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Mitochondrial DNA and Y Chromosome-Specific Polymorphisms in the Seminole Tribe of Florida

Abstract

Mitochondrial DNA (mtDNA) sequence variation was examined in 37 Seminoles from Florida by polymerase chain reaction amplification and high resolution restriction endonuclease analysis. The Y chromosome *Taq*I restriction fragment length polymorphisms detected by the probes 49a, 49f, and 12f2 were examined in the 26 males of this group. Analysis of the mtDNA revealed that all four Native American haplogroups (A, B, C and D) were present in the Seminoles encompassing about 95% of the Seminole mtDNAs. No European mtDNAs were found among the Seminoles, but two mtDNAs (about 5%) were members of the African-specific haplogroup L1, thus indicating that a limited number of African women were incorporated in the Seminole tribe. Analysis of Y chromosome haplotypes supports the hypothesis that haplotypes 18 and 63 are the most likely founding Native American Y chromosome haplotypes from Asia. However, 11% of the Seminole Y chromosomes represented haplotypes generally attributed to Europeans, though none harbored standard African haplotypes. These findings support historical evidence that the Seminole tribe has integrated individuals of European and African ancestry, but suggests that the sex ratio of nonnatives from different continents may have varied.

Introduction

The Seminole tribe of Florida consists of 2,300 individuals who reside on five federal-trust reservations in South Florida which include Hollywood, Big Cypress, and Brighton. Approximately 600 tribal members live on each of these three large reservations. Two smaller reservations are at Immokalee and Tampa. The Miccosukee tribe of Florida, the only other federally recognized tribe in the state, consists of about 800 Seminoles who broke away politically from the Seminole tribe in the 1960s and live on federal land deep in the Everglades.

There were hundreds of Native American tribes living across the land the Spaniards named La Florida when the Spaniards first entered southeastern North America in the early sixteenth century. The most numerous were the culturally similar Maskóki speakers, who were the ancestors of today's Seminoles. Following European arrival, their numbers began to decline rapidly due to the effects of European-introduced pathogens, the establishment of English colonies in the Southeast after 1670, English slave raiding, Anglo-Spanish conflicts, and the sociodemographic effects of the English deerskin trade.

By 1821, at least 5,000 Maskóki speakers from numerous tribes – including Abalache, Yamasee, Yuchi, Abalachicola, Calusa, Timuguana, Coca, Oconî, Ochisi, and others – were scattered across the peninsula. English-speakers applied several misnomers to these peoples by calling them, generically, 'Creeks', 'Mikasukî', and 'Siminolie'. By the end of the three Seminole Wars in 1858, about 3,500 of these Native Americans had been forcibly removed to Oklahoma Territory, leaving as few as 300 in Florida. These had taken refuge in the Everglades, and despite the genetic tenuousness of such a small population, they not only survived but increased demographically. Their descendants emerged in the early twentieth cen-

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tury when American settlement again encroached upon them.

Today they are collectively called 'Seminoles', a misnomer derived from a transliteration of the Spanish word cimarrón, originally an Arawak word meaning 'free peoples'. Within the Seminole tribe of Florida today, members still recognize their tribal clans, which represent matrilineal, extended-family affiliations comprising both blood and fictive kinship. Seminoles speak two distinct languages belonging to the North Penutian group of the Amerind linguistic family: Maskóki and Mikasukî [1]. Maskóki is a descendant of the parent language and is spoken predominantly by the Maskóki people (the so-called Creeks), who reside almost exclusively at Brighton, whereas Mikasukî is a linguistic descendant of Hitchíti (a Maskóki dialect), and is spoken by the majority of tribal members, although many Seminoles speak both languages.

Since 1510, the Seminoles have experienced some European genetic admixture, albeit limited by strong traditional proscriptions. From 1638 onward, the peninsular tribes lived in proximity to runaway African American slaves who fled the English and the Americans into the sanctuary of Florida. During the period 1817–1858, the period of the Seminole Wars, the history of the two groups became directly intertwined as the trickle of escapees or 'maroons' (also from the Arawak-Spanish word, cimarrones), turned into a flood. The Seminole-African relationship was generally a symbiotic one, however, with runaways permitted to form villages adjacent to Native American villages in return for tributary payments in the form of agricultural products. Some Native Americans purchased slaves outright in the manner of the Americans. The African Americans also served as translators and councilors for the Natives and fought beside them against removal. There was undoubtedly some genetic admixture, and this has continued into the twentieth century [2, 3].

To learn more about the genetic history of the Seminoles, we examined the mitochondrial DNA (mtDNA) and Y chromosome variation from 37 individuals representing several clans. Human mtDNA and Y chromosome variation encompasses a spectrum of continent-specific polymorphisms which permit estimation of the extent of female and male contributions to a Native American population by African and European individuals.

