

Geographic variation of Africanized honey bees (*Apis mellifera* L.) in Brazil: multivariate morphometrics and racial admixture

José Alexandre Fellzola Diniz-Filho¹ and Osmar Malaspina²

ABSTRACT

The correspondence between morphometric and isozymic geographic variation patterns of Africanized honey bees in Brazil was analyzed. Morphometric data consisted of mean vectors of 19 wing traits measured in 42 local populations distributed throughout the country. Isozymic data refer to allelic frequencies of malate dehydrogenase (MDH), and were obtained from Lobo and Krieger (*Heredity* 68: 441-448, 1992). The two data sets were analyzed through canonical trend surface, principal components and spatial autocorrelation analyses, and showed north-south clines, demonstrating that Africanized honey bees in southern and southeastern Brazil are more similar to European honey bees than those found in northern and northeastern regions. Also, the morphometric variation is within the limits established by the racial admixture model, considering the expected values of Africanized honey bee fore wing length (WL) in southern and northeastern regions of Brazil, estimated by combining average values of WL in the three main subspecies involved in the Africanization process (*Apis mellifera scutellata*, *A. m. ligustica* and *A. m. mellifera*) with racial admixture coefficients.

INTRODUCTION

The history of the introduction of African honey bees in Brazil in 1956 has been widely discussed, especially since their descendents arrived in the United States, after a very fast process of colonization and population growth in the Neotropical region (Michener, 1974; Taylor, 1977; Rutner, 1988; Moritz and Southwick, 1992). During this colonization process, honey bees were affected by distinct evolutionary pressures, such as adaptation to new ecological conditions (including new climatic characteristics, predators and floral resources), a population bottleneck

and racial admixture with European honey bees previously introduced in southern and southeastern Brazil in the 19th century. The honey bees resulting from these processes are known as Africanized honey bees, and are more similar to their African parents than to their European parents in many morphological, behavioral, ecological, biochemical and genetic characters (Daly, 1991; Winston, 1992; Villa *et al.*, 1993).

In recent years, several studies, especially those based on isozymic variation, have stressed that what is usually called the Africanized honey bee does not constitute a single and uniform population, but instead many local populations with distinct degrees of racial admixture between African and European subspecies (Stort and Bueno, 1985; Lobo *et al.*, 1989; Del Lama *et al.*, 1988, 1990; Sheppard *et al.*, 1991; Rinderer *et al.*, 1991; Lobo and Krieger, 1992). Morphological and behavioral patterns were not analyzed in detail in these studies

¹ Departamento de Biologia Geral, Instituto de Ciências Biológicas, Universidade Federal de Goiás, Caixa Postal 131, 74001-970 Goiânia, GO, Brasil. Send correspondence to J.A.F.D.-F.

² Departamento de Biologia, Instituto de Biociências, Universidade Estadual Paulista (UNESP), Caixa Postal 199, 13506-900 Rio Claro, SP, Brasil.

because of environmental effects and non-additive genetic variation usually associated with these traits, making it difficult to detect patterns of genetic variation because of the large climatic differences in distinct regions of Brazil.

However, in a recent paper, Oldroyd *et al.* (1991) found a high proportion of additive genetic variance for several morphometric traits used to discriminate between European and Africanized honey bees in Neotropical regions, demonstrating that multivariate morphometric analysis of honey bee populations is still a valid approach to detect changes in the genetic structure of populations, influenced by selection, drift or racial admixture. Under this assumption, Diniz-Filho and Malaspina (1995) analyzed geographic patterns of the morphometric variation of Africanized honey bees in Brazil using multivariate and spatial data analyses, and found north-south clines similar to those obtained with isozymes. Geographic structure of morphometric data, associated with genetic within-population correlation analysis, gave support to the racial admixture hypothesis proposed by Lobo and Krieger (1992), associated with a northward demic diffusion process.

The basic purpose of this paper is to present a detailed analysis of the correspondence between morphometric and isozymic data for Africanized honey bees in Brazil, using spatial data analysis in an attempt to test the hypotheses recently developed to explain the Africanization of *Apis mellifera* in the Neotropics.

MATERIAL AND METHODS

Material analyzed

Morphological data used in this paper consisted of mean vectors of 19 wing characters measured in 42 populations of Africanized honey bees collected in Brazil (Figure 1), in a total of 990 individual bees from 180 colonies. Each mean vector was defined by the following characters: fore wing length and width (WL and WW), lengths *a* and *b* of the cubital vein (CV A and CV B), hind wing length and width (HWLN and HWWD), number of hamuli in the hind wing (HA) and ten venation angles of the fore wing (A29, A30, A31, A32, A33, A34, A35, A36, A38 and A39). These characters have been used in several recent studies of Africanized honey bee morphometrics (Buco *et al.*, 1987; Rinderer *et al.*, 1990, 1991; Sheppard *et al.*, 1991). For this work, we included two other fore wing characters, the length of the radial cell (RcL) and the length of apical portion of this cell (PRcL). Details of sampling

