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Original article

Morphometric differences in a single wing cell can discriminate *Apis mellifera* racial types[1]

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Abstract – Morphometry is a very powerful, though often laborious and time-consuming, tool for the identification of bee species and subspecies. In an attempt to develop a simplified methodology for such work, we marked five easily identified landmarks of digitalized images of the right forewing radial cell in 50 workers of each of three different racial groups of *Apis mellifera.* Software was developed to calculate angles between the landmarks, cell area, continuous curvature, and arc lengths (total of 11 characters). Based on multivariate analysis, significant differences were detected between commercial USA Italian bees, German Carniolan bees and Africanized honey bees (a polyhybrid that is predominantly *Apis mellifera scutellata*). A single wing cell carried enough information to discriminate nearly 99% of the individuals. Most of the classifications gave $P > 0.99$, and only three Africanized bees were misclassified. We concluded that the features measured in a single wing cell are sufficient to discriminate these racial groups.

***Apis mellifera ligustica* / *Apis mellifera carnica* / Africanized honey bee / morphometry / multivariate statistics / subspecific taxonomy**

1. INTRODUCTION

Because of its great variability and importance, the subspecific taxonomy of *Apis mellifera* L. has been very extensively studied. This species covers a large distribution area, extending throughout almost all of Africa and Europe and part of Asia. Along this distribution, *Apis mellifera* occupies quite varied ecological niches, from desert zones to tropical rain forests and from mountainous regions to swamps (Smith, 1961). According to Ruttner (1988), there are at least 24 *Apis mellifera* subspecies grouped into three or four evolutionary branches, based on morphometric data. These branches were later confirmed with other markers, including mitochondrial DNA and microsatellites (Estoup et al., 1995; Franck et al., 2000). A few of these subspecies were introduced into the New World, where they have freely crossed and originated a poly-hybrid, now called the Africanized honey bee (Kerr, 1967; Gonçalves, 1974, 1982).

The first attempts to classify bee subspecies were based mainly on color and size (Ruttner et al., 1978); later, with the help of univariate statistics, the discriminations were improved (Alpatov, 1929; Goetze, 1940). However, these discriminations remained imprecise because the ranges of the features that were measured often overlapped (Ruttner, 1988). DuPraw (1964, 1965a, b) was the first to apply discriminant

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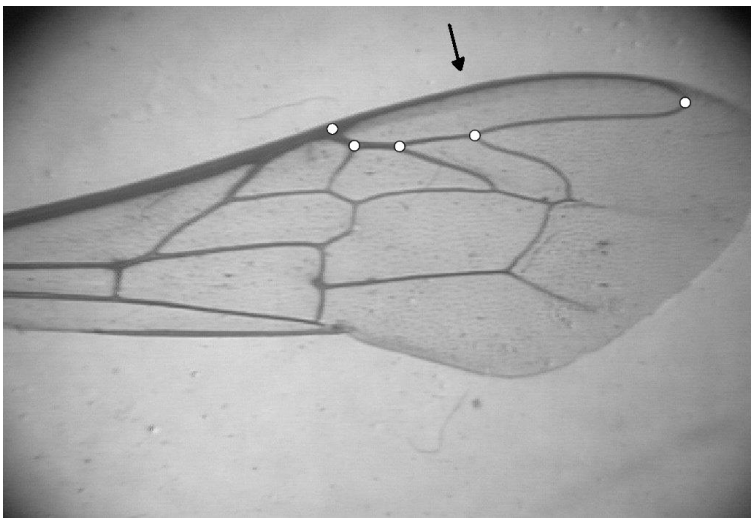

Figure 1. Right forewing of an Africanized honey bee. The black arrow indicates the radial cell. Four of the landmarks on the radial cell (shown as open circles) are at the intersections of veins and cross veins, and one is at the apical margin of the radial cell.

