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Original article

Genetic differentiation estimated by isozymic analysis of Africanized honeybee populations from Brazil and from Central America

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Summary — Adult *Apis mellifera* workers were electrophoretically examined in at least 7 enzyme loci. Five loci were found to exhibit polymorphism (Est-1, Est-3, PGM-1, HK-1 and MDH-1) in the Africanized bee populations from Brazil and from Central America (Costa Rica and Honduras). Genetic variation was not observed for Est-1 and HK-1 in the bee samples from Italy and Germany, and for PGM-1 in the Italian bee sample. Genotypic frequencies at the MDH-1 and HK-1 loci in the Honduras sample are in disagreement with that expected for Hardy–Weinberg equilibrium, suggesting an incipient process of mixture between pre-existing European bees in this country and the Africanized swarms that came from South America. Racial admixture estimates based on MDH-1 data indicate that the Africanization level of Central American honeybees is less than for bees from Southeast and Northern Brazil.

Africanized honeybee / genetic variation / genetic differentiation / gene frequency / isozyme

INTRODUCTION

Before 1956, the populations of *Apis mellifera* L existing in South America were of European origin. In that year, queens of *Apis mellifera adansonii* (or *Apis mellifera scutellata* according to Ruttner, 1981) were introduced to the Rio Claro region, State of São Paulo, Brazil. After the accidental escape of 26 African queens, the formation of "Africanized bees" started to occur in the South American continent (Michener, 1975). These original swarms must have intercrossed with the bees of European origin, since Kerr and Bueno (1970) showed that African and Italian bees were not reproductively isolated. The expansion and occupation of new territories by these Africanized swarms occurred rapidly and led to a widespread replacement by Africanized bees of populations originating from European bees.

A relatively larger proportion of genes from European races is expected in Africanized hybrid populations in regions of Latin America in which apiculture was more active before the introduction of Africanized bees, as is the case for Southern Brazil, Uruguay and Northern Argentina.

This gene flow originating from European bees may also modify the gene pool of the present migratory populations in Central America. Some investigators believe that the relative importance of the gene flow originating from European races as a mechanism in the genetic interpopulation differentiation of Africanized bees is small or absent (Cornuet, 1986; Ruttner, 1986). Morphometric (Daly and Balling, 1978), isozymic (Nunamaker and Wilson, 1981) and nuclear and mitochondrial DNA (Hall, 1988; Hall and Muralidharan, 1989; Smith *et al,* 1989) studies showing few differences between Africanized bees and samples of *Apis mellifera scutellata* have given support to this assumption. However, similar studies based on the analysis of a larger number of polymorphic loci and colonies have clearly indicated that part of the variability observed among Africanized bee populations may be the consequence of the different levels of racial admixture shown by these populations throughout their area of distribution (Lobo, 1986; Del Lama *et al,* 1988; Lobo *et al,* 1989).

Among the enzyme polymorphisms exhaustively studied in *Apis mellifera,* MDH, HK and PGM have shown the highest levels of heterozygosity and therefore represent the genetic markers that reveal most clearly the genetic relationships among population clusters in this species. MDH in particular is an informative system showing distinct allelic frequencies in some subspecies of *Apis mellifera* (Contel *et al,* 1977; Badino *et al,* 1983, 1984, 1985; Nunamaker *et al,* 1984).

Two biochemical markers known for this species, MDH-1 and HK-1, are particularly useful for estimating the level of genetic differentiation between European and Africanized *Apis mellifera* populations. The MDH-1 locus has 3 variants, 100, 80 and 65, which exhibit markedly distinct frequencies in *Apis mellifera scutellata, Apis mellifera mellifera* and *Apis mellifera ligustica* (see Contel *et al,* 1977; Badino *et al,* 1983, 1984, 1985; Nunamaker *et al,* 1984).

The 100 variant of the HK-1 locus is virtually fixed in European populations (Sheppard and Berlocher, 1985; Sheppard and McPheron, 1986; Del Lama *et al,* 1988) and the 83 variant occurs at high frequency in Brazilian Africanized bees, indicating its probable African origin (Del Lama *et al,* 1988).

The objective of the present study was to compare the data concerning the gene frequencies of these markers among different populations of Africanized and European bees. Data for these systems obtained in bees from Central America (Costa Rica and Honduras) are reported for the first time.

