Hybrid Origin of Japanese Mice "*Mus musculus molossinus*": Evidence from Restriction Analysis of Mitochondrial DNA[1]

Hiromichi Yonekawa,† Kazuo Moriwaki,‡ Osamu Gotoh,* Nobumoto Miyashita,‡ Yoshibumi Matsushima,§ Liming Shi,‖ Wang Su Cho,# Xiao-Lan Zhen,** and Yusaku Tagashira**

*Department of Biochemistry, Saitama Cancer Center Research Institute, Japan; †Howard Hughes Medical Institute, Department of Microbiology, University of Texas Health Science Center; ‡Department of Cell Genetics, National Institute of Genetics, Japan; §Department of Laboratory Animal Sciences, Tohoku Dental University, Japan; ‖Kun-min Institute of Zoology, Academica, People's Republic of China; #Institute of Agricultural Sciences, Korea; and **Shanxi Cancer Institute, People's Republic of China

The Japanese mouse, *Mus musculus molossinus,* has long been considered an independent subspecies of the house mouse. A survey of restriction-site haplotypes of mitochondrial DNA (mtDNA) showed that Japanese mice have two main maternal lineages. The most common haplotype is closely related to the mtDNA of the European subspecies *M. m. musculus.* The other common haplotype and two minor ones are closely related to each other and to the mtDNA of an Asiatic subspecies, *M. m. castaneus.* Two other rare variants are probably the result of recent contamination by European *M. m. domesticus.* The *musculus* type of mtDNA is found in the southern two-thirds of Japan, whereas the common *castaneus* type is found in the northern third and the minor variants are found sporadically throughout Japan. The *castaneus* mtDNA lineage had a few minor variants, whereas the *musculus* lineage was completely monomorphic. By contrast, the native population of *M. m. castaneus* and the Chinese and Korean *musculus* populations were highly polymorphic. These results suggest that *M. m. molossinus* is a hybrid between ancestral colonies, possibly very small, of *M. m. musculus* and *M. m. castaneus,* rather than an independent subspecies.

Introduction

The taxonomy of mice was recently revised considerably. The 15 subspecies proposed by Schwarz and Schwarz (1943) were combined into five or six subspecies in revised taxonomies (Marshall 1981; Thaler et al. 1981). Even in these new taxonomies, the Far-East subspecies was considered independent and designated *Mus musculus molossinus.*

Kuroda (1940) considered Japanese mice a species of the genus *Mus,* and he recognized four subspecies of *M. molossinus.* His taxonomy showed that three of the four major islands of Japan were occupied by the subspecies *M. mol. molossinus,* Hokkaido by *M. mol. yesonis,* and two southern minor islands by *M. mol. orii* and *M. mol. yonakunii.* Kuroda found slight morphological differences in Japanese mice,

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Address for correspondence and reprints: Hiromichi Yonekawa, Department of Biochemistry, Saitama Cancer Center Research Insitute, Kitaadachi-gun, Saitama, 362, Japan.

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but his taxonomy was not supported by other taxonomists or geneticists (Tokuda 1969; Marshall 1981; Kondo 1983, pp. 108–124).

On the basis of an analysis of skull features and coat colors, Marshall (1981) classified museum specimens from all over Japan as *M. m. molossinus* and a single sample from Hokkaido as *M. m. castaneus.* All Korean samples were also classified as *molossinus,* whereas Chinese mice were divided among *M. m. molossinus, M. m. castaneus,* and *M. m. musculus* (*wagneri*). *M. m. molossinus* was said to closely resemble *musculus* and *castaneus.*

In a biochemical survey of Japanese mice, Minezawa et al. (1979, 1981) defined four populations—northern, Sea of Japan or eastern, Pacific or western, and southern—that differed in the allele frequencies of the allozymes studied but that were all considered members of a single subspecies. The northern population was the most distinct of the four, and it was suggested that the southern population had been invaded by other subspecies. Minezawa and his co-workers also suggested that *M. m. molossinus* is closely related to the European *M. m. musculus.*These findings were confirmed in an extensive analysis of both subspecies by means of many biochemical markers (Bonhomme et al. 1984).

