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# EVOLUTION AND POPULATION STRUCTURE OF AFRICANIZED HONEY BEES IN BRAZIL: EVIDENCE FROM SPATIAL ANALYSIS OF MORPHOMETRIC DATA

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*Abstract.*—In recent years, studies based on isoenzymatic patterns of geographic variation have revealed that what is usually called the Africanized honey bee does not constitute a single population. Instead, several local populations exist with various degrees of admixture with European honey bees. In this paper, we evaluated new data on morphometric patterns of Africanized honey bees collected at 42 localities in Brazil, using univariate and multivariate (canonical) trend surface and spatial autocorrelation analyses. The clinal patterns of variation found for genetically independent characters (wing size characters and some wing venation angles) are concordant with previous studies of malate dehydrogenase (MDH) allelic frequencies and support the hypothesis that larger honey bees in southern and southeastern Brazil originated by racial admixture in the initial phases of African honey bee colonization. Geographic variation patterns of Africanized honey bee populations reflect a demic diffusion process in which European genes were gradually lost because of the higher fitness of the African gene pool in Neotropical environmental conditions.

*Key words.*—Africanized honey bees, demic diffusion, multivariate morphometrics, population structure, spatial autocorrelation, trend surface analysis.

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Since their introduction in Brazil in 1956, African honey bees (*Apis mellifera scutellata*) and their descendents have colonized much of South and Central America, and have entered the southern regions of United States (Michener 1975; Taylor 1977, 1985; Winston 1992). During this colonization process, honey bees were affected by several evolutionary forces, including natural and artificial selection (Michener 1975; Ratnieks 1991) and racial admixture with European honey bees (*Apis mellifera ligustica* and *Apis mellifera mellifera*) (Lobo et al. 1989; Lobo and Krieger 1992) that were introduced into Brazil in the nineteenth century (Nogueira-Neto 1972). The bees resulting from these processes are known as Africanized honey bees and resemble their African parents more than their European parents in terms of morphology, behavior, population dynamics, hemolymph proteins, biochemistry of cuticular hydrocarbons, allelic frequencies of isoenzymatic systems and DNA RFLPs patterns (Sylvester 1982; Daly 1991; Winston 1992). A number of hypotheses have been proposed regarding the mechanisms underlying the "Africanization" process and the limited introgression of European genes into African-Africanized honey bee populations (Michener 1975; Taylor 1985; Hall and Muralidharan 1989; Smith et al. 1989; Moritz and Meusel 1992). However, despite interest in these bees, due to their defensive behavior and commercial importance, their population genetics are poorly understood (Moritz and Meusel 1992).

Isoenzymatic studies have shown that what is usually called the Africanized honey bee does not constitute a single population, but instead includes several local populations with distinct degrees of racial admixture (Lobo et al. 1989; Del Lama et al. 1988, 1990; Sheppard et al. 1991a; Lobo and Krieger 1992). Lobo et al. (1989) proposed that the geographic structure of malate dehydrogenase (MDH) allelic frequencies, reflecting distinct levels of racial admixture in Brazil, could be explained by two alternative hypotheses: (1) greater gene flow from European races in southern Brazil or (2) selection favoring the African gene pool in northeastern Brazil. Based on historical data about the Africanization process, the authors chose the first hypothesis as the most parsimonious (although they do not exclude the possibility of selection on MDH). Unfortunately, small samples of local populations were analyzed and significant geographic patterns were found only for MDH (although ten enzymatic systems were analyzed). Thus, inferences concerning microevolutionary processes based on spatial analysis of multilocus data are not reliable (Sokal and Jacquez 1991). Morphological and behavioral patterns of variation were also not analyzed in detail because of environmental effects and nonadditive genetic variation usually associated with these traits (Lobo et al. 1989). However, Oldroyd et al. (1991) found a high proportion of additive genetic variance for several morphological traits in honey bee populations worldwide (including Brazilian populations of Africanized honey bees). Thus, multivariate analysis of morphological variation can be useful in evaluating geographic patterns resulting from evolutionary processes at the populational level (Oldroyd et al. 1991). Indeed, morphometric variation patterns are concordant with isoenzymatic ones in zones of recent hybridization between Africanized and European populations in Mexico (Rinderer et al. 1991) and Argentina (Sheppard et al. 1991b).

The main objective of this paper is to investigate population structure and geographic variation of Africanized honey bees in Brazil, using techniques of spatial data analysis applied to new morphometric data. With these techniques it is possible to infer evolutionary processes from spatial patterns and to test the theories developed to explain the "Africanization" of *Apis mellifera* L. populations in the Neotropics.