MATERIALS AND METHODS

The *Apis mellifera* sample analyzed consisted of 4 population groups: 1), Africanized *Apis mellifera* collected from 9 geographical regions of Brazil: João Pessoa (Jop), Recife (Rec), Salvador (Sal), Vicosa (Vic), Luiz Antonio (Lua), Brasilia (Bra), Rio do Sul (Ris), São Joaquim (Sjo), and Taquari (Taq). Figure 1 represents the sites of the Brazilian bee samples used for electrophoretic studies in this work. 2), Africanized *Apis mellifera* from 2 regions in Central America: La Garita, Costa Rica, and Catacamas (Olancho), Honduras. These samples were collected in August 1987 and January 1988, respectively. 3), *Apis mellifera carnica* from Tübingen, FRG. Most of these bees are probably the result of hybridization of *carnica* queens with other races occurring at this site and therefore would not represent pure strains. The nests analyzed were obtained on 3 different occasions and the results were pooled: 4), *Apis mellifera ligustica* from 2 sites in Italy, *ie,* Bologna and Seregno (Milan).

The nests represent swarms collected in nature and maintained in apiaries. The number of colonies analyzed for each location is presented in table I. Except for São Joaquim, the number of colonies analyzed per location was 15 or more. Eight workers were analyzed per colony, except for João Pessoa (5 workers per colony).

Fig 1. Sites of origin of the Brazilian bee samples used for electrophoretic analysis.

Five enzyme systems corresponding to 7 gene loci (Est-1, Est-3, PGM-1, HK-1, SOD, MDH-1 and MDHm) were studied by starch gel electrophoresis in all populations sampled. Two additional gene loci (ME and a-GPDH) were studied in Africanized bees. In addition, ACON-2 and LAP were analyzed in Africanized populations of Brazil and Central America, respectively. For polymorphic loci, allozyme designations are based on relative mobilities, with decimal points omitted.

Four enzyme systems (esterases, phosphoglucomutase, hexokinase and superoxide dismutase) were studied using the Tris–EDTA–maleate–magnesium, pH 7.4 (Spencer *et al,* 1964) buffer system; the other enzymes were studied in the Tris–citrate, pH 8.0 (Detter *et al,* 1968) buffer system. Gel and sample preparation and the experimental conditions have been reported in previous papers (Del Lama *et al,* 1985, 1988). Enzyme activity was visualized by the techniques of Harris and Hopkinson (1976).

RESULTS

Of the gene loci studied, 5 proved to be polymorphic in Africanized populations from Brazil and Central America (Est-1, Est-3, PGM-1, HK-1 and MDH-1). Genetic variation was not observed for Est-1 and HK-1 in the bee samples from Italy and Germany, and for PGM-1 in the Italian bee sample.

Table I shows the gene frequencies for these markers. In agreement with previous studies (Del Lama *et al,* 1988; Lobo *et al,* 1989), MDH-1 and HK-1 loci proved to be highly polymorphic in all Africanized bee populations and showed marked differences in relation to the European populations. However, the Est-1, Est-3 and PGM-1 loci showed low heterozygosity levels.

Table II shows the levels of heterozygosity observed and expected at the MDH-1, HK-1 and PGM-1 loci from Hardy–Weinberg equilibrium for the Africanized populations of Brazil and Central America. The data demonstrate that these populations, with the exception of Recife and Honduras, are in equilibrium for the 3 systems studied. No equilibrium was observed for the MDH-1 and HK-1 loci for the Honduras population, and a significantly lower than expected intralocus heterozygosity was observed owing to the excess of homozygotes. This equilibrium was also reflected in the expected levels of mean heterozygosity estimated from these three loci.

Figure 2 presents a dendrogram constructed using the UPGMA method to illus-

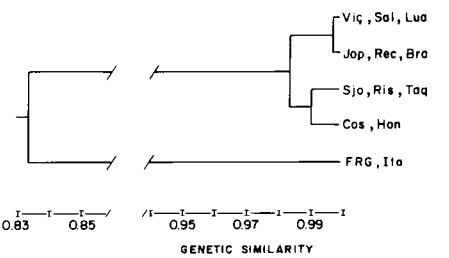

Fig 2. Clustering analysis of similarity coefficients (Nei, 1973) between European and Africanized populations of *Apis mellifera* using the UPGMA method of clustering.

