between the climatic regions and also to the migration flow.

Materials and Methods

Sampling

We sampled workers from 161 colonies of Africanized honey bees, in 19 localities from five states of northeast Brazil (Table 1), representing different climatic regions in these states. Bees were collected from September to December 2012 during the dry season, except for 37 colonies from Mossoró - RN, which were collected in March 2013 during the rainy season. All collected individuals were stored in 96% ethyl alcohol to preserve the samples for future molecular analysis.

Morphometric analyses

The right forewings of approximately five workers per colony were mounted between a microscope slide and coverslip and photographed with a digital camera connected to a stereomicroscope. The traditional morphometric analyses were based on a dataset of measures from wings as described by Ruttner et al. (1978). All measurements were performed with the aid of the software TSView version 7. To analyze the patterns of wing venation, a tps file was made from the images using the software tpsUtil version 1.70 (Rohlf, 2015). Nineteen landmarks were plotted on the wing vein intersections using tpsDig2 version 2.26 (Rohlf, 2015) according to Francoy et al. (2008).

Traditional morphometric analyses were carried out using Statistica7 software (Statsoft, 2004). Geometric morphometric analyses were conducted using the software MORPHOJ version 1.03 (Klingenberg, 2011). We first produced a Procrustes fit to eliminate variation caused by differences in size, position, and orientation of the wings. The residuals of this regression were used as "size free" variables in the following statistical analyses. Both morphometric data were used as input in canonical variant analyses (CVA) and discriminant function analyses (DFA). Bees were grouped according to their original sampling location and climate. A leave-one-out cross-validation test was performed to assess the accuracy of the data. We also calculated the morphological distances between the centroids of the groups' distribution and

Table 1. Localities (state and city) and number of Africanized honey bee colonies sampled in northeast Brazil, during the dry season (in 2012). N = number of colonies sampled. *37 colonies from Mossoró were collected in March 2013 during the rainy season.

State	Location	Latitude	Longitude	Climate	Mean Precipitation	N
Alagoas	Rio Largo	09° 28' 49'' S	35° 51' 29'' W	Humid coastal	1,634 mm	8
Alagoas	Barra de Santo Antônio	09° 24′ 58″ S	35° 30′ 33″ W	Humid coastal	1,634 mm	5
Alagoas	Murici	09° 18' 18'' S	35° 56' 30'' W	Humid coastal	1,309 mm	2
Alagoas	Maceió	09° 39′ 59″ S	35° 44′ 06″ W	Humid coastal	1,570 mm	1
Alagoas	Joaquim Gomes	09° 06' 60'' S	35° 44' 15'' W	Humid coastal	1,634 mm	3
Paraíba	João Pessoa	07° 06′ 55″ S	34° 51′ 40″ W	Humid coastal	1,874 mm	1
Paraíba	Baraúna	05° 04′ 14″ S	37° 37′ 02″ W	Semi-arid	750mm	2
Paraíba	Cuité	06° 28′ 54″ S	36° 08′ 59″ W	Semi-arid	735 mm	2
Paraíba	Taperoá	13° 32′ 18″ S	39° 06′ 01″ W	Semi-arid	503 mm	5
Paraíba	Maturéia	07° 15′ 59″ S	37° 20′ 57″ W	Semi-arid	726 mm	1
Piauí	São Raimundo Nonato	09° 00' 54'' S	42° 41' 50'' W	Semi-arid	697 mm	14
Piauí	Bela Vista	07° 58′ 58″ S	41° 52′ 40″ W	Semi-arid	847 mm	16
Piauí	Isaias Coelho	07° 44' 16'' S	41° 40' 45'' W	Semi-arid	847 mm	4
Piauí	Parnaíba	09° 06' 41'' S	45° 55' 50'' W	Tropical	1,064 mm	13
Rio Grande do Norte	Macaíba	05° 51′ 36″ S	35° 20′ 59″ W	Humid coastal	1,442 mm	2
Rio Grande do Norte	São Paulo do Potengi	05° 53' 44'' S	35° 45' 29'' W	Humid coastal	1,750 mm	5
Rio Grande do Norte	Mossoró	05° 11' 17'' S	37° 20' 39'' W	Semi-arid	765 mm	57*
Sergipe	Brejo Grande	10° 25' 38'' S	36° 28' 12'' W	Humid coastal	1,650 mm	7
Sergipe	São Cristóvão	11° 00' 49'' S	37° 13' 21'' W	Humid coastal	1,372 mm	13

