

Origin of Amerindian Y-Chromosomes as Inferred by the Analysis of Six Polymorphic Markers

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ABSTRACT We analysed the frequency of six Y-specific polymorphisms in 105 Amerindian males from seven different populations, 42 Caucasian males, and a small number of males of African, Chinese, and Melanesian origin. The combination of three of the six polymorphisms studied produced four different Y-haplogroups. The haplogroup A (non-variant) was the most frequent one. Eighty-five percent of Amerindians showing haplogroup A have the alphoid II (α hII) and the DYS19A Y-specific markers, an association that is found only in 10% of Caucasians and that has not been detected in Asiatics and Africans. Haplogroups C (YAP+) and D (YAP+ plus an A \rightarrow G transition in the *locus* DYS271) are of African origin. Four percent of Amerindians and \sim 12% of Caucasians showed haplogroup C; \sim 1% of Amerindians and \sim 2% of Caucasians had haplogroup D. Haplogroup B is characterized by a C \rightarrow T transition in nucleotide position 373 of the SRY gene domain; this haplogroup is found in Caucasians (\sim 12%) and Amerindians (\sim 4%). None of the Amerindians exhibiting the haplogroups B, C, or D show the haplotype α hII/DYS19A. By haplotyping the *Alu* insert and the DNA region surrounding the insert in YAP+ individuals, we could demonstrate that Amerindian Y chromosomes bearing African markers (haplogroups C and D) are due to recent genetic admixture. Most non- α hII/DYS19A Amerindian Y-chromosomes in haplogroup A and most cases in haplogroup B are also due to gene flow. We show that haplotype α hII/DYS19A is in linkage disequilibrium with a C \rightarrow T transition in the *locus* DYS199. Our results suggest that most Amerindian Y-chromosomes derive from a single paternal lineage characterized by the α hII/DYS19A/DYS199T Amerindian-specific haplotype. The analysis of a larger sample of native American Y-chromosomes will be required in order to confirm or correct this hypothesis. *Am J Phys Anthropol* 102:79–89, 1997. © 1997 Wiley-Liss, Inc.

The human Y-chromosome is formed by two distinct regions delimited by a boundary which contains an *Alu* repeat (Ellis et al., 1989). The pseudoautosomal region, located in the most distal part of Yp, undergoes recombination with a homologous region

placed in the distal end of Xp. Conversely, most of the Y-chromosome has no homolo-

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gous counterpart, is not reshuffled by recombination, and is patrilineally transmitted as a complete linkage group. These peculiarities have made the Y-specific chromosomal region a potential candidate for studies of human evolution.

Since the number of germ-cell divisions per generation is much larger in males than in females it has been proposed that mutation rates are also larger in males than in female germ cells (Haldane, 1947). Direct evaluation of mutation rates in regions flanking the non-conserved domain of the SRY gene among different species of rodents (Tucker and Lundrigan, 1993) and primates (Whitfield et al., 1993) showed rapid sequence evolution. Moreover, the interspecies analysis of divergence in intron and exon sequences of ZFX and ZFY genes among human, primates, and other mammal species showed that the frequency of base substitutions is about twofold larger in Y than in X sequences (Pamilo and Bianchi, 1993; Shimmin et al., 1993, 1994; Chang et al., 1994).

At the intraspecies level, the results differ depending on the gene and species studied. The analysis in 20 *Mus domesticus* specimens of a 1,063 bp corresponding to a non-coding region flanking the Sry gene showed two polymorphic base substitutions and two polymorphic insertion/deletion sites (Nachman and Aquadro, 1994). On the other hand, the sequencing of the last intron of the ZFY gene in 38 men from different geographical areas showed no nucleotide difference among them (Dorit et al., 1995). This lack of interindividual variation in humans was assumed to occur due to a process of genetic hitchhiking such as defined by Maynard-Smith and Haig (1974) and Kaplan et al., (1989).