Table I. Genic frequencies of 5 polymorphic isozymic systems analyzed in different populations of Africanized honeybees and European races of *Apis mellifera*. Numbers between parentheses represent the number of hives analyzed in each locality. * HK data (except Sjo, Cos and Hon) from Del Lama *et al* (1988).

Subspecies	*Africanized honeybees*											*carnica*	*ligustica*
Locality	*JOP* (17)	*REC* (21)	*SAL* (24)	*VIC* (24)	*LUA* (25)	*BRA* (26)	*RIS* (25)	*SJO* (07)	*TAQ* (19)	*COS* (29)	*HON* (19)	*FRG* (36)	*ITA* (15)
System													
Est-1													
f(100)	1.000	0.990	0.990	0.987	0.962	0.990	0.982	0.906	0.970	—	1.000	1.000	1.000
f(75)	0.000	0.010	0.010	0.013	0.038	0.010	0.018	0.094	0.030	—	0.000	0.000	0.000
Est-3													
f(130)	—	0.000	0.016	0.034	0.038	0.012	0.010	0.031	—	0.011	—	0.017	0.067
f(100)	—	1.000	0.984	0.966	0.962	0.988	0.985	0.969	—	0.985	—	0.950	0.933
f(70)	—	0.000	0.000	0.000	0.000	0.000	0.005	0.000	—	0.004	—	0.033	0.000
PGM-1													
f(100)	0.947	0.923	0.891	0.975	0.882	0.918	0.970	0.911	0.944	0.983	0.984	0.983	1.000
f(67)	0.047	0.068	0.091	0.003	0.100	0.034	0.018	0.053	0.013	0.017	0.016	0.017	0.000
f(50)	0.006	0.009	0.018	0.022	0.018	0.048	0.012	0.036	0.043	0.000	0.000	0.000	0.000
HK-1 *													
f(100)	0.470	0.473	0.396	0.385	0.348	0.464	0.515	0.554	0.553	0.586	0.600	1.000	1.000
f(87)	0.530	0.527	0.604	0.615	0.652	0.536	0.485	0.446	0.447	0.414	0.400	0.000	0.000
MDH-1													
f(100)	0.853	0.875	0.844	0.768	0.790	0.906	0.797	0.759	0.753	0.752	0.647	0.243	0.340
f(80)	0.118	0.092	0.151	0.228	0.195	0.087	0.200	0.241	0.227	0.088	0.225	0.108	0.009
f(65)	0.029	0.033	0.005	0.004	0.015	0.007	0.003	0.000	0.020	0.160	0.128	0.649	0.650

Table II. Estimates of observed and expected (within parentheses) intralocus heterozygosity and mean heterozygosity for three polymorphic systems analyzed in Africanized populations of *Apis mellifera*. Significant deviation from Hardy–Weinberg equilibrium at the 5% (*) and 1% (**) level.

Population	*MDH*	*HK*	*PGM*	*H*
João Pessoa	0.2823 (0.2576)	0.4705 (0.4982)	0.1058 (0.1009)	0.2862 (0.2855)
Recife	0.2261 (0.2248)	0.5773 ** (0.4985)	0.1428 (0.1433)	0.3154 (0.2888)
Salvador	0.2708 (0.2648)	0.4985 (0.4783)	0.2083 (0.1975)	0.3228 (0.3135)
Vicosa	0.3529 (0.3581)	0.4600 (0.4735)	0.0500 (0.0480)	0.2876 (0.2932)
Luiz Antonio	0.3600 (0.3376)	0.4550 (0.4550)	0.2250 (0.2117)	0.3466 (0.3343)
Brasilia	0.1778 (0.1715)	0.4759 (0.4974)	0.1634 (0.1538)	0.2723 (0.2742)
Rio do Sul	0.3437 (0.3247)	0.5500 (0.4995)	0.0600 (0.0580)	0.3179 (0.2940)
São Joaquim	0.3750 (0.3658)	0.5357 (0.4941)	0.1786 (0.1650)	0.3631 (0.3416)
Taquari	0.3684 (0.3810)	0.4473 (0.4943)	0.1118 (0.1068)	0.3112 (0.3273)
Costa Rica	0.3836 (0.4011)	0.4913 (0.4852)	0.0344 (0.0314)	0.3031 (0.3065)
Honduras	0.4782 * (0.5143)	0.3894 * (0.4800)	0.0315 (0.0314)	0.2997 (0.3419)

trate the clustering of African and European populations through the coefficients of genetic similarity (Nei, 1973). As expected, the dendrogram initially separates European and Africanized bees and later separates 2 groups of Africanized populations at higher levels of similarity: the first includes the populations from Southern Brazil and Central America (Sjo, Ris, Taq, Cos and Hon), and the second the populations from Southeastern and Northeastern Brazil (Vic, Sal, Lua, Jop, Rec, and Bra).