The extent and nature of mtDNA variation in the Native Americans has been the object of many studies [4–17]. These studies have revealed that Native American mtDNAs cluster in primarily four mtDNA haplogroups, named A, B, C and D, each defined by a specific set of mutations. The distribution and ages of these haplogroups in Amerind, Na-Dene, Siberian and Asian populations has been interpreted as indicating that the first human expansion from Siberia could have occurred between 26,000 and 34,000 years before present, giving rise to the Amerinds, while a more recent migration (7,000–10,000 years before present) gave rise to the Na-Dene of North America [18, 19].

In contrast, over 70% of Africans harbor a different mtDNA haplogroup defined by a *Hpa*I site at nucleotide pair (np) 3592 [20], and about 65% of Europeans harbor distinctive haplotypes labeled H, I, J, K, each delineated by distinctive restriction site polymorphisms [21]. These continent-specific markers, together with our extensive data on mtDNA variation of different Native American populations, now make it possible to investigate interrelationships of various tribes, and the effects of more recent female admixture of Europeans and Africans on Native American genetic diversity.

The analysis of the variation in the paternally transmitted Y chromosome provides complementary data on the origins of Native American males and the integration of European and African men into Native American tribes. Unlike the highly variable mtDNA, Y chromosome-specific sequences show a limited amount of nucleotide sequence diversity [22, 23]. However, a number of polymorphic Y chromosome-specific sequences have been identified [22, 24–28]. Many studies on Y chromosome-specific DNA variation have been performed with the 12f2 [29–32], and the p49a/p49f probes [31, 33–44]. Both 49f and 49a probes identify 18 *Taq*I fragments (named from A to R) which, with the exception of the K and L bands, are male-specific [34]. The p12f2 probe recognizes a restriction fragment length polymorphism identifiable by both *Eco*RI (allelic fragments of 5.2 and 3.2 kb) and *Taq*I (allelic fragments of 10 and 8 kb) that most likely represents an insertion/deletion polymorphism [29]. There is a strong linkage disequilibrium between the 49f-49a haplotypes and the 12f2 alleles [31], and between 49f haplotypes and other Y chromosome-specific markers [27, 42]. Both mtDNA and Y chromosome-specific sequences escape regular recombinational processes, and thus their variation is the result of the sequential addition of mutations through the generations.

Analysis of the Seminole's mtDNA and Y chromosome variation revealed that the Seminole harbor all four Amerind mtDNA haplogroups, and have experienced limited gene flow from African women. The Y chromosome data suggested a more substantial contribution from European males.

Subjects and Methods

Subjects

Blood samples were collected from 37 members of the Seminole tribe of Florida. Although clans are the basic social units within the tribe, they no longer cluster in discrete camps. Therefore, we sampled representatives of as many clans as possible, without regard to reservation. Because only a small number of individuals constituted the 'founding' gene pool of the modern tribe (i.e., the 1858 survivors), it is probable that many Seminoles are close relatives. Therefore, to maximize the detection of genetic diversity, we solicited samples from only those individuals who did not share a common kinship within the last one to two generations (such as parent/child, or full or half siblings).

Before sample collection, the subjects were interviewed and informed about the aim of the study, and pedigree information and informed consent were obtained from all participants. Blood samples were collected in ACD solution and kept cold until arrival at our laboratory. DNAs for mtDNA analysis were extracted from platelet pellets, and DNAs for Y chromosome analysis were extracted from buffy coats using standard procedures.

mtDNA Analysis

The entire mtDNA of each sample was amplified in nine partially overlapping fragments by the polymerase chain reaction (PCR) using the primer pairs and amplification conditions described [8]. Each PCR segment was digested with 14 restriction endonucleases (*Alu*I, *Ava*II, *Bam*HI, *Dde*I, *Hae*II, *Hae*III, *Hha*I, *Hin*cII, *Hin*fI, *Hpa*I, *Msp*I, *Mbo*I, *Rsa*I, *Taq*I). The resulting fragments were resolved through electrophoresis in NuSieve plus SeaKem agarose (FMC BioProducts) gels and visualized by UV-induced fluorescence. This restriction analysis screens about 15–20% of the mtDNA nucleotides and defines the mtDNA haplotype (Appendix). The Seminole mtDNA haplotypes were compared to each other and to those observed in other Native American populations [7, 8, 10, 11] through phylogenetic analysis using parsimony [45]. Maximum parsimony (MP) trees were generated through random addition of sequences using the tree bisection and reconnection (TBR) algorithm. Because of the large number of terminal taxa, thousands of MP trees could be obtained. We terminated our search at 1,050 trees after 1,000 replications, with no more than 10 MP trees saved for each replication. Consequently, shorter trees could exist, although none were observed in our analyses. A strict consensus tree of the 1,050 MP trees obtained by the TBR algorithm was also obtained. The phylogenetic dendrograms were rooted using a Senegalese mtDNA haplotype ('African outgroup') [8].