analysis to a set of quantitative characters of wing venation and he correctly classified many subspecies. In 1978, Daly and Balling successfully applied this method to differentiate Africanized and European honey bees in South America. Later, Daly et al. (1982) began to use digital measurements to investigate the morphometrics of honey bees, significantly reducing the time necessary for measuring, storing and analyzing the data. Since then, considerable research using multivariate methods has been published, focusing on a wide range of problems, especially involving Africanized honey bee identification (Rinderer et al., 1990, 1993; Crewe et al., 1994; Quezada-Euán et al., 2003). Steinhage et al. (1997) developed a semi-automated method to obtain wing measures that reduced the analysis time and improved precision for identifying bee species; this method was later improved to an automatic system (Steinhage et al., 2001). This new technique attains a precision of 99.8% for classifying bee species. Most researchers have used characteristics from various parts of the bee body, which requires time-consuming specimen preparation and measurement procedures. Our objective was to investigate whether we could simplify the procedures by determining if differences in a single wing cell carry enough information to make intraspecific discriminations among *Apis mellifera* workers belonging to different racial groups.

2. MATERIALS AND METHODS

We analyzed 50 workers from each of three different racial groups. Commercial Italian bees, predominantly *Apis mellifera ligustica* Spinola, were collected from the island of Fernando de Noronha, Brazil, where this subspecies was introduced in 1984 (Correa-Marques and De Jong, 2002). The original queens were artificially inseminated daughters of queens imported from two different places in the USA: California and Georgia (Malagodi et al., 1986). The Carniolan bees, predominantly *Apis mellifera carnica* Pollmann, were from already mated queens imported from the experimental apiaries of the University of Bonn, Germany, in 1972. The Africanized honey bees, a polyhybrid between an African subspecies, *Apis mellifera scutellata* Lepeletier, and European honey bees, were collected in Ribeirão Preto, SP, Brazil in 2004. Genetically, these bees are about 90% African (Sheppard et al., 1991). The right wings of all individuals were placed between microscope slides and were then photographed with a digital camera attached to a stereomicroscope. Five bees were taken from each of 10 colonies for each type of bee. We measured a set of characteristics of the forewing radial cell. All the measurements were made using the methodology developed by Prado et al. (2004), adapting its use to the forewing radial cell (Fig. 1). This software, run using MATLAB®, was originally designed to analyze *Drosophila* wings.

Five landmarks were manually plotted on the radial cell (Fig. 1), and measurements of angles between the landmarks, cell area, continuous curvature and arc length were made using software (available from the corresponding author) developed for

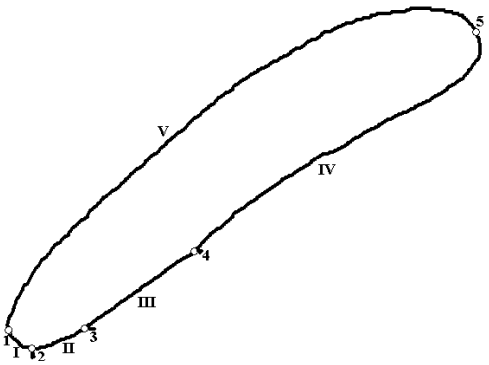

Figure 2. Digital radial wing cell image used in the measurements. The open circles and arabic numbers indicate the landmark positions, and the roman numerals indicate the arcs (between the landmarks) that were measured.

this purpose (Fig. 2, Tab. I). Continuous curvature is a geometric measure that expresses the rate of change of the angle between the tangent of the curve and the x-axis (Costa et al., 2004). The equalized curvature values are generated through standardization, by generating a sigmoid of the continuous curve (Costa and Cesar, 2000). This system gives maximum information about the curvature of the structure.

The data were analyzed using univariate and multivariate methods. The differences between the means of these types of bees were tested using one-way MANOVA. The significant univariate F values were used to identify the wing parameters that contributed most to the discrimination between the groups. These measures were then used to compare the three groups of bee samples. A preliminary exploration of the data set was undertaken, using principal component analysis, to determine if the Africanized, Italian and Carniolan bees formed distinct clusters. After the three racial types were plotted in three different clusters, a multivariate discriminant analysis was carried out to determine the distances between the groups.