Initial systematic searches for restriction fragment length polymorphisms (RFLP) suggested a paucity of Y-polymorphisms (Jakubicz et al., 1989; Malaspina et al., 1990). Recently, however, the use of the Y chromosome to study human origins and migrations started to gain momentum due to the finding of informative markers. Some of these polymorphisms are due to base substitutions (Ngo et al., 1986; Seielstad et al., 1994), while some others are size variations of a DNA fragment produced by duplications

(Spurdle and Jenkins, 1991), insertion of an *Alu* sequence (Persichetti et al., 1992), or by changes in the number of repeats in a microsatellite (Santos et al., 1993) or in the Y-centromeric alphoid block (Persichetti et al., 1992).

A new and rich source of Y-specific polymorphisms has been reported by Santos et al. (1995b, 1996a). These authors amplified by PCR variant alphoid satellite DNA subunits. When the amplified product was resolved by polyacrilamide gel electrophoresis, a complex pattern of bands was observed. Further analysis of these PCR fragments showed that polymorphic slow migrating bands were heteroduplexes formed from *loci* at the edges of the alphoid array which differed in size and sequence. Study of several human populations has disclosed the existence of 23 distinct heteroduplex phenotypes (α h-alphoid haplotypes), numbered from I to XXIII, which showed patrilineal inheritance (Pena et al., 1995; Santos et al., 1996a).

Recently, in a preliminary report we assessed the association of α h variants with the microsatellite alleles at *locus* DYS19 in chromosomes from human populations of wide geographical origin (Pena et al., 1995). The combination of the 23 variants with the five (A-E) DYS19 alleles may potentially produce 115 different Y-haplotypes. In the populations analysed we could detect 45 different haplotypes with African, Caucasian, and Asiatic populations having 13 to 23 distinct haplotypes (Pena et al., 1995; Santos et al., 1996a). On the other hand, the association of the α hII variant with the DYS19A allele was found in 74% of 54 Y-chromosomes belonging to 12 different Amerindian populations. By excluding the Amerindian population showing the highest level of non-Indian gene flow, the frequency of α hII/DYS19A increased to 91%, suggesting that this haplotype is the predominant one, or perhaps the only founder Y-haplotype in Amerindians (Pena et al., 1995).

In this report, to further explore this assumption, we correlate four Y-specific polymorphisms with the variant forms of α hI-XXIII and DYS19A-E in Caucasians, Amerindians, and in a small number of DNA samples from individuals of other geographi-

TABLE 1. Populations analysed

Populations	No. of cases	Genetic admixture	Linguistic group	Geographic origin
Mapuche	26	<12% \pm 0.09	Mapudugun	Province of Rio Negro, Argentina
Huilliche	12	<5% \pm 0.04	Mapudugun	San Juan de la Costa, Chile
Pehuenche	8	<2% \pm 0.03	Mapudugun	Province of Bio Bio, Chile
Wichi	32	<3% \pm 0.03	Mataco-Mataguayo	Province of Salta, Argentina
Lengua	10	<3% \pm 0.02	Mascoy	Southern Paraguay
Tehuelche	5	<14% \pm 0.08	Aoini-Ken	Pampa de Chalia, Chubut, Argentina
Mayan	12	?	Yucatec	Campeche, Peninsula of Yucatan, Mexico
La Plata Caucasian	26	<30% ¹	—	La Plata, Argentina
CEPH Caucasian	16	?	—	France
Chinese	3	—	—	San Francisco, CA
Melanesian	2	—	—	Bougainville (Coriell Institute)
African Pygmy	4	—	—	Zaire (Coriell Institute)
Total	156			

¹ Data on genetic admixture in La Plata Caucasians was taken from López-Camelo et al. (1995).

cal origin. The four Y-polymorphisms screened are: (1) the presence (+) or absence (–) of the *Alu* insert (YAP) reported by Persichetti et al. (1992); (2) an A \rightarrow G transition in the nucleotide position 168 of the DYS271 *locus* (Seielstad et al. 1994); (3) a C \rightarrow T transition detected by our group in the nucleotide position 373 of the SRY domain (Behlke et al., 1993; accession number L08063); and (4) a C \rightarrow T transition in the 181 bp position of the DYS199 *locus* (Underhill et al., 1996).

