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Y Chromosomal DNA Variation and the Peopling of Japan

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Summary

Four loci mapping to the nonrecombining portion of the Y chromosome were genotyped in Japanese populations from Okinawa, the southernmost island of Japan; Shizuoka and Aomori on the main island of Honshu; and a small sample of Taiwanese. The Y *Alu* polymorphic (YAP) element is present in 42% of the Japanese and absent in the Taiwanese, confirming the irregular distribution of this polymorphism in Asia. Data from the four loci were used to determine genetic distances among populations, construct Y chromosome haplotypes, and estimate the degree of genetic diversity in each population and on different Y chromosome haplotypes. Evolutionary analysis of Y haplotypes suggests that polymorphisms at the YAP (DYS287) and DXYS5Y loci originated a single time, whereas restriction patterns at the DYS1 locus and microsatellite alleles at the DYS19 locus arose more than once. Genetic distance analysis indicated that the Okinawans are differentiated from Japanese living on Honshu. The data support the hypotheses that modern Japanese populations have resulted from distinctive genetic contributions involving the ancient Jomon people and Yayoi immigrants from Korea or mainland China, with Okinawans experiencing the least amount of admixture with the Yayoi. It is suggested that YAP^+ chromosomes migrated to Japan with the Jomon people >10,000 years ago and that a large infusion of YAP^- chromosomes entered Japan with the Yayoi migration starting 2,300 years ago. Different degrees of genetic diversity carried by these two ancient chromosomal lineages may be explained by the different lifestyles (hunter-gatherer versus agriculturalist). of the migrant groups, the size of the founding populations, and the antiquities of the founding events.

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Introduction

The genetic material making up the nonrecombining portion of the Y chromosome is effectively haploid and inherited in a patrilineal manner. Therefore, polymorphisms in this region of the nuclear genome are valuable for investigating male-mediated gene flow and for complementing maternally based studies of mtDNA. The paucity of informative markers in this portion of the human Y chromosome has proven to be a major impediment in such investigations (Jakubiczka et al. 1989; Malaspina et al. 1990; Spurdle et al. 1994*a*). Furthermore, many of the known polymorphic probes identified so far detect repeated sequences on the Y chromosome, making it difficult to reconstruct historical events (Spurdle and Jenkins 1991). A simple polymorphism resulting from the recent insertion of an *Alu* element on the long arm of the Y chromosome has proved to be useful for human population studies (Persichetti et al. 1992; Hammer 1994; Spurdle et al. 1994*a*). Hammer (1994) presented mapping data showing that the *Alu* element, referred to as the Y *Alu* polymorphic (YAP) element, is present at a specific site on the Y chromosome in some individuals and absent in others. The frequency of Y chromosomes containing the YAP element (YAP^+) is highest in sub-Saharan African populations, followed by North African and European populations. Most Asian populations examined so far completely lack the YAP element. An exception to this pattern was the discovery of the YAP element in a small sample of Japanese subjects (Hammer 1994). The distribution of this polymorphism raises the possibility of tracing paternal lineages and male-mediated gene flow between largely separated geographical regions. The potential for such studies is bolstered by DNA sequencing studies that showed that the YAP element is present between the same two base pairs on eight Y chromosomes from different geographic origins (M. Hammer, unpublished data). This result supports the hypothesis that YAP^+ chromosomes from Africa and Japan are identical by descent.

There have been at least two major migration events that brought modern human populations from the Asian continent to the Japanese archipelago. The Jomon people arrived in Japan >10,000 years ago, although the exact timing and geographic origin of this migration event are still puzzling (Turner 1990; Nei, in press). The

Yayoi, originally from northeast Asia, started migrating to Japan from the Korean peninsula ~2,300 years ago. One of the main problems in Japanese prehistory is understanding the extent to which the aboriginal Jomon and the more recently migrating Yayoi contributed genetically to modern Japanese populations (Chard 1974, p. 114). There are two contemporary ethnic groups that appear to be distinct from the Japanese populations living on the centrally located islands of Honshu, Shikoku, and Kyushu. These are the Ainu, inhabiting the northern island of Hokkaido, and the Ryukyuans living in the southernmost island of Okinawa. There is some agreement that these two groups represent the modern descendants of the aboriginal people in the neolithic Jomon Age (Horai et al. 1987; Kozintsev 1993).

This study was initiated to examine the paternal relationships of populations from the main island of Honshu and the southernmost island of Okinawa and to use this information to test hypotheses for the origin of modern Japanese populations. The frequency of the YAP element was measured in a sample of 133 males from Japan as well as in a smaller sample of Taiwanese and Koreans. In addition, variation at three other Y-specific loci was examined, including the RFLP detected by the probe p47z (DXYS5Y) (Nakahori et al. 1989), complex restriction patterns detected by probe p49f (DYS1) (Ngo et al. 1986), and variable numbers of GATA repeats at the DYS19 locus (Roewer et al. 1992).

Subjects and Methods

Subjects and DNA Extraction

DNA was previously extracted from 45 individuals from Okinawa, 27 individuals from Aomori, 61 individuals from Shizuoka, and 21 Taiwanese males of Chinese ancestry (Horai et al. 1987). Buccal cells were collected from Chinese and Korean volunteers at the University of Arizona (where the study was approved by the human subjects committee), and buccal samples were provided by Dr. Wook Kim (Dankook University, Seoul). DNA was isolated from buccal cells according to the procedure of Richards et al. (1993). DNA isolated from additional Chinese volunteers was provided by Dr. Mark Stoneking.

DNA Probes and Hybridization

The YAP element at the DYS287 locus (referred to as the YAP locus) was detected with a 0.5-kb probe that is released from the pYAP-2.8 vector on digestion with *Eco*RI and *Bgl*II (Hammer 1994). The probes p47z and p49f were purchased from the American Type Culture Collection. All enzymes were purchased from New England Biolabs. After isolation from low-melting-temperature agarose gels, fragments were labeled with ^{32}P by the method of Feinberg and Vogelstein (1984).