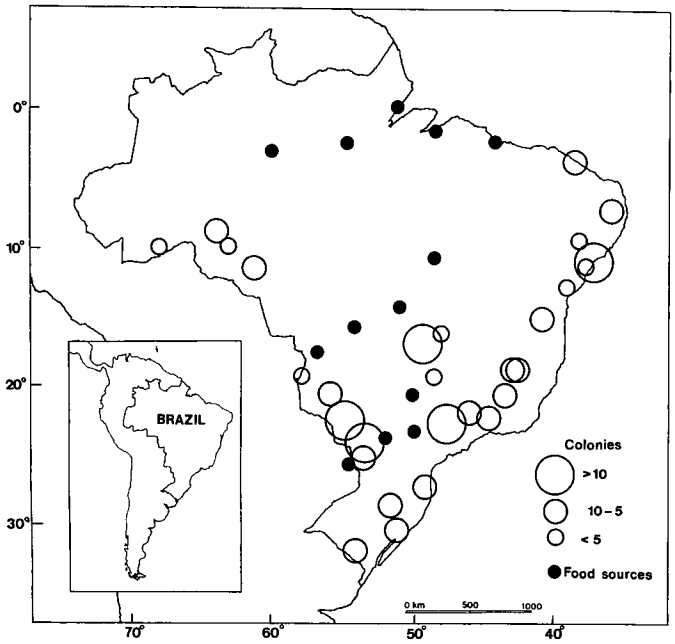


FIG. 1. Forty-two localities sampled in Brazil. Closed circles indicate samples collected from food sources, and open circles (with different sizes indicating number of colonies) indicate samples collected from apiaries.

## MATERIALS AND METHODS

### *Sampling Procedures and Morphometrics*

Nineteen wing characters were measured on randomly collected honey bee workers taken from apiaries and food sources at 42 localities in Brazil. Samples were obtained from 1989 and 1993 (fig. 1). Characters measured included forewing length and width (WL and WW), lengths a and b of cubital vein (CV A and CV B), hindwing length and width (HWLN and HWWD), number of hamuli (HA), and ten venation angles of forewing (A29, A30, A31, A32, A33, A34, A35, A36, A38, and A39). These characters are part of a set of 25 characters used in multivariate morphometrics of Africanized and European honey bees (Daly and Balling 1978; Buco et al. 1987; Rinderer et al. 1990, 1991; Sheppard et al. 1991a,b; Daly 1991). Wing venation angles were obtained by trigonometric procedures based on 22 linear measurements of wing veins. Two new forewing characters were also included here, the length of the radial cell in the forewing (RcL) and the length of apical portion of the radial cell (PRcL).

Approximately 25 worker honey bees were measured from each locality ($\bar{x}$ = 23.786, median = 25), for a total of 990 bees and 180 colonies. The number of colonies sampled in each locality ranged from 3 to 20 colonies (median = 5 colonies) (fig. 1). We avoided sampling colonies of recognized European origin or included in breeding programs, such that our collections are representative of local average phenotypes (Sheppard et al. 1991a,b). Because in 13 localities (especially in the Amazon and Central regions of Brazil) honey bees were collected from food sources (fig. 1), a multivariate analysis of covariance (MANCOVA) (Johnson and Wichern 1992) was used to detect differences between mean vectors of the two sampling procedures (from apiaries or food sources), whereas the geographic coordinates (latitude and longitude) were held constant.

### *Data Analysis*

Both univariate and multivariate analyses of variance (ANOVA and MANOVA) (Sokal and Rohlf 1981; Johnson and Wichern 1992) were used to establish differences among local populations. Because the purpose of this study is to evaluate geographic variation of Africanized honey bees (excluding European honey bees), samples were initially classified using the discriminant function of Daly and Balling (1978) (set 4, for 16 wing characters). For comparative purposes, a local population of honey bees was collected in Montevideo (Uruguay), which is in the sourthern limit of Africanized honey bee distribution in America (Sheppard et al. 1991b) and included in this analysis. Mean vectors of each local population were combined with discriminant coefficients from Daly and Balling (1978), producing scores that were compared with Africanized and European centroids using Mahalanobis $D^2$ distances. Although this discriminant function was originally developed to identify samples from a single colony, we used it to evaluate the "degree of Africanization" of the average phenotype in each local population.