3. RESULTS

The distributions of the variables, based on the means plus or minus one standard deviation, overlapped among the three types of bees for almost all radial cell measures, which prevented us from using them individually for separating these groups. Nevertheless, based on the univariate F values, there were significant differences between almost all the bee groups for all of the variables ($P < 0.001$) except for angle 2, which gave $P = 0.17$. The two

Table I. Descriptive statistics of the radial cell (Fig. 1) variables in three different racial types of *Apis mellifera*. Angle 1 = angle formed by landmarks 5, 1 and 2. Angle 2 = angle formed by landmarks 1, 2 and 3. Angle 3 = angle formed by landmarks 2, 3 and 4. Angle 4 = angle formed by landmarks 3, 4 and 5. Angle 5 = angle formed by landmarks 4, 5 and 1 (see Fig. 2). SD = standard deviation. The angles are in radians, total arc lengths are in mm and cell areas are in mm^2.

Character	Africanized		Italian		Carniolan	
	mean	SD	mean	SD	mean	SD
Cell curvature mean	–0.33	0.01	–0.32	0.01	–0.31	0.01
Cell curvature SD	0.99	0.06	0.88	0.05	0.94	0.07
Equalized curvature mean	–0.22	0.01	–0.24	0.01	–0.21	0.01
Equalized curvature SD	0.44	0.01	0.41	0.02	0.42	0.02
Continuous curvature	23.65	0.75	24.53	0.85	25.88	0.88
Cell area	9.24	0.48	10.97	0.52	10.93	0.48
Angle 1	2.41	0.12	2.35	0.11	2.39	0.10
Angle 2	0.61	0.13	0.66	0.11	0.64	0.10
Angle 3	0.16	0.02	0.18	0.03	0.14	0.03
Angle 4	0.03	0.02	0.03	0.02	0.05	0.02
Angle 5	3.08	0.01	3.06	0.01	3.07	0.01

SD = standard deviation.

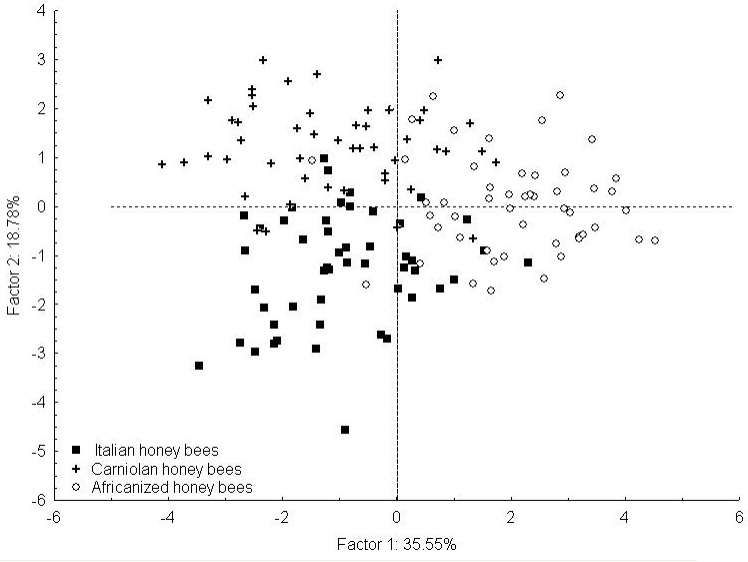

Figure 3. Scatterplot of the principal component analysis of 150 worker bees (50 of each type) measured for 11 radial wing cell characteristics, showing three clusters.

size-related characteristics, total arc length and the area of the radial cell, were significantly smaller in the Africanized bees than in the Italian and Carniolan bees ($P < 0.01$). All the other features involving radial cell form, including curvatures and angles, also differed among these racial types, but the lowest value was not always that of the Africanized honey bees (Tab. I).