Approximately 2 μg of DNA were digested with restriction endonucleases, were electrophoresed on 0.8% agarose gels, and were alkaline-transferred to nylon membranes. Hybridization with radioactively labeled probes was carried out according to the procedure of Hammer and Silver (1993). The probes pYAP-0.5 and p49f were hybridized to nylon membranes containing DNA digested with *Eco*RV, and the probe p47z was hybridized to membranes containing *Stu*I-digested DNA.

PCR and PAGE

The YAP element was detected also by PCR amplification using primers designed to sequences flanking the insertion site of the *Alu* element—YAP.1: 5′-CAGGGGAAGATAAAGAAATA-3′ and YAP.2: 5′-ACTGCTAAAAGGGGATGGAT-3′. These primers amplify either a 455-bp (YAP^+) or 150-bp (YAP^-) fragment that can be resolved by electrophoresis on 2% agarose. The reactions were carried out in a total volume of 25 μl containing 50 ng of genomic DNA, 0.12 μM each primer, 0.2 mM each dNTP, 2.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), and 0.5 U AmpliTaq DNA polymerase (Perkin-Elmer). The cycling conditions were 94°C for 2 min, and then 30 cycles of 94°C for 1 min, 51°C for 1 min, and 72°C for 1 min.

Polymorphism in the number of GATA repeats at the DYS19 locus was analyzed as described by Roewer et al. (1992) with the following modifications. The reactions were performed in 25 μl with 50 ng genomic DNA, 0.12 μM each primer, 0.2 mM each dNTP, 3 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), and 0.5 U AmpliTaq DNA polymerase. A total of 40 cycles were performed, each cycle consisting of 1 min at 94°C, 1 min at 54°C, and 1 min at 72°C. The samples were resolved on 20 × 35 × 0.15 cm 6% polyacrylamide/0.15% bisacrylamide gels in TBE buffer (50 mM Tris-borate/EDTA [pH 8.3]) for 8 h at 300 V. Following electrophoresis, the gel was stained with ethidium bromide, and the fragments were visualized by UV.

Data Analysis

Levels of paternally inherited genetic variation within and between populations were used to assess the effects of genetic drift and the evolutionary relationships among populations. The method of Cavalli-Sforza and Edwards (1967) was used to calculate genetic distances. Genetic distance variances were determined by jackknifing over loci (Sokal and Rohlf 1981). In this method, the genetic distances are calculated in the complete sample, and then they are computed with each of the loci left out in turn. Pseudovalues, jackknifed estimates, and variances of the jackknifed estimates were computed using the equations of Sokal and Rohlf (1981, p. 796). F_{st} values were calculated by the method of Reynolds et

al. (1983). Neighbor-joining clustering diagrams (Saitou and Nei 1987) were generated from genetic distances based on all pairwise F_{st} values between populations. Haplotypic diversity, used as a measure of genetic variability within populations and as a measure of genetic variation carried by different Y haplotypes, was determined by two methods. Equation 8.5 of Nei (1987, p. 179) was used to obtain an unbiased estimate of haplotype diversity (h), or the probability that two randomly chosen Y chromosomes from a population are different. The variance of h was determined as described in Nei (1978). The diversity values can range between 0 and 1, with 0 representing the minimum value (e.g., a single haplotype fixed in a population) and 1 representing the maximum genetic variability (e.g., an equal frequency of each of many haplotypes). In addition, the Shannon-Weiner information measure (Wilson and Bossert 1977, p. 144) was used to calculate diversity based on the relative abundance of each Y chromosome haplotype. Haplotype diversity variances were estimated by jackknifing as described above, except that each of the haplotypes was left out in turn.

Results

YAP

The hybridization of the probe pYAP-0.5 to genomic DNAs digested with *Eco*RV is shown in figure 1*A*. The frequency of the YAP element was determined in a sample of 153 individuals from Japan and Taiwan (table 1). The YAP element was present in 55 (42%) of the 132 Japanese males and absent in all 21 individuals from Taiwan. The YAP element was also absent in a sample of 13 Korean males. This result is consistent with previous surveys that showed the YAP element to be polymorphic in Japan and absent in other Asian and Oceanic populations (Hammer 1994; Spurdle et al. 1994*b*). In Japan the frequency of the YAP element ranges from 33% in Shizuoka to 56% in Okinawa, with an intermediate frequency of 39% in Aomori. The frequency is significantly higher in Okinawa than in Shizuoka (Fisher's exact test, $P = .0284$), but the Okinawa frequency is not significantly different from the Aomori frequency ($P = .2196$). However, the frequency in Okinawa is significantly higher than in the combination of the two Honshu prefectures ($P = .0256$).

All pairwise F_{st} values were calculated on the basis of YAP allele frequencies in Japan and Taiwan, as well as in 13 other populations (Hammer 1994; Spurdle et al. 1994*b*). The neighbor-joining method was used to generate a clustering diagram (fig. 2). All Asian and Oceanic populations, except the Japanese, form a single group that is closely allied with the European populations. The greatest genetic distance is the one that separates these Eurasian populations from the Japanese and African