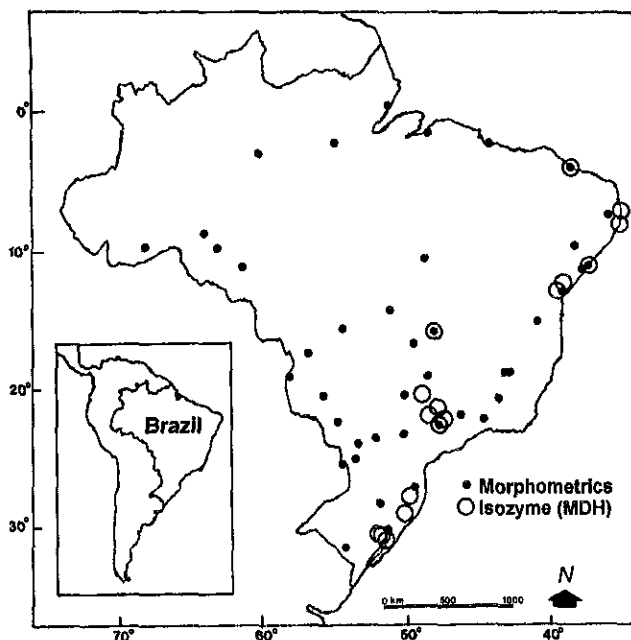


Figure 1 - Localities analyzed for morphometric and isozymic variation of Africanized honey bees in Brazil. Open circles indicate isozymic data, and closed circles morphometric data.

procedures and character definition are given in Diniz-Filho and Malaspina (1995).

Isozymic data were obtained from Lobo (1991), and basically consisted of allelic frequencies estimated for 17 local populations in Brazil of allele A of malate dehydrogenase (p_A) (Figure 1). When more than one frequency was estimated in the same locality (in different years), we used only the more recent value. The data used dated from 1984 to 1990. This selection is important since Lobo (1991) and Lobo and Krieger (1992), who studied bees collected from 1977 to 1990, found a significant increase of allelic frequency in some localities when the older samples were included in the analysis.

Spatial data analysis

A canonical trend surface analysis (CTS) (Wartenberg, 1985) was applied to the morphometric data, in an attempt to obtain a linear combination of the original characters in such a way that its correlation with geographic location (latitude and longitude) was as large as possible (Wartenberg, 1985; Bocquet-Appel and Sokal, 1989). This technique is a multivariate generalization of trend surface analysis (Unwin, 1976; Davis, 1986), a multiple regression in which a dependent variable (morphometric characters or allelic frequencies) is adjusted to geographic location parameters (latitude, longitude and their polynomial expansions). The projection of the original data on this

linear combination produced canonical scores, which were mapped using a distance weighted least squares (DWLS) algorithm from SYSTAT/SYGRAPH (Wilkinson, 1989). Residuals from this multivariate linear model were analyzed using spatial autocorrelation (Sokal and Oden, 1978a,b), in an attempt to discover significant structure in the data after large-scale geographic trends had been removed by CTS. Spatial correlograms were obtained by calculating Moran's I coefficient for 12 distance classes, whose upper limits (in km) are: 471, 700, 918, 1099, 1486, 1713, 1968, 2202, 2407, 2688 and 3527. Class intervals were automatically established in such a way that approximately the same number of locality pairs is used to estimate each autocorrelation coefficient. Overall significance of correlograms was established using Bonferroni criterion (Oden, 1984). Recent applications of spatial autocorrelation in honey bee systematics can be found in Daly *et al.* (1991) and Diniz-Filho *et al.* (1993).

We also obtained principal components from a correlation matrix among characters, estimated using mean vectors of local populations (among-population correlations). Principal components with eigenvalues larger than 1.0 were extracted and, if necessary, rotated to a new varimax solution (Harris, 1975; Johnston and Wichern, 1992). Principal component scores were also mapped using DWLS and submitted to univariate trend surface and spatial autocorrelation analyses. These two general multivariate procedures were used to evaluate the general patterns of geographic variation of morphometric characters of Africanized honey bees in Brazil.

Isozymic data were analyzed using a simple linear regression between p_A and latitude and spatial autocorrelation analysis. DWLS interpolated bivariate surface was not applied because of the low point density and spatial aggregation. Due to the small number of localities, correlograms were established using five distance classes, whose upper limits (in km) are: 480, 984, 1591, 2213 and 3229.

To evaluate the correspondence between morphometrics and isozymic data, we plotted the values of first canonical axis against malate dehydrogenase (MDH) allelic frequencies (using data from Lobo, 1991). To overcome the differences in sample size and sampling localities, we used 12 values in each data set, estimated for 12 geographic lags by DWLS against latitude (Sokal *et al.*, 1989).

Concordance with racial admixture patterns

Lobo *et al.* (1989) and Lobo and Krieger (1992) proposed that Africanized honey bees are a mix of three

basic genotypes (*Apis mellifera ligustica*, *A. m. scutellata* and *A. m. mellifera*), and estimated distinct levels of racial admixture for two regions of Brazil based on isozymic data.