Four factors with eigenvalues greater than one were extracted in the principal component analysis: factor one included cell curvature mean, cell curvature standard deviation, continuous curvature and cell area; factor two comprised equalized curvature mean, angle 3 and angle 1; factor three included angles 4 and 5; and factor 4 included equalized curvature standard deviation, continuous curvature and angle 1. Together, these four components explained 81.6% of the data set variability. The two main principal components extracted from the principal component analysis explained 54.3% of the data set variability. The first principal component explained 35.6% of the variability and the second principal component explained 18.8% of the variability among the features (Fig. 3). Despite the fact that the clusters plotted relatively close to each other, it was easy to distinguish the three groups. The Africanized honey bees were placed mainly in the upper and in the lower-right-hand quadrant, *A. m. ligustica* mainly in the lower-left-hand quadrant and *A. m. carnica* mainly in the upper-left-hand quadrant of the plot. Once we established that these measures divided the bees into three clusters, a discriminant analysis was performed. There was a significant difference between the three groups (Wilk's $\lambda = 0.0453$, $P < 0.0001$), clearly showing that data on these characteristics were sufficient to distinguish among the three groups. An analysis of Mahalanobis distances between the centroids of the groups revealed that each group was significantly different from the other two groups (Fig. 4). The discriminant analysis correctly classified 98% of all individuals. The linear discriminant functions were able to distinguish 94% of the Africanized, 100% of the *A. m. ligustica* and 100% of the *A. m. carnica* bees with a probability of $0.85 \leq P \leq 1.00$; with $P > 0.99$ for most of the classifications. One Italian bee was classified $P > 0.61$, one Africanized bee $P > 0.53$, and three Africanized bees were misclassified.

4. DISCUSSION

Generally speaking, Africanized bees are smaller than honey bees of European origin. Among 63 morphological characters of the head and thorax of worker bees, Gonçalves

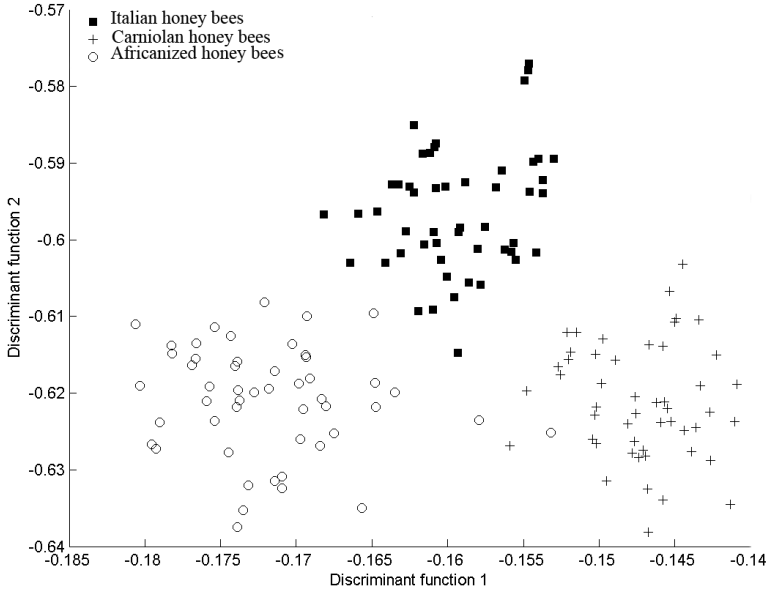

Figure 4. Discriminant analysis of the three subspecies. The Mahalanobis distances between centroids were as follows: Africanized to Italian = 18.840, Africanized to Carniolan = 31.902, Italian to Carniolan = 18.567.