Geographic variation patterns were initially analyzed with a canonical trend surface analysis (CTS) (Lee 1969; Wartenberg 1985). In univariate trend surface analysis, a multiple linear (or polynomial) regression is applied to each variable, using geographic coordinates (latitude and longitude and their polynomial expansions) as independent variables (Davis 1986). However, because morphometric characters are not independently distributed, it seems biologically and statistically more appropriate to analyze them joinly using CTS (Bocquet-Appel and Sokal 1989). The objective of the method is to obtain a linear combination of several biological variables maximizing its correlation with geography, using a standard canonical correlation analysis (Cooley and Lohnes 1971; Harris 1975; Johnson and Wichern 1992). Thus, it is possible to evaluate which portions of morphometrics and geography covary at a large geographic scale (Wartenberg 1985). Residuals of this multivariate linear model were analyzed using spatial autocorrelation analysis (see below). A map of the canonical surface was produced using the interpolation algorithm DWLS (distance weighted least squares) from SYSTAT/SYGRAPH (Wilkinson 1989).

Spatial autocorrelation analysis (Sokal and Oden 1978a,b; Sokal 1983, 1986) was also applied to describe spatial patterns both in the original data and in the residuals of CTS, evaluating the existence of local structure independent of large-scale geographic trends. Moran's I coefficients were calculated using 12 distance classes, whose upper limits (in kilometers) are 471, 700, 918, 1099, 1286, 1486, 1713, 1968, 2202, 2407, 2688, and 3527. The significance of the entire correlogram was established using Bonferroni criterion (Oden 1984).

Correlation structure among the 19 characters, within and among local populations, was also compared. Narrow-sense heritabilities for wing characters of *Apis mellifera* are usually very high (Oldroyd et al. 1991), such that the pooled within-

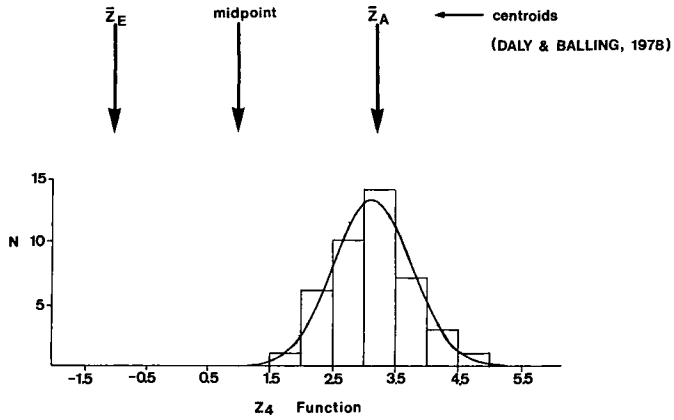


FIG. 2. Frequency distribution of the average phenotypes of the 42 local populations along the discriminant function (set 4), with values of centroids for Africanized, European, and midpoint as defined by Daly and Balling (1978).

population phenotypic correlation matrix derived from MANOVA is a reasonable approximation to the additive genetic correlation matrix, because

$$r_P = h_X h_Y r_G + e_X e_Y r_E$$

where $r_G$, $r_P$ and $r_E$ are the genetic, phenotypic, and environmental (including nonadditive effects) correlation coefficients between characters $X$ and $Y$, respectively; $h_X$ and $h_Y$ are the square roots of narrow-sense heritability for characters $X$ and $Y$ and $e_i = (1 - h_i^2)^{1/2}$, where $i = X$ or $Y$ (Falconer 1981; Atchley 1983; Cheverud 1988). With this comparison, it is possible to understand which part of the correlation structure at populational level (correlation among spatially distributed characters) can be attributed to genetic effects (especially pleiotropy) acting within local populations (Sokal 1978; Sokal and Riska 1981; Sokal et al. 1991a).

All computations were performed with an AT-386 microcomputer. MANOVA, canonical correlation analysis, DWLS interpolated surfaces and correlation matrices were calculated using SYSTAT/SYGRAPH, version 5.01 (Wilkinson 1989), and spatial autocorrelation analysis was performed in SAAP, version 4.3 (Wartenberg 1989).

## RESULTS

All mean vectors were classified as Africanized according to Daly and Balling's (1978) discriminant function, with low probabilities of type-I error ($P < 0.05$). Mahalanobis $D^2$ distances between local population centroids and Africanized honey bee centroid were not significant, except for the local population from Montevideo (correctly classified as a European sample). The scores of average phenotypes of each local population along the discriminant function are normally distributed (fig. 2), with an average $Z$ for Africanized honey bees ($\bar{Z} = 3.148$) very similar to the value obtained by Daly and Balling (1978) ($\bar{Z} = 3.243$).