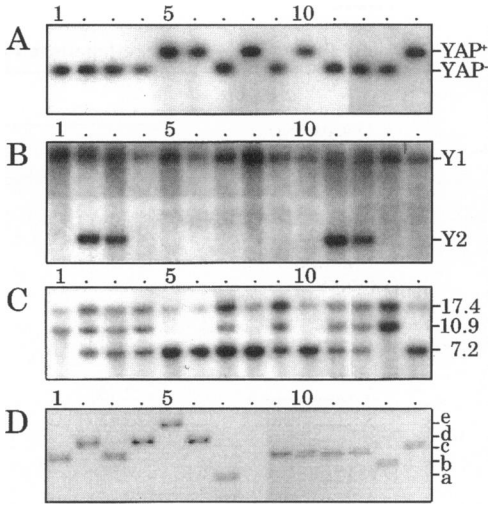

Figure 1 Allelic classes at each of four Y chromosome loci in 14 individuals from Aomori (lanes 1–14). *A*, Hybridization of the pYAP-0.5 probe to *Eco*RV-digested genomic DNAs. The 2.8- and 3.1-kb fragments represent the YAP^+ and YAP^- alleles, respectively. *B*, Hybridization with the p47z probe (DXYS5) to *Stu*I-digested DNAs identifies a 17.1-kb Y1 allele and a 5.3-kb Y2 allele. *C*, Hybridization with the p49f probe (DYS1) to *Eco*RV-digested DNAs identifies three patterns: pattern A, 17.4-kb and 10.9-kb fragments (e.g., lane 1); pattern B, 17.4-kb and 7.2-kb fragments (e.g., lane 5); and pattern C, all three fragments (e.g., lane 2). *D*, Polyacrylamide gel electrophoresis of fragments amplified with the primers Y-27H39.1 and Y-27H39.2 (DYS19). Five size classes shown here correspond to alleles A (186 bp), B (190 bp), C (194 bp), D (198 bp), and E (202 bp).

populations. The Okinawan and Honshu populations are separated; the former population clusters in the middle of the African groups, and the latter population clusters between the African and Eurasian samples. This pattern is distinct from those obtained in mitochondrial and nuclear DNA analyses that demonstrated a primary division between African and non-African populations (Wainscoat et al. 1986; Cann et al. 1987; Excoffier et al. 1987; Bowcock et al. 1991; Horai et al. 1991; Nei and Roychoudhury 1993; Mountain and Cavalli-Sforza 1994).

DXYS5Y Alleles

The probe p47z detects variation at loci on the short arm of the Y (DXYS5Y) and the long arm of the X (Xq21) chromosomes (Nakahori et al. 1989). Digestion with the restriction endonuclease *Stu*I identifies a two-allele, Y-specific polymorphism of 17 kb (Y1) and 5.3 kb (Y2) (fig. 1*B*). The frequency of the Y2 allele in Okinawa (11%) is marginally lower than in Honshu (25%) (Fisher's exact test, $P = .071$) (table 1). The frequencies in Honshu are very similar to the results of Nakahori et al. (1989), where 28% of the Japanese had the Y2 allele. Nakagome et al. (1992) found that the Y2 allele was present in 4 of 41 males from Korea, but

Table 1

Allele Frequencies in Three Japanese Populations and Taiwan

Locus and Allele/Pattern	No. (%) in				
	Okinawa	Shizuoka	Aomori	Honshu	Taiwan
DYS287 (YAP):					
–	20 (44)	41 (67)	16 (61)	57 (66)	21 (100)
+	25 (56)	20 (33)	10 (39)	30 (34)	0
DXYS5Y:					
Y1	40 (89)	45 (76)	19 (73)	64 (75)	17 (100)
Y2	5 (11)	14 (24)	7 (27)	21 (25)	0
DYS1:					
A	2 (5)	4 (7)	2 (8)	6 (7)	0
B	25 (57)	20 (33)	10 (38)	30 (35)	0
C	17 (39)	36 (60)	14 (54)	50 (58)	9 (100)
DYS19:					
A	3 (7)	5 (8)	2 (7)	7 (8)	1 (7)
B	1 (2)	1 (2)	2 (7)	3 (3)	4 (29)
C	16 (36)	35 (57)	13 (48)	48 (55)	4 (29)
D	14 (31)	13 (21)	5 (19)	18 (20)	4 (29)
E	10 (22)	7 (11)	5 (19)	12 (14)	1 (7)
F	1 (2)	0	0	0	0

was absent in a survey of 70 Chinese, 33 Jewish, 26 Caucasian, and 21 African American males. In another study, the Y2 allele was present in 1 Asian male out of 10 examined, and absent in 53 Europeans, 18 Africans, 5 Oceanians, and 2 Native Americans (Mathias et al. 1994).

DYS1 EcoRV Patterns

The probes p49a and p49f are different subclones of cosmid 49 (Bishop et al. 1983), and both hybridize to ~15 Y-specific *Taq*I bands corresponding to a low-copy-number sequence (Ngo et al. 1986). At least 8 of the 15 Y-specific bands have been shown to be present, absent, or variable in length and are considered to be independent loci (Ngo et al. 1986; Guerin et al. 1988; Lucotte et al. 1990; Torroni et al. 1990). This probe has also been shown to detect complex *Pvu*II polymorphisms involving ≥10 polymorphic fragments (Spurdle and Jenkins 1991). In this survey, three male-specific fragments of 17.4 kb, 10.9 kb, and 7.2 kb were detected by hybridizing p49f to *Eco*RV-digested DNAs. The 17.4-kb band is present in all individuals, whereas the 10.9-kb and 7.2-kb bands are either present or absent (fig. 1C). There are also quantitative differences in the intensity of the bands, but no attempt was made to determine the density of each fragment. The three observed patterns are referred to as A (17.4 kb/10.9 kb), B (17.4 kb/7.2 kb), and C (17.4 kb/10.9 kb/7.2 kb).

The frequency of B in Okinawa (57%) is marginally higher than in the Honshu prefectures (36%); this is balanced by a lower frequency of C in Okinawa (χ^2 test, $P = .057$). Pattern A is found at low frequencies (<10%) in all populations. All nine Taiwanese individuals typed had the C pattern (table 1).

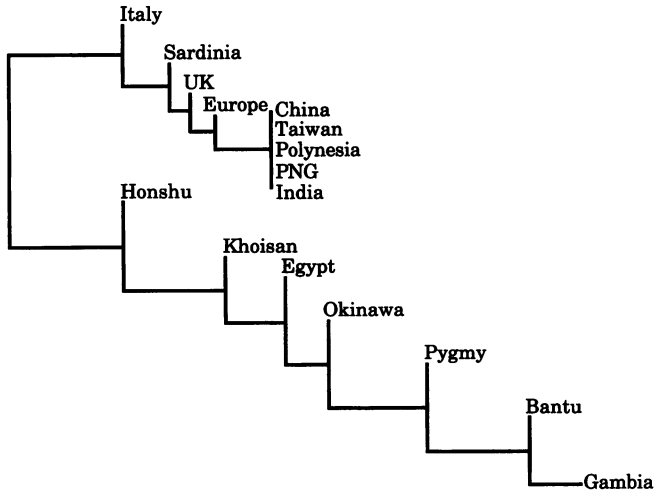

Figure 2 Neighbor-joining tree for 14 populations on the basis of allele frequencies at the YAP locus. PNG = Papua New Guinea; and UK = United Kingdom.