(1970) found lower values for Africanized honey bees compared to those of European bees, except for the number of hamuli, width of basitarsus, and diameter of the ocelli, which were larger in the Africanized bees. Various such traits are necessary to provide adequate discrimination among the various types of honey bees, especially when the workers of the bee races are similar in size. Most attempts to differentiate honey bee groups based on morphological data have used multiple body characteristics, including (worker) body size, hair length, wing length and width, pigmentation, and proboscis length (Buco et al., 1987; Ruttner, 1988; Rinderer et al., 1990, 1993; Crewe et al., 1994; Ftayeh et al., 1994; Diniz-Filho and Malaspina, 1995; Quezada-Euán et al., 2003); such studies require time-consuming mounting and measuring of various body parts. We were able to discriminate the three honey bee racial types based on information concerning a single wing cell (radial cell), using a digitalized wing image, greatly facilitating and speeding analysis. We conclude that measurements of a small part of the entire bee body can be sufficient to discriminate among honey bee racial groups. This methodology is simple and it could be extended to finer discriminations between types of bees with the addition of further landmarks.

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Résumé – Discrimination des sous-espèces d'*Apis mellifera* par les différences morphométriques d'une seule cellule alaire. La morphométrie a joué depuis longtemps un rôle important dans les études de taxonomie. Lorsqu'elle est couplée aux statistiques multivariées, c'est un outil très puissant pour les analyses discriminantes et donc pour l'identification des espèces. Chez *Apis mellifera* de nombreuses différences entre sous-espèces sont quantitatives et les diverses études menées jusqu'à présent ont toujours utilisé les mesures de diverses parties du corps de l'abeille. Afin de simplifier et d'accélérer les analyses, nous avons mesuré 11 caractéristiques d'une seule cellule de l'aile antérieure pour savoir si les différences dans cette cellule permettaient de discriminer les trois sous-espèces *A. mellifera ligustica, A. m. carnica* et les abeilles africanisées du Brésil. Une analyse en composantes principales a été effectuée sur les mesures afin de déterminer si notre corpus de données formait trois groupements distincts (Fig. 3). Ceux-ci ont été

vérifiés par une analyse discriminante (Fig. 4). Les caractéristiques retenues forment bien trois groupes significativement différents ($P < 0{,}00001$) et la fonction discriminative linéaire a pu classer correctement 98,7 % des échantillons. Ces résultats montrent que des mesures d'une petite partie du corps de l'abeille sont suffisantes pour discriminer les sous-espèces.

***Apis mellifera ligustica* / *Apis mellifera carnica* / abeille africanisée / morphométrie / analyse multivariée / taxonomie infra-spécifique**

Zusammenfassung – Unterscheidung von *Apis mellifera* Rassentypen anhand morphometrischer Unterschiede einer einzigen Flügeladerzelle. Bei *Apis mellifera* sind viele der Unterschiede zwischen den Rassen quantitativ und in den unterschiedlichen bisher durchgeführten Untersuchungen wurden generell Messungen von unterschiedlichen Körperteilen verwendet. Um die Untersuchungen zu beschleunigen, haben wir 11 Charakteristika einer einzigen Flügeladerzelle des Vorderflügels durchgeführt (Tab. I), die durch eine Software aus 5 leicht zu identifizierenden Landmarken gewonnen wurden. Hierdurch wollten wir feststellen, ob die Unterschiede in dieser Zelle ausreichen, drei Bienengruppen aus unterschiedlichen Rassen (Italienische Bienen, Carnicabienen und Afrikanisierte Honigbienen aus Brasilien) zu unterscheiden. Die Messungen wurden in einer Hauptkomponentenanalyse ausgewertet um zu sehen, ob der Datensatz drei abgegrenzte Kluster ausbildet (Abb. 3), diese wurde mit einer Diskriminanzanalyse überprüft (Abb. 4). Die Proben bildeten drei signifikant unterschiedene ($P < 0{,}0001$) Gruppen aus, und die lineare Diskriminanzanalyse konnte 98 % der Proben korrekt reklassifizieren. Die Vermessung eines Flügelteils kann daher ausreichen, um zwischen Gruppen aus unterschiedlichen Rassen von *Apis mellifera* zu unterscheiden.

***Apis mellifera ligustica* / *Apis mellifera carnica* / Afrikanisierte Honigbienen / Morphometrie / multivariate Statistik**

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