

# Further evidence for the existence of a major founder Y-chromosome haplotype in Amerindians

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## ABSTRACT

We haplotyped human Y-chromosomes with two different PCR-based DNA polymorphisms. The first was the tetranucleotide microsatellite *DYS19* (*Y-27H39*) while the other was based on sequence variation in alphoid repeats located in the Y centromeric region, typed by heteroduplex analysis. There are 23 different alphoid haplotypes ( $\alpha$ h) that, together with *DYS19*, enable us to distinguish at least 37 different Y-chromosome haplotypes worldwide. Previously we studied 12 different Amerindian populations of diverse geographical origins (ranging from Argentina to Mexico) and from several linguistic groups. The haplotype IIA (combination of  $\alpha$ h II and *DYS19* allele A) was seen in the great majority of the individuals studied. We describe here results for 37 further Amerindians belonging to five tribes from the Amazon Basin and Central Brazil. Again, haplotype IIA was found in most individuals (87%), thus confirming its nature as a major, perhaps single, founder haplotype of Amerindians.

## INTRODUCTION

DNA polymorphisms in the human genome are excellent markers of human individuality because of the diploidy and high recombination rate characteristic of our species (Pena *et al.*, in press). Newly arising mutations are quickly shuffled by recombination and involved in complex phenomena such as gene conversions, sister chromatid exchanges, unequal cross-over, etc., to generate further variability and genetic uniqueness. This situation contrasts markedly with two very special compartments of the human genome, the Y chromosome and the mitochondrial DNA,

both of which exhibit a haploid state and lack of recombination. Thus, both remain unaltered from generation to generation, establishing genetic lineages that remain stable until a mutation supervenes. Specifically, human mitochondrial DNA (mtDNA) is transmitted by females only (through the oocyte cytoplasm) to all their progeny, establishing matrilineages. On the other hand, Y chromosomes are transmitted by males to their male offspring and establish patrilineages. Thus, by the study of polymorphisms of mtDNA or Y chromosomes of a given biological sample one can establish with great precision that it belongs to a certain lineage, but not to any specific individual in that lineage. That is why these polymorphisms have been called "lineage markers" (Pena *et al.*, in press).

Recently we reported that the study of twelve widely different Amerindian populations from South and Central America disclosed the existence of a major, perhaps single, Y chromosome founder haplotype (Pena *et al.*, in press). We haplotyped Y-chromosomes with two different PCR-based DNA polymorphisms. The first was the tetranucleotide microsatellite *DYS19* (*Y-27H39*) that has at

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least eight alleles worldwide (Santos *et al.*, in press, a) and a gene diversity of 0.66 in Caucasians (Santos *et al.*, 1993). The other polymorphism, called  $\alpha h$ , is a PCR-based system that amplifies divergent units from the alphoid centromeric region (DYZ3) of the human Y chromosome, causing the formation of heteroduplex molecules between products with slightly different sequences (Santos *et al.*, in press, b). The polymorphic bands are detected by heteroduplex analysis, and at least 23  $\alpha h$  haplotypes can be discriminated worldwide (Santos *et al.*, in press, b). In 73 Amerindian individuals from twelve different tribes ranging from Mexico to Argentina (Pena *et al.*, in press) we identified the presence of haplotype IIA in 74% (91% if we excluded Mapuches, who have a high degree of admixture).

## MATERIAL AND METHODS

### Population samples

Blood was collected from 37 subjects belonging to the Waiwai, Gavião, Zoró, Suruí and Xavante tribes. Their linguistic group, geographical location and degree of admixture are indicated in Table I. Despite the fact that the Gavião, Suruí and Zoró Amerindians belong to the same Tupi linguistic group, each tribe speaks a distinct language. DNA was prepared by standard methods.