Table III lists racial admixture data estimated for some Africanized populations by the maximum-likelihood method of Krieger *et al* (1965), using the data for allele frequencies at the MDH locus. In the present study, we used estimates of MDH from Badino *et al* (1983) for *ligustica* and the estimates of Nunamaker *et al* (1984) for *mellif-*

Table III. Estimates of racial admixture in different populations of Africanized bees from Central America and from Brazil. * Mean (± SD) admixture estimated by the method of Krieger *et al* (1965).

Population	*Proportion of admixture * of* A m scutellata	*Proportion of admixture * of European races*
Recife	0.865 ± 0.019	0.135 ± 0.021
João Pessoa	0.844 ± 0.028	0.156 ± 0.032
Brasilia	0.903 ± 0.014	0.097 ± 0.016
Vicosa	0.766 ± 0.022	0.234 ± 0.019
Taquari	0.746 ± 0.025	0.254 ± 0.028
São Joaquim	0.757 ± 0.026	0.243 ± 0.019
Costa Rica	0.710 ± 0.023	0.290 ± 0.026
Honduras	0.611 ± 0.027	0.389 ± 0.033

era and *scutellata* (see Lobo *et al,* 1989). It can be seen that the populations from the Center-West (Bra) and Northeast (Rec and Jop) of Brazil have a larger proportion of African genes than the populations from Southern Brazil (Sjo and Taq) and from Central America (Cos and Hon).

DISCUSSION

The present results show a high level of genetic similarity among Africanized bee populations, in agreement with observations on populations resulting from a recent introduction, with a short time for expectations for subsequent differentiation. Under these conditions, genetic markers with high levels of polymorphism and racial differentiation should be expected to be able to reveal incipient processes of population differentiation.

Ruttner (1986) suggests that the distribution of MDH gene frequencies in Europe and Africa agrees with the hypotheses existing on the center of origin and infraspecific diversification of *Apis mellifera.*

The most important observation in the present analysis of Africanized bee populations from Brazil and Central America is that the most frequent MDH and HK alleles in European races show slightly higher frequencies in the samples from Central America and Southern Brazil than in the samples from Northern and Southeast Brazil. This tendency causes the separation of these 2 population groups into different clusters upon analysis of genetic similarity and leads to higher estimates of admixture of European races for Central America and Southern Brazil. Similar observations have been reported in a previous study on Brazilian samples (Lobo *et al,* 1989).

These results may be explained by raising the hypothesis that the rapid migration rate of Africanized bees during the initial phase of expansion (Kerr *et al,* 1982) may have favored a phenomenon of interracial hybridization of different rates in different geographic regions. The migratory waves that first reached the Brazilian Northeast and Center-West found, in the local climatic conditions (favorable annual cycles of humidity and temperature) and in the limited local beekeeping activity, favorable conditions for a rapid replacement of preexisting honeybees and for a smaller gene flow from European races. In the Brazilian South and Southeast, the hybridization process may have lasted over a larger number of generations as a result of different factors such as climatic conditions that permitted lower growth rates for Africanized populations, the existence of a well-developed beekeeping industry, especially in Rio Grande do Sul (Nogueira-Neto, 1972) and attempts at artificial hybridization after the expansion of Africanized bees by massive rearing and distribution of European queens (Goncalves *et al,* 1972).

In Central America, estimates of racial admixture such as those observed here may be explained by progressive hybridization of the bees during their migration through the Northern part of South America and through Central America. Despite uncertainly over the mechanism responsible for the racial admixture, we believe that the genetic similarities of these populations justify the clustering observed. Other explanations, such as similar selective pressures, would meet serious difficulties since they involve marked differences in climatic pattern between Honduras–Costa Rica and Southern Brazil, especially with respect to the existence of a prolonged cold season in the latter.

