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Research Article

Microsatellite studies on an isolated population of African descent in the Brazilian state of Bahia

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Abstract

The African descent population of the Bananal community in the Brazilian state of Bahia (BA) was characterized as a genetic isolate and analyzed for some short tandem repeat (STR) microsatellite autosomic polymorphic loci (*CSF1PO*, *TH01, TPOX, F13A1, FESFPS* and *vWA*). These genetic variants were further compared to data obtained from an urban sample from the town of Jequié (BA) regarding demographic and anthropogenetic aspects. The Bananal sample comprised 32 unrelated individuals whereas Jequié was represented by 76 individuals. The Bananal Negroid Phenotypic Index (NPI) was 0.98 and the Negroid Cultural Index (NCI) 0.24. Consanguineous marriages occurred at a frequency of 34.61% and the F value was 0.0126. All six loci studied were in Hardy-Weinberg Equilibrium ($p > 0.05$). The genotypic and allele frequencies of the *CSF1PO* and *vWA* loci were similar. In the Bananal population the genic diversity of the *THO1* locus was 66.8% and that of the *F13A1* locus was 83.7%. The estimated ethnic racial admixture was 81% African and 19% Amerindian. The multiple correlation coefficient (R^2) indicated adequate adaptation (99%). Total genetic variation for the six loci was 82.9% with an index of 6.7% for population subdivision ($G_{ST}' = 0.067$). Anthropologic data and results obtained from the allele frequencies of the loci studied are indicative of a genetic isolate in Bananal, reminiscent of the a `quilombo community' (*i.e.* one founded by run away slaves).

Key words: microsatellite, African-descendant isolate.

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Introduction

This study was undertaken in the Bananal community located in the Rio de Contas district of the Chapada Diamantina region of the Brazilian state of Bahia, past studies having identified this community as a genetic isolate (Barbosa, 2003).

Oral and historical information shows that the origin of the Bananal community is closely related to that of the Rio de Contas district (area 895 km^2), a small community formed in the Seventeen Century due to the discovery of gold and diamonds in the Chapada Diamantina (literally, the plateau of diamonds) region of Bahia (Reis and Gomes, 1996). However, Messeder and Martins (1991) point out that public archives show that before the discovery of mineral resources this region was agricultural and there were communities of African slaves who had escaped from farms, such communities being known as quilombo communities (or simply quilombos) from the African word *quilombo* meaning `a run away slave village'. The word Bananal is Portuguese, meaning a banana grove or plantation and thus indicates the agricultural origin of the community.

Our work focused on the populational parameters and anthropogenetic aspects of the Bananal quilombo community in order to understand the processes that transformed this population into a genetic isolate. To recreate and understand the history of this group, we estimated the allele fre-

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quencies of selected short tandem repeat (STR) microsatellite autosomic polymorphic loci (*CSF1PO, TPOX, TH01, F13A1, FES/FPS* and *vWA*) and compared them to those observed in a population of African descent from the town of Jequié in the same region and those observed in other populations. We also estimated the ethnic admixture and genetic distances in the Bananal community and investigated the distribution of surnames and the past consanguinity of this group.

Material and Methods

Population

The African descent Bananal community (13°36'00" S; 41°48'10" W) is located in a valley between Serra das Almas and Malhado at a distance of approximately 15 km from the city of Rio de Contas in the southern Chapada Diamantina region of the Brazilian state of Bahia.

The Bananal community is composed of 26 small households, a church, and a district school. Houses are made of a clay (sometimes mixed with straw) and are built collectively by the members of the community. Electricity was partially installed in 1996 but is not present in all households. There is no water system or sewage facilities and the streets are unpaved. The population consist of 82 individuals (42 men and 40 women), according to data from 1995-1997.

The town of Jequié in the same region has a population of 147,115 of mixed descent and is located at 13°51'50" S; 40°04'54" W.

Population analysis

Data from the families belonging to the Bananal community were obtained both from direct observation and interviews which took place in the households with the aid of a questionnaire which was applied to one of the adults (husband or wife) present. A pedigree of the Bananal community was reconstructed which considered the information obtained in the households as well as additional data obtained at the official registry office and at the parish level. The populational genetic parameters considered in this study were the reproductive population (Nr), effective population (Ne), effective migration (me), inbreeding coefficient (f), size of the isolate, and isolation index (i).