used it to construct a dendrogram of morphological proximity based on the Neighbor-Joining algorithm using MEGA 5.2 (Tamura et al., 2011). We ran a Mantel test using in TFPGA software (Miller, 1997) on the morphological distances between the centroids of the groups and the geographic distances among the sampling locations (measured by Google Earth 6.1.0.5001/2011).

Results

Analyses were carried out with data collected by traditional and geometric morphometrics of collected bees during the dry season. The workers from different climates presented a subtle separation for both traditional and geometric morphometrics (Fig 1A-B). Furthermore, once we added workers collected in the rainy season, we observed a change in the distribution pattern, with the homogenization of samples from the semi-arid region and humid coastal (Fig 1C-D). Therefore, we classified the individuals according to the climate, assigning Mossoró two classifications (rainy and dry seasons), and we made further statistical analyses for both morphometric methods.

Traditional morphometrics

Using the data sets from traditional morphometric measurements, we found a subtle separation of bees according to the climates, with workers from Mossoró entirely differentiated according to the sampling period, grouping oppositely in the scatter plot (Fig 2A). The bees from Mossoró collected during dry season were placed within the semi-arid group, while bees collected during rainy season grouped separately from the others. The two first canonical variate functions were more influenced by length wings, which affected most of the first axis (CVA1), and angle 5, which influenced most of the second axis (CVA2). We also observed that the differences in size were significantly different according to climatic groups (Table S1, Supplementary material). The bees from