We used the coefficients estimated by Lobo *et al.* (1989) and Lobo and Krieger (1992) to establish the expected amplitude of morphological variation of Africanized honey bees in Brazil. For this purpose, it is important to know the average value of the character analyzed in the three ancestral populations involved in the admixture process and determine if the morphological character chosen is highly genetically determined (possesses narrow-sense heritability close to 1.0), in such a way that environmental effects do not disturb the estimates of racial admixture. The character analyzed that seems to be the most adequate for our purposes is the fore wing length (WL), for the following reasons: 1) according to Rinderer (1977), Moritz and Klepsch (1985), Oldroyd *et al.* (1991) and Diniz-Filho (1994), this character possesses elevated heritability in several populations all around the world; 2) it is strongly correlated with clinal variation found for Africanized honey bees in Brazil (Diniz-Filho and Malaspina, 1995); 3) it displays large differences between Africanized and European honey bees (Daly and Balling, 1978; Rinderer *et al.*, 1990) and; 4) it is possible to estimate its average value in all the three ancestral populations, using data from Ruttner (1988). These values (followed by standard deviations and sample size), expressed in millimeters, are:

A. m. scutellata - 8.660 ± 0.160 (N = 19 colonies)

A. m. ligustica - 9.208 ± 0.175 (N = 35 colonies)

A. m. mellifera - 9.344 ± 0.111 (N = 10 colonies)

So, it is possible to combine these average values with the racial admixture coefficients and obtain the expected values for WL in the two regions (WL_{EXP}). The expected amplitude of morphological variation was obtained by recalculating the admixture coefficients by adding one standard error to the coefficient of the race with the largest coefficient and subtracting the same value from the coefficient of the race with smaller value, for each region.

We used a subset of our morphometric data to test the adequacy of the admixture model. This was necessary because honey bees used for this evaluation needed to be collected in localities close to the ones used by Lobo *et al.* (1989) and Lobo and Krieger (1992) to estimate the admixture coefficients. When an exact correspondence between sampling stations was not possible (since many sampling stations were not coincident in both studies), we used samples from

localities no more than 250 km apart. This pairing can be considered effective for analytical purposes, since WL displays strong positive spatial autocorrelation up to 1000 km (Diniz-Filho and Malaspina, 1995).

RESULTS AND DISCUSSION

Out of two possible canonical surfaces, only the first was significant at the 5% level, with a canonical correlation equal to 0.941. The map of canonical scores (CS1), mapped using DWLS, permits to observe a clear south-north trend, with higher scores in the southern regions (Figure 2). The loadings of this canonical surface (Table I) showed important contributions from wing size characters (WL, WW, RcL, PRcL and HWWD) and three venation angles (A29, A32 and A34). Indeed, since this canonical axis is correlated with the first principal component of the among-localities correlation matrix ($r = 0.536$; $P < 0.01$), it can be considered an expression of multivariate size (Diniz-Filho and Malaspina, 1995). Larger honey bees (with a larger European genetic component) can be found in southern and southeastern regions of Brazil. Nevertheless, all mean vectors were classified as "Africanized" honey bees. Spatial correlograms for residuals of CTS were often non-significant, indicating that the most important spatial patterns were removed with this technique.

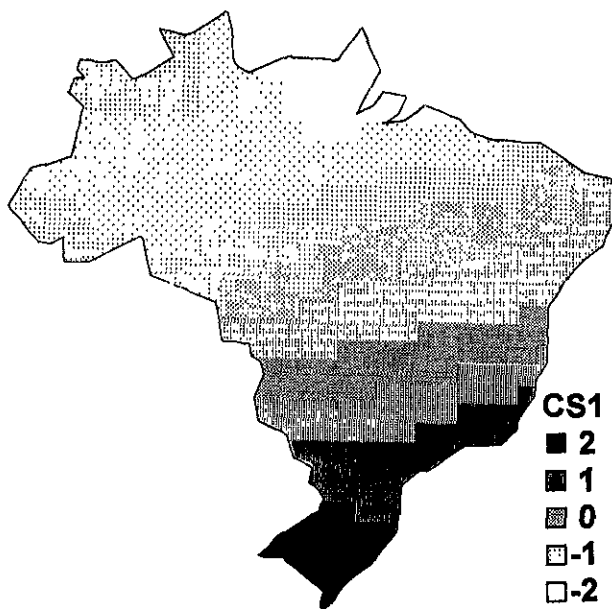


Figure 2 - Distance weighted least squares (DWLS) contouring map of the scores of the first canonical surface (CS1). The surface is based on 42 data points, and higher values on the multivariate axis are represented by increasing darkness of shading.