Individuals belonging to the Bananal community were considered of African descent in accordance with the morphological `racial' classification proposed by Krieger *et al.* (1965) and modified by Azevêdo (1975). Surnames were classified according to Tavares-Neto and Azevêdo (1977, 1978), names having a religious connotation being important cultural markers related to African ancestry in Northeastern Brazil (Barbosa *et al.,* 1997).

The Negroid Phenotypic Index (NPI) is obtained by counting all individuals classified as blacks and mulattos in relation to the total number of individuals that compose the sample, while the Negroid Cultural Index (NCI) takes into consideration the frequency of individuals who carry a surname with religious connotation, and the Amerindian Cultural Index (ACI) is given by the frequency of surnames referring to plants and animals (Azevêdo *et al.*, 1982).

Data on consanguineous marriages were obtained during the interviews and all of them were confirmed in the official registry documents. Such data were further confirmed by the surname analysis. There is no divergence between these three information.

Laboratory analyses

Venous blood samples were obtained from subjects after informed consent, the study previously having been approved by the ethical committees of the institutions involved. From each individual we collected 5 ml of venous blood in EDTA tubes and obtained high molecular weight DNA by the *salting-out* method (Miller *et al.,* 1988) as modified by Alonso *et al.* (1997). We also used *Chelex* 100 (Walsh *et al*., 1991) for DNA extraction.

We genotyped unrelated individuals of African descent, 68 from the Bananal community and 76 from Jequié, for the following human short tandem repeat (STR) microsatellite autosomic polymorphic loci and their alleles: the *CSF1PO* CSF-1 receptor gene c-fms proto-oncogene (Edwards *et al.* 199; Hammond *et al*. (1994); the *TPOX* thyroid peroxidase gene (Anker *et al*., 1992); the *TH01* tyrosine hydroxylase gene (Polymeropoulos *et al.*, 1991a); the *vWA* von Willebrand factor gene (Kimpton *et al.*, 1992); the *F13A1* coagulation factor XII subunit gene (Polymeropoulos *et al.*, 1991b); and the *FES/FPS* c-fes/fps proto-oncogene (Polymeropoulos *et al.*, 1991c).

Polymerase chain reaction (PCR) DNA amplification was accomplished using the *Gene Print* kit (STR Systems, USA) and the FFv and CTT Multiplexes from Promega (USA) according to suggested protocols (Schumm *et al*., 1994). Amplified fragments were separated on denaturing polyacrylamide gel according to instructions from the manufacturer (Anon. 1999) and genotype was assigned after staining the gells with 6% (w/v) aqueous silver nitrate.

Statistical analysis

Allele and genotype frequencies were tested for the Hardy-Weinberg equilibrium by exact tests (Raymond and Rousset, 1995b) using version 3.2 of the GENEPOP program (Raymond and Rousset, 1995a). Intrapopulational (H_S) and interpopulational (G_{ST}') Nei's genic diversity index were estimated using the program FSTAT (Goudet, 1999). Ethnic proportions were calculated by the genic identity method (Chakraborty, 1985) using the ADMIX 3 and ADMIX 2 programs. The allele frequencies representative of the African, European and Amerindian populations were the mean frequencies of other reports in the literature, adjusted by the sample size (Table 1). To calculate genetic distance, the ancestral African, European and Amerindian frequencies from the six loci studied were compared to those observed in our sample. The dendrogram was generated by the neighbor-joining method (Saitou and Nei, 1987) using the DISPAN program (Ota, 1993) and 1,000 bootstrap replicates.

Results and Discussion

For the Bananal sample 71.8% were phenotypic classified as black, and 28.2% were of mulattos phenotype consisting of 2.3% light-colored, 11.8% medium-colored and 14.1% dark-colored. There were no individuals classified as Amerindian or Caucasian by phenotype in this commu-

Table 1 - Distribution of allelic frequencies for some short tandem repeat (STR) microsatellite loci and their alleles occurring in populations of African descent in the Brazilian towns of Bananal and Jequié (both in the state of Bahia) and in the parental populations. The frequencies of the most common alleles are in bold type. Asterisks indicate alleles.