MATERIALS AND METHODS

Populations studied

We analysed a total of 105 Amerindian, 42 Caucasian, 3 Chinese, 2 Melanesian, and 4 African pygmy Y-chromosomes. The geographic and ethnic origin of donors and the linguistic group and level of genetic admixture of Amerindian populations are given in Table 1. Gene flow was calculated through the ADMIX program (Chakraborty, 1975) by using alleles of the ABO, Rh, Kell, and Lutheran systems, with the allelic frequencies estimated by MAXLIK program (Reed and Schull, 1968).

The La Plata Caucasians were students from the University of La Plata (Province of Buenos Aires). It has been estimated that Amerindian and Negroid gene admixture in the geographical area of influence of the University of La Plata is 33 and 5%, respectively (López-Camelo et al., 1995). However, since we selected donors with Italian surnames, the level of Amerindian admixture in the paternal lineage of our series of La

Plata male Caucasians is probably much lower than 33%.

DNA samples from Mapuches, Huilliches, Wichis, Lengua, Tehuelches, Pehuenches, and La Plata Caucasians were obtained from the DNA bank at the IMBICE. All samples from this bank come from donors who gave informed consent to the use of their DNA. CEPH samples were generously provided by Dr. H.M. Cann from the Centre d'Etude du Polymorphisme Humain, Paris, France; individuals included in this study belonged to the following families: CEPH 02, 12, 17, 21, 23, 35, 37, 45, 66, 102, 104, 884, 1331, 1333. Mayan DNA samples were provided by Dr. R. Herrera from the College of Arts and Sciences, University Park, Miami, FL. African, Chinese, and Melanesian samples were provided by Coriell Institute for Medical Research, Camden, NJ.

Detection of Y-polymorphisms

YAP, DYS19A-E, and α hI-XXIII markers were amplified by PCR and identified by electrophoresis. The primers used and the PCR and electrophoresis conditions have been reported elsewhere (Hammer and Horai, 1995; Santos et al., 1993, 1995a, 1996a). The DYS271A \rightarrow G (168 bp) transition was identified by PCR amplification and *Not*III digestion as described by Seielstad et al. (1994). The C \rightarrow T transition at base position 181 of the DYS199 *locus* was identified by allele specific PCR using the primers and PCR conditions reported by Underhill et al. (1996). pSRYC \rightarrow T 373 bp transition was detected as follows.

We amplified by PCR a fragment of 390 bp delimited by bp positions 244 and 634 of the SRY domain (Belhke et al., 1993; accession number L08063). The primers used were pSRY244 (forward) 5' CGC GGC TTT GAA TTT CAA GCT CTG 3'; pSRY634 (reverse) 5' CCA GGG CCC CGA GGG ACT CTT 3'. The PCR mixture was 6 pmol of each primer, 200 μ M dNTP, 0.625U Taq polymerase (GIBCO-BRL, Gaithersburg, MD), 50–100 ng DNA in a final volume (25 μ l) of 1 \times Taq buffer. Cycling conditions were 94°C 1 min, 63°C 1 min, 72°C 1 min, for 30 cycles followed by 5 min at 72°C. The PCR product was resolved in 2% NuSieve agarose gel electrophoresis. The fragment was isolated from the gel and eluted in 100 μ l of distilled water. One microliter of the eluted fragment was reamplified in two PCR reactions. One reaction contained the primer pSRY244 and the reverse primer pSRY486, 5' GGG AGT GAC AAC CAA GAA G 3'; the fragment produced by this reamplification was 242 bp long. The second PCR reaction contained the reverse primer pSRY634 and the forward primer pSRY478, 5' GAG GTT CCT CTT CTT GGT TG 3'; the fragment generated was 156 bp long. PCR conditions for reamplifications were as indicated above, except for the annealing temperature that was 55°C.

