Afro-Derived Amazonian Populations: Inferring Continental Ancestry and Population Substructure

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Abstract A panel of Ancestry Informative Markers (AIMs) was used to identify population substructure and estimate individual and overall interethnic admixture in 294 individuals from seven African-derived communities of the Brazilian Amazon. A panel of 48 biallelic markers, representing the insertion (IN) or the deletion (DEL) of small DNA fragments, was employed for this purpose. Overall interethnic admixture estimates showed high miscegenation with other ethnic groups in all populations (between 46% and 64%). The proportion of ancestral genes varied significantly among individuals of the sample: the contribution of African genes varied between 12% and 75%; of European genes between 10% and 73%; and of Amerindians genes between 8% and 66%. The obtained data reveal a high contribution of Amerindian genes in these communities, unlike in other African-derived communities of the Northeast and the South of Brazil. In addition, the majority of the Amerindian contribution may result from the preferential inclusion of indigenous women in the African descent groups. High heterogeneity of the proportion of interethnic admixture among analyzed individuals was found when the proportion of ancestral genes of each individual of the sample was estimated. This heterogeneity is reflected in the fact that four populations can be considered as substructured and that the global African descent sample is possibly formed by two subpopulations.

The Brazilian population is the product of the miscegenation of three main ethnic groups: Amerindians, Europeans, and Africans. It is estimated that approximately four million Africans were brought by force to Brazil as slaves between 1550 and 1870 (Klein 1986). The distinct geographical regions of Brazil received different numbers of African slaves, being greater in the Northeast and lower in the North (Santos and Guerreiro 1995). The arrival of African slaves to the Amazon began in the mid-17th century (1750). It is estimated that at least 53,000 Africans arrived in the Brazilian Amazon during the slavery period (Curtin 1996).

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Human Biology, October 2011, v. 83, no. 5, pp. 627–636.
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KEY WORDS: AMAZON, AIM, AFRICAN DESCENT, INDEL, POPULATION SUBSTRUCTURE.

These slaves frequently escaped into the forest because of the living conditions they experienced and they formed settlements known today as "*quilombos*" (Acevedo and Castro 1998). Initially, the fugitive slaves remained relatively genetically isolated when the *quilombos* were formed, but later they mixed with other population groups, including Amerindians and Europeans (Ribeiro-dos-Santos et al. 2002; Kiyoko et al. 2004).

In Brazil, various studies have been conducted on Afro-derived groups in an attempt to estimate the contribution of parental populations to the formation of these populations, using classic genetic polymorphisms (Bortolini et al. 1995; Arpini-Sampaio et al. 1999; Guerreiro et al. 1999), polymorphisms of nuclear DNA (Bortolini et al. 1999; Silva Jr. et al. 1999; Cayres-Vallinoto et al. 2003); mitochondrial DNA markers (Bortolini et al. 1999; Ribeiro-dos-Santos et al. 2002; Carvalho et al. 2008) and Y chromosome markers (Bortolini et al. 1999; Kiyoko et al. 2004).

These studies demonstrate that, depending on the genetic marker used, the contribution of African genes in different communities can vary greatly and that the contribution of European and Amerindian genes is significant (Schneider et al. 1987; Guerreiro et al. 1999; Ribeiro-dos-Santos et al. 2002; Carvalho et al. 2008), implying that population stratification may be present in all Afro-derived communities studied. Stratification occurs when a population is formed from a relatively recent admixture of subpopulations (European, African, and indigenous, for example) and when the proportion of this admixture varies between the individuals that make it up (Hoggart et al. 2003).

The types of genetic markers used in previous studies allow estimating only the global interethnic admixture of the population analyzed. More recently, a series of published studies have used panels of Ancestry Informative Markers (AIMs), that is, genetic polymorphisms that demonstrate significant differences ($>$40%) in the allele frequencies between populations of distinct geographical origins (Parra et al. 1998; Bastos-Rodrigues et al. 2006; Santos et al. 2010). This approach is much more accurate and gives reliable estimates of the proportion of ancestral genes in each individual of the population under study (Parra et al. 2001; Shriver et al. 2003; Bastos-Rodrigues et al. 2006; Benn-Torres et al. 2007; Santos et al. 2010).

In this study, a panel of 48 AIMs was used to estimate individual and global interethnic admixture in unrelated individuals in seven Afro-derived communities of the Brazilian Amazon. All of the markers used were INDEL that represent the insertion (IN) or the deletion (DEL) of small DNA fragments.

