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The Split of the Arara Population: Comparison of Genetic Drift and Founder Effect

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Abstract
The total genetic diversity of the Amerindian population is as high as that observed for other continental human populations because a large contribution from variation among tribes makes up for the low variation within tribes. This is attributed mainly to genetic drift acting on small isolated populations. However, a small founder population with a low genetic diversity is another factor that may contribute to the low intratribal diversity. Small founder populations seem to be a frequent event in the formation of new tribes among the Amerindians, but this event is usually not well recorded. In this paper, we analyze the genetic diversity of the Arara of Laranjal village and the Arara of Iriri village, with respect to seven tandem repeat autosomic segments (D1S80, ApoB, D4S43, vW1, vW2, F13A1 and D12S67), two Y-chromosome-specific polymorphisms (DYS19 and DYS199), and mitochondrial DNA (mtDNA) markers (restriction fragment length polymorphisms and sequencing of a segment of the D loop region). The occurrence of a single Y chromosome and mtDNA haplotype, and only 1–4 alleles of the autosomic loci investigated, corroborates historic and demographic records that the Arara of Iriri were founded by a single couple of siblings who came from the Arara of Laranjal, the largest group. Notwithstanding this fact, the genetic distance and the molecular variance between the two Arara villages were greater than those observed between them and other Amazonian tribes, suggesting that the microevolutionary process among Brazilian Amerindians may be misinterpreted if historic demographic data are not considered.

Introduction

The genetic diversity of human populations depends on the interaction of many factors, the more important being the genetic composition of the founders, the demic history, especially the population size, bottlenecks, expansion periods, degree of isolation, gene flow, and the selective effect of environmental factors. The low intratribal diversity observed among Amerindians is attributed

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to genetic drift acting on small, isolated populations. A small founder population with a low genetic diversity is another factor that may contribute to the low intratribal diversity. Small founder populations seem to be a frequent event in the formation of new tribes among the Amerindians, within the fission model, but this process and its consequence for the differentiation of the tribes is not easily recorded.

The factors related to village fission events, as well as the effects of these processes on the genetic divergence between villages, have been investigated in detail particularly for the Yanomama and Makiritare tribes [1–4]. Basically, three models of village fission were identified to occur with some regularity, as a consequence of ecological factors, political relationships and/or internal organizational features. Two of them, named random fission (random genetic assortment at the time of fission) and lineal fission (involving related individuals), often involve mutual hostility between the factions, so that subsequent gene flow between daughter villages is minimal. A third example of fission results from the amiable split of a village, accompanied by a free genetic exchange between the fission products.

In the history of the Arara Indians, a Karib-speaking group, there are records of several conflicts with other tribes and particularly with non-Indians (settlers and lumbermen) who invaded their territory, especially during the construction of the Transamazonica highway (a highway that crossed the Amazon forest). As a result, the Arara's territory and population size were drastically reduced, followed by successive events of fission and fusion.

The Arara of Iriri village probably constitute an example of lineal fission that occurred in an ancestral village, as observed for the Yanomama's villages [1]. The Arara of Iriri were first contacted in 1987, and oral reports indicate that the 43 individuals who constitute this village today are descendants from a single couple of siblings who were expelled from the largest Arara village. The initial expansion of the Iriri group occurred through the mating of closely related persons (parents-sibs) and later by marriages between relatives somewhat less close, such as uncle-niece, aunt-nephew and first cousins.

In this paper, we report the results of an evaluation of the genetic diversity of the two Arara villages, today named the Arara of Laranjal and the Arara of Iriri, and these data are compared with those previously obtained for the Arara of Laranjal village [5–8].

Materials and Methods

Population Sampled

Samples were obtained from the 43 individuals who constitute the Arara of Iriri village. They belong to the Karib linguistic group and are located on the right border of the Iriri river, a tributary of the Xingu river, in the state of Pará, Brazil.

Sample Preparation

Blood samples were collected with EDTA anticoagulant, refrigerated shortly afterwards, and flown in this condition to Belém, where they were processed.