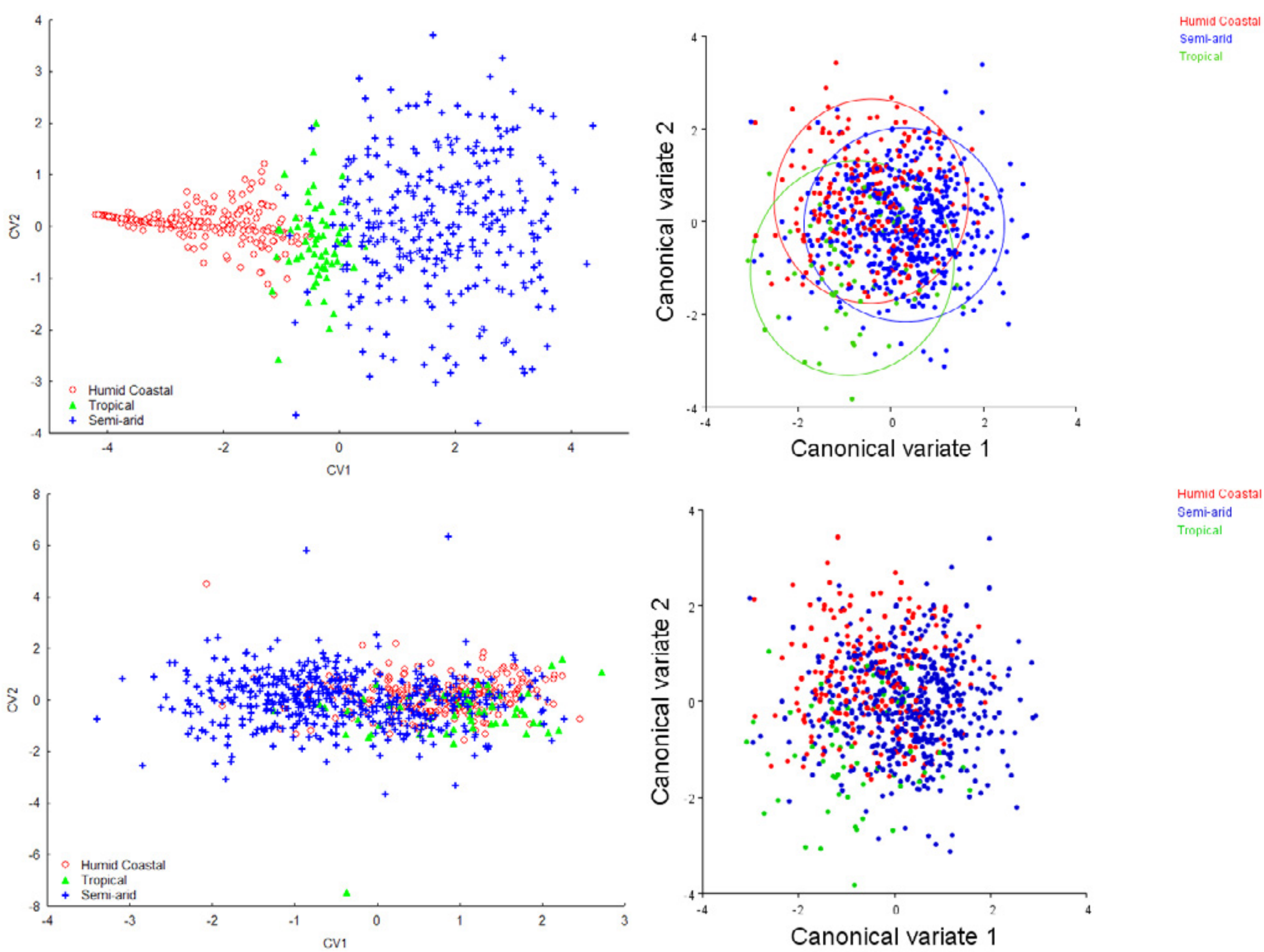

Fig 1. Africanized honey bee distribution to according climatic variation in Northeast Brazilian. Canonical variant analyses on the data of all morphometric traits produced by traditional and geometric morphometrics. (**A**) CVA on samples from Northeast collected during dry season by traditional morphometric; (**B**) CVA on samples from Northeast collected during dry season by geometric morphometric; (**C**) CVA on samples from Northeast collected during dry and rainy seasons by traditional morphometric; (**D**) CVA on samples from Northeast collected during dry and rainy seasons by geometric morphometric.

Mossoró collected during the rainy season were the largest in both wings length and width (Table S2, Supplementary material). The Mahalanobis distances among the centroids in the CVA of the climatic groups were significant (Table 2), and through these morphological distances, we confirmed that workers from Mossoró diverged utterly according to the sampling period, positioning oppositely in the dendrogram (Fig 2B). Besides, bees sampled during the rainy season were more differentiated among the groups (Table 2). Assignment of the specimens to groups correctly classified approximately 53.2% of the total analyzed individuals (Table 3). The cross-validation test also showed the highest accuracy in classifying bees from Mossoró during the rainy season, around 96.3%. On the other hand, the bees collected during the dry season in Mossoró exhibited the lowest accuracy (11.5%) and a high error rate (35.7%) when we compared to the specimens from semi-arid regions.

Geometric morphometrics

Using 19 Cartesian landmarks generated from the right wings, we also found a subtle divergence between semi-arid and humid coastal in which the tropical climate overlaps with semi-arid (Fig 2C). The two first canonical variate functions were significant for the discrimination of the climate ($p < 0.001$) and explained 84.73% of the total data variability (CVA1 accounted for 69.22%; CVA2 accounted for 15.51%, Fig 2C).