Y Chromosome Analysis

Analysis of the Y chromosome polymorphisms was performed for the 26 males. About 8 μg of total DNA were digested with 5 U/μg of *Taq*I. The fragments were separated by electrophoresis on 0.85% BRL agarose gels and blotted to nylon membranes (MagnaGraph MSI). The filters were hybridized first with a mixture of the 49f and 49a probes and autoradiographed. They were then stripped and rehybridized with the 12f2 probe [31, 36]. Probes 49f (2.8 kb) and 49a (0.9 kb) are subfragments, 6 kb apart [46], localized at Yq11.2 [47]. Probe 12f2 (2.3 kb) is a derivative of the 12f probe which was isolated from a partial human Y DNA library [46] and was assigned to Yq11.1-11, 21 [29]. Y probes were ^{32}P-labeled by random priming to a specific activity of about 2×10^9 cpm/μg of DNA, hybridized to filters, and the filters washed twice for 30 min at 50°C in 2 × SSC (0.3 *M* NaCl; 0.03 *M* trisodium citrate) plus 0.1% SDS.

Table 1. mtDNA haplotypes in the Seminole

Haplotype	Haplogroup	Seminole (n = 37)
AM1*	A	2 (5.4)
AM9*	A	6 (16.2)
AM13*	B	4 (10.8)
AM32*	C	2 (5.4)
AM43*	C	1 (2.7)
AM63*	A	1 (2.7)
AM88*	D	2 (5.4)
AM125	A	1 (2.7)
AM126	A	8 (21.6)
AM127	A	1 (2.7)
AM128	A	2 (5.4)
AM129	A	1 (2.7)
AM130	B	1 (2.7)
AM131	B	3 (8.1)
AM132	L	2 (5.4)

Asterisks indicate haplotypes previously observed in other Amerind populations [8, 10, 11]. Haplogroup L is an African-specific haplogroup [20]. Numbers in parentheses indicate percent frequencies.

Results

mtDNA Haplotype Analysis

Fifteen mtDNA haplotypes (AM1, AM9, AM13, AM32, AM43, AM63, AM88 and AM125–AM132) were observed among the Seminoles (table 1) as defined by 29 polymorphic restriction sites and the 9-base pair COII-tRNA^{Lys} intergenic deletion [48–51] (Appendix). Thirty-five out of the 37 mtDNAs analyzed (94.6%) fell into one of the four haplogroups A, B, C and D which characterize Amerind populations [8]. The remaining two mtDNAs (5.4%) harbored an identical haplotype (AM132) which was previously reported only in sub-Saharan Africans. None of the Seminole mtDNA haplotypes belonged to any of the European-specific haplogroups [21].

Haplogroup A is defined by a *Hae*III site at np 663 and encompassed eight (AM1, AM9, AM63, AM125–AM129) of the 15 haplotypes observed in this study and 59.5% of the Seminole mtDNAs. Haplotypes AM1, AM9 and AM63 have been previously described in other Native American tribes. Haplotypes AM1 and AM9 are broadly distributed among numerous tribes, but haplotype AM63 was previously reported only in the Ojibwa of Northern Ontario [8]. The remaining five haplogroup A