Both reamplified fragments were analysed by SSCP (Orita et al., 1989) in a 0.5 \times MDE gel run at 3 W for 8 h at room temperature; fragments were visualized by silver staining. All fragments showing a variant migration with the SSCP method were sequenced with the Cyclist Exo-Pfu DNA sequencing kit (Stratagene, La Jolla, CA) using the corresponding forward and reverse primers for each one of the complementary DNA strands. All variant SSCP bands showed the same pattern, and all cases exhibiting this pattern had a C \rightarrow T transition in bp position 373, which produces the loss of a *BanI* site. In a group of 22 males (Mayan, African, Chinese, and Melanesian) the transition was detected by PCR amplification and *BanI* restriction. In the 129 remaining cases the base substitution was diagnosed by SSCP, sequencing, and *BanI* digestion. *BanI* restriction was also employed to confirm the presence of C

in 373 bp position in 26 Mapuches and 12 Huilliches with non-variant SSCP patterns.

Alu haplotyping

Alu fragments from YAP+ individuals were amplified by PCR, isolated from agarose gels and restricted with *AluI*, *HaeIII*, *EcoRI*, *DdeI*, *SspI*, *HhaI*, *MboI*, *FokI*, *NlaIII*, *BanI*, *HpaII*, *HindI*, and *TaqI*. The length of the polyA tail and the identification of nucleotides at positions 338, 1682, and 1926 of the *Alu* insert domain were determined as indicated in Hammer (1995).

RESULTS

Alu insert

YAP+ individuals have an *Alu* insert in the *locus* DYS214 (interval 50, Vollrath et al., 1992) of the Y-chromosome (Hammer, 1994). It has been proposed that this insertion occurred and spread in sub-Saharan African populations before the migration of humans from Africa to other continents (Hammer, 1994). Support for this hypothesis comes from the high frequency of YAP+ individuals in Negroid (78%) and Khoisan (68%) populations (Hammer, 1994; Spurdle et al., 1994). In Caucasians the frequency of YAP+ cases ranges from 4 to 11% depending on the population studied (Spurdle et al., 1994). The frequency of YAP+ chromosomes in Japanese range from 10 to 30% (Spurdle et al., 1994; Hammer and Horai, 1995). Conversely, only YAP-chromosomes were observed in other Asian or Oceanian populations (Spurdle et al., 1994; Hammer and Horai, 1995).

In our series we found two Mapuches, one Pehuenche, one Wichi, and one Mayan with the *Alu* insert, representing a frequency of 4.8% YAP+ in Amerindians (the frequency falls to 3.8% if we exclude the Mayan which also bears an additional polymorphism; see below) (Table 2). The frequency of YAP+ cases in Caucasians was 11.5 and 12.5% for La Plata and CEPH samples, respectively. Two out of the five African pygmies studied were also YAP+ (Table 2).

DYS271 locus

An A \rightarrow G transition in bp position 168 of the DYS271 *locus* has been recently reported by Seielstad et al. (1994). In a total of

TABLE 2. Population frequencies of Y-specific markers

Y polymorphisms	Populations ¹											
	Map 26*	Hui 12	Peh 8	Teh 5	Wic 32	Len 10	May 12	Cau ² 26	Cau ³ 16	Afr 5	Chi 3	Mel 2
YAP												
+	2 (7.7)	—	1 (12.5)	—	1 (3.1)	—	1 (9)	3 (11.5)	2 (12.5) ⁴	2 (40)	—	—
—	24 (92.3)	12 (100)	7 (87.5)	5 (100)	31 (96.8)	10 (100)	11 (91)	23 (88.5)	14 (87.5)	3 (60)	3 (100)	2 (100)
DYS271												
G	—	—	—	—	—	—	1 (9)	—	1 (6.5)	1 (20)	—	—
A	26 (100)	12 (100)	8 (100)	5 (100)	32 (100)	10 (100)	11 (91)	26 (100)	15 (93.8)	4 (80)	3 (100)	2 (100)
pSRY373												
T	—	—	4 (50)	1 (20)	—	—	—	3 (11.5)	2 (12.5)	—	—	—
C	26 (100)	12 (100)	4 (50)	4 (80)	32 (100)	10 (100)	12 (100)	23 (88.5)	14 (87.5)	5 (100)	3 (100)	2 (100)

¹ Map = Mapuche; Hui = Huilliche; Peh = Pehuenche; Teh = Tehuelche; Wic = Wichi; Len = Lengua; May = Mayan; Cau² = La Plata Caucasian; Cau³ = CEPH Caucasian; Afr = African; Chi = Chinese; Mel = Melanesian. Numbers below population acronyms are individuals; numbers in parentheses are percentages.