The markers were selected based on three main criteria: (1) great differences in allele frequencies ($\delta > 40\%$) between African, European, and/or Native American populations; (2) mapping to different chromosomes or to different physical regions of the same chromosome; and (3) variable size between 3 and 40 base pairs (bp) to permit simultaneous genotyping of multiple markers. The selection process was based on data from Weber et al. [2002] and from the online database at www.marshfieldclinic.org/mgs/.

This panel was previously used by Santos et al. (2010) and has been successfully used to assess parental genetic contribution in different populations (Ota et al. 2010; Tarazona-Santos et al. 2011).

The data obtained were also used to identify population substructure in different Afro-derived community as well as to estimate the genetic distances between them (F_{ST}), in an attempt to understand the history of the formation and expansion of these communities.

Materials and Methods

Populations Samples. This study was carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association. Blood samples of 294 unrelated individuals from seven Afro-derived communities of the Brazilian Amazon were collected under informed consent, with the following distribution: Pacoval (21 individuals), Pitimandeua (64), Marajó (48), Trombetas (40), Curiaú (43), Mazagão (45), and Pontal (33). The geographical coordinates of the communities studied are given in Figure 1.

To estimate the individual and global interethnic admixture in the samples, previously obtained data (Santos et al. 2010) relating to the same 48 genetic markers in populations considered to be ancestral to the populations studied here were used. These samples consisted of 189 sub-Saharan Africans individuals (from Ivory Coast, Angola, Mozambique, Zaire, and Cameroon), 161 European individuals (the majority from Portugal) and 243 individuals originating from seven indigenous tribes of the Brazilian Amazon.

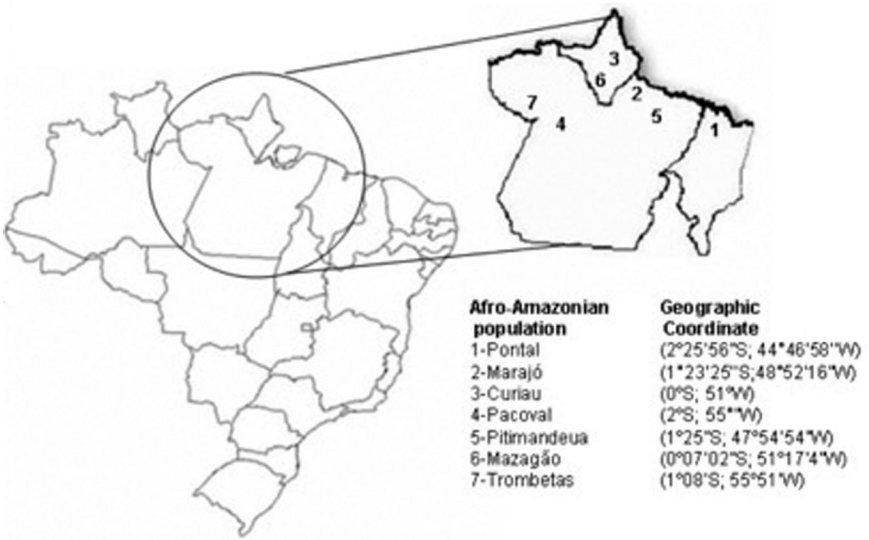

Figure 1. Geographical coordinates of the investigated Afro-derived populations.

DNA Analysis. DNA samples were genotyped for the 48 biallelic INDEL by means of three 16-plex PCR amplifications. The PCR thermocycling conditions were: 10 min at 95°C followed by 1 min at 94°C, 1 min at 60°C and 2 min at 70°C for 10 cycles, followed by 1 min at 90°C, 1 min at 60°C and 2 min at 70°C for 17 cycles, and one final period of 60 min at 60°C.

Samples with 1.0 μl of the amplified product were prepared for capillary electrophoresis, added to 8.5 μL of deionized formamide (Applied Biosystems, Foster City, CA, USA) and 0.5 μL 500 LIZ (Applied Biosystems). The samples were analyzed using the software ABI PRISM 3130 Genetic Analyzer (Applied Biostystems). The results were analyzed using the software GeneMapper v3.1 (Applied Biosystems).

Statistical Analysis. The statistical analysis for estimating the allele frequencies, the tests of Hardy-Weinberg Equilibrium, the analysis of genetic distances between populations (F_{ST}), and the exact test of population differentiation (Raymond and Rousset 1995) were achieved using the Arlequin v.3.1 software package (Excoffier et al. 2005). F_{ST} genetic distances were visualized in two-dimensional space using the Multi-Dimensional Scaling (MDS) method, included in the software SPSS v. 14.0 (SPSS Ins. Chicago, IL, USA).