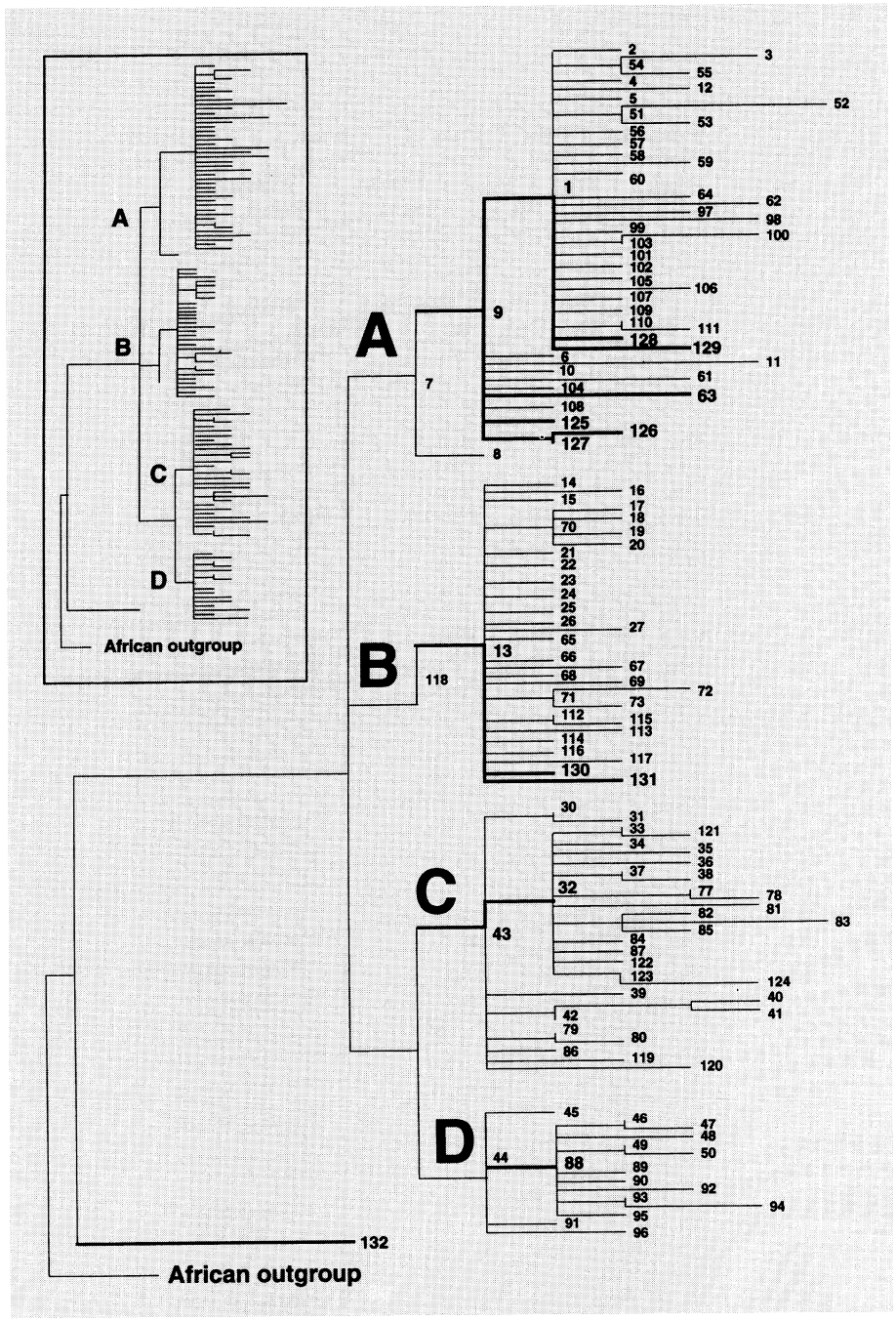

Fig. 1. MP tree which includes the 15 haplotypes (AM1, AM9, AM13, AM32, AM43, AM63, AM88, AM125–AM132) found in the Seminole, and the other haplotypes (AM1–AM27, AM29–AM73, AM77–AM124) previously observed in other Native American tribes [8, 10, 11]. Haplotypes AM28, AM29 and AM74–AM76 were not included because of their probable European origin [52]. The capital letters A–D indicate the four haplogroups. Haplotypes observed in the Seminole are indicated by bold lines. The numbers at the end of each branch indicate different mtDNA haplotypes. The horizontal branch lengths are proportional to the number of mutational events that separate the haplotypes. The inset shows the strict consensus tree of the 1,050 MP trees generated with the TBR branch swapping algorithm. This dendrogram is 217 steps in length, with consistency and retention indices of 0.414 and 0.781, respectively. Each of the 1,050 MP trees was 185 steps in length, with consistency and retention indices of 0.552 and 0.874, respectively.

haplotypes observed in the Seminoles (AM125–AM129) were found here for the first time. The most common haplogroup A haplotypes were AM126 and AM9, which encompassed 36.4 and 27.3%, respectively, of the Seminole haplogroup A mtDNAs.

Haplogroup B is defined by the COII-tRNA^{Lys} intergenic deletion in association with the *Hae*III np 16517 site gain. This haplogroup encompassed three haplotypes (AM13, AM130, AM131) and 21.6% of the Seminole mtDNAs. Half of the Seminole haplogroup B mtDNAs were haplotype AM13. This haplotype is the most common haplogroup B haplotype in most Amerind populations, and has been proposed as the founding haplotype of haplogroup B [7, 8]. Haplotypes AM130 and AM131 are new haplotypes and encompassed 12.5 and 37.5%, respectively, of the Seminole haplogroup B mtDNAs.