DYS19 Alleles

Polymorphism at the DYS19 locus on the short arm of the Y chromosome is due to differences in the number of GATA tandem repeats (Roewer et al. 1992). Primers designed to unique flanking sequences amplify fragments ranging in size from 186 bp to 202 bp in 4-nt

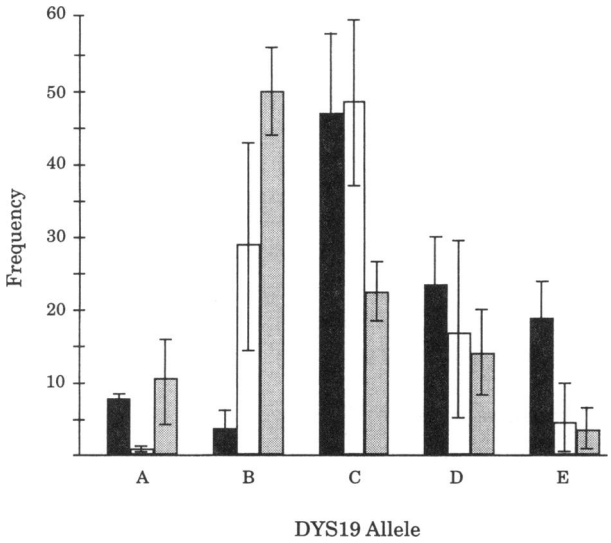

Figure 3 Frequency of DYS19 alleles in Japanese (dark gray), Asian (white), and Western European (light gray) populations. One-standard-deviation-error bars are indicated by vertical lines.

increments and corresponding to 10–14 copies of the GATA motif. In previous surveys of Europeans and Asians, five allele classes (A–E) were reported (Roewer et al. 1992; Santos et al. 1993; Gomolka et al. 1994; Muller et al. 1994). In this survey of alleles at the DYS19 locus, all five alleles were identified, as well as a sixth allele (206 bp) in a single Okinawan individual.

Among the three Japanese populations examined here, there were no significant differences in allele frequencies (table 1). The C allele is the most frequent (47% ± 6.1%), followed by the D (24.0% ± 3.8%) and E (17.7% ± 3.0%) alleles. In contrast, as is shown in figure 3, the B allele is the most frequent in Western European populations (50.0% ± 3.3%), followed by the C (22.3% ± 2.2%) and D (14% ± 3.1%) alleles (Roewer et al. 1992; Santos et al. 1993; Muller et al. 1994). There is significant differentiation between the Japanese and Western European populations (χ^2 test, P = .0001). The major determinants of this significant difference are the much higher frequency of the B allele in Europeans and the higher frequencies of the C and E alleles in the Japanese.

The group mean frequencies of DYS19 alleles also distinguish Japanese populations from nine Asian populations (χ^2 test, P = .0001), including the Chinese samples reported here and eight populations reported by Gomolka et al. (1994). The major determinants of this differentiation are the higher frequencies of the A and E alleles in the Japanese and the higher mean frequency of the B allele in the Asian samples (fig. 3). The Japanese are similar to seven of eight Asian populations surveyed by Gomolka et al (1994), in having a higher frequency of the C allele. Among Asian groups, the Japanese have the lowest frequency of the B allele, which is characteristically high in Western European populations (Roewer et al. 1992; Santos et al. 1993; Muller et al. 1994).

Genetic Distances among Japanese Populations

Genetic distance analyses based on the frequencies of alleles at all four Y chromosome loci indicate a slightly greater affinity between the Shizuoka and Aomori populations than between the Okinawa population and either of the other populations (table 2). F_{st} values (Reynolds et al. 1983) based on allele frequencies at the YAP, DXYS5Y, and DYS1 loci gave very similar results (table 2). The low F_{st} values in modern Japanese populations are comparable to those for populations within Italy (Persichetti et al. 1992). This differentiation of Okinawans relative to populations on the main island is similar to results based on mtDNA (Horai et al. 1987), salivary proteins (Tsuchida et al. 1989), red cell antigens (Misawa et al. 1974), and morphological comparisons (Hanihara 1984; Hanihara 1991).

Associations among Y Chromosome Alleles and Haplotypes

As expected for simple polymorphisms in the nonrecombining portion of the Y chromosome, the allelic systems at the YAP and DXYS5Y loci are not randomly associated: no subject was found to carry the allelic combination $YAP^+/Y2$ (table 3). Considering allelic associations among the YAP, DXYS5Y, and DYS1 loci (table 3), we found that there are five haplotypes: (1) YAP^-/Y1/C, (2) YAP^-/Y2/C, (3) YAP^-/Y2/A, (4) YAP^-/Y1/A, and (5) YAP^+/Y1/B. The mean frequencies of these five haplotypes in Japan are 33%, 19%, 1%, 5%, and 42%, respectively. The DYS1 B pattern is always associated with the $YAP^+/Y1$ combination, whereas the C pattern is associated with both the $YAP^-/Y1$ and $YAP^-/Y2$ combinations. The A pattern is mainly associated with the $YAP^-/Y1$ combination; a single Y chromosome was identified with the A pattern in association with the $YAP^-/Y2$ combination. Comparisons among the three

Table 2

Genetic Distances (above Diagonal) and F_{st} Values (below Diagonal) among Three Japanese Populations

	Okinawa	Shizuoka	Aomori
Okinawa		.049 ± .019	.037 ± .015
Shizuoka	.078 ± .020		.010 ± .005
Aomori	.057 ± .010	.008 ± .001	

NOTE.—Genetic distances were calculated by the chord method of Cavalli-Sforza and Edwards (1967) and standard errors were determined by jackknifing over four loci. F_{st} values were calculated by the method of Reynolds et al. (1983), based on YAP, DXYS5Y, and DYS1 only.

