EVIDENCE FOR GENETIC DIFFERENCES IN THE AFRICANIZED HONEY BEE POPULATIONS OF SOUTH AND NORTH AMERICA

By

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To the Faculty of Washington State University:

The members of the Committee appointed to examine the thesis of FATIMAH ALHAMLAN find it satisfactory and recommend that it be accepted

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Chair

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DEDICATION

I would like to dedicate this work to my husband, Abdullah Assiri, for his understanding and assistance. His support and patience during the past two years did not go unnoticed or unappreciated. Without his strength and encouragement, I know I could not have accomplished this. Abdullah managed to hold our household together as I pursued the Master degree. He took on the job of housekeeper, cook and caregiver for our daughter in addition to his own busy schedule. His kindness, generosity, responsibility, and love were an essential part of this project. He is my partner and soul mate and his love and encouragement gave me the stamina to withstand the pressures and stress of graduate school. Thank you, Abdullah.

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Abstract

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The arrival of Africanized honey bee *Apis mellifera* to the United States less than fifty years after its original release in Brazil is an interesting phenomenon. To better understand the population genetic aspects of this phenomenon, genetic variability and differentiation among Africanized honey bee populations were investigated. Mitochondrial and microsatellite DNA markers were used to compare the genetic composition of several populations of Africanized honey bees from Brazil, Central America, and Texas. Using mitochondrial DNA markers, three haplotypes were identified. One haplotype was European (C1) and the others were African (A1 and A4). A major shift in the frequencies of African mitochondrial DNA haplotypes A1 and A4 was detected across the range of Africanized honey bees. The A1 haplotype increased northward to Texas and largely replaced the A4 haplotype. The direction of the clinal distribution of A1-A4 in South America and perhaps even Africa appears opposite to the findings of the present study. It was reported that A1 was predominant in the more equatorial regions of Africa and South America, while the frequency of A4 increases in

the more temperate (southern region). Selection along a temperature/climate gradient has been suggested as the reason for an observed A1-A4 cline in South America. However, in our study, the best explanation for the A1 northward incline would be selection for some geneotypes. The microsatellite DNA analysis revealed that the three populations displayed high genetic diversity with an average of expected heterozygosity per locus 0.82. Furthermore, private alleles found in the Texas population suggest ongoing hybridization with non-Africanized honey bees.

TABLE OF CONTENTS

Page

Introduction

The Old World honey bee, *Apis mellifera*, has been classified into 26 subspecies, and four evolutionary lineages based on morphological characters (Ruttner 1988; Sheppard 1997; Engel 1999; Sheppard & Meixner 2003). The evolutionary lineages are supported by data from molecular studies conducted with mitochondrial DNA (Smith 1991b; Cornuet *et al*. 1991a; Cornuet & Garnery 1991b; Garnery *et al*. 1992; Garnery *et al*. 1993; Arias & Sheppard. 1996; Garnery *et al*. 1998a), allozymes (Del Lama *et al*. 1988; Lobo *et al*. 1989; Sheppard *et al*. 1991b) and microsatellite markers (Estoup *et al*. 1995; Garnery *et al*. 1998b; Franck *et al*. 2001; De la Rua *et al*. 2001; Solignac *et al*. 2003). The lineages include: M, corresponding to western European subspecies; C, that includes subspecies from southeastern Europe; A, corresponding to African subspecies and O, corresponding to subspecies from western and central Asia (Ruttner 1988; Meixner *et al*. 1994; Sheppard & Meixner 2003). Historically, a number of honey bee subspecies from various lineages were introduced into the Americas for beekeeping. The New World descendents of one subspecies imported from Africa have been particularly newsworthy and are commonly known as Africanized honey bees. Africanized honey bees (AHB) are descended from African queen bees released in the mid 1950's in southeastern Brazil from hives populated by the subspecies *A. m. scutellata* (Kerr 1967; Kerr & Bueno 1970). The subspecies *A. m. scutellata* (Lepeletier) is highly adapted to tropical conditions (Taylor 1985). The purpose of its introduction to South America was to cross-breed it with imported European bees already present in Brazil to obtain honey bees better adapted to tropical conditions with the hope of increasing honey production.