Table 2. Y chromosome-specific polymorphisms in Amerind populations

49a/49f haplotype[a]	49a/49f polymorphic fragments					12f2 probe alleles		Seminole (n = 26)	Mixtec[b] (n = 11)	Zapotec[b] (n = 6)	Mixe[b] (n = 14)	Total (n = 57)
	A	C	D	F	I	8 kb	10 kb					
1	0	0	0	1	1	–	+	–	–	–	1 (7.1)	1 (1.8)
5	2	0	0	1	1	–	+	–	–	–	1 (7.1)	1 (1.8)
8	2	0	1	1	1	–	+	4 (15.4)	–	–	–	4 (7.0)
12	3	0	1	1	0	–	+	1 (3.8)	–	–	–	1 (1.8)
13	3	0	1	1	1	–	+	–	–	2 (33.3)	2 (14.3)	4 (7.0)
15	3	1	2	1	1	–	+	3 (11.5)	1 (9.1)	1 (16.7)	–	5 (8.8)
18	4	0	1	1	1	–	+	8 (30.8)	5 (45.5)	1 (16.7)	8 (57.1)	22 (38.6)
54	2/3	0	0	1	0	–	+	1 (3.8)	–	–	–	1 (1.8)
56	2/3	1	0	1	1	–	+	1 (3.8)	–	–	–	1 (1.8)
63	5	0	1	1	1	–	+	4 (15.4)	2 (18.2)	–	2 (14.3)	8 (14.0)
64	2/3	0	2	1	1	–	+	1 (3.8)	–	1 (16.7)	–	2 (3.5)
65[c]	6	0	1	1	1	–	+	–	–	1 (16.7)	–	1 (1.8)
66[c, d]	5	0	1	1	1	–	+	–	1 (9.1)	–	–	1 (1.8)
67[c]	4/5	0	0	1	1	–	+	–	1 (9.1)	–	–	1 (1.8)
68[c, e]	2/6	0	1	1	0	–	+	–	1 (9.1)	–	–	1 (1.8)
69[c]	4*[f]	0	1	1	1	–	+	2 (7.7)	–	–	–	2 (3.5)
70[c]	5/6	0	1	1	1	–	+	1 (3.8)	–	–	–	1 (1.8)

Figures in parentheses indicate percentage.

[a] Nomenclature follows the numbering order of Torroni et al. [10, 36] and Santachiara-Benerecetti et al. [31]. An alternative numbering system was adopted by Spurdle and Jenkins [41] and Spurdle et al. [27].

[b] From Torroni et al. [10].

[c] Haplotypes not described in Old World populations.

[d] Has an additional band of about 6.6 kb in the region of the D fragments.

[e] Lacks fragment B.

[f] The size of the fragment A4* observed in haplotype 69 is smaller than that observed in a typical A4 fragment (fig. 2).

Haplogroup C is defined by the concurrent loss of the *Hin*cII site at np 13259 and *Alu*I site gain at np 13262 in association with the *Dde*I np 10394 and *Alu*I np 10397 site gains. This haplogroup encompassed two haplotypes (AM32 and AM43) and 8.1% of the Seminole mtDNAs. Both haplotypes AM32 and AM43 have been previously seen in various Amerind tribes, and AM43 has been proposed as the founding haplotype of haplogroup C [8].

Haplogroup D is defined by the *Alu*I site loss at np 5176 in association with the *Dde*I np 10394 and *Alu*I np 10397 site gains. This haplogroup encompassed only one Seminole haplotype (AM88), encompassing 5.4% of the Seminole mtDNAs. Haplotype AM88 has been described in other Amerind tribes and has been proposed to be the founding haplogroup D haplotype [8].

The genetic relationships among the 15 mtDNA haplotypes (AM1, AM9, AM13, AM32, AM43, AM63, AM88, AM125–AM132) observed in the Seminoles and the 119 haplotypes (AM1–AM27, AM30–AM73, AM77–AM124) described for other Native American populations [7, 8, 10, 11] were further defined through parsimony analysis. The strict consensus tree of 1,050 MP trees is also shown in figure 1.

The two Seminole mtDNAs which did not cluster in any of the four Native American haplogroups (fig. 1) harbored the same haplotype, AM132. This haplotype possesses the *Hpa*I np 3592 and the *Hin*fI np 10806 site gains, which define the African-specific haplogroup L1. Interestingly, haplotype AM132 is identical to the African haplotype AF71 observed among the Mandenka, the Wolof, and the Pular from Senegal [20]. The presence of this African haplotype in the Seminole indicates that at least one African woman was integrated in the Seminole population.