Among the six principal components with eigenvalues larger than 1.0, only the first two had spatial patterns according to univariate trend surfaces and spatial autocorrelation analyses applied to principal component scores, as shown in Table I. Rotations did not improve the results, and the simplest (unrotated) solution was retained. Scores displayed north-south trends similar to Figure 2, but with distinct east-west orientations (Figure 3A and B), and their loadings indicated that both components express distinct parts of CS1 (Table I). In fact, their correlations with CS1 are equal to 0.536 and 0.508, respectively ($P < 0.01$). Because of this relationship and considering that these components are orthogonal by definition, we can conclude that they both contain the same geographic information (north-south clines), and that orthogonality is generated by residual (non-geographic) effects. Wartenberg (1985) points out that canonical trend surface analysis is less sensitive to non-geographic informations in data matrix than standard (non-spatial) multivariate techniques, and the different results for the two techniques can be explained in these terms (Diniz-Filho, 1995).

Table I - Loadings of canonical trend surface analysis (CS1) and principal components (PC1 and PC2) of morphometric variation in Africanized bees (*Apis mellifera*) from Brazil. The R^2 and F statistics are from univariate trend surface analysis and include the significance level of a correlogram based on Moran's I coefficient (P_c), both applied to the first two principal components.

Character	CS1	PC1	PC2
WL	0.753	0.879	0.110
WW	0.537	0.720	-0.120
RcL	0.515	0.833	0.063
PRcL	0.662	0.747	0.505
CV A	0.178	0.386	0.040
CV B	0.241	-0.313	0.546
HWLN	0.212	0.826	-0.223
HWWD	0.346	0.663	-0.334
HA	-0.092	0.452	-0.259
A29	-0.415	-0.287	-0.675
A30	0.242	0.663	0.151
A31	-0.104	0.366	-0.364
A32	0.522	0.477	0.591
A33	0.139	-0.148	0.515
A34	0.304	-0.168	0.454
A35	0.118	-0.125	0.327
A36	-0.124	0.132	-0.581
A38	0.016	0.028	-0.149
A39	0.180	0.276	0.256
Eigenvalue		5.158	2.778
Eigenvalue (%)		27.15	14.62
R^2 (%)		25.40	31.50
F		6.652**	8.955**
P_c		0.009	< 0.001

** $P < 0.01$.

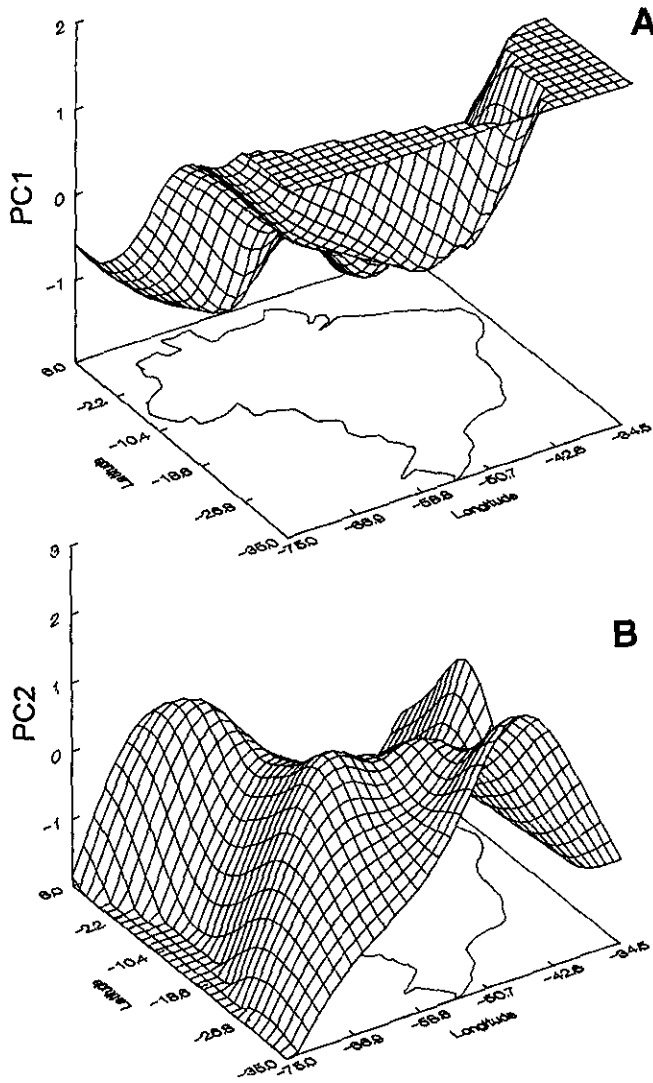


Figure 3 - Distance weighted least squares (DWLS) surfaces of the scores of the first two principal components, showing clines for PC1 (A) and PC2 (B). The interpolated surfaces are based on 42 data points.

Spatial correlograms for the first two principal component scores confirm the north-south clines, with positive significant autocorrelations in the first two distance classes (up to 700 km), and negative significant autocorrelations in the last two distance classes (2688-3527 km) (Figure 4).