After their introduction, AHB spread rapidly through Brazil at a rate of approximately 350 km/year (Lobo *at al*. 1989). In 1990, the leading edge of the Africanized honey bee population was detected in Texas (Sugden and Williams 1990). By 1999, Africanized honey bees were established in four states: Arizona, California, Nevada, and New Mexico (Sheppard *et al*. 1999, Boyce *et al*. 2002), and in 2005, Africanized honey bees were discovered in Florida (Sanford & Hall 2005).

For several decades, the northward expansion of Africanized honey bees was extensively studied using morphological, behavioral, and biochemical methods. Studies of neotropical feral populations suggested that Africanized honey bees expanded primarily by maternal migration (Taylor 1985; Hall & Muralidharan 1989; Smith *et al*. 1989; Hall & Smith 1991b; Smith 1991a; Hall& McMichael 2001). Therefore, hybridization between African and European lineage honey bees in tropical South America most likely resulted from paternal (drone) contributions (Del Lama *et al*. 1988; Lobo *et al* 1989; Del Lama *et al*. 1990; Sheppard *et al*. 1991a). Moreover, microsatellite DNA (Franck *et al*. 1998; Clarke *et al*. 2002) showed unequal introgression of nuclear European and African genes in hybrid populations. In nesting biology and behavior, AHB populations within tropical areas remained essentially African (Schneider *et al*. 2004).

Although Africanized honey bees expanded their range to occupy a large portion of South America, they have not successfully colonized latitudes further south than 35º (Diniz *et al*. 2003). Apparently, there has been a relatively stable distribution of Africanized honey bee populations in Argentina since the mid 1970's (Kerr *et al*. 1982; Sheppard *et al*. 1991b; Diniz *et al*. 2003). Temperate climates appear to represent a

natural limit to the expansion of Africanized honey bee populations (Rinderer *et al*. 1993b). In early 1990, the Rinderer group studied the hybridization process between European and Africanized honey bees in the neotropical Yucatan peninsula, where the invading front of AHB encountered large populations of European honey bees. Thirty percent of the colonies in Yucatan peninsula displayed African-derived mitochondrial DNA, which suggested that introgressive hybridization had occurred (Rinderer *et al*. 1991). Twelve years after the arrival of Africanized honey bees in Yucatan peninsula, the percentage of the African-derived mitochondrial DNA increased to 87% indicating a substantial gene flow from Africanized honey bee queens to the European colonies (Clarke et al. 2002). A recent study by Pinto *et al*. (2004) reported the replacement of a feral European honey bee population by Africanized honey bees in southern Texas over an 11 year period. The study found a situation similar to that on the Yucatan peninsula, due to the invasion of AHB populations. By 2001, 90% of the feral colonies in the study site were composed of African-derived mitochondrial DNA, supporting the hypothesis that AHB queens replaced most of the wild European honey bee queens. However, interpreting the Texas results was complicated due to different factors. The first factor was the presence of feral European honey bee colonies that allowed the hybridization to take place. The second factor was the presence of the parasitic mite V*arroa destructor* (Anderson and Trueman) that caused an asymmetrical loss, primarily of European honey bee colonies (Pinto *et al*. 2004).

The hybrid zone in North America has not been fully studied during the AHB invasion process. In this research, I characterized the genetic composition of several populations of Africanized honey bees from Brazil to the southern United States using

molecular approaches. These data were used to address alternative hypotheses concerning the AHB expansion: 1) AHB populations that reached Texas have a higher proportion of African ancestry than AHB population in Brazil ("migrant front" hypothesis) or, 2) Texas AHB has a higher proportion of European ancestry ("hybridization" hypothesis).

The "migrant front" hypothesis suggests that the most highly African-like bees could be found in the expanding "front" populations. The bees along this portion of the range would have expanded without extensive hybridization with European honey bees. As a result, AHB populations that reached Texas could contain a higher proportion of African genes than older established AHB populations. The hybridization hypothesis suggests that genetic mixing between African and European bees occurred during the expansion process. Therefore, the bees that reached Texas should have a higher proportion of European hone bee genes than the established AHB populations in Brazil.