Restriction-site haplotypes of mitochondrial DNA (mtDNA) have proved to be useful diagnostic markers for identification of subspecies (Yonekawa et al. 1980, 1981, 1982; Ferris et al. 1982, 1983*a,* 1983*b;* Moriwaki et al. 1984; Boursot et al. 1984); only near a subspecies boundary are mice sometimes found with mtDNA of a subspecies different from their nuclear type (Yonekawa et al. 1982; Ferris et al. 1983*a;* Boursot et al. 1984). We found that mice collected in the central and southern parts of Japan all had the same monomorphic type of mtDNA that was unique to *M. m. molossinus* (Yonekawa et al. 1981); later we realized that this mtDNA is closely related to that of *M. m. musculus* from Bulgaria (Yonekawa et al. 1982), and we proposed that Japanese mice are not an independent subspecies but rather a local race of *M. m. musculus.* To test this hypothesis, we greatly expanded our geographical survey of mouse mtDNA and included mice from northern Japan.

Material and Methods

1. Animals

The wild mice used were collected in the localities shown in figure 1 and listed in table 1.

2. Enzymes and Chemicals

Restriction endonucleases and Klenow fragments (large fragment of *Escherichia coli* DNA polymerase I) were all purchased from Bethesda Research Laboratories. ^{32}P-α-dNTP's were obtained from Amersham. DNase I and ethidium bromide were from Millipore Corporation and Sigma Chemical Company, respectively. Seakem agarose was from Marine Colloids, Incorporated, and acrylamide and bis-acrylamide were from Wako Company. Other chemicals were reagent grade.

3. Preparation and Restriction Analysis of Liver mtDNA

mtDNAs were prepared by means of the cleared-lysate method described elsewhere (Yonekawa et al. 1980, 1981). After digestion of mtDNA with 6-base restriction enzymes, the DNA fragments were separated by electrophoresis in 1% agarose gels, and their patterns were examined by staining with 0.1 μg ethidium bromide/ml as described elsewhere (Yonekawa et al. 1978). After digestion of mtDNA with 4- or 5-base re-