⁴ The two CEPH YAP + individuals (01) belong to families 66 and 102.

TABLE 3. *Y-chromosome haplogroups*

Populations	Haplogroups				N
	A YAP – DYS271A pSRYC	B YAP – DYS271A pSRYT	C YAP + DYS271A pSRYC	D YAP + DYS271G pSRYC	
Huilliche	12	0	0	0	12
Mapuche	24	0	2	0	26
Wichi	31	0	1	0	32
Tehuelche	4	1	0	0	5
Pehuenche	3	4	1	0	8
Lengua	10	0	0	0	10
Mayan	11	0	0	1	12
Melanesian	2	0	0	0	2
Chinese	3	0	0	0	3
Zaire Pygmy	3	0	1	1	5
CEPH Caucasian	12	2	1	1	16
La Plata Caucasian	20	3	3	0	26
Total	135	10	10	2	157

149 human Y-chromosomes from wide geographical origins, the authors found the base substitution in 19 (12.8%) males. All those cases were also YAP+, indicating that the A → G transition very likely occurred as a single event in a YAP+ individual. Eighteen of the 19 YAP+/DYS271G individuals were sub-Saharan Africans, while one Mayan out of 31 Amerindians analysed was also YAP+ (Seielstad et al., 1994).

We found the YAP+/DYS271G haplotype in one African pygmy, one Mayan Amerindian, and in individual 01 of the CEPH family 102 (Table 2). The male progeny from this individual also showed the same polymorphism (only the proband is included in Tables 2 and 3). We consulted the Coriell Institute which provided our African samples and Dr. Herrera who donated the Mayan samples and it is possible that the African and Mayan cases in Table 2 are the same reported by Seielstad et al. (1994). It is worth mentioning here that in a series of seven Tobas from Chaco, Argentina, not included in this report, we detected an additional YAP+/DYS271G Y-chromosome.

pSRY locus

In the human SRY domain, 2,500 bp upstream of the SRY open reading frame, close to the boundary region, there is a 456 bp DNA segment corresponding to a pseudogene having 86% homology with the cDNA of an autosomal or X-linked gene (Belhlke et al., 1993) (GeneBank accession number L08647). As pseudogenes are prone to muta-

tions, we decided to study the frequency of base substitutions in a segment of 390 bp representing 87% of the pseudogene extension. Besides the 157 males analysed in this report we also studied 30 DNA samples from females. Under the PCR conditions specified in Materials and Methods, primers pSRY244/pSRY634 failed to produce amplification in PCR mixtures containing female DNA. Accordingly, we concluded that the pSRY390 bp fragment produced by the above primers was Y-specific.

The SSCP method was used to make a population screening for mutations in the pSRY fragment. By using this procedure we were able to identify a C → T transition in the 373 bp position of the pseudogene (Fig. 1) which produces the loss of a *BanI* site (Fig. 2). The C → T transition was detected in 11.5 and 12.5% of La Plata and CEPH Caucasians, respectively (Table 2). In the two cases from the CEPH (family 02 individual 01, and family 35 individual 01) we also detected the mutation in male relatives of the proband, confirming the patrilinear inheritance of the polymorphism. In Amerindians the pSRY transition was found in one out of five Tehuelches and in four out of eight Pehuenches (Table 2). The finding of the pSRYT → C (373 bp) transition in four of the eight Pehuenches strongly suggests that these variant individuals had a common and recent male ancestor in spite of the fact that family names were different. This assumption is further supported by the finding of a common αhII variant in the four cases and

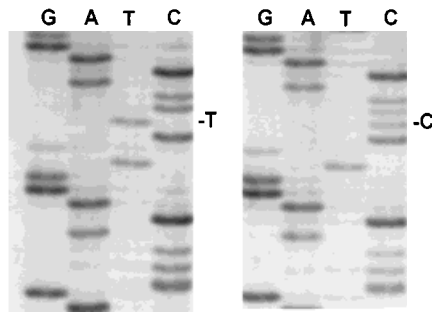


Fig. 1. Sequencing of the pSRY fragment showing a C \rightarrow T transition in bp position 373.