Materials and Methods

Samples and DNA extraction

A total of 232 colonies of *Apis mellifera* from presumptive AHB populations were collected between 1991 and 1993 from different regions of Brazil, Central America, and Texas. There were 178 colonies from Brazil divided as follow: (92 colonies from Sao Paulo, 11 colonies from Rio Do Sul, 11 colonies from Vicosa, 23 colonies from Brasilia, 22 colonies from Bahia and 19 colonies from Pernambuco). There were 20 colonies from Central America 10 from Honduras and 10 from Costa Rica. Finally, there were 44 colonies of AHB from Texas. Collecting locations are reported in Table 1. The Texas

samples were collected between 1991 and 1993, and were determined to be "Africanized" using the full morphometric protocol developed for this purpose (USDA-ID) (Rinderer *et a*l. 1993a). The bees were maintained at -80 ºC or in 70% ethanol until they were processed in the laboratory. Total DNA was extracted from single bee legs from each sample in 150µl 10% Chelex and 5µl proteinase K (10 mg/ml) (modified from Walsh *et al*. 1991). The legs and the extraction solution were incubated for 1 hour at 55 °C, 15 minutes at 99 °C, 1 minute at 37 °C and 15 minutes at 99 °C. Extracted DNA was stored at -80 °C until use in mtDNA restriction fragment length polymorphism (RFLP) and microsatellite analysis.

Mitochondrial DNA Restriction Fragment Length Polymorphism (RFLP)

Oligonucleotide primers E2 and H2 and published amplification procedures (Cornuet *et al*. 1991a) were used to PCR-amplify the COI-COII intergenic region of the honey bee mitochondrial DNA. To determine total PCR product size, a fraction of the amplified products was run on 1% agarose gel electrophoresis. Polymorphisms were scored for different lengths as previously described; Q (200 bp), PQ (250 bp), PQQ (450 bp), and PQQQ (650 bp) (Garnery *et al*. 1993). A 20µl aliquot from each amplification was digested with 5 units of *Dra*I restriction enzyme (Promega, Madison, WI) at 37 °C for 6 hours. Digested fragments were separated by electrophoresis on a 10% polyacrylamide gel and DNA fragments were visualized under UV light after staining with ethidium bromide. Haplotypes A, C and M refer to the African, west European and north Mediterranean mitochondrial lineages, respectively. Resulting haplotypes were scored according to Garnery *et al*. (1993).

Subspecies belonging to the C and O evolutionary lineages were characterized as mitochondrial haplotype using *Dra*I restriction enzyme methodology (Granery *et al*. 1993).

Microsatellite DNA analysis

Ten polymorphic microsatellite loci were analyzed in this study: A7, A24, A28, A88, A113, B124 (Estoup *et al*. 1995), Ap43, (Garnery *et al*. 1998b), Ap55, Ap66, and Ap81 (Garnery *et al*. unpublished data) . The amplifications were carried out using two multiplex reactions. Extracted DNA was amplified by PCR in 10 µl reactions containing 1 µl extracted DNA, 1X reaction buffer (Promega, Madison WI), 3 mM dNTP mixture, 1.0- 4.0 mM labeled primers, 0.001mg bovine serum albumin (BSA), and 1.5 units of Taq polymerase (Promega, Madison WI). The concentration of $MgCl_2$ was adjusted to 1.5 mM for loci A24, 128, A88, Ap66, and B124 and 1.2 mM for loci A7, A113, Ap43, Ap55, and Ap81. The reaction conditions for the PCR were: one 7 minutes cycle at 95 °C, 30 cycles of 95 °C for 30 seconds, 54 °C for 30 seconds, 72 °C for 30 seconds and 60 minutes cycle at 72 °C (Kandemir *et al*. 2006). The locus-specific primers were fluorescently labeled and the products were separated and sized using an Applied Biosystems ABI 3730 automated DNA sequencer. The resulting electrophorograms were scored using GeneMapper™ software. The genetic diversity among our samples was assessed by using the GENETIX software package (Belkhir *et al*. 2001). The allele numbers, allele frequencies, and the expected and observed heterozygosity at each microsatellite locus per population were calculated. The GENEPOP program version 3.4 (Raymond & Rousset 1995) was used to calculate the departure from Hardy-Weinberg

equilibrium (HWE), test the linkage disequilibrium (LD), calculate the number of migrants using private alleles (Nm), and the genetic differentiation among the populations (F_{ST}).