The $F$-statistic derived from Wilks' lambda obtained in multivariate analysis of convariance (MANCOVA), 1.842, indicated that samples from apiaries and food sources were not different ($P > 0.05$) when latitude and longitude were held constant. Thus, the two samples were pooled in geographic variation analysis. The $F$-statistic obtained in multivariate analysis of variance (MANOVA), however, indicated significant differences among mean vectors of local populations ($F = 3.47$, $P < 0.01$), permiting us to reject the null hypothesis of spatial homogeneity among populations. All 19 characters contributed to this differentiation ($P < 0.01$), with univariate $F$-statistics derived from univariate analysis of variance (ANOVA) ranging from 1.891 to 7.992. Many recent papers have discussed the effects of spatial autocorrelation on type-I error rates of $F$-statistic obtained in ANOVA (Legendre et al. 1990; Sokal et al. 1993). In our data, several characters possess positive spatial autocorrelations in the short-distance classes (see below), such that significance levels of $F$-statistics from ANOVA and MANOVA are biased. Because these autocorrelations are usually positive, the significance levels obtained here are expected to be conservative ($F$-values are smaller than they should be in the absence of spatial autocorrelation) (Sokal et al. 1991a).

TABLE 1. Results of univariate and canonical trend surface analyses applied to 42 populations of Africanized honey bees in Brazil. $R^2$ and $F$-statistics† refer to univariate surfaces, and $r$ is the Pearson product-moment correlation‡ between each character and first canonical surface (CS 1).

| Character | $R^2$ | $F$ | $r$ |
|---|---|---|---|
| WL | 0.502 | 19.651** | 0.753** |
| WW | 0.255 | 6.678** | 0.537** |
| RcL | 0.240 | 6.143** | 0.515** |
| PRcL | 0.388 | 12.344** | 0.662** |
| CV B | 0.088 | 1.880 | 0.178 |
| CV A | 0.132 | 2.962 | 0.241 |
| HWLN | 0.050 | 1.023 | 0.212 |
| HWWD | 0.107 | 2.327 | 0.346** |
| HA | 0.013 | 0.259 | −0.092 |
| A29 | 0.244 | 6.298** | −0.415** |
| A30 | 0.053 | 1.100 | 0.242 |
| A31 | 0.022 | 0.445 | −0.104 |
| A32 | 0.254 | 6.641** | 0.522** |
| A33 | 0.026 | 0.528 | 0.139 |
| A34 | 0.142 | 3.215* | 0.304* |
| A35 | 0.028 | 0.564 | 0.118 |
| A36 | 0.058 | 1.079 | −0.124 |
| A38 | 0.022 | 0.448 | 0.016 |
| A39 | 0.029 | 0.585 | 0.180 |

* $0.05 > P > 0.01$; ** $P < 0.01$.
† $F$-statistic with 2 and 39 df.
‡ $r$ with 40 df.

In univariate linear trend surfaces, six characters showed significant $F$-statistics at $P < 0.01$, with $R^2$ ranging from 0.240 to 0.502 (table 1). The wing venation angle A34 also had a significant $F$-statistic at $P < 0.05$, with $R^2$ equal to 0.142. These low $R^2$-values indicate a large amount of local (non-geographic) morphometric differentiation.

Out of two possible canonical surfaces, only the first was significant (Bartlett sphericity test $X^2 = 91.916$; $P < 0.01$) (Johnson and Wichern 1992). The canonical correlation of this surface was 0.941, and the distribution of eigenvalues indicates that it explains 88.55% of the covariation between morphometrics and geography. However, the redundancy coefficient of this surface (Cooley and Lohnes 1971; Wartenberg 1985), was equal to 11.69%, indicating that canonical surface explains a very small part of the total variance of morphometric data. This low value confirms that there is a

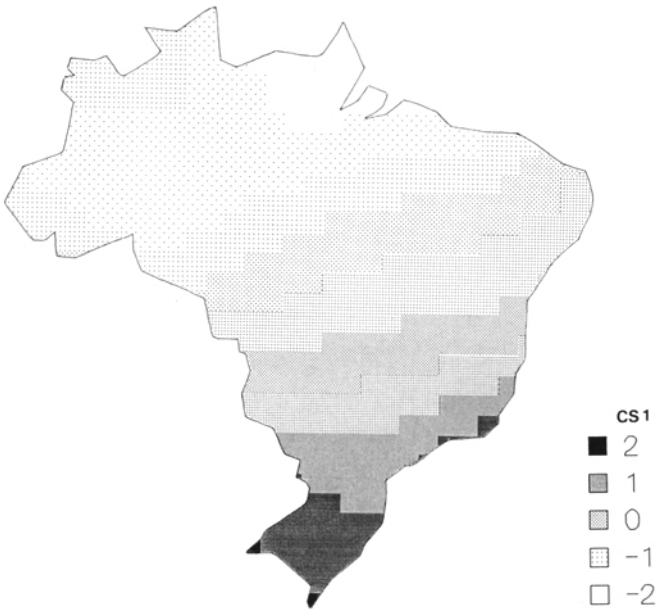


FIG. 3. Distance weighted least-squares (DWLS) contouring map of the first canonical surface (CS 1). Higher values on the multivariate axis are represented by increasing darkness of shading.