Global admixture proportions were estimated by the ADMIX95 program (www.genetica.fmed.edu.uy/software.htm), which is based on the gene identity method (Chakraborty 1985).

The estimates of individual interethnic admixture and analysis related to population substructure were achieved using the software STRUCTURE v. 3.2, available free of charge at http://pritch.bsd.uchicago.edu/software.html. The basic algorithm was described by Pritchard et al. (2000) and extensions to the method were published by Falush et al. (2003 and 2007).

Results and Discussion

The variability present in seven Afro-derived communities of the Brazilian Amazon was investigated with regard to 48 autosomal INDEL. The observed allele frequencies are presented in Table 1. The observed genotype distribution was tested for Hardy-Weinberg equilibrium. After correction for multiple tests (Bonferroni correction), all of the studied markers were considered to be in Hardy-Weinberg equilibrium, except MID1923 in the Marajó community.

Despite the fact that all 48 investigated INDEL are Ancestry Informative Markers (AIM), the average heterozygosity is high in all surveyed populations, ranging from 39.3% (Curiau) to 42% (Trombetas). We interpreted this fact as due to the intense process of miscegenation that originated the current African-derived populations of the Amazon, that in only ten generations reduced to half the African genes contribution (see Table 2).

Differentiation between Populations. The genetic distance (F_{ST}) between the seven Afro-derived communities studied is 2.81%. The greatest differentiation

Table 1. Allele Frequencies (S allele) Observed of the Seven Afro-Derived Amazonian Populations

INDEL	*PACOVAL*	*PITIMANDEUA*	*MARAJÓ*	*TROMBETAS*	*CURIAÚ*	*MAZAGÃO*	*PONTAL*
MID1357	0.738	0.369	0.388	0.484	0.383	0.545	0.220
MID273	0.428	0.391	0.597	0.453	0.372	0.568	0.470
MID1684	0.404	0.478	0.402	0.312	0.441	0.636	0.602
MID818	0.357	0.510	0.388	0.328	0.279	0.386	0.500
MID1172	0.309	0.413	0.250	0.359	0.209	0.295	0.411
MID1176	0.547	0.641	0.666	0.531	0.709	0.681	0.661
MID1358	0.666	0.565	0.736	0.531	0.534	0.727	0.602
MID1785	0.190	0.489	0.339	0.312	0.604	0.386	0.540
MID1271	0.547	0.445	0.476	0.343	0.279	0.500	0.348
MID789	0.785	0.652	0.694	0.546	0.511	0.613	0.455
MID494	0.500	0.510	0.597	0.359	0.430	0.295	0.397
MID625	0.690	0.717	0.536	0.468	0.696	0.545	0.470
MID1379	0.785	0.934	0.875	0.812	0.639	0.772	0.647
MID2011	0.404	0.565	0.444	0.484	0.151	0.568	0.352
MID1726	0.404	0.532	0.527	0.406	0.593	0.590	0.382
MID473	0.547	0.597	0.416	0.687	0.651	0.500	0.661
MID619	0.833	0.586	0.541	0.812	0.732	0.590	0.647
MID1448	0.214	0.065	0.138	0.125	0.139	0.227	0.220
MID1923	0.119	0.478	0.416	0.375	0.244	0.204	0.470
MID856	0.261	0.478	0.444	0.562	0.440	0.613	0.568
MID99	0.619	0.728	0.833	0.734	0.581	0.818	0.867
MID93	0.357	0.336	0.263	0.343	0.220	0.409	0.338
MID1716	0.428	0.500	0.486	0.421	0.627	0.659	0.500
MID682	0.642	0.553	0.817	0.515	0.500	0.840	0.529
MID1039	0.285	0.281	0.263	0.228	0.186	0.386	0.264
MID1780	0.595	0.467	0.402	0.406	0.511	0.500	0.558
MID1470	0.166	0.402	0.194	0.156	0.186	0.250	0.176
MID132	0.500	0.369	0.236	0.468	0.370	0.454	0.367
MID1098	0.355	0.412	0.331	0.367	0.332	0.402	0.338
MID1558	0.300	0.259	0.277	0.171	0.174	0.272	0.161
MID217	0.071	0.119	0.222	0.125	0.232	0.159	0.117
MID568	0.142	0.119	0.180	0.156	0.104	0.250	0.220
MID1952	0.312	0.334	0.287	0.406	0.267	0.272	0.147
MID476	0.285	0.152	0.305	0.234	0.232	0.272	0.132
MID275	0.540	0.487	0.583	0.500	0.662	0.636	0.735
MID152	0.500	0.217	0.537	0.265	0.290	0.270	0.333
MID196	0.452	0.521	0.361	0.531	0.430	0.409	0.529
MID778	0.714	0.728	0.597	0.593	0.709	0.590	0.617
MID1603	0.612	0.790	0.388	0.484	0.614	0.681	0.818
MID1386	0.285	0.358	0.316	0.280	0.159	0.360	0.142
MID660	0.571	0.347	0.537	0.421	0.430	0.454	0.367
MID575	0.350	0.222	0.333	0.296	0.093	0.090	0.102
MID216	0.640	0.759	0.597	0.453	0.488	0.681	0.627
MID481	0.160	0.195	0.152	0.093	0.069	0.156	0.157
MID913	0.119	0.010	0.035	0.093	0.061	0.065	0.054
MID350	0.119	0.043	0.148	0.171	0.174	0.159	0.088
MID988	0.400	0.313	0.402	0.484	0.197	0.272	0.250
MID184	0.448	0.391	0.583	0.500	0.325	0.500	0.480