Results and Discussion

MtDNA data

Among the 242 analyzed colonies, three previously reported mitochondrial haplotypes were observed, A1, A4 and C1 (Garnery *et al*. 1993). The haplotypes A1 and A4 originate from the African evolutionary lineage and C1 belongs to the southeastern European lineage. The haplotype frequencies found in Brazil were as follows: Sao Paulo, A4 (98%), A1 (1%), C1 (1%); Rio Du Sol, A4 (91%), A1 (9%); Vicosa, A4 (91%), A1 (9%); Brasilia, A4 (70%), A1 (26%), and C1 (4%); Bahia, A4 (72%), A1 (23%), and C1 (5%); and Pernambuco, A4 (68%), A1 (21%), and C1 (11%). The haplotype frequencies found in Central America were as follows: Costa Rica A4 (70%), A1 (20%), C1 (10%); Honduras A4 (40%), A1 (40%), C1 (20%). The Texas samples yielded haplotype frequencies A4 (23%) and A1 (77%) (Table 1). The frequency of haplotype A4 declined from Brazil to Texas. The A4 haplotype, predominant in Brazil and Central America, occurred in a frequency of only 23% in Texas, while the A1 haplotype increased to 77%. The frequency distributions of the three haplotypes are given in Figure 1.

The most obvious change between the samples from South America and Texas was the major frequency shift in the two African mitochondrial haplotypes. The shift occurred gradually starting from Rio Du Sol (South) through Sao Paulo (South East) through the North regions of Brazil including Vicosa, Bahia, and Pernambuco through

Central America to Texas (Figure 1). There are several possible explanations for this change in frequency from Brazil to Texas. One of these could be the simple effects of genetic drift due to repeated bottlenecks as the AHB moved northward from Brazil. Alternatively, the haplotype A1 could be linked to a migrant-prone genotype, consistent with the migrant front hypothesis. Selection along a temperature/climate gradient has been suggested as the reason for an observed A1-A4 haplotype cline in South America (Collet *et al*. 2006). However, the direction of the clinal distribution of A1-A4 in South America and perhaps even Africa (Moritz *et al*.1994; Garnery *et al*. 1995; Franck *et al*. 2001) appears to be opposite to that found in the present study. It was reported that A1 was predominant in the more equatorial regions of Africa and South America (Collet *et al*. 2006), while the frequency of A4 increased in the more temperate (southern) region. In our study, the frequency of A1 was highest in Texas, the most temperate area sampled and higher than has been reported for any Brazilian populations (Collet *et al*. 2006). The linkage with a migrant-prone genotype cannot be ruled out, although there is an ample evidence of subsequent hybridization in the Texas population.

Microsatellite DNA

The allele numbers, allele frequencies, and the expected and observed heterozygosity at each microsatellite locus per population were calculated using GENETIX software. As shown in Table 3, the allelic variability of the ten loci ranged from 24 alleles (locus Ap66) to 8 alleles (locus A24) with an average of 13.9 alleles per locus. The total number of alleles detected in Brazil for the 10 loci was 162, and for Texas it was 146. Only 106 alleles were found in Central America, although this was not comparable to Brazil and Texas due to the smaller number of colonies sampled. The

allele frequencies for the ten loci are detailed in Table 2. Overall, the average expected heterozygosity per locus as a measure for gene diversity was high across all populations (D= 0.82). Unbiased estimates of Hardy-Weinberg exact (HWE) test were conducted on 5 loci that yielded non-0 values in the preliminary analysis of the three populations. All Brazilian locations, with the exception of Brasilia, were in Hardy-Weinberg equilibrium. This was expected since the Brazilian population has been Africanized for more that 40 years. However, the Texas and Central American populations deviated from Hardy-Weinberg equilibrium.