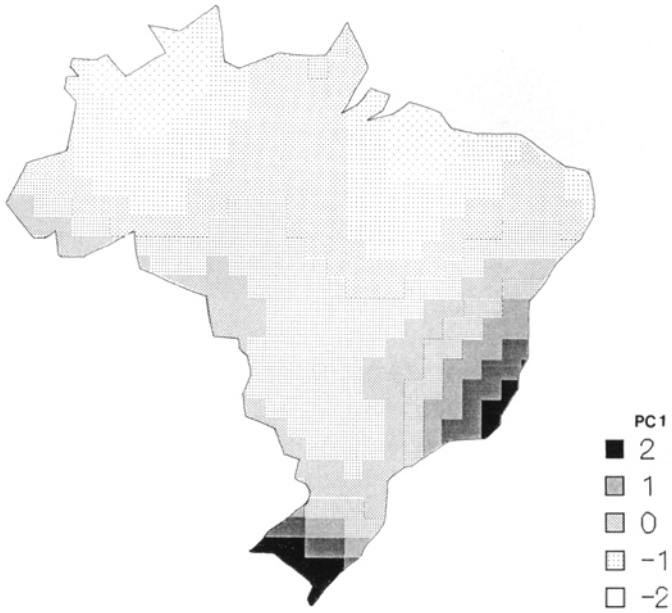


FIG. 4. Distance weighted least-squares (DWLS) contour map of the first principal component (PC 1). Higher values on the multivariate axis are represented by increasing darkness of shading.

large residual morphometric variance at the multivariate level, which is expected because only 7 of 19 characters possess significant ($P < 0.05$) $F$-statistics in univariate trend surfaces, with relatively low $R^2$-values. Coefficients of canonical surface (table 1) indicate that wing size characters (WL, WW, RcL, PRcL, and HWWD) and some venation angles (A29, A32, and A34) were the characters with significant spatial linear patterns. Coefficients displayed are structure coefficients and were estimated by the Pearson product-moment correlation between scores of canonical surface with original characters (Cooley and Lohnes 1971; Johnson and Wichern 1992).

A contour map of canonical surface CS 1, interpolated using the algorithm distance weighted least squares (DWLS), indicates that localities with highest scores on this multivariate axis occur in southern and southeastern Brazil and that these values decrease with latitude to the north, forming a cline (fig. 3). According to canonical coefficients (table 1), this multivariate axis is an expression of wing size (but excluding HWLN). Indeed, the correlation between individual scores of canonical surface and first principal component (PC 1) derived from among-population correlations (representing the size variation of honey bees among local populations and explaining 27.15% of the morphometric variability) was equal to 0.536 ($P < 0.01$). Thus, the principal direction of morphometric (linear) variation across geographic space (CS 1) corresponds to the principal direction of morphometric variation among local populations (PC 1). The map of the first principal component, also interpolated with DWLS (fig. 4) shows that clines obtained from CS 1 and PC 1 are similar, although the multivariate similarity between southern and southeastern regions of Brazil is more evident on PC 1.

The autocorrelation patterns were concordant with results derived from trend surface analyses. Out of 228 possible Moran's I coefficients derived from original data (12 distance classes × 19 characters), 42 (18.42%) were significant at the 5% level. Eight correlograms were significant according to the Bonferroni criterion (fig. 5A). As expected, the correlograms of characters that were more related to canonical surface show significant positive autocorrelations in the first or second distance classes, associated with significant negative autocorrelations in the last two distance classes (clinal pattern). A34 is significantly correlated with canonical surface (table 1) and also shows a clinal pattern of variation, but possesses marginally significant correlograms ($P < 0.10$) by the Bonferroni criterion. Two characters (CV A and A38) showed significant correlograms, but were only marginally correlated with canonical surface ($P < 0.10$). CV A shows a strong positive autocorrelation in the first distance class associated with significant negative autocorrelations in the seventh and tenth distance classes. A38 shows a single significant negative autocorrelation in the seventh distance class.