Table 3

Associations among Four Y Chromosome Polymorphisms in Japanese Populations

	Haplotype[a]				
	1	2	3	4	5
Locus:					
YAP	−	−	−	−	+
DXYS5Y	Y1	Y2	Y2	Y1	Y1
DYS1	C	C	A	A	B
	No. of Chromosomes[b]				
DYS19 allele:					
A	6	0	0	4	0
B	2	0	0	1	0
C	17	21	1	1	23
D	11	4	0	1	15
E	5	0	0	0	16
F	0	0	0	0	1

[a] Associations of alleles at three loci (YAP, DXYS5Y, and DYS1). Haplotype 1 = −/Y1/C; haplotype 2 = −/Y2/C; etc.

[b] No. of chromosomes with DYS19 alleles (A–F) in association with haplotypes 1–5.

Japanese populations indicate that the frequency of haplotype 5, the only haplotype associated with the YAP element, is significantly higher in Okinawa (57%) compared with the other prefectures (Fisher's exact test, $P = .024$) (fig. 4).

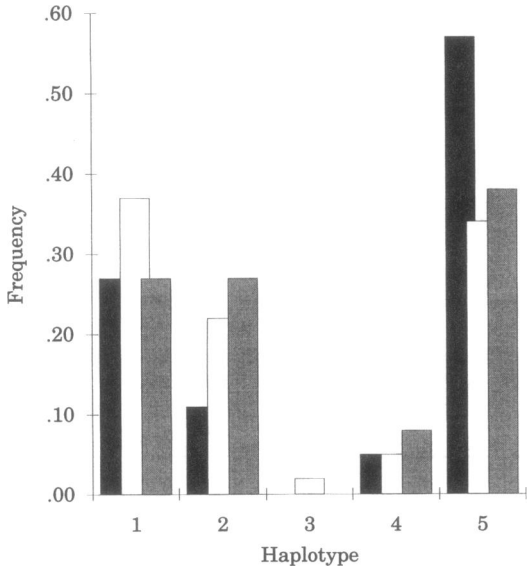

Figure 4 Frequency of combination haplotypes on the basis of variation at the YAP, DXYS5Y, and DYS1 loci in Okinawa (blackened bars), Shizuoka (unblackened bars) and Aomori (gray bars). Three-locus haplotypes 1–5 are described in the text.

The associations of DYS19 alleles with the five three-locus haplotypes (YAP/DXYS5Y/DYS1) are shown in the lower part of table 3. Several of the allelic classes are associated with more than one haplotype; however, the associations are not completely random (i.e., of the 30 possible combinations of alleles and haplotypes, only 16 were found in Japan). These 16 haplotypes are referred to as 1A–E, 2C–D, 3C, 4A–D, and 5C–F (table 3; fig. 5). Only haplotype 1C significantly differs in frequency among the three Japanese populations (χ^2 test, $P = .04$): 20% in Shizuoka versus 5% and 8% in Okinawa and Aomori, respectively (fig. 5).

Y Chromosome Haplotype Diversity

The genetic diversity carried by each of the five three-locus haplotypes was determined on the basis of the number and relative abundance of DYS19 alleles associated with each haplotype (table 3). Haplotype 1 has the highest diversity value (.735 ± .006) because it is associated with roughly equal proportions of all five common DYS19 alleles (fig. 6). The levels of genetic diversity carried by haplotypes 5 (.678 ± .003) and 2 (.237 ± .022) are significantly lower (Bonferroni tests, $P < .001$) (fig. 6). It is interesting that haplotype 4, found in only 5% of the Japanese subjects, has a high level of diversity (.714 ± .068). It is suggested that these differences in levels of diversity reflect differences in the age of each haplotype (see below).

Among the 16 four-locus haplotypes (YAP/DXYS5Y/DYS1/DYS19), 12 are associated with YAP^- chromosomes, while only four are associated with YAP^+ chromosomes (table 3). YAP^- chromosomes are significantly more variable than YAP^+ chromosomes in the Japanese population as a whole, as well as in each of the subpopulations (*t* tests, $P < .0001$). These results were confirmed using the Shannon-Weiner diversity measure and haplotype diversity variances calculated by the jackknife procedure.

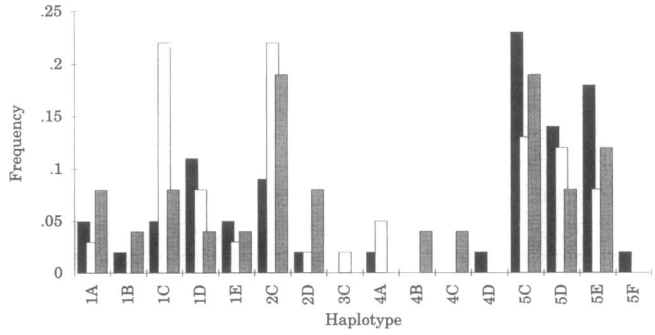

Figure 5 Frequency of combination haplotypes based on variation at all four Y chromosomal loci in Okinawa (blackened bars), Shizuoka (unblackened bars), and Aomori (gray bars). Haplotypes 1A–5F are described in the text.

Haplotype:	1	2	3	4	5
YAP:	-	-	-	-	+
DXYS5Y:	Y1	Y2	Y2	Y1	Y1
DYS1:	C	C	A	A	B
Frequency:	33%	19%	1%	5%	42%
Diversity:	0.735	0.237	0.0	0.714	0.678
SE:	0.006	0.022	0.0	0.068	0.003

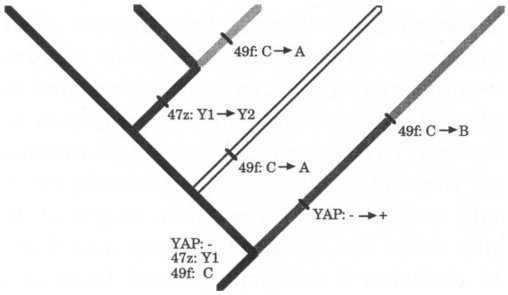

Figure 6 Evolutionary tree of Y chromosome haplotypes (1–5) based on allelic associations at the YAP, DXYS5Y, and DYS1 loci. The crossbars on the branches of the tree indicate a change in allelic state at one of the three loci. The allelic states at each locus, frequency (in the Japanese population), and diversity of each haplotype are shown (see text).