The genetic differentiation among the three populations was low (F_{ST} = 0.014). Based on a pairwise F_{ST} test, the genetic differentiation between Brazil and Texas was higher (F_{ST} = 0.020) than the genetic differentiation between Brazil and Central America (F_{ST} = 0.004), probably as a result of increased admixture within the Texas population. In the Brazilian populations, the pairwise F_{ST} test revealed significant differences between populations from southern and northern Brazil (Table 4). For instance, the highest F_{ST} value (F_{ST} = 0.09) was detected between Sao Paulo (South East Brazil) and Pernabmuco (North Brazil), followed by a F_{ST} value of 0 .07 between Sao Paulo (South East Brazil) and Rio Do Soul (south Brazil).

F_{IS} (inbreeding coefficient), and linkage disequilibrium (pairwise association among loci) gave us valuable information about the mating patterns and rates of admixture. The F_{IS} value between the Brazilian population and Texas was (- 0.04) indicating a low level of inbreeding. By calculating the average of F_{IS} among Brazilian samples alone, the value was 0.08. Multiple probability test by pair of loci across all

populations (Fisher's method) detected significant linkage disequilibrium between loci A88 and A113 (P= 0.05), and between loci A113 and Ap43 (P= 0.05).

To estimate the gene flow among the three populations, Nm (N is the effective population size, and m is the proportion of migrants/generation of the total population) was calculated with the GENEPOP program. For all three populations, the Nm value was 3.11. However, by calculating the Nm value for Brazilian populations only, the value was high (Nm= 4.62). This result is unsurprising, because the gene flow between populations is limited compared to the gene flow within a population.

The total number of alleles in the Brazilian populations was higher than the Texas population with 162 and 148 alleles, respectively (Table 3). Of the 162 alleles found in Brazil, 108 were found in Texas. However, 33 private alleles that were found in the Brazilian Africanized honey bee populations. In addition, the Texas population contained 31 private alleles. In Central America, 7 private alleles were detected. The loss of some alleles as a result of successive bottlenecks associated with range expansion and the detection of new alleles in the Texas AHB populations, supports a strong role for hybridization in defining the AHB population, at least near its climatic limits in North America. Stable introgression of European alleles into an AHB population has been reported from the Yucatan peninsula (Clarke *et al*. 2002). These authors concluded that, compared to the AHB of Brazil, the Yucatan AHB population was the result of additional hybridization with European-derived honey bees.

Conclusion

The ability of Africanized honey bee populations to retain largely African behavioral traits is an interesting phenomenon that occurred throughout much of the range of the honey bee in South America. This study showed that the genetic make up of three AHB populations was composed mainly of African-derived mitochondrial DNA. However, the unique pattern of A1-A4 mtDNA haplotypes and the loss of microsatellite alleles in Texas that originated in Brazil and the gain of microsatellite alleles in the population in North America suggest that successive bottlenecks and hybridization have been important in determining the genetic composition of AHB populations in North America. Furthermore, the large number of private alleles that detected in Texas supports the existence of an ongoing hybridization of AHB populations near their temperate climatic limits.

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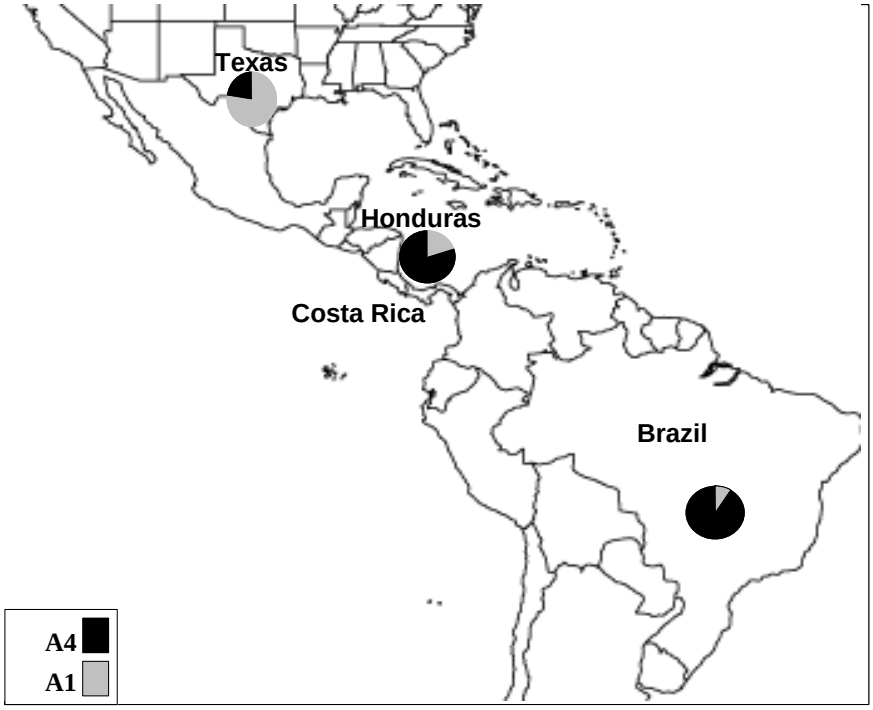