Y Chromosome Haplotype Analysis

Ten 49a-49f/*Taq*I haplotypes (8, 12, 15, 18, 54, 56, 63, 64, 69 and 70) were observed among the 26 Seminole males (table 2). These haplotypes are defined by the presence or absence of fragments C, D, and I, and by variation in size and number of the fragments of the A and D series

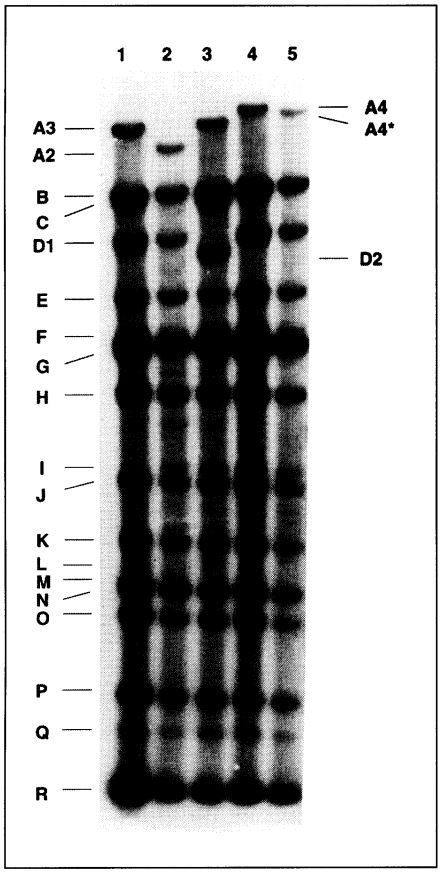

Fig. 2. Electrophoretic patterns of Seminole DNAs digested with *Taq*I and hybridized by p49a, f probes. With the exception of K and L, which are also present in female patterns, all other bands identified by these probes are Y chromosome-specific. Lane 1 represents haplotype 12 (30110), lane 2 haplotype 8 (20111), lane 3 haplotype 15 (31211), lane 4 haplotype 18 (40111), lane 5 haplotype 69 (4*0111).

(fig. 2). Eight of the haplotypes have been previously described in Old World populations, while two (69 and 70) are new haplotypes.

Overall, the A series showed the largest variation. Band A was represented by one of the variant fragments A2, A3, A4, A4*, A5 and A6 with the relative sizes of the series being A2 < A3 < A4* < A4 < A5 < A6. A4* fragment is a new A fragment whose size is slightly lower than the typical A4 fragment (fig. 2). Haplotypes 54, 56 and 64 had the concurrent fragments A2/A3, and haplotype 70 showed the concomitant presence of fragment A5/A6. None of the Seminole lacked the A bands (A0) (table 2), a feature found in several Old World populations and in one Mixe from southern Mexico [10]. Fragment A1, common only in African populations [31, 36, 41] was also not observed in the Seminole. The D series (D0, D1 and D2; D1 > D2) also showed the entire range of variation observed in other human populations. Fragment D1 was observed in haplotypes 8, 12, 18, 63, 69, 70, and fragment D2 in haplotypes 15 and 64. D fragments were absent in haplotypes 54 and 56. The fragment I was absent in haplotypes 12 and 54, and the fragment F was present in all Seminole similar to the Y chromosomes of Amerinds from southern Mexico, but different from Europeans and Africans.

Four of the haplotypes (haplotypes 15, 18, 63 and 64) were previously observed in the Mixtec, Zapotec and Mixe from southern Mexico. None of the Seminole harbored the African-specific haplotypes 3 and 4 which represent 70–80% of sub-Saharan African Y chromosomes [36].

Haplotype 18 was the most common Y chromosome haplotype among the Seminole with a frequency of 30.8%. This haplotype was also the most prevalent haplotype (45.2%) in the Amerind populations from southern Mexico [10]. Haplotype 18 has also been described at low frequencies in some European, African, and Polynesian populations [31, 41–44].

Haplotypes 8 and 63 were the next most common, both having an overall frequency of 15.4% in the Seminole. Haplotype 8 has not been observed in Native Americans from Mexico [10], while it has been reported in several European populations and some African populations, reaching particularly high frequencies (about 23%) in the Ashkenazi and Sephardi Jews [31, 33, 36, 41]. This haplotype is also very common (25.4%) in Polynesia [44]. Haplotype 63 was previously reported in about 13% of the Native Americans from Mexico (table 2). It is absent in Polynesians, and only rarely observed in some European populations [41, 42, 44].

Haplotype 15 was present at a frequency of 11.5% in the Seminole. This is the most common haplotype in European populations, ranging from 9 to 49%, but is rare or absent in Africans [31, 36, 41, 42]. A recent survey of Polynesians has revealed that haplotype 15 represents 22% of Polynesian Y chromosomes [44].