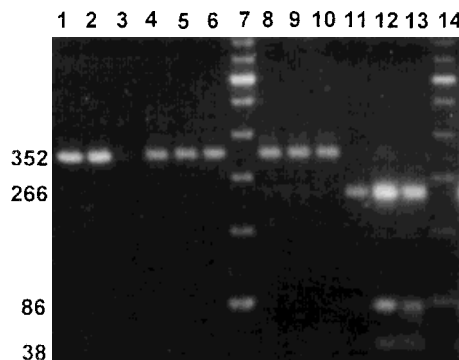


Fig. 2. Non-variant Y-chromosomes have *BanI* sites at 282 and 373 bp positions giving rise to fragments of 266, 86, and 38 bp after restriction. **Lanes 11–13:** Non-variant Amerindian samples. The loss of the 373 bp *BanI* (C \rightarrow T transition) produces fragments of 352 and 38 bp after restriction. **Lanes 1, 2:** La Plata variant Caucasians. **Lanes 4–6:** Related variant males from CEPH family 35. **Lane 8:** Variant Tehuelche. **Lanes 9, 10:** Variant Pehuenches. **Lane 3:** Negative control. **Lanes 7, 14:** DNA marker ladder of 100 bp.

by the demonstration of high levels of inbreeding in this Pehuenche population (Deka et al., 1995).

The five Tehuelches included in our series are aged individuals whose genealogies can be traced back for three generations to the end of the 19th century. The extant Tehuelche population from which we selected the five males included in this report exhibits relatively high levels of admixture with Caucasians (>14%). Most of this admixture comes from a Caucasian family that has lived and interacted with Tehuelches for approximately 80 to 90 years. We studied several Y-chromosomes from this family and we did not detect the pSRYT \rightarrow C transition. Therefore, although we cannot be absolutely

certain, it is probable that the pSRY transition in Tehuelches may not be due to admixture with this co-inhabiting Caucasian family. Moreover, this Tehuelche mutant Y-chromosome shows the α hIV heteroduplex pattern, indicating that it derives from a paternal lineage different from the Pehuenche pSRYT \rightarrow C lineage.

Haplogroups

The combination of YAP, DYS271, and pSRY polymorphisms produces the four different haplogroups depicted in Table 3 (these associations are considered haplogroups as Y-chromosomes clustering in each of the four classes are heterogeneous with regard to α hI-XXIII and DYS19A-E markers). None of the haplogroups detected is Caucasian- or Amerindian-specific.

Haplogroup A (non-polymorphic) is the most frequent (87% of cases). Eighty-five percent of Amerindians in this haplogroup are α hII/DYS19A, a combination that is only observed in 10% of Caucasians and is not observed in Asiatics or Africans (Pena et al., 1995).

Y-chromosomes in haplogroups C and D (Table 3) show different combinations of α hI-XXIII and DYS19A-E; however, none of these cases was α hII/DYS19A. All Caucasians in haplogroup B were α hII/DYS19B; the four Pehuenches showed the pattern α hII and the Tehuelche had the α hIV pattern. Unfortunately, due to the lack of DNA, we could not test the DYS19 marker in these five Amerindians.

Alu haplotyping

Alu inserts from YAP+ chromosomes of Caucasian, African, or Amerindian origin showed similar restriction patterns after digestion with 13 different endonucleases.

Sequencing of the *Alu* insert and a 2.6 kb segment encompassing the *Alu* insert in YAP+ and YAP- chromosomes of different geographical origin showed base polymorphisms at positions 338, 1682, and 1926 of the DNA fragment surrounding the insert and two forms of *Alu* inserts having a short (28 bases) and a long (45 bases) tail of polyA (Hammer, 1995). The combination of these polymorphisms generates five different haplotypes (1–5) with haplotype 1 being the

TABLE 4. Characteristics and correspondence of *Alu* haplotypes with haplogroups C and D