Fig. 1 The distribution of the African mitochondrial DNA lineage (A1 & A4). Colors indicate relative proportions of each mitochondrial haplotype for colonies with African maternal lineages.

Fig. 2 The distribution of the African and European mitochondrial DNA lineages (A4 & A1 and C1) among Brazilian populations.

Table 1. The origin of the samples and the number of haplotypes that were detected in each population.

Country	Locality	A4	A1	C1	Total
Brazil	Sao Paulo (MTI + Br+ LA)	90	1	1	92
	Rio Do Sul	10	1	0	11
	Minas Gerais (Vicosa)	10	1	0	11
	Goias (Brasilia)	16	6	1	23
	Bahia	16	5	1	22
	Pernambuco	13	4	2	19
Costa Rica		7	2	1	10
Honduras		4	4	2	10
Texas		12	32	0	44

Table 2. Allelic frequencies, expected heterozygosity (H exp.), and observed heterozygosity (H obs.) of honey bee populations. N is the number of samples. Bold cells represent the private alleles.

Locus A24 alleles	Brazil n= 170	Central America n= 31	Texas n= 183
90	0.003	0.016	0.003
92	0.103	0.097	0.030
94	0.332	0.306	0.481
96	0.023	0.048	
98	0.112	0.081	0.125
100	0.106	0.145	0.060
102	0.064	0.048	0.065
104	0.020	0.161	0.163
106	0.017	0.080	0.008
108	0.005		0.005
124	0.002	0.016	
128	**0.008**		

	132	0.014		0.054
	138			**0.002**
H exp.		0.807	0.831	0.714
H obs.		0.864	0.774	0.7374

Locus A28	alleles	Brazil n= 169	Central America n= 29	Texas n= 136
	102	0.003		0.007
	104	0.006		0.007
	118			**0.004**
	122	0.003		0.011
	124	0.213	0.224	0.279
	126	0.109	0.086	0.055
	128	0.275	0.293	0.194
	130	0.017	0.034	0.011
	132	0.269	0.206	0.250
	134	0.059	0.069	0.033
	135	0.005	0.017	0.007
	136	0.014	0.069	0.058
	138	0.023		0.077
	150			**0.003**
H exp.		0.790	0.802	0.808
H obs.		0.828	0.586	0.853

Locus A88	alleles	Brazil n= 170	Central America n= 29	Texas n= 158
	128			**0.006**
	132	0.002		0.034
	134	**0.017**		
	136	0.132	0.120	0.047
	138	0.326	0.310	0.395
	140	0.008	0.082	0.003
	142	0.017	0.034	0.038
	143	**0.005**		
	144	0.029	0.017	0.022
	145	0.002		0.006
	146	0.091	0.086	0.123
	148	0.176	0.104	0.180
	150	0.044	0.120	0.047
	152	0.141	0.120	0.094
	154	**0.002**		
H exp.		0.813	0.833	0.780
H obs.		0.859	0.827	0.854