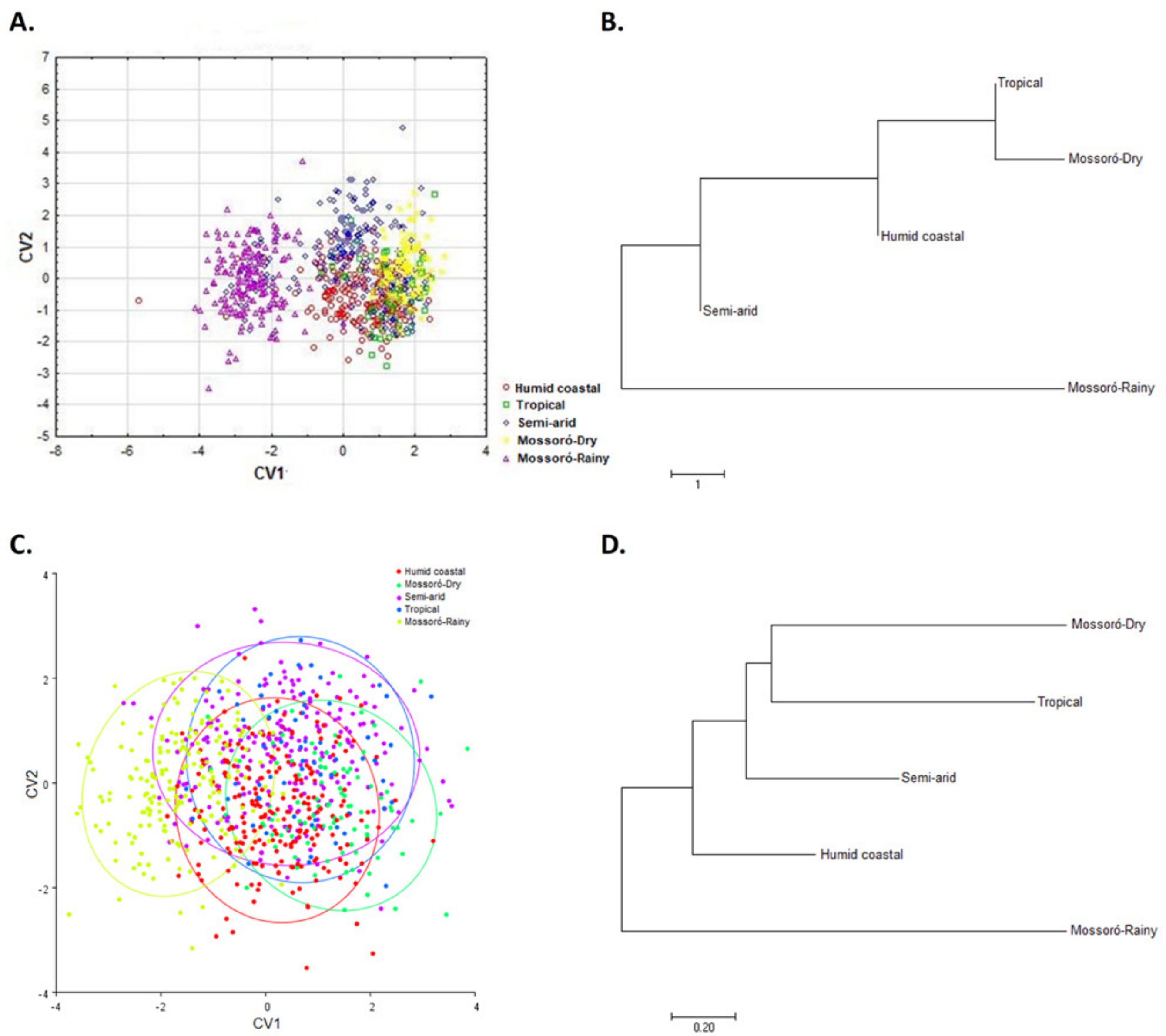

Fig 2. The dispersion patterns of Africanized honey bee along the seasons in Northeast Brazilian. Analyses on data collected by traditional and geometric morphometrics. (**A**) CVA on samples from Northeast collected during dry and rainy seasons by traditional morphometric; (**B**) Dendrogram of clustering of samples from Northeast collected during dry and rainy seasons by traditional morphometric; (**C**) CVA on samples from Northeast collected during dry and rainy seasons by geometric morphometric; (**D**) Dendrogram of clustering of samples from Northeast collected during dry and rainy seasons by geometric morphometric. Mossoró – Rainy, bees collected during the rainy season; Mossoró – Dry, bees collected during the dry season.

