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ORIGINAL INVESTIGATION

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Y chromosome polymorphisms in Native American and Siberian populations: identification of Native American Y chromosome haplotypes

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Abstract We have initiated a study of ancient male migrations from Siberia to the Americas using Y chromosome polymorphisms. The first polymorphism examined, a C→T transition at nucleotide position 181 of the DYS199 locus, was previously reported only in Native American populations. To investigate the origin of this DYS199 polymorphism, we screened Y chromosomes from a number of Siberian, Asian, and Native American populations for this and other markers. This survey detected the T allele in all five Native American populations studied at an average frequency of 61%, and in two of nine native Siberian populations, the Siberian Eskimo (21%) and the Chukchi (17%). This finding suggested that the DYS199 T allele may have originated in Beringia and was then spread throughout the New World by the founding populations of the major subgroups of modern Native Americans. We further characterized Native American Y chromosome variation by analyzing two additional Y chromosome polymorphisms, the DYS287 Y *Alu* polymorphic (YAP) element insertion and a YAP-associated A→G transition at DYS271, both commonly found in Africans. We found neither African allele associated with the DYS199 T allele in any of the Native American or native Siberian populations. However, we did find DYS287 YAP+ individuals who harbored the DYS199 C allele in one Native American population, the Mixe, and in one Asian group, the Tibetans. A correlation of these Y chromosome alleles in Native Americans with those of the DYS1 locus, as detected by the p49a/p49f (p49a,f) probes on *Taq*I-digested genomic DNA, revealed a complete association of DYS1 alleles (p49a,f haplotypes) 13, 18, 66, 67 and 69 with the DYS199 T allele, while DYS1 alleles 8 and 63 were associated with both the DYS199 C and T allele.

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Introduction

In the past decade, many researchers have attempted to trace human genes throughout history by detecting and characterizing polymorphisms in the mitochondrial DNA (mtDNA). Its unique genetic properties, including maternal inheritance, lack of interlineage recombination (Giles et al. 1980), and a high mutation rate (Miyata et al. 1982; Wallace et al. 1987), have resulted in a high degree of mtDNA sequence variation, which has accumulated along radiating female lineages. A number of these mutations have defined continent-specific and population-specific mtDNA haplotypes, which have proved invaluable in the study of human origins and subsequent migrations (Cann et al. 1987; Chen et al. 1995; Torroni et al. 1992, 1993a, 1994b, d; Wallace 1995).

The male counterpart of the mtDNA is the Y chromosome, which exhibits strict paternal transmission, and encompasses large regions of nonrecombining sequence, thus offering the possibility for studies of male migration. Although the Y chromosome nucleotide sequence evolution rate is slower than that of the mtDNA, the Y chromosome contains various repeat elements (Affara et al. 1994), which mutate rapidly. Hence, a combination of base sub-

stitutions and repeat element variants on the Y chromosome can provide useful haplotypes for tracing male migration (Jobling and Tyler-Smith 1995).

One of the first Y chromosome polymorphic systems utilized for population studies was the DYS1 locus detected by probe49a/probe49f Southern hybridization. These probes recognized up to 18 fragments in *Taq*I-digested human genomic DNA, 8 of which could be present, absent, or present in different sizes (Lucotte and Ngo 1985; Ngo et al. 1986). The combinations of fragments present on individual Y chromosomes define p49a,f "haplotypes", referred to here as DYS1 alleles. Unfortunately, the mechanism for generating DYS1 diversity has not been elucidated, and there is evidence that identically sized fragments arose more than once (Torroni et al. 1990; Spurdle and Jenkins 1991), thereby making it difficult to determine the mutational sequence which produces these different alleles. Furthermore, although there are clear differences in the frequencies of these alleles amongst populations, many are shared between continentally distinct populations (Torroni et al. 1990; Spurdle and Jenkins 1991; Spurdle et al. 1994a, b; Torroni et al. 1994a). Thus, unambiguous phylogenetic relationships are difficult to define.

Recently, three polymorphic loci for which the causative mutational events are well-defined have been identified. These markers can be screened by polymerase chain reaction (PCR)-based assays, allowing for rapid screening of a large number of individuals. The first is the DYS287 polymorphism, an *Alu* element insertion (Y *Alu* polymorphic element or YAP) at Yq11. This insertion is present at a high frequency (78%) in African Negroids (Hammer 1994; Spurdle et al. 1994a), at an intermediate frequency (46%) in African Khoisan populations (Spurdle et al. 1994a), and at a low frequency (0 to 11%) in European populations (Hammer 1994; Persichetti et al. 1992; Spurdle et al. 1994a). In Asia, YAP-containing (YAP+) chromosomes have been detected in the Japanese (Hammer 1994; Hammer and Horai 1995) and in Tibetans, Mongolians, and Siberian Altayans (Hammer et al. 1996).