<i>Alu</i> domain haplotypes	YAP	PolyA tail	Base at position			Geographical origin	Haplogroup B and C
			338	1682	1926		
1	—		C	C	G	African, Caucasian Asiatic, Australian	
2	—		C	C	A	African	
3	+	Long	C	C	G	African, Asiatic	C (African Pygmy and Cauca- sian CEPH, Pehuenche)
4	+	Short	C	T	G	Australian, Caucasian	C (La Plata Caucasian, Wichi, Mapuche)
5	+	Short	T	T	G	African	D (African Pygmy, Maya, Toba)

ancestral one (Hammer, 1995). Table 4 details the characteristics and geographic origins of the haplotypes reported by Hammer (1995). All individuals of our series belonging to haplogroups C and D (with the exception of one Mapuche of haplogroup C) were tested for the variants reported by Hammer (1995). The results obtained are indicated in the last column of Table 4.

DYS199 locus

Underhill et al. (1996) reported a C → T transition in base position 181 of the DYS199 locus. The DYS199T allele was observed in 90% of Amerindians, in a high proportion of Nadene and Eskimo, and was absent in African, Asian, and Caucasian populations that showed the non-variant DYS199C allele (Underhill et al., 1996).

In a total of 35 male Amerindians studied, we found thirty Y chromosomes having the α hII/DYS19A haplotype and the DYS199T allele. Four Mapuches from the haplogroup A were, respectively, α hII/DYS19B/DYS199T (1 case), α hII/DYS19B/DYS199C (2 cases), and α hIII/DYS19C/DYS199C (1 case). Moreover, one YAP+ Wichi (haplogroup C) had the haplotype α hXII/DYS19A/DYS199C.

DISCUSSION

Y-chromosomes analysed in this report are distributed in four haplogroups (A–D) such as defined by YAP, DYS271A → G, and pSRYC-T polymorphisms (Table 3). Eighty-one of the 95 Amerindian chromosomes in haplogroup A show the allele A of DYS19 and the variant α hII, an association that is observed at low frequency in Caucasians and that has not been detected in Africans or Asiatics (Pena et al., 1995). From the 14 Amerindians in haplogroup A that were not

α hII/DYS19A, eight were α hII/DYS19B, three were α hIII/DYS19B, one was α hIII/DYS19C, and one was α hV/DYS19B. The α hII/DYS19B haplotype is prevalent in Caucasians, the α hV/DYS19B association has been detected in Caucasians and African pygmies, and α hV/DYS19B has been found in Caucasians and Asiatics (Pena et al., 1995). Accordingly, most non- α hII/DYS19A Y-chromosomes in Amerindians from haplogroup A are very likely the result of gene flow.

As mentioned before, Y-chromosomes in haplogroups C and D (Fig. 3) are assumed to have appeared in Africa after the divergence of humans and big apes and before the divergence of major African groups (Hammer, 1994; Seielstad et al., 1994). These African haplotypes may be present in Amerindians due to genetic admixture with Caucasians or with individuals of African ancestry, or alternatively, they may have entered the Americas from Asia during the early colonization of the continent. The characterization of *Alu* inserts and neighbour DNA regions provides clues to decide between these two possibilities. Table 4 shows that the *Alu*-domain haplotype 4 is detected in Australian Aborigines and Caucasians, while haplotype 5 is of African origin. Since these haplotypes are not found in Asiatics, it is valid to conclude that Y-chromosomes from Wichi and Mapuche Amerindians in haplogroup C and the Toba and Mayan Y-chromosomes in haplogroup D derive from males of African or European ancestry (Table 4). The *Alu* haplotype 3 may be of African or Asiatic origin (Table 4). Therefore, although the Pehuenche chromosome having the *Alu* haplotype 3 and belonging to haplogroup C is probably due to gene flow from non-

Indians, it is not possible to rule out the origin of this chromosome in Asia.

Y-chromosomes in haplogroup B (pSRYT) were found in ~12% of Caucasians and in one Laotian male out of 20 Asiatic individuals tested for pSRYT and not included in this report. Therefore, although Amerindian Y-chromosomes in haplogroup B are very likely the result of admixture with Caucasians, we cannot discard the possibility of an Asiatic origin for some of them.