Table 2. Mahalanobis square distances (below diagonal) and statistical significance (above diagonal) among the populations obtained from canonical variant analysis of traditional morphometric. Mossoró – Rainy, bees collected during the rainy season; Mossoró – Dry, bees collected during the dry season. (α = 0.05).

	Tropical	Humid coastal	Semi-arid	Mossoró – Rainy	Mossoró – Dry
Tropical		<.0001	<.0001	<.0001	<.0001
Humid coastal	0.83		<.0001	<.0001	<.0001
Semi-arid	2.77	1.58		<.0001	<.0001
Mossoró – Rainy	15.17	10.17	7.47		<.0001
Mossoró – Dry	1.04	2.21	2.90	18.54	

Table 3. Percentage of correct classifications of individuals in the cross-validation test from traditional morphometric. Mossoró – Rainy, bees collected during the rainy season; Mossoró – Dry, bees collected during the dry season. Values marked with * are assignments of the specimens to groups correctly classified.

	Humid coastal	Tropical	Semi-arid	Mossoró – Dry	Mossoró – Rainy	Total
Humid coastal	45.4*	24.8	10.6	15.6	3.6	100
Tropical	21	48.4*	17.7	12.9	0	100
Semi-arid	11.9	16.7	59.5*	11.3	0.6	100
Mossoró – Dry	11.1	35.4	35.7	11.5*	6.3	100
Mossoró – Rainy	1.3	0	1.2	1.2	96.3*	100

The Mahalanobis squared distances among the centroids of the climates were also significantly different from each other (Table 4). Workers from Mossoró diverged completely according to the sampling period, placing oppositely in the scatter plot (Fig 2C) being more differentiated among the groups (Table 4), similar to results from traditional morphometric. Although bees from Mossoró collected during the rainy season were the most differentiated among the groups, these bees were close to the bees from humid coastal climate, while the bees from Mossoró collected during dry season were close to the semi-arid group (Fig 2D). Assignment of the specimens to groups correctly classified approximately 52.2% of the total analyzed bees (Table 5). The cross-validation test showed high accuracy about the bees from Mossoró during the rainy season, around 76%. Like traditional morphometric, the bees collected during the dry season in Mossoró exhibited lower accuracy than bees from the same place but sampled in during another period, and a high error rate (34.4%) when compared to the specimens from semi-arid regions.

Discussion

Both traditional and geometric morphometrics indicated similar results regarding a subtle morphological variation in agreement to the climatic patterns. It suggests that groups may be relatively adapted to these environmental conditions, although there is probably a gene flow between them.

Table 4. Mahalanobis square distances (below diagonal) and statistical significance (above diagonal) among the populations obtained from discriminant function analysis of geometric morphometric. Mossoró – Rainy, bees collected during the rainy season; Mossoró – Dry, bees collected during the dry season. (α = 0.05).

	Tropical	Humid coastal	Semi-arid	Mossoró – Rainy	Mossoró – Dry
Tropical		<.0001	<.0001	<.0001	<.0001
Humid coastal	1.5074		<.0001	<.0001	<.0001
Semi-arid	1.4851	1.1687		<.0001	<.0001
Mossoró – Rainy	2.9128	2.3535	2.0903		<.0001
Mossoró – Dry	1.9457	1.5432	1.4874	3.6632	

Table 5. Percentage of correct classifications of individuals in the cross-validation test from geometric morphometric. Mossoró – Rainy, bees collected during the rainy season; Mossoró – Dry, bees collected during the dry season. Values marked with * are assignments of the specimens to groups correctly classified.