Levels of haplotype diversity were also determined for each of the three populations (table 4). Total haplotype diversity, as well as that for YAP^- chromosomes, is highest in Aomori (Bonferroni test, $P < .001$), followed by Okinawa, which has a higher haplotype diversity than does Shizuoka ($P < .01$). In contrast, diversity levels of YAP^+ chromosomes do not differ among the three populations.

Discussion

Evolution of Y Chromosome Haplotypes

A hypothesis for the evolution of the five three-locus Y chromosome haplotypes found in Japan is presented in figure 6. The absence of the YAP element is ancestral: the insertion event most likely occurred after the divergence of humans from African apes (Hammer 1994). The frequency and distribution of this polymorphism are consistent with the hypothesis that the YAP element originally inserted on the Y chromosome of a sub-Saharan African (Hammer 1994). The implication is that both the YAP^- and YAP^+ lineages existed in other parts of the world before populations that gave rise to modern Japanese migrated to Japan.

Although the allelic state at the DXYS5Y locus is not known in great apes, the Y1 allele probably predates the Y2 allele for the following reasons. First, the Y2 allele has a limited geographic distribution relative to the Y1 allele (Nakagome et al. 1992; Mathias et al. 1994). Second, Y chromosomes carrying the Y2 allele (haplotypes 2 and 3) have very low levels of diversity compared with haplotypes bearing the Y1 allele (fig. 6). Mutations that have arisen recently are expected to have more restricted geographic distributions (Templeton 1993), and haplotypes created by recent mutations will be less diverse. These data are consistent with the hypothesis of Nakagome et al. (1992) that the Y2 allele originated after the divergence of the Japanese/Korean group from other ethnic groups. We suggest that the mutation giving rise to the Y2 allele occurred on haplotype 1 (fig. 6) and that this chromosome migrated from Korea to Japan during the Yayoi period (see below).

For the DYS1 locus, the B pattern is in complete linkage disequilibrium with YAP^+ chromosomes in Japan. This situation is different from that found in two African populations genotyped at the same loci. In Egyptian and Pygmy populations there are high frequencies of YAP^+ chromosomes that are mainly associated with the DYS1 C pattern (M. Hammer, unpublished data). Although there are a few cases of associations between YAP^+ and DYS1 A, DYS1 B appears to be absent in these African populations. Therefore, we suggest that the B pattern originated after the insertion of the YAP element on a chromosome with the DXYS5 Y1 allele and DYS1 C pattern. Although this putative ancestral haplotype (YAP^+/Y1/C) is present in Africa, it has not been identified in Japan.

Although the frequencies of haplotypes with the DYS1 C pattern are much higher than those with the A pattern in Japan, the diversities of C- and A-bearing chromosomes are not significantly different. Because the haplotype diversities of these chromosomes are high and the same restriction patterns have been identified in African populations, it is likely that the DYS1 C and A patterns represent ancient polymorphisms (relative to their arrival in Japan). It is not possible to determine from these data which of the two patterns is ancestral. However, it is clear that there has been more than one origin for either the C or A pattern. If one assumes that the C pattern is ancestral (fig. 6), then the A pattern must have originated twice: one time recently in an event the gave rise to haplotype 3 from haplotype 2 and one time in the more distant past when haplotype 1 gave rise to haplotype 4. It is important to note that even if the A pattern is assumed to be ancestral, at least two

Table 4

Haplotype Diversity in Three Japanese Populations

Population	Total	YAP^-	YAP^+
Japan	.889 ± .001	.838 ± .003	.678 ± .003
Okinawa	.887 ± .004	.889 ± .011	.707 ± .009
Shizuoka	.872 ± .002	.782 ± .006	.689 ± .010
Aomori	.917 ± .006	.892 ± .015	.689 ± .033

NOTE.—Mean haplotype diversity (±SE) is calculated as $h = n(1 - \Sigma x_i^2)/(n - 1)$, where n is the no. of chromosomes sampled and x_i is the frequency of the ith haplotype (Nei 1987, p. 179).

independent mutations to the C pattern would be required. The results presented here are similar to other studies utilizing the p49f probe at the DYS1 locus (Torroni et al. 1990; Spurdle and Jenkins 1991; Spurdle et al. 1994*a*). In these studies, some of the polymorphic *TaqI* fragments were shown to arise more than once and to evolve in a convergent manner in human populations. In the case reported here, an enzyme that produces a much simpler pattern also yields restriction fragments that are subject to the process of recurrent mutation.

A similar phenomenon is occurring at the DYS19 locus. Because several of the DYS19 alleles are associated with more than one of the five three-locus haplotypes (table 3), there is evidence that the microsatellite locus is undergoing multiple hits and evolving in a convergent manner. For example, both microsatellite alleles C and D are associated with haplotype 2. This recently derived haplotype (see above) is mainly associated with DYS19 allele C, and so it is reasonable to assume that haplotype 2D (DYS19 allele D) resulted from a mutation on haplotype 2C. However, DYS19 allele D is also associated with haplotypes 1, 4, and 5, implying that the 198-bp allele class (i.e., 13 copies of the GATA repeat) has originated independently on different chromosomal lineages.