Locus Ap66	alleles	Brazil n= 113	Central America n= 21	Texas n=97

90			**0.010**
92	0.017	0.023	
93	0.110	0.214	0.293
94	0.287	0.195	
95	0.048	0.047	0.144
97	0.031	0.071	0.097
98	0.070	0.119	0.005
100	0.208	0.142	0.221
101			**0.005**
102	0.031	0.023	0.010
103	0.017	0.023	0.015
104	0.097	0.023	0.030
105			**0.005**
106		0.008	0.010
107		0.005	0.004
108	0.035	0.047	0.046
113			**0.020**
115			**0.005**
122	0.008	0.047	0.020
128		0.013	0.010
129			**0.005**
130			**0.005**
132		0.004	0.005
133			**0.010**
135			**0.005**
138	0.004	0.023	0.010
H exp.	0.841	0.868	0.830
H obs.	0.637	0.428	0.618

Locus B124 alleles	Brazil n= 165	Central America n= 28	Texas n=127
204		**0.017**	
206			**0.007**
212	0.003		0.011
214	0.027	0.035	0.031
216	0.066	0.107	0.082
218	0.097	0.125	0.063
220	0.149	0.221	0.178
222	0.181	0.078	0.071
224	0.127	0.017	0.169
226	0.063	0.084	0.089
228	0.012		0.007
230	**0.018**		
232		0.033	0.007
234	0.060	0.107	0.023
236	0.136	0.178	0.173
238	0.003	0.035	
240	0.003		0.011
242	0.024	0.017	0.011
244	0.003	0.017	
246			**0.003**

H exp.	0.882	0.880	0.870
Hobs.	0.927	0.857	0.890

Locus A7 alleles	Brazil n= 131	Central America n= 21	Texas n=160
92	0.026	0.095	
94	0.126	0.071	0.528
96	0.095	0.003	
98	0.106	0.047	0.121
100	**0.019**		
102	0.022	0.142	0.009
104	0.137	0.095	0.065
106	0.137	0.166	0.078
108	0.251	0.142	0.101
110	0.087	0.071	0.034
112	0.003		0.031
114	0.045		0.009
116		0.023	0.003
118			**0.003**
122			**0.009**
124	**0.003**		
126	0.003	0.047	
130	**0.011**		
134	**0.007**		
148	**0.003**		
226	**0.003**		
H exp.	0.860	0.890	0.683
H obs.	0.557	0.571	0.350

Locus A113 alleles	Brazil n= 137	Central America n= 21	Texas n=84
200		**0.095**	
202	0.062	0.119	0.119
204	0.010		0.006
206	0.003		0.041
208	0.025	0.047	0.083
210	0.025	0.047	
212	0.124	0.071	0.035
214	0.120	0.023	0.154
216	0.219	0.261	0.148
218	0.058	0.023	0.065
220	0.200	0.214	0.244
222	0.032	0.023	0.065
224	0.080		0.006
226	0.032		0.029
230		**0.023**	
232	**0.003**		
250		**0.047**	

H exp.	0.864	0.848	0.861
H obs.	0.861	0.810	0.762

Locus Ap43	alleles	Brazil n= 103	Central America n= 13	Texas n=44
	122	**0.024**		
	127	**0.009**		
	131	0.393	0.153	0.079
	133	0.082	0.153	0.272
	134	0.121	0.076	
	135	0.150	0.076	0.159
	136	0.009	0.076	
	137	**0.019**		
	139	0.014		0.045
	140	0.009		0.038
	141	**0.019**		
	145	0.019	0.192	
	146	**0.009**		
	147	0.009		0.090
	149			**0.011**
	153	**0.009**		
	156			**0.011**
	157	**0.009**		
	160	0.115		0.011
	161	0.014		0.272
	167			**0.011**
	169	0.019		0.034
	171	**0.019**		
	173	0.034		0.038
	212		**0.076**	
H exp.		0.796	0.875	0.808
H obs.		0.301	0.230	0.296

Locus Ap55	alleles	Brazil n= 119	Central America n= 19	Texas n=58
	152	**0.013**		
	164	**0.004**		
	167	0.113	0.105	0.172
	169	0.004		0.052
	170	**0.008**		
	171	0.096	0.105	0.060
	173	0.054	0.026	0.164
	174	**0.004**		
	175	0.319	0.289	0.129
	177	0.067	0.026	0.026