Frequently associated with the DYS287 YAP insert is the DYS271 polymorphism, an A→G transition resulting in an *Nla*III site loss. This polymorphism has been found only on African YAP+ chromosomes (Seielstad et al. 1994), and is thought to have arisen once during modern human evolution on a YAP+ African chromosome.

A third polymorphism, a C→T transition mutation at the DYS199 locus, has been found to be present on a significant proportion of Native American Y chromosomes (Underhill et al. 1996). The T allele was shown to exist at frequencies of 62% to 100% in six Native American populations, although the sample sizes for each population were small, and significant linkage disequilibrium was observed with certain microsatellite length polymorphisms at DYS19. In contrast, the T allele was not found in any African, European or Asian populations. Additionally, the T allele was found to be present in all three putative Native American linguistic stocks – Amerind, NaDene, and Eskimo-Aleut. These data were interpreted as indicating a single origin for Native Americans, followed by subsequent haplotype differentiation within indigenous populations (Underhill et al. 1996). However, the authors could not rule out the possibility that the original mutation occurred in a Siberian or Beringian population that was the source, or had contact with the source, of different population expansions leading to present day Native Americans.

In this paper, we report the occurrence of the DYS199 T allele at significant frequencies in two northeast Siberian populations, as well as in five other Native American populations. Additionally, this marker was always found on Y chromosomes that lacked both the DYS287 YAP insert and the DYS271 G allele, yet was highly correlated with DYS1 alleles 13, 18, 66, 67 and 69. These combinations of markers therefore define several Native American Y chromosome haplotypes. Moreover, since the DYS199 T allele has been found in the northeasternmost Siberian regions (which at the end of the Pleistocene were part of western Beringia), it is possible that these Y chromosomes arose in Beringia prior to the first migration of Asians into the Americas, and were maintained in this area until the submergence of the Bering land bridge. If so, this could explain the broad distribution of the DYS199 T allele in all Native American populations.

Materials and methods

Subjects

Native Americans. Mixtec, Mixe, and Zapotec samples were collected in highland Oaxaca, Mexico (Torroni et al. 1994a) and genomic DNA was extracted from lymphoblastoid cell lines. Seminole samples were collected in Southern Florida (Huoponen et al. 1996) and Navajo samples were collected in Albuquerque, New Mexico (Torroni et al. 1992), with the DNA being extracted from buffy coats.

Siberians and Asians. Evenk, Nivkh, and Udegey (Torroni et al. 1993b) DNA was extracted from buffy coats (see Fig. 1 for geographical location of Siberian populations), and Siberian Eskimo and Chukchi (Starikovskaya et al. 1997) DNAs were extracted from lymphoblastoid cell lines. Korean (Ballinger et al. 1992), Tibetan (Torroni et al. 1994c), and Northern Altayan and Ket (Sukernik et al. 1996) DNAs were extracted from buffy coats. Koryak and Itel'man blood samples were collected in the summer of 1996 in the villages of Voyampolka and Kovran, respectively, in the Tigilsky District, Koryak Autonomous Region, Kamchatka Province, Russia; genomic DNA was extracted from buffy coats. Informed consent was obtained from all individuals.

Methods

DYS199 alleles were typed by PCR amplification followed by digestion with the restriction enzyme *Mun*I. The PCR amplifications were performed with the previously published DYS199 forward primer (Underhill et al. 1996) and a novel reverse primer (5′-TTTCATTTTAGGTACCAGCTCTTCCCAATT-3′). This reverse primer introduces a A→G change in the DYS199 sequence at nucleotide position (np) 186, which, along with a C at np 181, creates a *Mun*I restriction site. For all samples, 211-bp products were amplified by 30 cycles of PCR performed on a Perkin-Elmer 9600 model thermal cycler under the following conditions: 94° C for 30 s, 53° C for 30 s, and 72° C for 30 s. The uncut T alleles (211 bp) were resolved from the cut C alleles (181 + 30 bp) by elec-