The HK and MDH data observed in the Honduras sample suggest that this is a migratory population undergoing a more recent colonization process than the Costa Rica sample. The lack of Hardy–Weinberg equilibrium in the Honduras population, with the simultaneous absence of heterozygotes in the 2 systems, agrees with the pattern expected during the first generations resulting from the migration of new genes in the population (Cavalli-Sforza and Bodmer, 1981).

It is important to note that a possible difference in gene frequency between sexes results in a decrease in the rate at which equilibrium is reached in haplodiploid systems (see Hartl, 1972). Thus, the Honduras population can be considered as a population in search of equilibrium, in contrast to the Costa Rica population which seems to be more stable in terms of the effects of migration of new African genes. Data on more northerly regions along the present migratory front (Guatemala and Mexico) may provide more information regarding this question.

Even though the present data demonstrate that the gene flow of European races is more significant in Central American samples (Costa Rica and Honduras) than in populations from Northern and Central Brazil, they do not permit us as yet to select with precision which of the 2 possible phenomena may be occurring in this region:

— The populations of Africanized bees currently colonizing Central America are different from the Africanized swarms that appeared after the process of Africanization started from São Paulo State in 1957, due to a progressive hybridization during their migration;

— The migratory populations of Africanized bees currently colonizing Central America may have large percentages of African admixture, with levels almost equal to those of the original African bees, and the higher degree of racial admixture in-

volving European races observed here is a transitory phenomenon caused by an incipient period of population growth. When growth reaches saturation the percentages of admixture now observed will be substantially modified. Thus, the Africanization process currently under way in Central America may be very similar to that which took place in the State of São Paulo and in Southeastern Brazil 30 years ago.

As there is ample information available on the biology of the honey bee and history of its introduction, it should be possible to improve our understanding of evolutionary processes underlying the genetic structure of Africanized bee populations. New information concerning other genetic markers and populations could be used to test the assumptions presented here.

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Résumé — Estimation de la différenciation génétique de populations d'abeilles africanisées du Brésil et d'Amérique centrale par l'analyse des isozymes. Les loci de 7 enzymes au moins ont été étudiés par électrophorèse sur des échantillons d'abeilles africanisées provenant de 9 lieux différents du Brésil (188 colonies) et d'Amérique centrale (Honduras : 19 colonies, Costa Rica : 29 colonies) et sur des échantillons d'abeilles européennes (*Apis mellifera ligustica* d'Italie : 15 colonies et *Apis mellifera carnica* d'Allemagne fédérale : 36 colonies).

L'électrophorèse a été réalisée sur des homogénats bruts et individuels d'ouvrières adultes. Huit ouvrières ont été analysées par colonie. Neuf systèmes enzymatiques ont été testés par électrophorèse sur gel d'amidon : estérase (Est), phosphoglucomutase (PGM), hexokinase (HK), superoxide dismutase (SOD), malate déshydrogénase (MDH), enzyme malique (ME), aconitase (ACON), α-glycérophosphate déshydrogénase (α-GDPH) et leucine aminopeptidase (LAP), mais tous n'ont pas été analysés dans toutes les populations. Deux systèmes tampons ont été utilisés : tris-EDTA-maléate-magnésium (pH 7,4) et tris-citrate (pH 8,0). La préparation du gel et de l'échantillon et les conditions expérimentales ont été décrites dans des articles précédents (Del Lama *et al,* 1985; Del Lama *et al,* 1988).

Cinq loci ont présenté un polymorphisme (Est-1, Est-3, PGM-1, HK-1 et MDH-1) chez les populations d'abeilles africanisées du Brésil et d'Amérique centrale. On n'a pas observé de variabilité génétique pour Est-1 et HK-1 dans les échantillons d'abeilles italiennes et carnoliennes, ni pour PGM-1 dans les échantillons d'italiennes. L'observation la plus importante de cette étude en ce qui concerne l'abeille africanisée est que les allèles de MDH et d'HK les plus fréquents chez les races européennes présentent des fréquences légèrement plus élevées dans les échantillons d'abeilles africanisées d'Amérique centrale et du sud du Brésil que dans ceux du nord et du sud-est du Brésil. Cette tendance provoque la séparation de ces 2 groupes de populations en nuages différents lors de l'analyse de similarité génétique et conduit à des estimations plus élevées de mélange des races européennes en Amérique centrale et dans le sud du Brésil.