A similar north-south cline was found by Lobo *et al.* (1989) and Lobo and Krieger (1992) for allele A of MDH, and the linear regression between p_A and latitude is shown in Figure 5. The spatial correlogram for p_A is also significant ($P < 0.01$), with significant positive autocorrelations in the first and second distance classes (Moran's I equals to 0.55 and 0.24; $P < 0.01$), followed by a significant negative coefficient in the last distance class ($I = -0.99$; $P < 0.01$). The scatterplot showing the correspondence between the two data sets (measured by 12 latitudinal lags) reveals

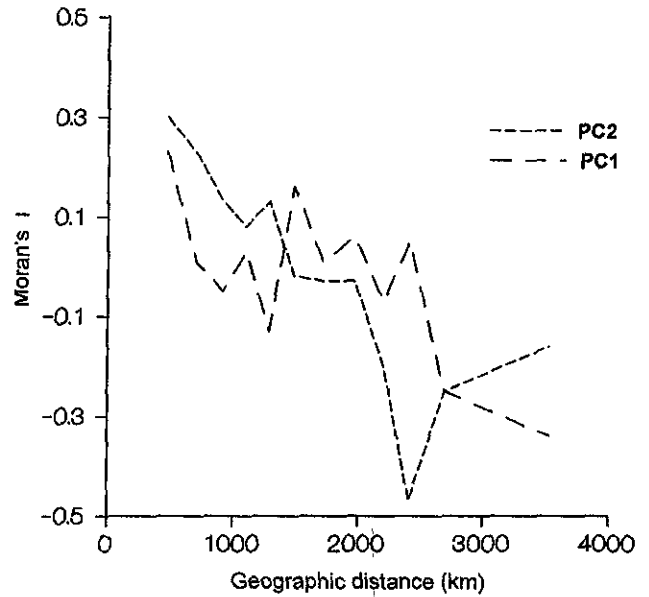


Figure 4 - Spatial correlograms for the first two principal components (PC1 and PC2).

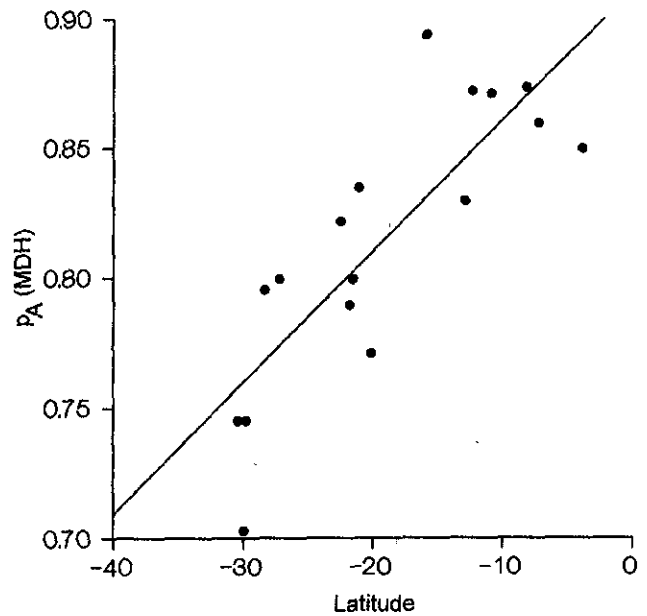


Figure 5 - Relationship between frequencies of allele A from MDH (p_A) and latitude (data from Lobo, 1991).

a similarity of geographic variation patterns (Figure 6). This relationship is not linear, since lags in northern Brazil show a relatively small variation in MDH frequencies compared to morphological variation, and the opposite situation seems to occur in southern Brazil. The relationship between p_A and CS1 is better described by a quadratic function of the form $p_A = 0.833 - 0.043 (CS1) - 0.024 (CS1)^2$, with R^2 equal to 0.959. However, there are problems in these latitudinal

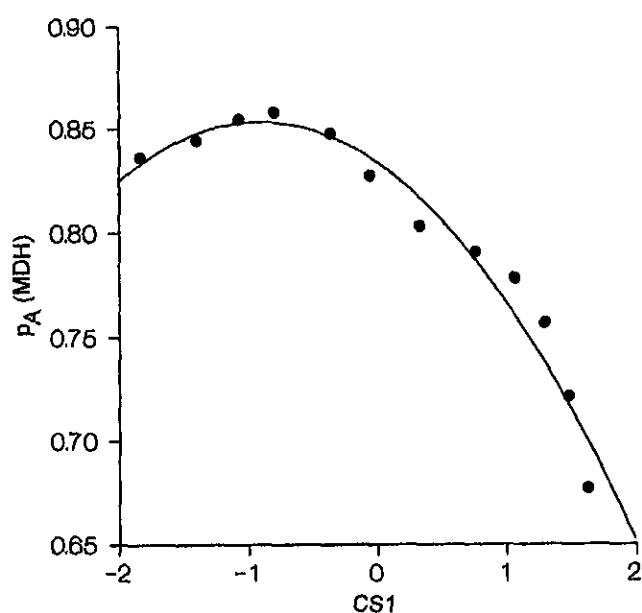


Figure 6 - Relationship between morphometric (CS1) and isozymic variation (p_A - MDH) of Africanized honey bees along 12 lags of latitude in Brazil.

extremes, since MDH data were not collected from several locations in the northern region. On the other hand, morphological data were collected from a small number of localities in the far south. Therefore, it is better to evaluate the concordance between the two data sets by intermediate lags (which show a linear relationship between geographic patterns).