	Allelic frequencies of the different populations studied				
Loci/* allele	Bananal	Jequié	African[1]	European[2]	Amerindian[3]
CSF1PO	(n = 49[#])	(n = 132[#])	(n = 783)	(n = 408)	(n = 322)
*6	0.000	0.000	0.003	0.000	0.000
*7	0.042	0.053	0.057	0.000	0.000
*8	0.021	0.053	0.067	0.005	0.000
*9	0.062	0.023	0.057	0.028	0.003
*10	0.167	0.205	0.311	0.282	0.284
*11	0.229	0.333	0.210	0.316	0.220
*12	0.396	0.280	0.249	0.303	0.393
*13	0.062	0.045	0.033	0.053	0.099
*14	0.021	0.000	0.012	0.009	0.000
*15	0.000	0.008	0.001	0.001	0.000
TH01	(n = 60[#])	(n = 132[#])	(n = 939)	(n = 4639)	(n = 323)
*5	0.000	0.000	0.000	0.001	0.000
*6	0.150	0.038	0.388	0.204	0.388
*7	0.517	0.008	0.446	0.170	0.446
*8	0.183	0.409	0.000	0.139	0.000
*9	0.133	0.197	0.002	0.198	0.002
*10	0.017	0.045	0.164	0.275	0.164
*11	0.000	0.258	0.000	0.013	0.000
*12	0.000	0.045	0.000	0.001	0.000
TPOX	(n = 56[#])	(n = 132[#])	(n = 143)	(n = 908)	(n = 321)
*6	0.018	0.189	0.105	0.002	0.002
*7	0.018	0.326	0.017	0.003	0.000
*8	0.268	0.212	0.392	0.498	0.215
*9	0.196	0.174	0.210	0.102	0.006
*10	0.107	0.023	0.088	0.061	0.012
*11	0.357	0.076	0.182	0.300	0.470
*12	0.036	0.000	0.007	0.033	0.294
*13	0.000	0.000	0.000	0.001	0.000
F13A1	(n = 57[#])	(n = 122[#])	(n = 289)	(n = 1050)	(n = 322)
*3	0.035	0.057	0.000	0.000	0.000
*3.2	0.000	0.000	0.136	0.089	0.407
*4	0.053	0.189	0.057	0.035	0.169
*5	0.333	0.270	0.377	0.202	0.261
*6	0.140	0.189	0.104	0.279	0.025
*7	0.140	0.164	0.185	0.341	0.115
*8	0.123	0.057	0.062	0.001	0.023
*9	0.000	0.008	0.016	0.000	0.000
*10	0.035	0.016	0.003	0.001	0.000
*11	0.018	0.008	0.005	0.000	0.000
*12	0.070	0.000	0.007	0.003	0.000
*13	0.035	0.016	0.017	0.003	0.000
*14	0.018	0.016	0.016	0.008	0.000
*15	0.000	0.008	0.011	0.016	0.000
*16	0.000	0.000	0.000	0.008	0.000
*17	0.000	0.000	0.000	0.005	0.000
*18	0.000	0.000	0.000	0.001	0.000
FES/FPS	(n = 55[#])	(n = 152[#])	(n = 290)	(n = 1967)	(n = 321)
*7	0.000	0.007	0.000	0.000	0.000
*8	0.055	0.059	0.100	0.010	0.008
*9	0.073	0.039	0.100	0.003	0.009
*10	0.127	0.263	0.266	0.342	0.022
*11	0.255	0.362	0.349	0.358	0.631
*12	0.345	0.217	0.150	0.238	0.204
*13	0.145	0.046	0.033	0.042	0.045
*15	0.000	0.007	0.000	0.006	0.098
vWA	(n = 52[#])	(n = 144[#])	(n = 706)	(n = 1259)	(n = 318)
*11	0.000	0.000	0.001	0.000	0.000
*12	0.000	0.000	0.002	0.000	0.000
*13	0.000	0.021	0.016	0.001	0.000
*14	0.173	0.097	0.081	0.113	0.024
*15	0.154	0.250	0.190	0.116	0.146
*16	0.346	0.222	0.245	0.223	0.358
*17	0.135	0.250	0.210	0.273	0.415
*18	0.115	0.104	0.160	0.189	0.072
*19	0.077	0.056	0.069	0.073	0.078
*20	0.000	0.000	0.023	0.010	0.006
*21	0.000	0.000	0.002	0.002	0.000