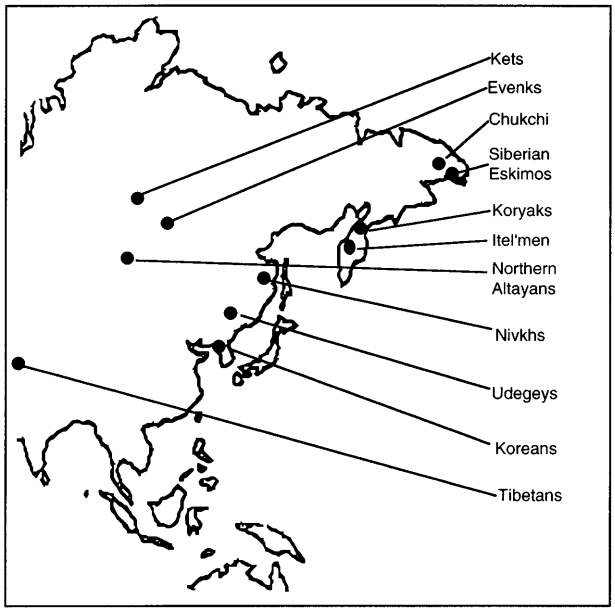

Fig. 1 A map of Northeast Siberia indicating the approximate locations of the Asian populations analyzed in this study. Note the two most northeastern populations, the Siberian Eskimo and the Chukchi, in which the DYS199 T allele was detected

trophoresis on ethidium bromide-stained 3% agarose gels. Selected PCR products were additionally purified and sequenced to confirm results of the PCR/RFLP screening.

The DYS287 (YAP) locus was PCR amplified using flanking primers (Hammer and Horai 1995) that amplify either a 150-bp product (YAP-) or an approximately 455-bp product (YAP+), both of which were resolved on 2% ethidium bromide-stained agarose gels. All YAP+ PCR products and selected YAP– products were directly sequenced utilizing both flanking primers.

The DYS271 locus was PCR amplified as a 209-bp fragment, which was then digested with the restriction enzyme *Nla*III (Seielstad et al. 1994). The fragments were resolved on 2% ethidium bromide-stained agarose gels.

Results

Distribution of Y chromosome polymorphisms

The DYS199 T allele was found in all five Native American populations examined (Mixe, Mixtec, Zapotec, Seminole, and Navajo), at frequencies ranging from 48% to 86% (Table 1). In most Siberian populations (Koryaks, Itelmen, Udegeys, Nivkhs, Evenks, Kets, and Northern Altayans), as well as in Tibetans and Koreans, only the C allele was detected. However, the DYS199 T allele was found in two Siberian populations from the Chukotka peninsula, the Siberian Eskimos and the Chukchi, with the T allele occurring in these groups at frequencies of 21% and 17%, respectively.

DYS287 YAP+ Y chromosomes were observed in one Native American and one Asian population. Two Mixe Indians from Southern Mexico exhibited the *Alu* element insertion. These individuals also exhibited DYS1 alleles previously observed in other non-Native American populations (Torroni et al. 1994 a), and they were the only two Mixe to harbor the DYS199 C allele.

Of 22 Tibetans, 8 were classified as YAP+ based on PCR analysis. All YAP+ PCR products amplified from Tibetan DNA were of equal size, but slightly larger than the PCR products of the Mixe YAP+ loci. Direct sequencing of the PCR products confirmed that the insertions in the Tibetans differed from the Mixe YAP insertions in the length of the 3′ oligo(dA) tail of the *Alu* element. The Tibetan insertions all contained a tail of approximately 45 adenine residues, whereas the Mixe insertions contained 28 adenine residue tails. These size variants appear to correlate with Hammer's (1995) description of long (L) and short (S) YAP elements.

No DYS271 G alleles were observed in any of these Asian or Native American populations.

Table 1 Frequencies of Y chromosome-specific polymorphisms in Native American and Asian populations

Population	*n*	DYS199 "T"	DYS287 (YAP+)	DYS271 "G"
Native Americans				
Mixe	14	85.7	14.3	–
Mixtecs	10	70.0	–	–
Zapotecs	6	50.0	–	–
Seminoles	25	48.0	–	–
Navajo	9	55.5	–	–
Asians/Siberians				
Siberian Eskimos	34	20.6	–	–
Chukchi	24	16.7	–	–
Koryaks	27	–	–	–
Itelmen	19	–	–	–
Nivkhs	19	–	–	–
Udegeys	20	–	–	–
Evenks	31	–	–	–
Northern Altayans	9	–	–	–
Kets	12	–	–	–
Tibetans	22	–	36.4	–
Koreans	4	–	–	–