Autocorrelation patterns of canonical trend surface (CTS) residuals indicate that most geographic variation was removed from the data set by the multivariate linear surface. Out of 228 possible Moran's I coefficients, only 23 (10.09%) were significant at the 5% level. Using Bonferroni criterion to establish the significance of the correlograms as a whole, only PRcL, CV A, and A38 still showed significant spatial pattern after removing large-scale linear trends. In the correlograms for residuals of the eight characters with significant patterns in the original data matrix (fig. 5B), only the correlogram for CV A possesses a significant positive coefficient in the first distance class (0–471 km), indicating patches of local variation independent of linear clines.

Comparison of correlation structures at distinct analytic levels (within and among local populations) (fig. 6) shows that high within-population correlation coefficients (both with negative and positive signals) corresponded to high among-population correlation coefficients of the same sign.

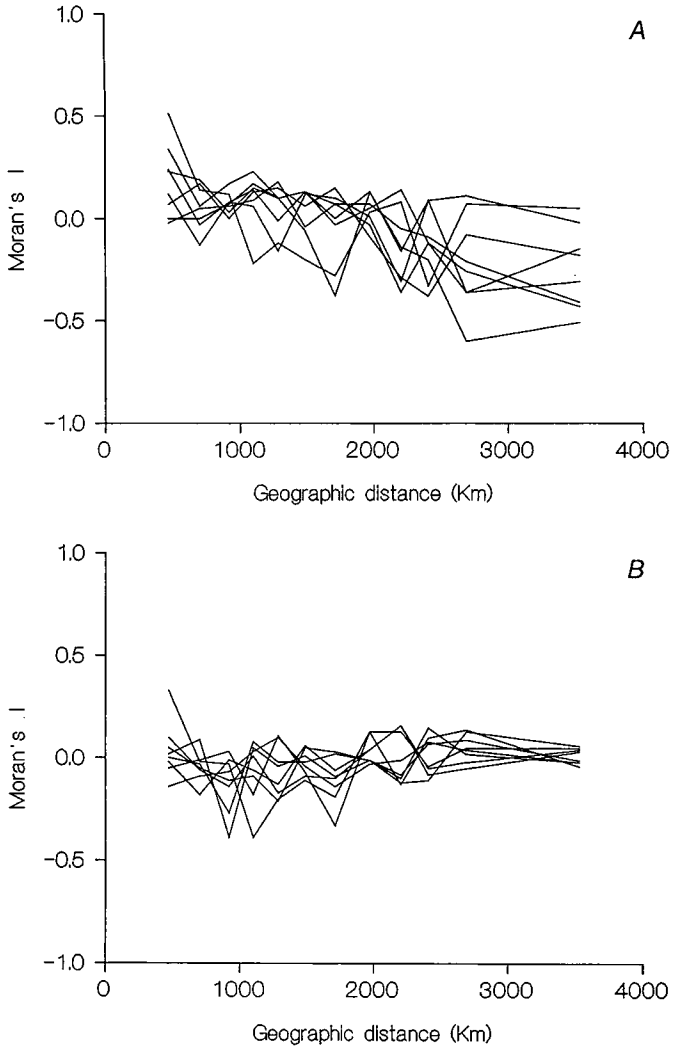


FIG. 5. Significant spatial correlograms based on original characters (A) and on the residuals of canonical trend surface for the same characters (B).

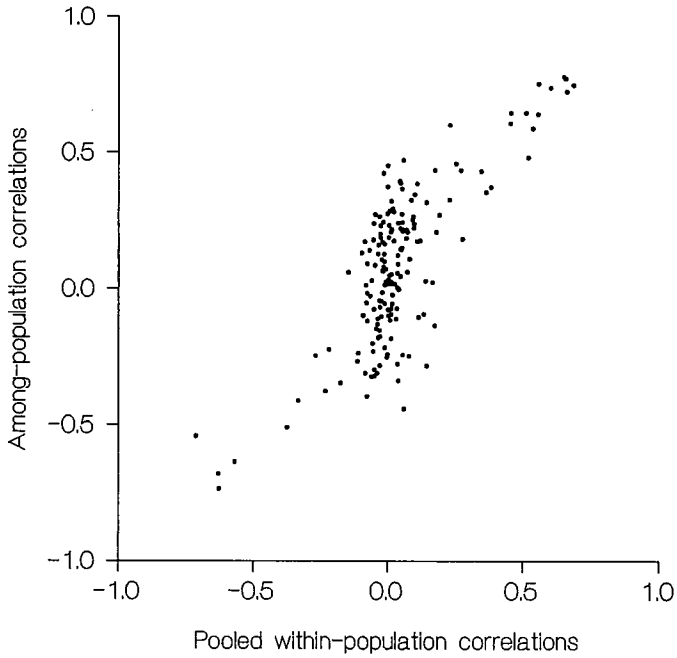


FIG. 6. Comparison of among-population correlations with pooled within-population correlations.