The genetic polymorphisms analyzed were: (1) seven variable number of tandem repeats (VNTRs) at the autosomic segments (D1S80, ApoB, D4S43, vW1, vW2, F13A1 and D12S67); (2) a short tandem repeat (STR) and a single-base-change polymorphism of the Y DNA (DYS19 and DYS199), and (3) mitochondrial DNA (mtDNA), by restriction fragment length polymorphism (RFLP) and direct sequencing of the D loop region.

DNA samples were obtained from leukocytes by phenol-chloroform extraction and ethanol precipitation [9]. Thirty amplification cycles were used, and polymerase chain reaction (PCR) products were analyzed by 6–9% polyacrylamide gel, except for sequencing.

Autosomic VNTRs and STRs

The autosomic segments analyzed were tandem repeats loci for which the primers, PCR reaction and electrophoresis conditions have been previously described: D1S80 [10], ApoB [11]; D4S43 [12]; von Willebrand factor VNTR-1 [13]; von Willebrand factor VNTR-2 [14]; F13A1 [15]; D12S67 [16].

Y DNA

The DYS19 locus, a tetranucleotide repeat, was analyzed by PCR amplification followed by 9% polyacrylamide gel electrophoresis and silver staining [16]. The two alleles produced by the C→T transition at the DYS199 locus were identified by allele-specific amplification [17], and the products were analyzed on 7% polyacrylamide gels.

mtDNA

Four mtDNA restriction sites that define the major haplogroups of Amerindians were analyzed. Each fragment was amplified with primers previously described [5], followed by digestion with a restriction endonuclease (*Hae*III, *Hin*cII and *Alu*I), except for the V region (COII-tRNALys), characterized by a 9-bp deletion. The presence of the *Hae*III site (at position 663) defines the haplogroup A; the 9-bp deletion in the V region defines the haplogroup B; the absence of the *Hin*cII site at position 13,259 defines the haplogroup C, whereas the haplogroup D is characterized by the absence of the *Alu*I site at position 5,176.

The sequence of 358 bp of the D loop of the mtDNA, between positions 16,047 and 16,405, was determined for 28 individuals as previously described [5].

Statistics

The genetic distance was estimated based on the studies of the tandem repeats loci, using the GENEPOP program, version 3.1 [18]. The molecular variance was estimated using the method described by Michalakis and Excoffier [19], obtained by mtDNA analysis.

Table 1. Frequencies of tandem repeats for individuals of the Arara of Iriri and Laranjal populations

Loci	Frequency		Alleles		Locus heterozygosity	
	Laranjal	Iriri	Laranjal	Iriri	Laranjal	Iriri
D1S80*18	33.3	100.0	3	1	0.6086	0.000
*23	14.3	–				
*30	52.4	–				
ApoB *34	14.3	–	3	2	0.4866	0.3330
*36	69.0	79.5				
*46	16.7	20.5				
D4S43*1L	78.6	83.9	2	2	0.3449	0.4773
*11	21.4	16.1				
F13A1*01	14.3	14.3	3	3	0.3995	0.6234
*02	76.2	42.8				
*03	9.5	42.8				
vW1*06	66.7	60.7	3	3	0.4704	0.5617
*10	2.4	16.1				
*12	30.9	23.2				
vW2*01	–	35.2	–	4	–	0.8140
*03	–	31.5				
*04	–	13.0				
*07	–	20.3				
D12S67*07	14.7	–	5	4	0.7504	0.7322
*08	23.5	29.6				
*09	5.9	33.3				
*10	41.2	16.7				
*11	14.7	20.4				
DYS19*186	100.0	100.0	1	1	0.0000	0.0000
DYS199*C	0.000	100.0	1	1	0.0000	0.000

Results and Discussion

Autosomal VNTRs and STRs

The frequencies of the seven tandem repeats and other measurements of genetic diversity observed in the Arara of Iriri and Laranjal villages are shown in table 1. All the alleles observed in the Arara of Iriri have been described previously in other Amerindian populations from the Amazon region, except for the D12S67 locus, for which there are not yet data available for the Amerindian population. The locus heterozygosity calculated for each tandem repeat for the Arara of Iriri varied from 0.000 for the D1S80 and DYS19 loci to 0.814 for the vW2 locus. The autosomic and sexual loci showed a limited number of alleles, varying from a single allele in the D1S80 and DYS19 loci to four alleles in the D12S67 and vW2 loci. In the Laranjal village, the locus heterozygosity varied from 0.000 in DYS19 to 0.7504 in D12S67, with a maximum of five alleles.