Table 2 Y chromosome haplotypes in Native American populations

DYS1 allele[a]	p49a/f polymorphic fragments					No. of individuals with DYS199 allele		DYS287 allele	
	A[b]	C	D[c]	F	I	C	T	YAP+	YAP–
1	0	0	0	1	1	1	0	1	0
5	2	0	0	1	1	1	0	1	0
8	2	0	1	1	1	3	1	0	4
12	3	0	1	1	0	1	0	0	1
13	3	0	1	1	1	0	4	0	4
15	3	1	2	1	1	5	0	0	5
18	4	0	1	1	1	0	20	0	20
54	2/3	0	0	1	0	1	0	0	1
56	2/3	1	0	1	1	1	0	0	1
63	5	0	1	1	1	3	5	0	8
64	2/3	0	2	1	1	2	0	0	2
65	6	0	1	1	1	1	0	0	1
66	5[d]	0	1	1	1	0	1	0	1
67	4/5	0	0	1	1	0	1	0	1
68	2/6	0	1	1	0	1	0	0	1
69	4*[e]	0	1	1	1	0	2	0	2
70	5/6	0	1	1	1	1	0	0	1

[a] Nomenclature follows the numbering order of Torroni et al. (1990, 1994a) and Huoponen et al. (1997)
[b] The sizes of the A band series are: A2<A3<A4<A5<A6. A0 indicates absence of the A band
[c] The sizes of the D band series are: D1 > D2. D0 indicates absence of the D band
[d] Has an additional band of about 6.6 kb in the region of the D fragments
[e] The size of the A4* band in haplotype 69 is smaller than the typical A4 fragment (Huoponen et al. 1997)

Correlation of DYS199 alleles with DYS1 alleles

DYS199/DYS1 haplotypes were constructed (Table 2) by correlating our DYS199 results with previously published DYS1 alleles of Southern Mexicans (Torroni et al. 1994a) and Seminoles (Huoponen et al. 1997). Allele 18, the most common Native American DYS1 allele, was observed in 20 of 55 individuals, and was always found on chromosomes containing the DYS199 T allele. Four other DYS1 alleles, namely 13, 66, 67, and 69, were also found to exist only with the DYS199 T allele, although the number of individuals was low for each. Alleles 63 and 8, the second and third most common alleles in these populations (15% and 7%, respectively), were observed on both DYS199 C and T backgrounds. DYS1 allele 15, found in both the southern Mexicans and the Seminoles, is the most common European allele and was associated only with the DYS199 C allele. DYS1 alleles 1 and 5, found in the Mixe, have been detected at low and high frequencies, respectively, in both African and European populations. Both of these DYS1 alleles were detected on chromosomes containing the DYS287 YAP insert and the DYS199 C allele.

Discussion

Origin of the DYS199 T allele

The DYS199 T allele has previously been observed in linguistically diverse populations of Native Americans (Underhill et al. 1996). However, this initial study included only a limited sample of Asian populations, with no native Siberian groups represented amongst them. Consequently, Underhill et al. (1996) could not determine whether the DYS199 T allele arose in some ancestral population prior to migration into the New World or, later, in the subsequent expansions of the first Native Americans.

To expand the search for chromosomes containing the DYS199 T allele, we screened nine native Siberian populations, as well as five Native American and two Asian populations, for the presence of this marker. We found the T allele to be present at high frequencies (48–86%) in all Native American populations, including the Navajo who belong to the NaDene linguistic subdivision (Table 1). The presence of the T allele in the Navajo (Underhill et al. 1996; this paper) indicates that this polymorphism is either an ancient and shared feature of all NaDene populations or that it has been acquired only by southern Athapaskans (Navajo and Apache) through recent admixture with surrounding Amerind populations in the southwestern United States. However, the high frequency (55%) of the DYS199 T allele in the Navajo makes it unlikely that its presence is due solely to recent admixture with Amerinds, unless hypothesizing that most Navajo Y chromosomes are of Amerindian origin. Such an extensive gene flow from Amerinds into southern Athapaskans is not compatible with previous estimates obtained from nuclear and mtDNA studies (Schell and Blumberg 1988; Torroni et al. 1992; Shields et al. 1993).