Table 2. Global Estimate of Continental Ancestry in Seven Afro-Derived Amazonian Populations

Afro-Derived Populations	*African (%±SE)*	*European (%±SE)*	*N. American (%±SE)*
Pacoval	38.2 ± 0.0003	29.7 ± 0.0004	32.1 ± 0.0003
Pitimandeua	48.7 ± 0.0027	32.3 ± 0.0025	19.0 ± 0.0030
Marajó	41.1 ± 0.0014	27.8 ± 0.0016	31.1 ± 0.0012
Trombetas	52.8 ± 0.0078	19.6 ± 0.0085	27.6 ± 0.0086
Curiaú	58.3 ± 0.0004	21.6 ± 0.0004	20.1 ± 0.0004
Mazagão	38.2 ± 0.0002	41.0 ± 0.0003	20.8 ± 0.0002
Pontal	60.5 ± 0.0075	26.1 ± 0.0059	13.4 ± 0.0077
All populations	48.5 ± 0.0032	28.9 ± 0.0025	22.6 ± 0.0031

occurs between Pacoval and Pontal (5.9%). The Pacoval population shows the greatest genetic distance (3.9%), followed by Curiaú (3.6%) and Marajó (3.2%). An exact differentiation test (Raymond and Rousset, 1995) was applied to compare the samples under study, demonstrating that there are no significant statistical differences ($p > 0.05$) between the analyzed populations.

Genetic distances (F_{ST}) were visualized in two-dimensional space using the MDS method (Figure 2). We included in this analysis European, African and Native Americans parental populations. In this figure it is possible to identify groups which are formed depending on the ethnic composition of the populations: Pacoval and Marajó, two populations with greater contribution of Amerindian ancestry (both with 32%) are grouped in the Native Americans quadrant; Pontal, Curiau and Trombetas, populations with greater contribution of African genes (54%, 52%, and 46%, respectively) are grouped in African quadrant; Pacoval and Marajó (32% and 33% of European genes, respectively) are grouped in European quadrant.

Continental Ancestry. The estimates of interethnic admixture of each of the Afro-derived populations are given in Table 2. There is high miscegenation with other ethnic groups in all of the populations, ranging between 40% (Pontal) and 60% (Pacoval and Mazagão). The greatest contribution of Amerindian genes was found in the Pacoval (32%) and Marajó (31%) communities and the lowest in Pontal (13%). The whole estimated population contributions are: African genes, 48.5%; Europeans, 28.9%; and Amerindians, 22.6%.

Comparing the data obtained from the autosomal markers in the Amazonian Afro-derived populations and other Brazilian Afro-derived populations, it is possible to see that the Amazonian populations have a lower contribution of African genes than the Afro-derived populations of south (mean of 69%) and northeast (mean of 62%) of Brazil (Bortolini et al. 1995; Bortolini et al. 1998; Bortolini et al. 1999; Souza and Culpi 2005).

The estimated contribution of Amerindian genes to the Amazonian Afro-derived populations is high (23%). Previous studies using classic markers and/or autosomal STRs also suggest a high contribution of Amerindian genes in the