The genetic distance was greater between the two Arara villages than between the Arara of Iriri and seven other Brazilian Amerindian populations (Katuena, Zoé, Arara of Laranjal, Wayana-Apalai, Waiãpi, Yanomama and Kayapó from the Kokraimoro village) previously reported [6, 20] (table 2).

DYS19 and DYS199 Loci

In the 14 males investigated from the Arara of Iriri for the DYS19 locus, only the 186-bp allele, which is the most common in south American natives, was found [6, 8, 17, 21].

Only the DYS199*C allele was found in these 14 males. The DYS199*C allele has previously been found in other Brazilian Amazon Amerindians (Zoé, Awá-Guajá, Katuena, Xikrin, Urubu-Kaapor, Suruí, Wayana-Apalai and Yanomama), with frequencies ranging from 0.06 to 0.54 [8, 17]. The fixation of the DYS199*C allele among Native American populations is not a common event, but has been reported for the Ojibwa of Canada [22]. Only the DYS199*C allele, which is the most common among the Amerindians, was observed in 8 males from the Arara of Laranjal [7].

In this study, we observed the fixation of a single DYS199 allele, in accordance with oral reports about the

Table 2. Matrix of genetic distances ($\times$ 10,000), on the basis of seven tandem repeats autosomic segments, between seven populations of Amazonian Amerindians using F statistic estimated by the method of Weir and Cockerham [23]

	Arara of Iriri	Katuena	Zoé	Arara[2]	Waiãpi	Yanomama	Kayapó[1]
Arara of Iriri							
Katuena	1045						
Zoé	1299	0503					
Arara[2]	1493	1164	1412				
Waiãpi	2214	1185	1778	2212			
Yanomama	2242	0807	1080	1378	1563		
Kayapó[1]	1931	0917	1250	1571	1307	0506	

Systems considered: DYS19, D1S80, D4S43, ApoB, vW1, F13A1 and D12S67. Sources: Zago et al. [6]; Vallinoto [20]; present study.

[1] Kokraimoro village.

[2] Arara of Laranjal.

Table 3. The mtDNA haplotype identified in 28 individuals of the Arara of Iriri compared with the haplotypes from the Arara of Laranjal

Sample	Mutation (np)													
	16,111	16,189	16,217	16,223	16,249	16,290	16,292	16,298	16,312	16,319	16,325	16,327	16,344	16,362
Anderson et al. [24][1]	C	T	T	C	T	C	C	T	A	G	T	C	T	T
Arara 01[2]	–	C	C	–	C	–	–	–	G	–	–	–	C	–
Arara 02[2]	–	C	–	T	–	–	T	C	–	–	C	T	–	C
Arara 03[2]	–	–	–	T	–	–	T	C	–	–	C	T	–	C
Arara 04[2]	–	C	–	–	C	–	–	–	G	–	–	–	–	–
Arara of Iriri	**T**	**–**	**–**	**T**	**–**	**T**	**–**	**–**	**–**	**A**	**–**	**–**	**–**	**C**

Data for nucleotide sequences in the D loop region.

[1] Reference sequence.

[2] Arara of Laranjal [5].

origin of this population. Since the fixation of the DYS199*C allele in Amerindians is not a common event, if the probable origin of the Arara of Iriri were not known, this finding would conceivably be attributed to a bottleneck or prolonged periods of reduced population size. On the other hand, the absence of the DYS199*T allele among the Iriri group could suggest admixture with non-Amerindians. However, this seems not to be the case, since the haplotype DYS19*186/DYS199*C, which is fixed in the Arara of Iriri, is the second most common haplotype in Amerindians. In fact, the haplotype DYS19*186/DYS199*T is present in 71% of Amerindians, including the Arara of Laranjal, whereas the haplotype DYS19*186/DYS199*C was found in 11% of the males investigated in twelve tribes [7, 8, 17, 22]. The combination DYS19*186/DYS199*C was absent only in two tribes (Surui and Karitiana). According to Vallinoto et al. [8], these data would be in accordance with the suggestion that the C→T mutation arose in North America or in the ancestral population from which all Native Americans descend [7, 17].