In contrast, only the C allele was detected in Siberian and Asian populations, with the only exceptions being the two Chukotkan populations, the Siberian Eskimo and the Chukchi, in which the T allele was observed at frequencies of 21% and 17%, respectively. Since southeastern Siberia and Mongolia have been proposed as a potential geographic source for ancestral Native American populations (Neel et al. 1994; Merriwether et al. 1996; Sukernik et al. 1996), the absence of the T allele in eastern, western, and southern Siberian ethnic groups suggests that the original C→T mutation occurred in the putative ancestral

Native American population(s) after it left these geographic areas. The lack of DYS199 T alleles in the Tibetans further suggests that this Y chromosome marker did not arise in Central Asia.

The origination of the DYS199 T allele before the first New World colonization event is consistent with its presence in all of the major linguistic subdivisions of Native Americans. Under this model, the T allele would have been maintained in the ancestral Beringian population(s), which subsequently became separated from the populations living further south in the Americas due to glacial barriers (Rogers et al. 1991). This Beringian population(s), in turn, became ancestral to modern day Eskimo-Aleuts and Chukchi, who are postulated to have had a Beringian origin based on their genetic affiliation with other circumpolar populations (Szathmary 1984; Starikovskaya et al. 1997). Thus, the presence of the DYS199 T allele in the Chukchi may be attributable to its presence in the ancestral population of the Paleoasiatic-speaking groups of northeastern Siberia.

This scenario is consistent with the age estimate of 30 000 YBP for the original C→T mutation based on linkage with the DYS19 tetranucleotide repeat locus (Underhill et al. 1996) and a microsatellite mutation rate of 1.5×10^{-4} (Jin et al. 1994). In addition, an early human entry into the Americas from Asia has been favored by mtDNA RFLP data (25 000–38 000 YBP; Torroni et al. 1993 a, b, 1994 d; Starikovskaya et al. 1997), mtDNA control region sequence data (20 000–25 000 YBP; Forster et al. 1996), and nuclear genetic variation studies (31 000 YBP; Cavalli-Sforza et al. 1995). Thus, multiple independent estimates of genetic divergence times for Native American populations using different genetic systems provide strong support for a primary entry into the New World prior to the last glacial maximum, and are compatible with a Beringian origin of the DYS199 polymorphism.

Underhill et al. (1996) acknowledged, however, that the specific mutation rate at DYS19 is unknown and that the use of Weber and Wong's (1993) estimate of 2.1×10^{-3} would predict that the DYS199 C→T transition occurred as recently as 2147 YBP. Although this estimate appears to severely underestimate the age of the T allele given its frequency and distribution among Native American populations, it raises the possibility that this marker arose in a Beringian or American population after the initial entry into the New World. Since the Chukchi, Eskimos, and NaDene Indians trace their origins to the latest inhabitants of Beringia, and the Amerinds to an earlier expansion into the New World (Forster et al. 1996; Starikovskaya et al. 1997), the broad distribution of the T allele in the Americas would have to be due to extensive gene flow between native populations of diverse linguistic groups. Furthermore, acceptance of a much later origin of the DYS199 polymorphism requires the additional assumption that its presence in northeastern Siberia is due to an independent, identical C→T transition at this locus or that the distribution results from back migration and gene flow from Alaskan Eskimos westward across the Bering Strait, as suggested by Karafet et al. (1997) in a recent report, which also detected the T allele in three Siberian Eskimos, one Chukchi, and one Evenk individual. Arguing against this hypothesis is the fact that gene flow during the expansion of the Chukchi in northeastern Siberia over the last two centuries has occurred predominately through Chukchi males taking Eskimo female mates, as indicated by regional history (Bogoras 1909; Menovshichikov 1959), by their pedigrees and family histories (R. I. Sukernik, unpublished data), and by Gm typing data (Sukernik and Osipova 1982; Sukernik et al. 1986 a, b). This would not account for the large paternal admixture into the Chukchi necessary to create the present distribution and frequency of Y chromosome types. Hence, the observed distribution of this marker has most likely resulted from a single origin of the DYS199 T allele at a time when North America was contiguous with Beringia and the New World had not yet been populated by the ancestors of modern Native Americans. However, this question cannot be fully answered until better estimates of microsatellite mutation rates and more extensive Y chromosome haplotypes are available.

Interestingly, the distribution of the DYS199 T allele mirrors that of the np16111 C→T transition observed within mtDNA haplogroup A. This polymorphism occurs in populations of each major linguistic subdivision of Native Americans, but is found only in the Siberian Eskimo and Chukchi among eastern Siberian populations (Shields et al. 1993; Ward et al. 1993; Torroni et al. 1993 b). The divergence time of haplogroup A mtDNAs was estimated to be 28 000–35 000 YBP for both Siberian and Native American populations (Torroni et al. 1993 a; Starikovskaya et al. 1997), concordant with the early estimate of 30 000 YBP for the origin of the DYS199 C→T mutation. Thus, both paternal and maternal genetic systems provide support for theories of early entry into the New World around 30 000 YBP, and for the maintenance and further divergence of lineages in isolated Beringian populations.