The procedures described above allow evaluation of the similarity between geographic variation patterns for two distinct sets of data from Africanized honey bees in Brazil. However, it is also important to determine if morphological variation is within the limits established by the racial admixture model. This approach is an improvement over other studies comparing morphological and molecular data, and it is only possible because of the historical information about the evolutionary process of racial admixture and the quantitative genetic analysis of morphometric variation patterns.

Coefficients of racial admixture defined by Lobo and Krieger (1992) are shown in Table II. By combining the values of WL in the three ancestral races with these racial admixture coefficients, it is possible to determine the expected values of WL (plus their standard errors) in Africanized honey bees from the two regions. Analysis of WL data in some local populations close to those used by Lobo and Krieger (1992) to estimate racial admixture (Table III), shows that average observed values in each region are very close (within one standard error) to those expected by the admixture model of Lobo and Krieger (1992) (Table II).

Table II - Coefficients of racial admixture of Africanized honey bees (*Apis mellifera*) in Brazil estimated by isozymic data, and expected (EXP) and observed (OBS) morphological variation (fore wing length - WL).

Regions	Southern	Northeastern
Racial admixture coefficients (*)		
<i>A. m. scutellata</i>	0.728 ± 0.061	0.853 ± 0.033
<i>A. m. ligustica</i>	0.000 ± 0.028	0.003 ± 0.013
<i>A. m. mellifera</i>	0.272 ± 0.061	0.144 ± 0.035
Morphological variation		
WL _{EXP}	8.843 ± 0.041	8.759 ± 0.023
WL _{OBS}	8.837 ± 0.042	8.746 ± 0.019
Number of localities	4	5

*Racial admixture coefficients are from Lobo and Krieger (1992).

Table III - Local population means used for estimation of regional means of fore wing length (WL ± standard deviation) in Africanized honey bees (*Apis mellifera*).

Localities	State	WL (± SD)
<i>Southern Brazil</i>		
Porto Alegre	RS	8.850 ± 0.143
Bagé	RS	8.896 ± 0.164
Lagoa Vermelha	RS	8.888 ± 0.105
Blumenau	SC	8.713 ± 0.097
<i>Northeastern Brazil</i>		
Campina Grande	PB	8.705 ± 0.091
Paulo Afonso	BA	8.708 ± 0.143
Boquim	SE	8.758 ± 0.090
Aracaju	SE	8.714 ± 0.177
Cruz das Almas	BA	8.804 ± 0.131

There is a strong correspondence between morphological and isozymic variation for Africanized honey bees in Brazil, not only in the shape of spatial patterns of variation (north-south clines), but also in the sense that morphological variation is within the limits defined by the model of racial admixture based on isozymes. Since the two types of characters (morphological axis and MDH allelic frequencies) follow the same geographic pattern and probably do not possess a causal genetic relationship (are not correlated within local populations) (but see Oldroyd *et al.*, 1995), the best explanation for variation (and covariation) is demic diffusion, or northward/southward migration after the secondary contact (Sokal and Menozzi, 1982; Sokal, 1986; Diniz-Filho and Malaspina, 1995). Larger bees can be found today in southern and southeastern Brazil because the initial

expansion front of African/Africanized bees continuously encountered, both in time and space, new European populations in these regions. On the other hand, the northeastern and northern regions of the country were almost free of European populations, in such a way that African/Africanized bees rapidly occupied these regions and maintain today a smaller European genetic component. A detailed evolutionary scenario for this admixture/diffusion model was recently proposed by Diniz-Filho and Malaspina (1995).

Although our analyses support the admixture/diffusion model initially proposed by Lobo and Krieger (1992), there is a continuous debate about selective processes acting on clines of MDH worldwide (Nielson *et al.*, 1994; Oldroyd *et al.*, 1995). The evidence supporting these two hypotheses (selection versus admixture), however, is still open to discussion, especially because of the methodological difficulties to establish a causal basis for two spatial processes (Diniz-Filho and Bini, 1994; Oldroyd *et al.*, 1995). In fact, it seems to be that without a solid multi-locus data base it is not possible to definitively choose one of these two alternative (but not mutually exclusive) hypotheses by simple mapping of genetic variation (Sokal and Jacquez, 1991). More importantly, the next step in this research program should be (despite the operational difficulties) the evaluation of evolutionary and ecological processes within local populations, especially population dynamics parameters, and establish their relationships with genetic markers such as MDH alleles and quantitative morphometric characters that have been analyzed on a larger geographic scale.

ACKNOWLEDGMENTS

The authors are grateful to several researchers, beekeepers and friends from all around the country who helped to obtain samples for this study. This paper is part of a Doctoral thesis concluded at the Instituto de Biociências of UNESP - Rio Claro by the first author, and we wish to express our thanks to S.F. dos Reis, A. Malavasi, A.E.E. Soares, M.A. Del Lama, R.R. Sokal, W.E. Kerr, M. Petre Jr., H.G. Fowler, M.I.B. Pignata, L.M. Bini and A.A. Zacaro for discussions and suggestions throughout the project. This work was supported by Doctoral and Research Grants from CNPq and FUNDUNESP. Publication supported by FAPESP.