Sources: (1) Africans - (Deka *et al.*, 1999; Destro-Bisol *et al.*, 2000; Huguet *et al.*, 1998; Bosh *et al.*, 2001; Gene *et al.*, 2001; Alves *et al.*, 2001; Brinkmann *et al.*, 1996; Brinkmann *et al.*, 1998; Rolf *et al.*, 1998; Wang *et al.*, 2002; Pérez-Lezaun *et al.*, 1997; Yunis *et al.*, 2000; Corte-Real *et al.*, 2000; Dios *et al.*, 1998); (2) Europeans (Pérez-Lezaun *et al.*, 2000; Anjos *et al.*, 2000; Lourdes-Pontes *et al.*, 1998; Asmundo *et al.*, 1998; Santos *et al.*, 1996; Souto *et al.*, 1996, Souto *et al.*, 1998; Alves *et al.*, 2001; Pinheiro *et al.*, 1996; Amorim *et al.*, 1996; Pereira *et al.*, 1996; Gusmão *et al.*, 1997; Espinheira *et al.*, 1996; Miranda *et al.*, 1998; Prata *et al.*, 1998); (3) Amerindians (Mendes-Jr, 2001; Wanderley-Santos, 2001).

[#] The sample size varies between different loci in the same population due to the impossibility of genotyping all samples.

nity. This date on phenotypic ethnic classification is based on facial morphological characteristics and skin color in body regions not exposed to the sun (Azevêdo, 1980).

As regards anthropologic characteristics, the Bananal community NCI was 0.24, a median value compared to that expected for an African derived population (Azevêdo *et al.*,1982), possibly due to a founder effect related to the fact that few surnames with religious connotation were present in the initial composition of the community. The Bananal NPI was 0.8, higher than the 0.54-0.79 reported in other African derived populations (Azevêdo *et al.*, 1982), possibly due to the fact that the Bananal community was isolated for for at least seven generations due to geographic, historical and social causes. The Bananal inbreeding coefficient (F) was 0.0126, higher than those reported in other Brazilian isolates (Magalhães and Arce-Gomes, 1987; Muniz, 1978; Freire-Maia and Cavalli, 1978) and probably due to the small size of the isolate favoring consanguineous marriages.

The populational genetic parameters considered in this study were: reproductive population (Nr = 29), effective population (Ne = 14), effective migration (me = 19.2%), inbreeding coefficient (F = 0.0126), size of the isolate and isolation index (i = 2.688).

The anthropologic and genetic data obtained represent important evidence that Bananal is reminiscent of a quilombo community. Information gathered in questionnaires as well as from the historical narrative of the oldest inhabitants are consistent with this and coherent with the documented historical facts (Viana-Filho, 1988; Alencastro, 2000; Tavares, 2001).

The microsatellite frequencies found in the Bananal and Jequié communities were in Hardy-Weinberg Equilibrium and no new allele was detected (Table 1).

The most frequent *CSF1PO* allele in Bananal was *12 (0.396) while *8 and *14 were rare (0.021 each) and *15 was not detected, the most frequent *CSF1PO* allele in the Jequié sample being *11 (0.333) and the least frequent *15 (0.008). Comparing our results with published data, we found that the frequency of the *CSF1PO**12 allele in Bananal was similar to the 0.301 reported for African-American populations (Budowle *et al.,* 1997) and the 0.341 reported for populations from Mozambique (Alves *et al.*, 2001). The *CSF1PO**10, *11 and *12 alleles were the most frequent *CSF1PO* alleles seen in the different populations (Table 1). The *11 *CSF1PO* allele is the most common (0.314) in Portuguese population (Santos *et al.* 1996). We also found that the same was true for African descents of Jequié for which the *11 frequency was (0.333). This data indicates that there was greater European gene flow into the African descent of Jequié population as compared to the African descent Bananal isolate.