Underhill et al. (1996) found that 90% of male Amerindians showed a C → T transition in the *locus* DYS199 that is in linkage disequilibrium with the allele A of DYS19. We detected that DYS199T is also in linkage disequilibrium with α hII. Thus, the allele DYS199T should be added to the list of markers defining a predominant Amerindian founder lineage. These results lend additional support to our previous hypothesis that most male Amerindians may derive from a single paternal lineage (Pena et al., 1996) characterized by the haplotype α hII/DYS199T/DYS19A. The findings of Santos et al. (1996b) and Underhill et al. (1996) who detected this haplotype in Y chromosomes from Amerindian populations of North America and also in Y chromosomes from Nadene and Eskimo populations (Underhill et al., 1996), further bolster the above hypothesis. However, given the small sample sizes the assumption of a prevalent, or even single native American founder Y chromosome is still preliminary and will require the analysis of a larger number of male samples and the use of additional Y chromosome markers for definitive confirmation.

The assumption of a specific and highly predominant Amerindian Y-chromosome is quite intriguing specially when this finding is contrasted with the presence of several Amerindian mitochondrial matrilineages (Bailliet et al., 1994; Bianchi et al., 1995). It has been estimated that approximately 56 million people died as a result of the European colonization of America. This gave rise to a reduction of the Indian population size to only 10% of its former level (Black, 1992). This dramatic decrease in size has been proposed to be the main cause explaining the limited genetic variability in the HLA system of Amerindians (Solomon, 1992).

However, it is very unlikely that massive post-Columbian mortality may have played any decisive role in producing a single Amerindian Y-haplotype. Even if only 10% of Amerindians survived, it is difficult to envisage a mechanism by which a single variety of Y-chromosomes persisted and all the other variants were eliminated. Thus, we should conclude that the Amerindian-specific Y-chromosome must have resulted from concurrent causes that acted on the Beringian population from which the first New World colonizers stemmed.

In mammalian mating pairs, there is one Y-chromosome for every two pairs of homologous autosomes and every three X-chromosomes. For this reason, the effective population size of Y-chromosomes is only $\frac{1}{4}$ that of autosomes or $\frac{1}{3}$ that of X-chromosomes. Polygyny and increased male mortality due to warfare and hunting activities (Walker and Lambert, 1989, 1991) are two other factors that may decrease the number of males relative to the number of females in human populations. Moreover, due to the lack of recombination, any advantageous mutation in a gene located in the Y-specific region will move toward population fixation, and will drag along with it all neutral mutants in linkage disequilibrium. The marked decrease in variability that then occurs has been called "genetic hitch-hiking" by Maynard-Smith and Haig (1974) and Kaplan et al. (1989). The combination of a decrease in the effective population size and genetic hitch-hiking may have been the cause producing a single variety of Y-chromosomes in the earliest ancestors of extant Amerindians. This phenomenon may have been restricted to a particular ancestral population inhabiting a specific geographical area (for instance the Beringian region from which the migration wave/s into America originated). Furthermore, the prevalent Y-haplotype found in such a population may occur at much lower frequency, or even be absent in other populations living elsewhere, or under different conditions. Such conditions in ancestors may, in fact, explain the lack of Amerindian Y-chromosomes in a sample of 46 Mongolians studied by us (Pena et al., 1995), in a sample of 40 Asiatics screened by

Underhill et al. (1996) and in the three Chinese reported here.

Chakraborty (1992) has established that for a single autosomal allele to be found in a sample of 100 individuals with 0.01 level of confidence, it needs to have a population frequency higher than 0.0228. DYS19A, DYS199T, and α hII alleles are in linkage disequilibrium. Therefore, the Y haplotype produced by the association of these markers will behave as a single hemizygous allele that will appear at a 0.01 level of confidence in a sample of 100 Y-chromosomes only when its population frequency is higher than 0.0456. The absence of Amerindian Y markers in the sample of 89 Asiatics screened for these polymorphisms allows us to infer that the frequency of the α hII/DYS19A/DYS199 haplotype in extant Asiatic populations should be lower than 0.05. The finding of an Asiatic population exhibiting a high rate of this haplotype will suggest that this population, as well as Amerindians, derive from the same ancestor population.

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