### PCR reactions and electrophoresis

For *DYS19* the reaction was performed as described in Santos *et al.* (1993), but increasing the number of cycles to 35. Polyacrylamide gel electrophoresis was performed as described in Santos *et al.* (in press, ab). For the  $\alpha h$  system, PCR reactions and electrophoresis were carried out as described in Santos *et al.* (in press, b), with some modifications. For PCR we used a *Taq* polymerase provided by Cenbiot (Porto Alegre, RS, Brazil) and the amplification program was extended to 45 cycles, to allow a better formation of heteroduplexes. The electrophoresis in MDE 1X polyacrylamide (AT Biochem, Malvern, PA, USA) was run at 120 V for 15 hours. Both polyacrylamide gels were silver stained, as described previously (Santos *et al.*, 1993).

## RESULTS AND DISCUSSION

As shown in Table I, haplotype IIA was predominant, appearing in 32 out of the 37 Amerindians studied. This confirmed our previous identification of IIA as a major founder haplotype in this ethnic group.

An interesting finding was the presence of haplotype IA in five individuals from the Gavião tribe. The  $\alpha h$  haplotype I is characterized by the absence of heteroduplex bands due to the deletion of either left or right

**Table I** - Description of the populations studied and of their Y chromosome haplotypes.

Tribe	Number of individuals (N)	Linguistic group	Geographical location	Estimated admixture* (%)	Haplotypes (N/%)
Waiwai	5	Carib	Pará (57°55'W;0°40'S)	0	IIA (5)
Gavião	17	Tupi	Rondônia (61°8'W;10°10'S)	4	IIA (12/70%) I (L) A (3/18%) I (1) A (2/12%)
Zoró	5	Tupi	Mato Grosso (60°20'W;10°20'S)	0	IIA (5)
Suruí	5	Tupi	Rondônia (61°10'W;10°50'S)	0.5	IIA (5)
Xavante	5	Macro-Gê	Mato Grosso (51°40'W;13°20'S)	1	IIA (5)
TOTAL					IIA (32/87%) I (L) A (3/8%) I (1) A (2/5%)

\* Using Szathmary and Reed's (1978) method and considering 13 protein systems (blood groups: ABO, Kell, Rhesus; hemoglobin; erythrocyte enzymes: acid phosphatase, adenylate kinase, carbonic anhydrase, glucose-6-phosphate dehydrogenase, peptidase A, phosphogluconate dehydrogenase; serum proteins: ceruloplasmin, haptoglobin, transferrin. Unpublished results).

edge loci and can be further subtyped by artificial mixing experiments (Santos *et al.*, in press, b). To our surprise, the latter showed that the  $\alpha h$  haplotype I from these five individuals could be separated into two distinct genotypes, namely genotype I (L), where a deletion of the  $\alpha h$  right locus occurred, permitting the amplification of only the locus  $\alpha hL$ ; and genotype I (l), where the deletion occurred in the  $\alpha h$  left locus and the only remaining amplifying locus was  $\alpha hI$  (data not shown). Both  $\alpha hI$  (L) and  $\alpha hI$  (l) genotypes can be derived from  $\alpha hII$  by simple deletion events as depicted in Figure 1. However, it is remarkable that these two rare events occurred in a relatively small sample of the same Amazonian tribe. There is an alternative explanation. In contrast with haplotype IIA which has not yet been seen in Asians, both types of haplotype IA have been found in our studies of Mongolian (Pena *et al.*, in press) and Siberian (unpublished results) populations. Thus, the haplotypes IA could also represent ancestral founding haplotypes. But the absence of  $\alpha hI$  in other 93 Amerindians from fifteen tribes argues against an Asian origin for these genotypes. They could, for instance, have been introduced in the Gavião by non-Indian admixture (haplotype IA was found in 2% of 100 Brazilian Caucasians; Santos *et al.*, 1993). As is indicated in Table I, this tribe is the most admixed of the five, although its estimated level of admixture (4%) is not high. Information about three other DNA polymorphisms is available for these five individuals (beta-globin gene cluster, D1S80 and the sequence of the first 360 base pairs of the mtDNA major noncoding region). For two of them the data are inconclusive, but one of the carriers

of haplotype  $\alpha hI$  (l) has a beta-globin arrangement (no. 3, ----+) which is highly indicative of African ancestry (Bevilacqua *et al.*, in press). The genealogical information indicates that these individuals are not first degree relatives, but more remote degrees of relationship could not be excluded.