abeille africanisée / variabilité génétique / différenciation génétique / fréquence allélique / isozyme

Zusammenfassung — Genetische Differenzierung durch Isozymanalyse von afrikanisierten Honigbienen aus Brasilien und Mittelamerika. Proben von afrikanisierten Honigbienen aus neun verschiedenen Orten in Brasilien (188 Völker) und aus zwei Ländern Mittelamerikas (Honduras [19 Völker], Costa Rica [29 Völker]) und Proben aus europäischen Bienenvölkern (*Apis mellifera ligustica* aus Italien [15 Völker] und *Apis mellifera carnica* aus Deutschland [36 Völker]) wurden an mindestens sieben Enzymloci elektrophoretisch untersucht.

Material und Methoden. Die Elektrophorese wurde an Homogenisaten von einzelnen Arbeitsbienen durchgeführt. Es wurden 8 Arbeiterinnen pro Volk analysiert. Neun enzymatische Systeme wurden mit horizontaler Stärkegelelektrophorese getestet: Esterase, Phosphoglucomutase, Hexokinase, Superoxid Dismutase, Malatdehydrogenase, Malic Enzym, Aconitase, α-Glycerophosphat-Dehydrogenase und Leucin-Aminopeptidase. Jedoch wurden nicht alle Populationen auf alle Enzymsysteme analysiert. Zwei Puffersysteme wurden benutzt: Tris-EDTA-Maleat-Magnesium, pH 7,4 und Tris-Citrat, pH 8,0. Das Gel und die Aufbereitung der Proben sowie die experimentellen Bedingungen wurden bereits früher beschrieben (Del Lama *et al,* 1985; Del Lama *et al,* 1988).

Erbebnisse und Diskussion. An 5 Stellen (Est-1, Est-3, PGM-1, HK-1 und MDH-1) wurde Polymorphismus in der afrikanisierten Bienenpopulation aus Brasilien und Mittelamerika entdeckt. Est-1 und HK-1 zeigten jedoch in den Bienenproben aus Italien und Deutschland und PGM-1 in den italienischen Proben keine genetische Variation.

Die wichtigste Erkenntnis unserer gegenwärtigen Analyse der afrikanisierten Bienenpopulationen von Brasilien und Mittelamerika war, daß die in europäischen Bienenrassen am häufigsten vorkommenden Allele von MDH und HK etwas höhere Frequenzen in den Proben von Mittelamerika und Südbrasilien als in den Proben von Nord- und Südostbrasilien haben. Diese Tendenz verursacht bei einer Analyse der genetischen Ähnlichkeit die Trennung der beiden Populationen in verschiedene Cluster und führt zu höheren Schätzungen für die Durchmischung mit europäischen Rassen in Mittelamerika und Südbrasilien.

afrikanisierte Honigbiene / genetische Variation / genetische Differenzierung / Allelfrequenz / Isozyme

REFERENCES

Badino G, Celebrano G, Manino A (1983) Population structure and MDH-1 locus variation in *Apis mellifera ligustica. J Hered* 74, 443-446

Badino G, Celebrano G, Manino A (1984) Population genetics of Italian honeybee (*Apis mellifera ligustica* Spin) and its relationships with neighbouring subspecies. *Boll Mus Reg Sci Nat Torino* 2, 571-584

Badino G, Celebrano G, Manino A, Longo S (1985) Enzyme polymorphism in the Sicilian honeybee. *Experientia* 41, 752-754

Cavalli-Sforza LL, Bodmer WF (1971) *The Genetics of Human Populations.* WF Freeman, San Francisco

Contel EPB, Mestriner MA, Martins E (1977) Genetic control and developmental expression of malate dehydrogenase in *Apis mellifera. Biochem Genet* 15, 859-875

Cornuet JM (1986) Population genetics. *In: Bee Genetics and Breeding* (Rinderer TE, ed) Academic Press, Orlando, FL, 235-253

Daly MV, Balling SS (1978) Identification of Africanized honey bees in the western hemi-

sphere by discriminant analysis. *J Kansas Entomol Soc* 51, 857-869

Del Lama MA, Mestriner MA, Paiva JCA (1985) Est-5 and PGM: new polymorphisms in *Apis mellifera*. *Rev Bras Genet* 8(1), 17-27