Thus, these among-population correlation coefficients can be explained by genetic effects within local populations (correlations due to pleiotropy, linkage disequilibrium, or structural constraints). However, many nonsignificant within-population correlations (values close to 0) were associated with relatively high among-population correlations, ranging approximately from −0.50 to 0.50. These correlations cannot be explained by within-population genetic effects and must be viewed as a consequence of a widespread evolutionary process at the populational level.

## DISCUSSION

Geographic variation patterns, and especially clines, result from an interaction between history and current ecology (Endler 1977; Thorpe 1987). Morphological clines are frequently found for honey bees worldwide and have been primarily attributed to adaptive processes and natural selection (Ruttner 1988; Daly et al. 1991; Diniz-Filho et al. 1993). For Africanized honey bees, however, other evolutionary processes must be considered before infering adaptations. In Brazil, the morphological cline is very similar to that found for malate dehydrogenase (MDH) allelic frequencies, with larger honey bees (greater European genetic component) occurring in southern and southeastern regions. This north-south cline in MDH was recently explained by the initial admixture proportions and "hitchhiking" effect of neutral markers on adaptive alleles due to gametic disequilibrium in the first generations after contact between African and European honey bees (Lobo and Krieger 1992). However, clines can be produced by distinct evolutionary processes, including selection and secondary contact followed by isolation by distance or demic diffusion (Sokal 1986; Sokal and Jacquez 1991). How can our morphological data be used to discriminate among these alternative hypotheses?

Information exists concerning diffusion of Africanized honey bees in the Neotropical region and about geographic distribution of European honey bees prior to 1956. Moreover, recent studies using mitochondrial and nuclear DNA (Hall and Muralidharan 1989; Smith et al. 1989; Hall 1990) indicated that Africanized honey bees have continuous maternal lineages, spreading as swarms, in a demic diffusion process (Sgaramella-Zonta and Cavalli-Sforza 1973; Sokal and Menozzi 1982). This way, racial admixture provides a very robust model to explain geographic differentiation, and the question then consists of explaining the differences in admixture proportion by greater gene flow in southern and southeastern Brazil or by selective preassures favoring African gene pool in northeastern (Lobo et al. 1989).

The demic diffusion process predicts that gene flow accompanying population expansion at large geographic scales (Wijsman and Cavalli-Sforza 1984), and concordant spatial patterns should be found for genetically independent characters (Sokal and Menozzi 1982; Sokal 1986; Sokal et al.

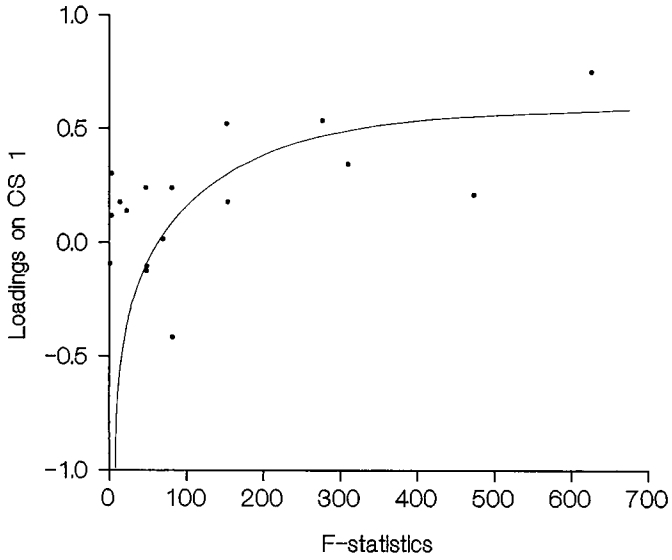


FIG. 7. Relationship between F-statistics comparing Africanized and European honey bees (according to Daly and Balling 1978) and loadings of CS 1.