For the *TH01* locus the most frequent allele was *7 (0.517) in the Bananal isolate. This allele was not polymorphic in African descendants from Jequié, in this sample the *TH01**8 being was the most frequent (0.409) allele. In Africans and African-Americans *TH01**7 was the most common, ranging from 0.262 to 0.434 (Budowle *et al.*,1997b; Dios *et al.*, 1998; Alves *et al.*, 2001).

In relation to the *TPOX* locus the *11 allele (0.357) was the most frequent in Bananal, this allele also being common (0.342-0.360) in Africans (Dios *et al.* 1998; Alves *et al.* 2001). In individuals of African descent from Jequié, the most frequent allele was *TPOX**7 (0.326), this frequency being higher than that observed individuals of African, Portuguese and Amerindian descent and in other populations (www.uni-duesseldorf.de/WWW/MedFak/Serology/database.html).

For the *F13A1* locus, the *5 allele was the most frequent in the Bananal (0.333) and Jequié (0.270) samples, this allele also being common in African-Americans (0.342) and Africans (0.337 to 0.500; Destro-Bisol *et al.*, 2000).

In the case of the *FES/FPS* locus the most frequent allele in Bananal was *12 (0.345), the frequency of this allele being higher in this population than in Africans, Portuguese or Amerindians (Table 1), probably due to genetic drift. The second most common allele in Bananal (*11; 0.255) was the most frequent *FES/FPS* allele in Jequié (0.362), African-Americans (0.355) and Africans (0.359 to 0.414; Destro-Bisol *et al.*, 2000).

For the *vWA* locus, *16 (0.346) was the most frequent allele in Bananal, whereas in Jequié the *15 and *17 alleles occurred at the same frequency (0.250), and the allele *16 being (0.222) the second most frequent. When compared to other populations, it was found that *16 is the most frequent *vWA* allele in Africans and African-Americans (0.261 to 0.371; Destro-Bisol *et al.*, 2000). In the African descendant population of São Gonçalo, Bahia (Sousa, 2001) the allele *16 was also the most frequent (0.286). For the Portuguese populations described by Santos *et al.* (1996) and Gusmão *et al.* (1997), allele *17 (0.255) and allele *16 (0.219) were also the most frequent *vWA* alleles, similar findings having been reported in the admixed Brazilian population described by Grattapaglia *et al.* (2001).

Of the six loci studied in individuals of African descent from Bananal and Jequié, four (*TH01*, *TPOX*, *FES/FPS* and *vWA*) presented heterogeneous frequencies between these two populations. In the Bananal population, genic diversity, measured as expected heterozygosity, for these six loci varied from 66.8% for the *TH01* locus to 83.7% for the *F13A1* locus, while in the Jequié population the values were of 72.8% for the *TH01* locus and 82.8% for *F13A1* (Table 2). The mean value for genic diversity was found to be very close for both populations (0.771 for Bananal and 0.776 for Jequié). After analyzing each locus individually, we concluded that for *TH01* and *TPOX*, values were higher in Jequié than in Bananal but for the other loci the opposite was true. We expected higher values in Jequié because this population was more exposed to ge-

Table 2 - Genic diversity (H_S) for the loci analyzed in the Bananal and Jequié populations of African descent.

Loci	Bananal	Jequié
CSF1PO	0.771	0.766
TH01	0.668	0.728
TPOX	0.762	0.782
F13A1	0.837	0.828
FES/FPS	0.788	0.750
vWA	0.803	0.802
Mean heterozygosity	0.771	0.776

netic influx and migration. The greater genic diversity in Bananal, a characteristic also observed in African populations, can be attributed to the historical fact that Bananal was founded by individuals of African descendant.

Total genic diversity for all six loci was in the order of 82.9% (Table 3). The heterozygosity index represents the frequency of heterozygotes for each locus in the population and can be related to the both the effective size of the population and to its degree of isolation (Bosch *et al*., 2001). These results are in accordance with those obtained for other African populations.