RFLP and Sequencing of mtDNA D Loop

Only one haplotype was observed for the 28 individuals, both by RFLP and D loop sequencing. The haplotype from the Arara of Iriri defined on the basis of RFLP could not be assigned to any of the common haplogroups (A–D) previously described for the Amerindians. On the other hand, the sequencing analysis identified four transitions (C→T, nucleotide position (np) 16,223 and np 16,290; G→A, np 16,319; and T→C, np 16,362) representing the motif of cluster III (corresponding to the haplogroup A as defined by RFLPs) (table 3). Similar haplotypes were previously reported for other Brazilian Amazonian tribes by Santos et al. [5]. The combined results of RFLP and sequencing suggest that these haplotypes probably repre-

Table 4. Matrix of analysis of molecular variance (× 10,000) between seven populations, estimated by the method of Michalakis and Excoffier [19]

	Arara of Iriri	Yanomama	Waiãpi	Arara[2]	Kayapó[1]	Katuena
Arara of Iriri	–					
Yanomama	5142	–				
Waiãpi	0656	4388	–			
Arara[2]	7363	1345	5733	–		
Kayapó[1]	4131	2209	3082	3450	–	
Katuena	4199	0824	2493	1670	1583	–
Zoé	4490	1678	3063	2563	1994	0434

System considered: sequencing the D loop of mtDNA.
[1] Kokraimoro, Kubenkokre and Pukany villages.
[2] Arara of Laranjal.

sent mitochondrial sequences of the haplogroup A, which may have suffered reverse mutation abolishing the *Hae*III$_{663}$ restriction site that defines this haplogroup by RFLP. It is interesting to observe that this particular haplotype observed among the Arara of Iriri was not detected among 20 individuals of the 'major' branch of the Arara [5].

When the mtDNA sequence found in the Arara of Iriri was compared with those reported for other Brazilian Amerindians (Yanomama, Waiãpi, Tiryo, Kayapó – Kokraimoro, Kubenkokre and Pukany villages – Arara, Katuena and Zoé) [5], the highest value of molecular variance was obtained between the two Arara villages, Iriri and Laranjal (table 4). When Mantel's test was used to compare the values of molecular variance with the genetic distances obtained from the autosomic VNTRs (table 2), a highly significant correlation ($c = 0.613$; $p = 0.0036$) was found.

Conclusions

The presence of a single Y DNA and mtDNA haplotype, and a variation of 1–4 alleles from the autosomic loci investigated, are in accordance with historic reports about the origin of the Arara of Iriri, which indicate that the village was founded by a single couple of siblings who came from another Arara group, the Laranjal village. The present study shows a drastic case of lineal fission from an ancestral village, resulting in a marked genetic cohesion in the daughter village, and great genetic differences between the daughter and ancestral villages. This specific model of fission is an additional factor contributing to allele fixation, as well as to the occurrence of private polymorphisms. Low intrapopulational and high interpopulational genetic diversity among the Amerindians may be caused not only by genetic drift acting on small isolated populations, but also by the process of tribe formation itself.

The present report illustrates that even the analysis of numerous loci may be insufficient to allow an interpretation of the microevolutionary process of these populations in the absence of historical data. On the other hand, biological information may be invaluable to correct historical interpretation based only on cultural and linguistic data, as in the case of two villages (Koatinemo and Trocara) from the Assurini tribe of the Tupi linguistic stock. Although they share linguistic and cultural features, classical genetic markers and mtDNA haplotyping do not support a common origin [5].

The occurrence of a dramatic founder effect may give rise to a misinterpretation of the evolutionary relationship of a population, if historic and demographic events are not taken into consideration. This fact was observed in the present work by comparing genetic measurements (genetic distance and molecular variance) obtained on the basis of the seven autosomic tandem repeats, Y DNA and mtDNA from seven Brazilian Amerindian tribes. In this case, the joint analysis of historical and biological data allowed a more realistic view of the tribe formation process and its genetic consequences. This is not an isolated example within the Amazonian Amerindians, and the present-day genetic similarities or dissimilarities observed for these populations may not reflect the evolutionary relationships between them, emphasizing the importance of considering ethnohistoric factors for populations under analysis.

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