RESUMO

Neste trabalho, a correspondência entre padrões de variação geográfica de caracteres morfométricos e de isoenzimas em abelhas africanizadas no Brasil foi analisada.

Os dados morfométricos referem-se a 19 caracteres das asas, mensurados em 42 populações locais. Os dados de isoenzimas foram obtidos no trabalho de Lobo and Krieger (*Heredity* 68: 441-448, 1992) e referem-se a frequências alélicas da desidrogenase málica (MDH). Os dois conjuntos de dados, analisados através de superfícies canônicas de tendência, componentes principais e autocorrelação espacial, apresentaram gradientes no sentido sul-norte, sendo que as abelhas africanizadas encontradas nas regiões Sul e Sudeste do país são mais semelhantes às formas européias do que as abelhas das regiões Norte e Nordeste. Além disso, a variação morfométrica encontrada está dentro dos limites estabelecidos pelo modelo de mistura racial, considerando os valores esperados do comprimento da asa anterior (WL) das abelhas africanizadas no Sul e no Nordeste do Brasil. Esses valores foram obtidos combinando-se linearmente os coeficientes de mistura racial com os valores médios de WL nas três principais subespécies de *Apis mellifera* envolvidas no processo de africanização (*Apis mellifera scutellata*, *A. m. ligustica* e *A. m. mellifera*).

REFERENCES

- Bocquet-Appel, J.P. and Sokal, R.R. (1989). Spatial autocorrelation analysis of trend residuals in biological data. *Syst. Zool.* 38: 333-341.
- Buco, M.S., Rinderer, T.E., Sylvester, H.A., Collins, A.M., Lancaster, V.A. and Crewe, R.M. (1987). Morphometric differences between South American Africanized and South African (*Apis mellifera scutellata*) honey bees. *Apidologie* 18: 217-222.
- Daly, H.V. (1991). Systematics and identification of Africanized honey bees. In: *The African Honey Bee* (Spivak, M., Fletcher, D.J.C. and Breed, M.D., eds.). Westview, San Francisco, pp. 13-44.
- Daly, H.V. and Balling, S.S. (1978). Identification of Africanized honey-bees in the Western Hemisphere by discriminant analysis. *J. Kansas Entomol. Soc.* 51: 857-869.
- Daly, H.V., Hoelmer, K. and Gambino, P. (1991). Clinal geographic variation in feral honey bees in California, USA. *Apidologie* 22: 591-609.
- Davis, J.C. (1986). *Statistics and Data Analysis in Geology*. John Wiley, New York, pp. 646.
- Del Lama, M.A., Figueiredo, R.A., Soares, A.E.E. and Del Lama, S.N. (1988). Hexokinase polymorphism in *Apis mellifera* and its use for Africanized honey bee identification. *Rev. Bras. Genet.* 11: 287-297.
- Del Lama, M.A., Lobo, J.A. and Soares, A.E.E. (1990). Genetic differentiation estimated by isozymic analysis of Africanized bee populations from Brazil and from Central America. *Apidologie* 21: 271-280.
- Diniz-Filho, J.A.F. (1994). Variação Geográfica de Abelhas Africanizadas (*Apis mellifera* L.) no Brasil. Doctoral thesis, UNESP, Rio Claro, SP.
- Diniz-Filho, J.A.F. (1995). Canonical trend surface analysis of morphometric variation in Africanized honey bees from Brazil. *J. Apic. Res* 34: 65-72.