Haplotypes 12, 54, 56 and 64 were all observed in only one Seminole. All of these except haplotype 64 have been reported in some European or African populations [31, 36, 41]. Haplotype 64 was previously detected only in one Zapotec and 1.7% of Polynesians [44]. Haplotypes 69 and 70 are new and were found in two and one Seminole, respectively. Haplotype 69 is characterized by the newly observed fragment A4* (fig. 2).

The results of the analysis with the 12f2 probe are also reported in table 2. Similar to the Native Americans from Mexico, all Seminole Y chromosomes were defined by the

10-kb allele, the 8-kb allele being completely absent. The 8-kb allele is also absent in Africans, whereas it reaches frequencies of more than 40% in some European populations [29–33].

Discussion

Origins of Seminole mtDNAs

The analysis of mtDNAs from Native American populations has revealed that four major haplogroups A, B, C and D primarily colonized the Americas from Asia [4, 5, 8, 19]. Analysis of mtDNA variation revealed that all but one of the 15 Seminole mtDNA haplotypes belonged to haplogroups A, B, C and D, and thus were of Native American origin. Only haplotype AM132 was nonnative in origin, being identical to the African haplotype AF71, which was previously observed in the Senegalese. Thus, some African women were integrated in the Seminole tribe, and African genes are present in the gene pool of modern Seminoles.

Of the 14 Amerind haplotypes, seven (AM125–AM131) are haplotypes which have not been previously reported in other Amerind tribes. One of these, AM126, was present in 21.6% of the samples and was the most common Seminole haplotype. The observation that a tribal-specific haplotype was also the most prevalent in a tribe is consistent with findings in other Amerind tribes, and indicates that founder effects and genetic drift have been important in formation of Native American tribes [8].

Of the remaining seven haplotypes found in other Native American tribes (AM1, AM9, AM13, AM32, AM43, AM63, and AM88), AM1, AM13, AM43 and AM88 have been proposed as founding Asian haplotypes of the Native American haplogroups A, B, C and D, respectively [8, 10, 11]. These four haplotypes are distributed throughout the range of Amerind populations.

The only Amerind populations living east of the Mississippi River which have been analyzed for mtDNA variation are the Seminole of Florida (the current study) and the Ojibwa of Northern Ontario. Comparison of their mtDNAs revealed some common features. Haplotype AM63, representing 2.7% of the Seminole mtDNAs, was previously described only in the Ojibwa at a frequency of 14.3% [8]. Similarly, the Seminole haplotype AM125 (2.7%) shared with the Ojibwa haplotype AM62 (35.7%) a *Hae*III site gain at np 2636. Since both AM125 and AM62 are members of haplogroup A, the np 2636 mutation probably predated the radiation of these two tribes. Alternatively, this and other similar mtDNAs of the Seminole and Ojibwa may have been the result of gene flow between the two populations.

Two of the Seminole haplogroup A haplotypes (AM127 and AM128) shared an *Rsa*I site loss at np 16049, yet differed by the presence of an *Hae*III site gain at np 16517 located in the D loop region. Since these haplotypes have only been observed in the Seminole, it is likely that the *Hae*III site polymorphism at np 16517 occurred recently. In previous studies, numerous other pairs of tribal-specific haplotypes have been found to differ in the presence of this same *Hae*III site. These pairs include AM5 and AM6 in the Dogrib, AM38 and AM39 in the Pima, AM40 and AM42 in the Ticuna, AM79 and AM81 in the Macushi, AM93 and AM94 in the Macushi, and AM103 and AM104 in the Mixtec. This confirms that the *Hae*III np 16517 site of the D loop region is particularly prone to mutations resulting in the distribution of this polymorphism among haplogroup A, C and D mtDNAs [8]. The *Hae*III np 16517 site gain is also highly variable in the haplogroups of Europe [21], and the same mutation has occurred at least eight independent times in a sample of 140 African individuals [20]. Thus, the instability of this *Hae*III site in most mtDNA haplogroups implies that it cannot be considered a phylogenetically reliable mtDNA marker for these haplogroups, and indicates that screening for this polymorphism in both modern and ancient populations cannot solve major issues concerning human origin and evolution [17, 52].

Origins of Seminole's Y Chromosome

The number and nature of the Y chromosomes which were brought from Asia to the Americas is currently unknown. Data on Asian Y chromosome variation for even the most commonly studied Y chromosome polymorphisms detected by probes 49a, 49f and 12f2 is unavailable. However, these polymorphic loci have been extensively characterized in European and African populations. Therefore, comparison of Native American Y chromosome variation with that of Africans and Europeans can provide some insights into the nature of the founding Native American Y chromosomes.