1989, 1991b). Adaptive hypotheses require that similar clines are found for groups of genetically related characters (due to general pleiotropic effects or linkage disequilibrium) or for characters that are tracking the same geographically patterned selective agent. Both alternatives are not good explanations for correspondence between clines in morphometric data and MDH allelic frequencies, because genetic correlations (within populations) between morphometric data and MDH electrophoretic phenotypes were not found. Thus, the correspondence between clines at large geographic scales supports to the hypothesis of diffusion proposed by Lobo and Krieger (1992). More importantly, the variability in wing venation angles, although heritable (Oldroyd et al. 1991), has been considered neutral in honey bee populations and is not genetically correlated with wing size (Wagner 1990). In our data, characters of wing size and some of these venation angles are all significantly correlated with the clines (figs. 3 and 4; see table 1). The most parsimonious explanation for these correlations is a simultaneous diffusion of all genetic systems determining morphometric patterns. The remaining question, however, is why only a few characters show significant correlations with the canonical surface? In general terms, clines will occur only for characters with large differences between populations in secondary contact (Sokal et al. 1989, 1991b). Indeed, we found a nonlinear relationship between *F*-statistics comparing divergence between Africanized and European populations, obtained from Daly and Balling's (1978) paper, and the loadings of CS 1 (fig. 7). Spearman rank correlation between these two vectors equaled 0.505 ($P < 0.025$). This significant correlation indicates that characters with larger differences between races involved in the Africanization process display more clear linear spatial patterns. Moreover, the $R^2$ values of univariate trend surfaces and redundancy coefficient of Canonical trend surface analysis (CTS) are relatively low and indicate that even for characters with significant linear north-south trend there is a great amount of local differentiation. This is expected for Africanized honey bees because of movements of apiculture activity, genetic drift, and local (not regional) new introductions of European bees. These factors increase the residual variance of regressions and disturb the analysis of clines at large geographic scales.

The comparison of within- and among-population correlation structures (fig. 6) summarizes the evidence about evolutionary events at the populational level. Many nonsignificant within-population correlations are paired with high among-population correlations, and they can be understood as a consequence of a widespread evolutionary process at the population level. Because these nonsignificant correlations refer mostly to wing venation angles, a common selective agent patterned in geographic space (favoring African pool in northeastern Brazil) is not an adequate explanation, and the best alternative is that differences in racial admixture accompanying range expansion produced a common spatial structure.

Our morphometric data and its congruence with malate dehydrogenase (MDH) allelic frequencies indicate that north-south clines can be explained by a diffusion process associated with distinct regional levels of interracial gene flow. Adding information about reproductive biology, population dynamics, and history of honey bee introductions in Brazil, it is possible to develop a general scenario to explain geographic structure of Africanized honey bees. Hybrids with a higher European component were originally located in southern and southeastern regions of the country, because of the previous introduction of European bees in these regions. Partial reproductive isolation between subspecies, which has been used to explain higher similarity between African and Africanized honey bees (Kerr and Bueno 1970; Michener 1975), was not found in regions recently colonized by both subspecies (Sheppard et al. 1991a,b; Rinderer et al. 1991). Moreover, initial hybridization seems to be a valid assumption. However, the African gene pool confers a higher viability and fertility (and consequently a higher colonization ability) in tropical and subtropical environments (Ratnieks 1991; Villa et al. 1993). In addition, those hybrids with a slightly higher African component (and associated higher colonization ability) dispersed faster and increased feral populations, in such a way that the migration front always kept a stronger African component than source local populations (confirmed by data from mitochondrial DNA [mtDNA]). Because the initial northward migration front encountered few feral European populations (up to Guianas and Venezuela), the process continued forming a north-south cline in Brazil. A similar phenomenon, but in a temporal dimension, was found by Boreham and Roubik (1987), analyzing Africanized honey bees in Panama from 1982 to 1985. This way, clines (including morphometric ones) can be explained by hitchhiking effects of neutral characters on adaptive loci (probably controlling swarming rates and other population dynamics parameters) before each range expansion event. Sometime after migration, local populations returned to genetic equilibrium because local panmixia eliminated gametic disequilibrium produced by hybridization. However, average values for each trait in local populations were already fixed and display a clinal pattern on geographic space.

We conclude that clinal patterns of variation in wing char-

acters confirm the hypothesis that Africanized honey bees in Brazil originated by racial admixture during the initial phases of African honey bee colonization, forming many populations with distinct levels of hybridization between African and European honey bees. In fact, our morphometric analysis is the first empirical test of the racial admixture hypothesis proposed by Lobo and Krieger (1992). The geographic variation patterns may be due to a demic diffusion process in which genetic changes resulting from hybridization between African and European honey bees in southern and southeastern Brazil were gradually lost because of the higher fitness of the African gene pool in Neotropical environmental conditions.

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