- Diniz-Filho, J.A.F. and Bini, L.M. (1994). Space-free correlation between morphometric and climatic data: a multivariate analysis of Africanized honey bees (*Apis mellifera* L.) in Brazil. *Glob. Ecol. Biogeogr. Let.* 4: 195-202.
- Diniz-Filho, J.A.F. and Malaspina, O. (1995). Evolution and population structure of Africanized honey bees in Brazil: evidence from spatial analysis of morphometric data. *Evolution* 49: 1172-1179.
- Diniz-Filho, J.A.F., Malaspina, O. and Pignata, M.I.B. (1993). Geographic variation in *Apis cerana indica* F.: a spatial autocorrelation analysis of morphometric patterns. *J. Apic. Res.* 32: 65-72.
- Harris, R. (1975). *A Primer of Multivariate Statistics*. Academic press, New York, pp. 332.
- Johnston, R.A. and Wichern, D.W. (1992). *Applied Multivariate Statistical Analysis*. Prentice-Hall Int., London, pp. 642.
- Lobo, J.A. (1991). Modelos para análise de frequências alélicas e mistura racial em *Apis mellifera*. Doctoral thesis, FMRP-USP, Ribeirão Preto.
- Lobo, J.A. and Krieger, H. (1992). Maximum likelihood estimates of gene frequencies and racial admixture in the Africanized honey bee (*Apis mellifera* L.). *Heredity* 68: 441-448.
- Lobo, J.A., Del Lama, M.A. and Mestriner, M.A. (1989). Population differentiation and racial admixture in the Africanized honey bee (*Apis mellifera* L.). *Evolution* 43: 794-802.
- Michener, C.D. (1974). The Brazilian bee problem. *Ann. Rev. Entomol.* 20: 399-416.
- Moritz, R.F.A. and Klepsch, A. (1985). Estimating heritabilities of worker characters: a new approach using laying workers of the cape honey bee (*Apis mellifera capensis* Esch.). *Apidologie* 16: 47-56.
- Moritz, R.F.A. and Southwick, E.E. (1992). *Bees as Superorganisms: an Evolutionary Reality*. Springer-Verlag, Berlin, pp. 395.
- Nielson, D., Page, R.E. and Crosland, M.W.J. (1994). Clinal variation and selection of MDH allozymes in honey bee populations. *Experientia* 50: 867-871.
- Oden, N.L. (1984). Assessing the significance of a spatial correlogram. *Geogr. Anal.* 16: 1-16.
- Oldroyd, B.P., Rinderer, T.E. and Buco, S. (1991). Heritability of morphological characters used to distinguish European and Africanized honeybees. *Theor. Appl. Genet.* 82: 499-504.
- Oldroyd, B.P., Cornuet, J.M., Rowe, D., Rinderer, T.E. and Crozier, R.H. (1995). Racial admixture of *Apis mellifera* in Tasmania, Australia: similarities and differences with natural hybrid zones in Europe. *Heredity* 74: 315-325.
- Rinderer, T.E. (1977). Measuring the heritability of characters in honeybees. *J. Apic. Res.* 16: 95-98.
- Rinderer, T.E., Daly, H.V., Sylvester, H.A., Collins, A.M., Buco, S.M., Hellmich, R.L. and Danka, R.G. (1990). Morphometric differences among Africanized and European honey bees and their F₁ hybrids. *Ann. Entomol. Soc. Am.* 83: 346-351.
- Rinderer, T.E., Stelzer, J.A., Oldroyd, B.P., Buco, S.M. and Rubink, W.L. (1991). Hybridization between European and Africanized honey bees in the Neotropical Yucatan peninsula. *Science* 228: 309-311.
- Ruttner, F. (1988). *Biogeography and Taxonomy of Honey Bee*. Springer-Verlag, Berlin, pp 284.
- Sheppard, W.S., Rinderer, T.E., Mazzoli, J.A., Stezer, J.A. and Shimanuki, H. (1991). Gene flow between African- and European-derived honeybee populations in Argentina. *Nature* 349: 782-784.
- Sokal, R.R. (1986). Spatial data analysis and historical processes. In: *Data Analysis and Informatics IV* (Diday *et al.*, eds.). Elsevier Publishers, Holland, pp. 29-43.
- Sokal, R.R. and Jacquez, G.M. (1991). Testing inferences about microevolutionary processes by means of spatial autocorrelation analysis. *Evolution* 45: 152-168.
- Sokal, R.R. and Menozzi, P. (1982). Spatial autocorrelation of HLA frequencies in Europe support demic diffusion of early farmers. *Amer. Nat.* 119: 1-17.
- Sokal, R.R. and Oden, N.L. (1978a). Spatial autocorrelation in biology. 1. Methodology. *Biol. J. Linn. Soc.* 10: 199-228.
- Sokal, R.R. and Oden, N.L. (1978b). Spatial autocorrelation in biology. 2. Some biological implications and four applications of evolutionary and ecological interest. *Biol. J. Linn. Soc.* 10: 229-249.
- Sokal, R.R., Harding, R.M. and Oden, N.L. (1989). Spatial pattern of human gene frequencies in Europe. *Amer. J. Phys. Anthropol.* 80: 267-294.
- Stort, A.C. and Bueno, O.C. (1985). Are *Apis mellifera* bees morphologically Africanized in Brazil? *Rev. Bras. Biol.* 45: 393-397.
- Taylor, O. (1977). The past and possible future spread of Africanized honey bees in the Americas. *Bee World* 58: 19-30.
- Unwin, D. (1976). An introduction to trend surface analysis. *Conc. Tech. Mod. Geogr.* 5: 1-40.
- Villa, J.D., Rinderer, T.E. and Collins, A.M. (1993). 'Overwintering' of Africanized, European and hybrid honey bees (Hymenoptera, Apidae) in the Andes of Venezuela. *Environ. Entomol.* 22: 183-189.
- Wartenberg, D. (1985). Canonical trend surface analysis: a method for describing geographic patterns. *Syst. Zool.* 34: 259-279.
- Wilkinson, L. (1989). *SYSTAT/SYGRAPH: The System for Statistics*. Evanston, Systat Inc., pp. 822.
- Winston, M. (1992). The biology and management of Africanized honey bees. *Ann. Rev. Entomol.* 37: 173-193.

(Received June 27, 1994)