178			**0.017**
179	0.193	0.078	0.181
180	**0.004**		
181	0.063	0.236	0.155
183	0.025	0.026	0.008
186			**0.017**
190	**0.004**		
194			**0.017**
196	0.016	0.105	
199	**0.008**		
H exp.	0.826	0.818	0.861
H obs.	0.663	0.737	0.621

Locus Ap81	alleles	Brazil n= 119	Central America n= 16	Texas n=175
	106			**0.005**
	108	0.071	0.125	0.057
	112			**0.002**
	114			**0.602**
	121			**0.017**
	122			**0.002**
	123			**0.005**
	124		**0.031**	
	125	0.088	0.062	0.045
	127	0.197	0.065	0.054
	128		**0.033**	
	129	0.088	0.098	0.022
	131	0.012		0.002
	133	0.075	0.218	0.068
	134	0.121		0.008
	135	0.189	0.156	0.045
	136	0.004		0.011
	137	0.021		0.005
	138	0.046	0.156	0.017
	139	**0.004**		
	141	**0.004**		
	143	**0.004**		
	145	0.029		0.005
	147	**0.004**		
	149	0.008		0.031
	150	**0.008**		
	152	0.012	0.031	0.005
	154			**0.002**
	156			0.005
	158	**0.004**		
	162			**0.002**
H exp.		0.880	0.867	0.620
H obs.		0.714	0.937	0.417

Table 3. Number of alleles detected in the three populations, and the expected and observed heterozygosities. Numbers in brackets are the sample size.

Locus/ alleles	Brazil	Central America	Texas
A24	(170)	(31)	(183)
Alleles	13	10	11
Private alleles	1		1
H exp.	0.080	0.831	0.714
H obs.	0.864	0.774	0.737
A28	(169)	(29)	(136)
Alleles	12	8	14
Private alleles			2
H exp.	0.789	0.802	0.807
H obs	0.828	0.586	0.852
A88	(170)	(29)	(158)
Alleles	14	9	11
Private alleles	3		1
H exp.	0.812	0.832	0.779
H obs	0.858	0.827	0.854
Ap66	(113)	(21)	(97)
Alleles	17	13	24
Private alleles			9
H exp.	0.840	0.868	0.829
H obs	0.637	0.428	0.618
B124	(165)	(28)	(127)
Alleles	17	13	16
Private alleles	1	1	2
H exp.	0.882	0.880	0.869
H obs	0.927	0.857	0.889
A7	(131)	(21)	(160)
Alleles	17	11	13
Private alleles	6		2
H exp.	0.859	0.888	0.682
H obs	0.557	0.571	0.350
A113	(137)	(21)	(84)
Alleles	14	12	12
Private alleles	1	3	
H exp.	0.864	0.848	0.860
H obs	0.861	0.809	0.761
Ap43	(103)	(13)	(44)
Alleles	20	10	11
Private alleles	8	1	3
H exp.	0.796	0.875	0.807

H obs	0.301	0.230	0.295
Ap55	(119)	(19)	(58)
Alleles	17	12	12
Private alleles	7		3
H exp.	0.825	0.818	0.861
H obs	0.663	0.736	0.620
Ap81	(119)	(16)	(175)
Alleles	21	11	22
Private alleles	6	2	8
H exp.	0.880	0.867	0.619
H obs	0.714	0.937	0.417
Mean			
H exp	**0.836**	**0.851**	**0.783**
H obs	**0.721**	**0.676**	**0.639**
Alleles	**16.2**	**10.9**	**14.6**
Private alleles	**4.13**	**1.75**	**3.44**

Table 4 The pairwise F_{ST} comparison among Brazilian population. Bold cells show the highest F_{ST} values.

population	MTI	Br	Brasilia	Bahia	Pernabuco	Rio Du Soul
Br	0.007					
Brasilia	0.045	0.042				
Bahia	0.034	0.047	0.006			
Pernamuco	**0.071**	**0.092**	0.028	0.038		
Rio Du Soul	**0.058**	**0.074**	0.023	0.005	0.028	
Vicosa	0.039	0.048	0.012	0.006	0.021	0.019