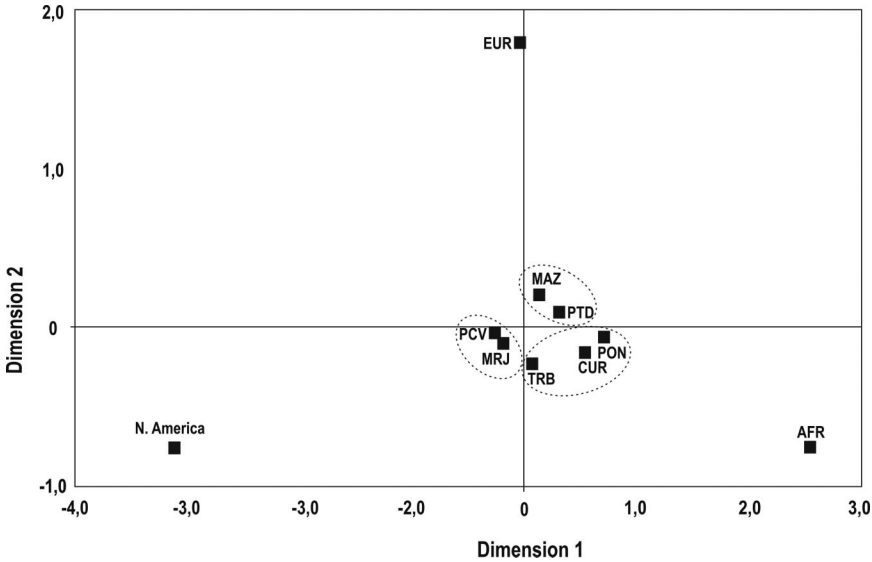

Figure 2. Multidimensional scaling (MDS) plot based on genetic differentiation (F_{ST}) values between afro-derived Amazonian populations and three ancestral populations. (Stress = 0.053; RSQ = 0.992) N. AMER (Native American); AFR (African); EUR (European); PCV (Pacoval); MRJ (Marajó); TRB (Trombetas); CUR (Curiaú); PON (Pontal); MAZ (Mazagão); PTD (Pitimandeua).

Amazonian Afro-descent (Schneider et al. 1987; Bortolini et al. 1995; Guerreiro et al. 1999; Cayres-Valinoto et al. 2003). However, the high contribution of Amerindian genes in Afro-derived communities is not common in other regions of Brazil. Other researchers have reported a small contribution of Amerindian autosomal genes (less than 10%) in the South (Bortolini et al. 1995; Souza and Culpi 2005), in the Central-West and the Northeast of Brazil (Silva Jr. et al. 1999).

Preferential Inclusion of Amerindian Women. The researchers involved in this study have conducted parallel research in at least six of the seven communities investigated here, using uniparental Y-DNA markers (nine STR loci—data available on the site www.yhrd.org) and mtDNA markers (Ribeiro-dos-Santos et al. 2002; Carvalho et al. 2008). The estimates obtained show very high (mean of 47%) Native American mtDNA and very low (mean of 5%) Native American Y-DNA contributions in the studied populations.

The observed differences between the Amerindian contribution based on the 48 autosomal markers (mean of 26%), intermediate between the values estimated using the mtDNA and Y-DNA markers, could be explained by two social facts: i) the integration of individuals belonging to neighboring social groups, such as happens in the indigenous tribes of the interior of the Amazon and which does not occur in other regions of Brazil, and ii) the preferential

inclusion of Amerindian women in contrast to Amerindian men as the Amazonian Afro-derived populations were formed.

The preferential inclusion of indigenous women into Amazonian groups of African descent was previously suggested by Ribeiro-dos-Santos et al. (2002). These facts suggest that studies using uniparental markers (mtDNA and Y-DNA) should be conducted to investigate whether this practice was specific to the Amazonian Afro-derived populations or also occurred during the formation of Afro-derived populations in other regions of Brazil.

Population Substructure. The data produced in this study was used together with the data of ancestral populations from previous studies (Santos et al. 2010) to estimate the interethnic admixture present in each individual in the sample. From the estimates, it can be seen that the individuals are largely heterogeneous. The contribution of African genes varies between 12% and 75%, of European genes, from 10% to 73%, and of Amerindian genes, between 8% and 66%.

In light of the clear evidence of differentiation between individuals, the data obtained was used to try to identify whether or not population stratification is present in the Amazonian Afro-derived communities. The results show no evident substructures in three communities (Pacoval, Trombetas and Marajó) and evident substructures (K = 2, where K stands for the number of distinct subpopulations on a genetically stratified population) in four populations (Curiau, Mazagão, Pitimandeua and Pontal). When the individuals are taken together as a single population, it is possible to identify population stratification with two (K = 2) subpopulations in the sample of the Amazonian Afro-derived populations.

Contract Grant Sponsor. Universidade Federal do Para (UFPA); Conselho Nacional de Desenvolvimento Cientifico e Tecnológico(CNPq); Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES); Financiadora de Estudos e Projetos (FINEP).

Received 28 February 2011; revision accepted for publication 16 May 2011.

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