	Humid coastal	Tropical	Semi-arid	Mossoró – Dry	Mossoró – Rainy	Total
Humid coastal	54.0*	4.8	24.6	6.3	10.3	100
Tropical	28.2	17.2*	35.9	10.9	7.8	100
Semi-arid	16.0	3.8	52.0*	11.3	16.9	100
Mossoró – Dry	28.1	8.3	34.4	27.1*	2.1	100
Mossoró – Rainy	14.0	0.6	9.4	0	76.0*	100

Previous studies indicated morphological variation gradients regarding geographical distribution. Souza et al. (2009) showed two different morphometric groups of *A. mellifera* in northeast Brazil. Nunes (2012) found morphologically differentiated groups of honey bees regarding geographical distribution in five Brazilian macro-regions (North, Northeast, South, Southeastern, and Midwest). Through traditional morphometric, we found significant size differences among groups, with bees from humid coastal climate being larger than others, while samples collected during the dry season in semi-arid areas are the smallest. Besides, it is possible to observe that bees from semi-arid areas present a greater form variation among the individuals, whereas samples collected in humid coastal areas present the smallest variation. The areas characterized by a semi-arid climate presented more adverse environmental conditions than humid coastal areas (Freitas et al., 2007). Hence, bees collected in semi-arid areas are under greater environmental stress than samples from humid coastal areas. Environmental stressors directly affect the development of organisms, what may imply changes in the wing shape (Debat et al., 2003).

These morphometric differences might also be related to the phenotypic plasticity linked to the environmental differences found in the different climatic regions. For instance, when wings of *Heliconius erato phyllis* (Fabricius) were analyzed using geometric morphometric, results showed a variation of wing size and shape among individuals fed with different plants, suggesting an environmental effect on the development of these individuals (Jorge et al., 2011). Environmental factors such as temperature, relative air humidity, food supply, and density have been related to phenotypic plasticity (Batista et al., 2013). The morphometric differences in *Triatoma brasiliensis* Neiva may be related to the phenotypic plasticity, once the variations in the wing size and shape would be associated to different ecotypes resulted of the conditions in each microhabitat (Batista et al., 2013).

The morphometric data indicated that the two populations of Mossoró (dry and rainy) are different from each other, with an important similarity observed between the sampling during the dry season and samples from semi-arid areas. It is noteworthy that the city of Mossoró is located in the semi-arid region. However, geometric morphometric emphasizes that samples collected in Mossoró during the rainy season would be more similar to bees from humid coastal areas. Freitas et al. (2007) monitored the flow of arrival and exit of swarms of two localities in northeastern Brazil, one in the interior and another on the coast of Ceará, in Northeast Brazil. They suggest that colonies migration of Africanized honey bees is closely related to the seasons. Honey bee colonies would nest in the semi-arid region only during the rainy season, absconding these areas during the dry season. Only a few colonies would remain in this area during the entire year due to the scarcity of natural resources during the dry season (Freitas et al., 2007). On the other hand, excessive precipitation would probably drive Africanized honey bee colonies back to the semi-arid during the rainy season (Freitas et al., 2007). Our results suggest samples collected during the dry season (November) in Mossoró were those that remained in their colonies even under adverse conditions and did not abscond like most of the other nest. The samples from the same place, but collected during the rainy season (March), were nests that settled in bait hives after the rainy period started when the conditions were once again favorable to the bees. The morphological proximity of these two groups (rainy and dry) respectively to the populations from humid coastal areas and semi-arid areas probably reflect the dispersion patterns of these bees along the seasons.

The morphological differences found between bees that resisted drought and did not swarm and bees that migrated also reflect behavioral differences and could be used in future artificial selection studies to develop lineages with a low tendency to swarm. Indeed, more studies are needed before this step of selection, but these data bring an exciting possibility for future works.

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Supplementary Material

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Authors' Contribution

CJ Moretti: Designed the experiment, performed experiments, and analysis, wrote the paper. CP Costa: Performed the analysis, wrote the paper. TM Francoy: Designed the experiment, performed the analysis and wrote the paper.

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