In a study of three isolated populations from southern Mexico (the Mixtec, Zapotec, and Mixe), the 12f2 locus was found not to be polymorphic, and the 49a/49f haplotypes which were most prevalent were 13, 18 and 63 (table 2).

Analysis of the Seminole's Y chromosomes confirmed the absence of polymorphism at the 12f2 locus and revealed ten 49a-49f/*Taq*I haplotypes (table 2). These

haplotypes together with those reported in the Mixtec, Zapotec, and Mixe bring the total number of haplotypes observed in Native Americans to 17. Six of these (65–70) have not been described in Old World populations, and probably represent new mutational events. The remaining 11 haplotypes have already been described in non-Native American populations. The most prevalent of the haplotypes shared with Old World populations is 18, representing 30.8% of the Seminole. Since the same haplotype was observed in 45.2% of southern Mexicans, it is likely to represent the predominant founding Native American Y chromosome. Another probable founding haplotype is 63. This haplotype represents 15.4% of the Seminole Y chromosomes and 12.9% of the southern Mexican haplotypes. The only other common haplotype is 15, which is found in 8.8% of Native Americans. This is also the most common haplotype in Europe, with a frequency greater than 40% in some European populations, but is virtually absent in Africans. Consequently, it is likely to be a European-specific haplotype, and its presence in the Seminole and southern Mexican tribes suggests that it may be due to genetic admixture with Spaniards. However, it recently has been observed that this haplotype encompasses 22% of Polynesian Y chromosomes, reaching a frequency of 33% in the Maori [44]. This high frequency of haplotype 15 in the Polynesians could be due to admixture with Europeans, or this haplotype might also be found in Asia. Consequently, additional studies of Asian and Siberian Y chromosomes will be necessary to determine whether haplotype 15 is a founding Native American haplotype or reflects genetic admixture with Europeans.

In conclusion, analysis of mtDNA variation reveals that the Seminole are a typical Amerind population which has acquired about 5% of nonnative mtDNAs through matrilineal gene flow from Africans. By contrast, analysis of Y chromosome variation did not reveal any African Y chromosomes, but raised the possibility that about 11% of the Y chromosomes present in the modern Seminoles could have been acquired by patrilineal gene flow from Europeans.

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Appendix

Polymorphic restriction sites observed in the Seminole mtDNA haplotypes

Sites	Haplotypes															
	1	9	1 3	3 2	4 3	6 3	8 8	1 2 5	1 2 6	1 2 7	1 2 8	1 2 9	1 3 0	1 3 1	1 3 2
1731	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
663e	1	1	0	0	0	1	0	1	1	1	1	1	0	0	0
1715c	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1
2349j	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
2636e	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0
2758k	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
3592h	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
4381k	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
4769a*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4793e	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5164k	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
5176a	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1
6618e	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
7025a	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
7055a	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
8858f*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10394c	0	0	0	1	1	0	1	0	0	0	0	0	0	0	1
10397a	0	0	0	1	1	0	1	0	0	0	0	0	0	0	1
10806g	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
13259o/**13262a**	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1
13325k	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1
13702e*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14199o*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14268g*	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
14279e	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
14268g*	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
15520a	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
16049k	1	1	1	1	0	1	1	1	1	0	0	1	1	1	1
16517e	0	1	1	0	1	1	0	1	1	1	0	0	1	1	1
Region V	2	2	1	2	2	2	2	2	2	2	2	2	1	1	2

Sites are numbered from the first nucleotide of the recognition sequence according to the published sequence [53]. Boldface numbers indicate site gains relative to the published sequence and nonboldface numbers indicate site losses. '1' indicates the presence of a site, and '0' indicates the absence of a site, except for the 9-bp deletion, where '1' indicates the presence of the deletion and '2' indicates the absence of the deletion. The restriction enzymes used in the analysis are designated by the following single-letter code: a = *Alu*I, b = *Ava*II, c = *Dde*I, e = *Hae*III, f = *Hha*I, g = *Hin*fI, h = *Hpa*I, i = *Msp*I, j = *Mbo*I, k = *Rsa*I, l = *Taq*I, m = *Bam*HI, n = *Hae*II, o = *Hin*cII. Sites separated by a diagonal line indicate either simultaneous site gains or losses for two different enzymes or a site gain for one enzyme and a site loss for another because of a single inferred nucleotide substitution. These sites are considered to be only one restriction site polymorphism in the parsimony analysis. Sites marked with an asterisk were found to be present in all samples contrary to the published sequence and were excluded from parsimony analyses.

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