Pooling the results of the Y chromosome haplotype frequencies reported here with those published previously (Pena *et al.*, in press), haplotype IIA is present in 78% of the Amerindians tested (N = 110). If we exclude the Mapuches and Huilliches, who have the highest degree of miscegenation, the frequency of haplotype IIA rises to 90% of the individuals, originating from a total of 14 Amerindian tribes. We are initiating molecular studies of North Amerindian populations to ascertain whether the reduction of variability in Y chromosome haplotypes occurred during migration through the Bering Strait or later, at the Panama isthmus.

### ACKNOWLEDGMENTS

This work was supported by grants from Conselho Nacional de Pesquisas (CNPq), the Centro Brasileiro-Argentino de Biotecnologia of the Brazilian Ministério de Ciência e Tecnologia, Financiadora de Estudos e Projetos (FINEP), Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS), the Wenner-Gren and MacArthur Foundations. The Fundação Nacional do Índio (FUNAI) gave permission to study the Indians and provided logistic help. Approval by the Indian leaders was also obtained before sample collection.

### RESUMO

Determinamos haplótipos do cromossomo Y humano com dois sistemas polimórficos baseados na Reação em Cadeia da Polimerase (PCR). Um dos sistemas foi o microsaturélite de tetranucleotídeo *DYS19* (*Y-27H39*) e o outro era baseado em variações nas repetições alélicas localizadas nas regiões centroméricas do Y, diagnosticadas por análise de heteroduplexes. Há 23 haplótipos alélicos ( $\alpha h$ ) diferentes, que somados ao sistema *DYS19* permitem diferenciar pelo menos 37 haplótipos do cromossomo Y na população mundial. Em nosso estudo prévio, investigamos 12 populações diferentes de ameríndios de diversas origens geográficas (da Argentina ao México) e de vários grupos lingüísticos. O haplótipo IIA (combinação de  $\alpha hII$  e o alelo *DYS19* A) foi visto na grande maioria dos indivíduos estudados. Descrevemos aqui nossos resultados com outros 37 ameríndios pertencentes a cinco tribos da Bacia Amazônica e do Brasil Central. Novamente, o haplótipo IIA foi encontrado na maioria dos indivíduos (87%), confirmando desta maneira sua característica de principal e talvez único haplótipo fundador em ameríndios.

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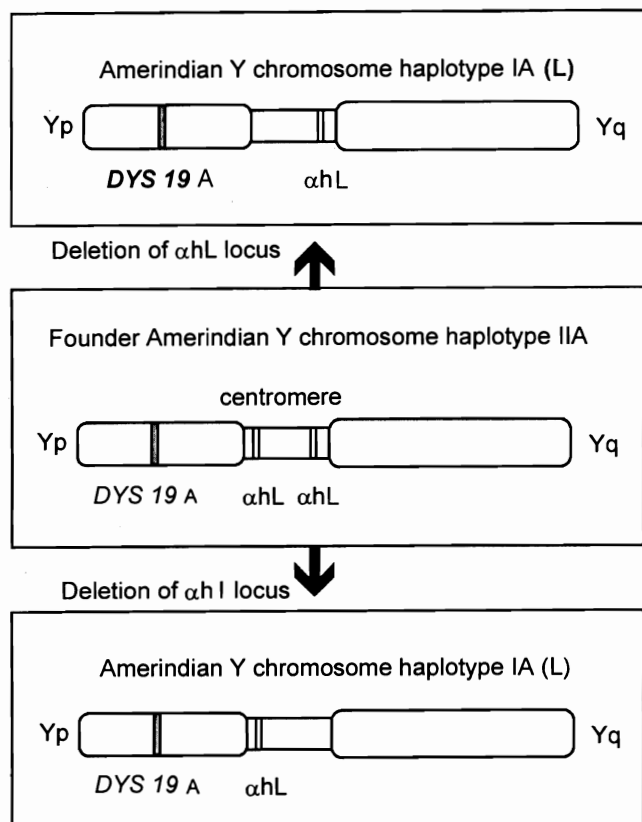


Figure 1 - Diagram indicating how the two types of haplotype IA are formed.

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(Received October 9, 1995)