Y Polymorphisms as Markers for the Peopling of Japan

There are several theories on the origins of modern Japanese populations, some of which were proposed >100 years ago (reviewed in Mizoguchi 1986). These theories attempt to explain the fairly large range of morphological, cultural, and genetic variation represented in modern Japanese populations and recognized in the archaeological and fossil records. Most authors agree that there have been at least two major migration events that brought people from the Asian continent to the Japanese archipelago. There is evidence for an early wave of migration that brought the Jomon culture to Japan >10,000 years ago (Chard 1974, p. 111). Some authors believe that modern humans entered Japan as early as 30,000 years ago when the islands were connected with continental Asia (Nei, in press). The Jomon people made pottery and lived a hunter-gatherer lifestyle in isolation for several thousand years before the Yayoi migration began. Entering from the Korean peninsula ~2,300 years ago, the Yayoi brought weaving, metalworking, and rice culture to Japan (Chard 1974, p. 172). The effects of this new culture are first seen in Kyushu, and then are seen to spread eastward. By ~300 A.D. Yayoi culture completed its spread, which resulted in the alteration of all local cultures south of Hokkaido. The main debate centers around the extent to which the ancestral Jomon made a genetic contribution to the historic Japanese (Chard 1974, p. 114). Current hypotheses can be classified into three groups: substitution, hybridization, and transformation (Suzuki 1981). Substitution theories involve the replacement of the aboriginal people of Japan by subsequent immigrants. This hypothesis predicts that contemporary populations should not contain a (major) genetic contribution from the aboriginal Jomon populations. Hybridization theories claim that modern Japanese are the result of admixture between different immigrant populations and predict that modern Japanese have genes deriving from both the Jomon and Yayoi people. Most transformation theories posit that the people of Japan gradually evolved from a single ancient population that migrated from south China at the end of the Pleistocene and that contemporary variation is primarily derived from variation that existed in that ancient migrant population. Divergence between descendant populations is explained by environmental differences.

Patterns of variation on the Y chromosome can be used to test some of these hypotheses from the perspective of male lineages. Based on the frequency and distribution of the YAP element it is inferred that the YAP existed in Japan before the Yayoi migration and that the YAP element is a marker of Jomon male lineages. The evidence for this inference is the absence of the YAP element in non-Japanese Asian populations (Hammer 1994; Spurdle et al. 1994*a*, 1994*b*), including the Taiwanese, Korean, and Chinese in this survey, and the finding that the frequencies in Okinawa are higher than on Honshu. These results lend support to the hybridization theory of the origin of modern Japanese. Substitution theories allege that the modern Japanese are primarily descended from neo-Mongoloid immigrants (i.e., Yayoi) from north China (Howells 1966; Turner 1976). If we assume that Y chromosome variation in contemporary populations from Korea and north China is representative of variation in the Yayoi of 2,300 years ago, substitution theories cannot account for the high frequency of the YAP element in modern Japanese populations.

A similar, but opposite, trend has been identified with the frequencies of the DXYS5 Y2 allele: there are higher frequencies in Honshu than in Okinawa (table 1; fig. 4). Transformation theories contend that genetic variation in modern Japanese populations derives solely from Jomon ancestors and does not reflect Yayoi admixture (Suzuki 1981; Mizoguchi 1986). Studies of the distribution of the DXYS5Y alleles in Africa, east Asia, and Europe indicate that the Y2 allele is restricted to Korea and Japan (Nakagome et al. 1992; Mathias et al. 1994). This is consistent with the hypothesis that the Y2 allele tracks male lineages that originated in Korea and migrated to Japan. Recently, the known range of DXYS5Y alleles was extended to other ethnic groups in east Asia: Evenks in central Siberia and Khalkhs in Mongolia had the Y1 allele only, whereas two groups living in Taiwan,

the Fo-lo and Hakka, had both the Y1 and Y2 alleles (Lin et al. 1994). However, the ancestors of all men with the Y2 allele were traced to the province of Henan in northern China. Therefore, the Y chromosome data support the hypothesis that migrants from northern China, moving through Korea, made an impact on the paternal genetic endowment of modern Japanese.

Thus, the hybridization theory is the most likely of the three alternatives. The frequencies of the YAP element and the Y2 allele are concordant with this historical reconstruction. Mixing between populations with high and low levels of the YAP element would lead to the observed lower frequencies of the YAP element in populations on Honshu. The frequency distribution of the Y2 allele implies derivation from the opposite direction. Both gradients are consistent with interpretations of clinal variation in several other data sets in Japanese populations. For example, present-day gradients southwest to northeast across the Japanese archipelago have been identified for cranial (Hanihara 1992) and dental (Hanihara 1991) morphology, ABO gene frequencies (Akaishi and Kudo 1975), transferrin polymorphisms (Nakanaga et al. 1991), and other characters. mtDNA studies have also revealed that the frequency of the Asian-specific 9-bp deletion in mainland Japanese is >15% (Horai and Matsunaga 1986), while that in the Ryukyuans (5%) as well as that in the Ainu (2%) is extremely low (Horai 1991; Harihara et al. 1992). The most pertinent explanation is that these trait distributions have resulted from ancient hybridization between the Jomon and Yayoi, with the Ainu and the Ryukyuans experiencing the least amount of Yayoi admixture (Hanihara 1991; Hanihara 1992).

Nei (in press) has used evidence from a survey of 18 polymorphic loci in Tokyoites, Ainu, Okinawans, and their surrounding populations to question the validity of the hybridization (dual structure) theory of Japanese origins. A phylogenetic analysis indicated that the Ainu and Okinawans are closest to each other and also closely allied with the Japanese from Tokyo and Koreans. However, the three Japanese populations were quite different from southeast Asians (southern Chinese, Taiwanese aborigines, Thais, Philippines). This does not support the hypothesis based on morphological characters that the ancestors of the Ainu and Okinawans (Jomon) are the direct descendants of southeast Asians (Turner 1976, 1990; Hanihara 1982). Nei (in press) favors a modification of the transformation theory (called the "out-of-northeast-Asia hypothesis") that posits: (1) modern humans first entered into Japan ~30,000 years ago from northeast Asia; (2) occasional gene flow from northeast Asia continued until 12,000 years ago when the Japanese islands were disconnected from continental Asia; and (3) the Yayoi migration, while making a large cultural contribution, made a minor impact on the modern Japanese gene pool.