Identification of Native American Y chromosome haplotypes

The DYS199 T allele was found to be correlated with specific DYS1 alleles in the Mixe, Mixtec, Zapotec, and Seminole Indians, confirming the discovery of Native American specific Y chromosome types. Two of the most common DYS1 alleles in Native Americans (13 and 18) were always associated with the DYS199 T allele, whereas two other common DYS1 alleles (8 and 63) were found to exist with the DYS199 C and T alleles. Additionally, the predominate European and African DYS1 alleles (15 and 5, respectively) were found only with the DYS199 C allele. These data suggest that the combinations of (DYS287) YAP–/DYS199 T allele/DYS1 allele 13, 18, 66, 67 or 69 define Native American Y chromosome haplotypes.

Based on these observations, we can speculate on the DYS1 allele state of the Y chromosome on which the

original C→T mutation occurred. One model of DYS1 allele differentiation states that the primary diversifying mechanism is the sequential loss of *Taq*I restriction sites due to the high frequency of base substitution at CpG dinucleotides (Ngo et al. 1986). Thus, smaller bands in the p49a,f allelic system should be ancestral to larger ones. Strict application of this model to our data suggests that DYS1 allele 8 is ancestral to all of the other DYS199 T allele-bearing Y chromosomes presented here, as they can all be derived from allele 8 by sequential *Taq*I site losses (see Table 2). This is consistent with DYS1 allele 8 being associated with both the DYS199 C and T alleles.

Another possibility, based on frequency and distribution data, is that the DYS199 T allele arose on a DYS1 allele 18 Y chromosome, which was previously suggested to be one of the founding Native American Y chromosome alleles (Torroni et al. 1994a). DYS1 allele 18 is the only DYS1 allele present in all four Native American populations in this study, and it is by far the most common allele in these populations, being present in 36% of these individuals. However, if a Y chromosome bearing the DYS1 allele 18 is the chromosome on which the DYS199 T allele arose, then both the DYS1 allele 8 and allele 63 must have arisen at least twice.

DYS1 allele 63, the second most common DYS1 allele in these Native American populations (15%), was found to be associated with both the C and T alleles at DYS199, raising the possibility that the DYS199 transition mutation may have occurred on a DYS1 allele 63 Y chromosome. However, as with an origin on a DYS1 allele 18 Y chromosome, this scenario also assumes that the 49a,f allelic system is prone to multiple reductions of band sizes, either through the restoration of *Taq*I sites or some other diversifying mechanism.

Y *Alu* polymorphic element and the origin of the Japanese

The question of Japanese origins has been debated for many years (reviewed in Nei 1995). Recently, Y chromosome variation analysis has shed new light on this subject. Studies have detected the DYS287 YAP element in Japanese populations (Hammer 1994; Spurdle et al. 1994a, b), and it has been speculated that the YAP element is a marker of Jomon male lineages (Hammer and Horai 1995), providing support for the hybridization theory of modern Japanese origins.

Here we report the finding of YAP+ Y chromosomes at a frequency of 36% in a Tibetan population. All YAP+ chromosomes in this population exhibited the same haplotype based on the markers analyzed (DYS199 C, DYS271 G). Additionally, the YAP elements in these Tibetans exhibited the long 3′ oligo-d(A) tail reported in the Japanese (Hammer 1995). Recently, Hammer et al. (1996) have also reported the presence of YAP+ Y chromosomes at a high frequency in Tibetans (53.3%) and at lower frequencies in Mongolians and Siberian Altayans (2.6% and 3.2%, respectively), as well as the lack of YAP+ chromosomes in 20 Southeast Asian and Oceanic populations. These findings suggest that the Tibetan and Japanese YAP+ Y chromosomes share a common origin and they provide strong support for the out-of-Northeast-Asia theory of Japanese origins (Nei 1995).

Conclusion

In conclusion, our results indicate that Native American males derive from a limited number of founders carrying defined Y chromosome haplotypes. Moreover, the discovery of the DYS199 T allele in the two northeasternmost Siberian populations suggests that the Native American Y chromosomes described here may have arisen in an ancestral Beringian population. However, additional Y chromosome markers must be studied in these and other populations to better order and define these events.

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