Del Lama MA, Figueiredo RA, Soares AEE, Del Lama SN (1988) Hexoquinase polymorphism in *Apis mellifera* and its use for Africanized honeybee identification. *Rev Bras Genet* 11 (2), 287-297

Detter JC, Ways PO, Giblett ER, Baughan MA, Hopkinson DA, Povey S, Harris H (1968) Inherited variations in human phosphohexose isomerase. *Ann Hum Genet* 31, 329-338

Goncalves LS, Kerr WE, Chaud-Neto J, Stort AC (1972) Relatorio final do grupo americano sobre a abelha africana. Anais do 2 Congresso Brasileiro de Apicultura. Sete Lagoas, Minas Gerais, Brazil

Hall HG (1988) Characterization of the African honey bee genotype by DNA restriction fragments. *In: Africanized Honey Bees and Bee Mites* (Needham GR, Page Jr RE, Delfinado-Baker M, Brown CE, eds), Ellis Horwood, Chichester, 287-293

Hall HG, Muralidharan K (1989) Evidence from mitochondrial DNA that African honey bees spread as continuous maternal lineages. *Nature* 339, 211-213

Harris H, Hopkinson DA (1976) *Handbook of Enzyme Electrophoresis in Human Genetics*. North Holland Co, Amsterdam American Elsevier, NY

Hartl DL (1972) A fundamental theorem of natural selection for sex linkage or arrhenotoky. *Am Nat* 106, 516-524

Kerr WE, Bueno D (1970) Natural crossing between *Apis mellifera adansonii* and *Apis mellifera ligustica*. *Evolution* 24, 145-148

Kerr WE, Del Rio SL, Barrionuevo MD (1982) Distribuição da abelha africanizada em seus limites ao sul. *Cienc Cult* 34, 1439-1442

Krieger H, Morton NE, Mi MP, Azevedo E, Freire-Maia A, Yasuda N (1965) Racial admixture in north-eastern Brazil. *Ann Hum Genet* 29, 113-125

Lobo JA (1986) Diferenciação genética e mistura racial em populações de abelha africanizada: sistemas isozímicos e dados morfométricos. University of São Paulo, Brazil, MSc dissertation

Lobo JA, Del Lama MA, Mestriner MA (1989) Population differentiation and racial admixture in the Africanized honeybee (*Apis mellifera* L). *Evolution* 43, 794-802

Michener CD (1975) The Brazilian bee problem. *Annu Rev Entomol* 20, 399-416

Nei M (1973) Analysis of gene diversity in subdivided populations. *Proc Natl Acad Sci USA* 70, 3321-3323

Nogueira-Neto P (1972) Notas sobre a història da apicultura brasileira. *In: Manual de Apicultura* (Camargo JMF, ed) Agronomica Ceres, São Paulo, Brazil, 17-32

Nunamaker RA, Wilson WT (1981) Comparison of MDH allozyme patterns in the African honey bee *(Apis mellifera adansonii)* and the Africanized populations of Brazil. *J Kansas Entomol Soc* 54, 705-710

Nunamaker RA, Wilson WT, Haley BE (1984) Electrophoretic detection of africanized honey bees *(Apis mellifera scutellata)* in Guatemala and Mexico based on malate dehydrogenase allozyme patterns. *J Kans Entomol Soc* 57, 662-631

Ruttner F (1981) Taxonomia de las abejas melíferas de Africa Tropical. XXVII International Congress of Apiculture. *Apimondia*, Acapulco, Mexico

Ruttner F (1986) Geographical variability and classification. *In: Bee Genetics and Breeding* (Rinderer TE, ed), Academic Press, Orlando, FL, 23-55

Sheppard WS, Berlocher SH (1985) New allozyme variability in Italian honeybees. *J Hered* 76, 45-48

Sheppard WS, McPheron BA (1986) Genetic variation in honey bees from an area of racial hybridization in western Czechoslovakia. *Apidologie* 17, 21-32

Smith DR, Taylor OR, Brown WM (1989) Neotropical Africanized honey bees have African mitochondrial DNA. *Nature* 339, 213-215

Spencer N, Hopkinson DA, Harris H (1964) Phosphoglucomutase polymorphism in man. *Nature* 204, 742-745