We found intrapopulational genetic diversity to be high and similar to that found in populations of African ascendant from different regions of the African continent. Our data also reveals that Bananal was formed by individuals coming from different ethnic groups of African origin, high genetic diversity being in accordance with historical facts from Bahia and Brazil in general which indicate that the traffic in African slaves involved trafficking individuals with diverse ethnic origins (Tavares, 2001).

A value of 6.7% (Table 3) for populational subdivision (G_{ST}' = 0.067) is indicative of group heterogeneity, reflecting the multifaceted African origin of Bananal and its historical isolation, this situation being different from that seen in the open population of Jequié which is composed of diverse ethnic groups such as Europeans, Amerindians, Arabs and Africans.

Table 3 - Estimators of variability according to Nei's methodology (1987).

Loci	Genic diversity		
	Intrapopulational		Interpopulational
	H_S	H_T'	G_{ST}'
CSF1PO	0.768	0.771	0.004
TH01	0.698	0.888	0.214
TPOX	0.772	0.870	0.113
F13A1	0.832	0.839	0.007
FES/FPS	0.769	0.787	0.023
vWA	0.802	0.818	0.019
Mean	0.774	0.829	0.067

The mean frequencies found for each locus in the ancestral populations (Table 1) were used to estimate the ethnic admixture by the genic identity method, initially using a trihybrid model for both the Jequié and Bananal samples. Using this model the African-derived Jequié urban population was shown to consist of 41.5% African, 35.8% European and 22.6% Amerindian ancestry with a multiple correlation coefficient (R^2) of 82.3%. However, for the Bananal data the trihybrid model was inconsistent but when a dihybrid model was applied considering the frequencies for both African and Amerindian ancestral populations the African contribution to the Bananal population was calculated to be in the order of 81% and the Amerindian contribution 19%, the multiple correlation coefficient (R^2) indicated a good fit (99%) to this model. This date is different from the phenotypic ethnic classification where no Amerindian phenotype was detected suggesting that the short tandem repeat (STR) microsatellite autosomic polymorphic loci studied herein are a more powerful tool on discriminating ethnic admixture. Gene flow and/or genetic drift may explain the Amerindian contribution to the Bananal community gene pool.

The high contribution of Africans to the gene pool of our African descent sample of Jequié differs from that seen in previous studies of northeastern Brazilian populations (Callegari-Jacques *et* al 2003; Ferreira *et al*. 2005), possibly due to the careful choice of African-descendants by the morphological ethnic classification (Azevêdo, 1980) used in our study.

Our results indicate that both the Jequié and Bananal populations were of African descent with the long isolation of the Bananal population explaining the differences found between them while the similarities resulted from the fact that their shared origin was both African and Amerindian, although the Jequié population showed a greater degree of ethnic admixture in that it also contained a European contribution.

Regarding any European contribution to the Bananal population, further studies using other autosomic microsatellite markers along with Y chromosome and mitochondrial markers would greatly contribute to our understanding of the contribution of Europeans to this community.

Nei's genetic distance was used to compare the ancestral and actual populations in relation to the loci studied and generate the neighbor-joining dendrogram shown in Figu-

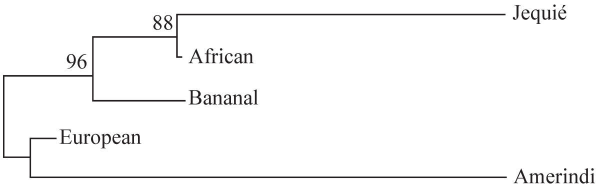

Figure 1 - Dendrogram generated by the *neighbor joining* method from Nei's genetic distances between the ancestral African, European and Amerindian populations and the Bananal and Jequié populations.

re 1 which shows a strong African component in both the Bananal and Jequié populations.

It is important to emphasize that our genic frequencies, NPI, NCI and populational data support the historical facts and information related to the populations studied and to the formation of Brazil as a nation. Our data also support the historical accounts that show that the Bananal community persisted as a genetic isolate reminiscent of the original quilombo communities.

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