The Y chromosomal polymorphisms discussed in this report provide a paternally based test of one aspect (3) of the out-of-northeast-Asia hypothesis. It is possible to estimate the proportion of Y chromosomes derived from the Yayoi migration if it is assumed that the present-day YAP frequencies in Okinawa and Korea/China are similar to frequencies in the Jomon and Yayoi populations, respectively (Reed 1969). Assuming that population mixture is the only process affecting the system, if q_O is the frequency of the YAP element in the Jomon ancestors of modern Japanese (.56), q_K is the frequency in the Yayoi ancestors (.0), and q_M is the frequency in modern Japanese on Honshu (.34), then M is the present proportion of Y chromosomes that are derived from the Yayoi:

$$M = q_M - \frac{q_O}{q_K - q_O}.$$

Substitution of YAP frequencies into this equation yields the following results: the proportion of Y chromosomes derived from the Yayoi is 39%, and the proportion derived from the Jomon is 61%. Therefore, the YAP data support the hypothesis that migrations during the Yayoi period and the subsequent protohistoric Kofun period made a significant paternal contribution to the modern Japanese gene pool (Yamaguchi 1982).

Y Chromosome Haplotypic Diversity and the Hybridization Hypothesis

The significantly higher levels of haplotypic diversity carried by YAP^- relative to YAP^+ chromosomes (table 4) is more easily explained under a hybridization model than either a transformation or substitution model. Under the hybridization model, YAP^+ chromosomes migrated to Japan with the Jomon people and not with the Yayoi; whereas, YAP^- chromosomes entered Japan with both the Jomon and Yayoi migrations. Neutral models of population genetics show that within-group variation is a function of population size, migration rates, and mutation rate (Crow and Kimura 1970). Assuming selective neutrality of Y chromosome variation, differences in levels of haplotype diversity between YAP^- and YAP^+ chromosomes may reflect differences in population sizes and levels of gene flow in ancient Jomon and Yayoi populations.

Contemporary levels of diversity carried by YAP^+ chromosomes should reflect the long period of genetic drift operating on Jomon populations isolated on the Japanese archipelago after the disappearance of the land bridges 10,000 years ago. The effective population size of this hunter-gatherer group was probably relatively low before and after the colonization of Japan because of the zero population growth typical of hunter-gatherer groups (Cavalli-Sforza et al. 1994, p. 106). Furthermore,

the original Jomon founders may have undergone a bottleneck on migration to the archipelago (Suzuki 1981).

In contrast, the diversity of YAP^- chromosomes in modern Japanese populations would be expected to be higher. Although YAP^- chromosomes were subjected to the same population-level forces as were YAP^+ chromosomes during the period of Jomon occupation, an infusion of new YAP^- haplotypes during the Yayoi migration should have resulted in an increase of YAP^- haplotypic diversity. Yamaguchi (1982) noted the importance of a possibly higher population-growth rate and density among the agriculturists from Asia than among the contemporary inhabitants of Japan. Even the earliest farming in east Asia permitted increases in population density of 5–50 times that of hunter-gatherers within the 1st millennium after agriculture was first introduced (Cavalli-Sforza et al. 1994, p. 107). Although levels of genetic diversity increase very slowly after population expansions (Rogers and Jorde 1995), one would expect contemporary populations that descended from ancestors practicing agriculture for several millennia to have elevated levels of neutral genetic variation relative to hunter-gatherer groups. For example, if the ancestors of the Yayoi people underwent a 10-fold increase in size in the 1st millennium after agriculture was first practiced in northern China (~7,000 years ago), levels of genetic diversity at the time of the Yayoi migration would be expected to be ~30%–40% higher than in the original hunter-gatherer group (Li 1977, eq. [6]; Rogers and Jorde 1995).

An alternative hypothesis is that levels of diversity associated with YAP^- chromosomes are higher in all geographic regions where both YAP^- and YAP^+ haplotypes are found. However, this is not the case for the two African populations examined so far (M. Hammer, unpublished data). Preliminary results indicate that haplotypic diversity is comparable between YAP^- and YAP^+ chromosomes in Egypt ($.56 \pm .03$ and $.54 \pm .03$, respectively) and that YAP^- diversity levels are significantly lower than for YAP^+ chromosomes in Pygmies ($.61 \pm .06$ and $.86 \pm .03$, respectively).

Prospects

As a direct test of the hypothesis that the YAP element is a marker of Jomon male lineages, it may be possible to survey the YAP element in DNA extracted from ancient Jomon and Yayoi bones and teeth as has already been done for mtDNA by Horai et al. (1991). Second, it is important to examine a much larger sample of contemporary populations from Korea and north China. This hypothesis would also gain support by finding high frequencies of the YAP element in the Ainu of Hokkaido. The Ainu are now recognized as a remnant population descended from the Jomon, which survived in Hokkaido in relative isolation from post-Jomon influences until the end of the past century (Howells 1966; Turner 1976; Ossenberg 1986). Comparisons based on several morphological traits and genetic polymorphisms attest to the affiliation of the Ainu and the Ryukyuans with ancient Jomon (Omoto and Misawa 1976; Ossenberg 1986; Hanihara 1991; Nei, in press). Therefore, this hypothesis predicts high frequencies of the YAP element in Hokkaido (similar to frequencies found in Okinawa) and lower frequencies in southwestern Japan, the region closest to the Korean peninsula. By sampling contemporary populations across the Japanese archipelago, it should be possible to test this hypothesis.

Finally, by genotyping these Y chromosome polymorphisms in several northeast and southeast Asian populations, it should be possible to distinguish between the out-of-northeast-Asia hypothesis (Nei, in press) and the hypothesis based on dental morphology, which posits that Jomon people migrated to Japan from southeast Asia (Turner 1976). Moreover, it should be possible to search for a genetic trail between Africa, the geographic site where YAP^+ chromosomes originated, and Japan. By examining the frequency of the YAP element in populations from southwest to east Asia, both north and south of the Himalayas, ancient male migration routes may be discovered.

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