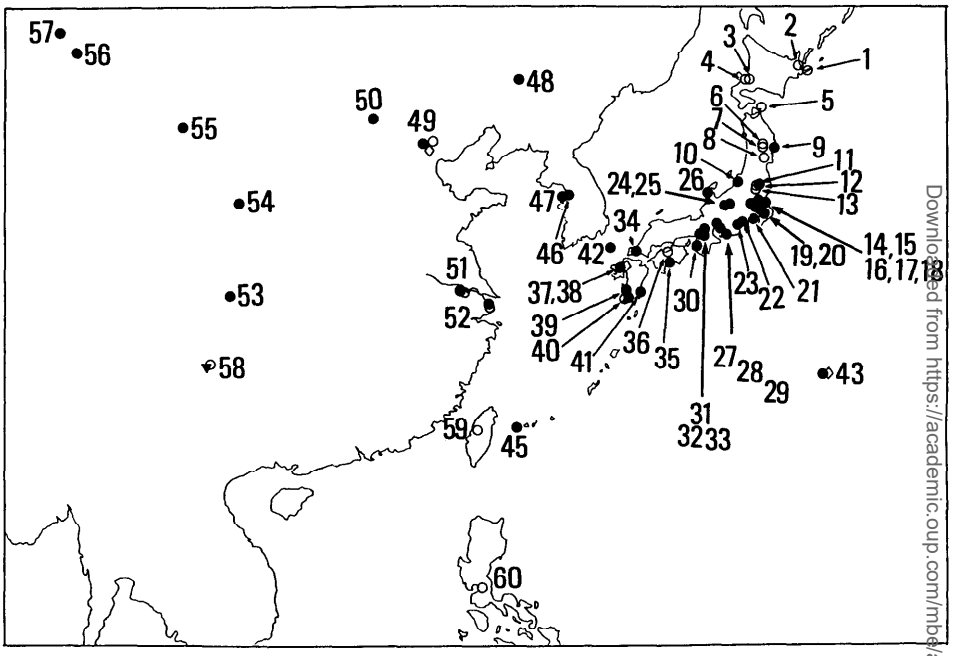


FIG. 1.—Localities where wild mice were collected. mtDNA types are indicated by symbols: ○ = *castaneus;* ● = *musculus;* ◇ = *domesticus;* and ▼ = *bactrianus.* Localities are numbered as in table 1.

striction enzymes, the fragments were labeled at their 3′ ends with a suitable ^{32}P-α-dNTP and Klenow fragments according to the method described by Brown (1980), and their electrophorectic patterns in 4% polyacrylamide gels were examined by means of autoradiography (fig. 2, panel A).

4. Preparation and Restriction Analysis of Blood, Liver, or Tail mtDNA

A sample (200–400 μl) of blood was taken from the retro-orbital venous plexus into heparinized glass capillary tubes. The blood was centrifuged, and the cell fraction was collected and mixed with 500 μl RSB (10 mM NaCl, 10 mM Tris-HCl, 25 mM ethylenediaminetetraacetate (EDTA), pH 7.5) and 1% (final concentration) sodium dodecyl sulfate. The mixture was then treated overnight with 100 μg proteinase K/ml (final concentration) at 37 C. After addition of 0.2 M NaCl (final concentration), the sample was extracted twice with phenol saturated with 3% NaCl, once with phenol-chloroform mixture saturated with 3% NaCl, and once with chloroform. Whole nucleic acids were precipitated with 2 vol ethanol and collected by means of centrifugation at 10,000 *g* for 20 min. The precipitation was repeated in the presence of 2 M ammonium acetate. The pellet of nucleic acids was washed with 90% ethanol, dried under vacuum, and finally dissolved in a solution of 0.1 mM EDTA (pH 8.0) to the same volume as the original blood sample. Samples of 45 μl nucleic acid solution were used for restriction analysis. Both digestion with restriction enzymes and agarose-gel electrophoresis were performed as described above. Southern blotting was performed on Pall Biodyne membranes (BNNG). The conditions for blotting, prehybridization, hybridization, and membrane washing were those recommended by the supplier. The mtDNA used as a probe was purified by means of two or three cycles of CsCl–ethidium

Table 1
Collection Localities of Wild Mice and 6-Base Restriction-Site Haplotypes of Their mtDNAs

Locality (No. in Fig. 1)	Male	Female	Organ	mtDNA Haplotype[a]
Japan:				
Main island Hokkaido:				
Nemuro (1)	1	1	Liver	CA1[b]
Nakasunbetsu (2)	1	0	Liver	CA1[b]
Sapporo (3)	1	1	Liver	CA1[b]
Teine (4)	1	0	Liver	CA1[b]
Main island Honshu:				
Oma (5)	1	0	Liver	CA1[b]
Yahaba (6)	1	1	Liver	CA2
Ajiro (7)	1	0	Blood[b]	CA1
Kurihara (8)	1	0	Liver	CA1
Ozuchi (9)	1	0	Liver	MU1[b]
Niigata[d] (10)	2	0	Liver	MU1[b]
Koriyama (11)	4	6	Liver	MU1[b], CA1[b]
Sukagawa (12)	2	0	Liver	CA1
Izumizaki (13)	0	2	Liver	CA1
Motegi (14)	0	1	Liver	MU1
Mito (15)	1	0	Tail[c]	MU1
Kuki (16)	1	0	Liver	MU1[b]
Inamachi (17)	1	1	Liver	MU1[b]
Omiya (18)	2	1	Liver	MU1[b]
Ichikawa[d] (19)	4	3	Liver	MU1[b]
Yotsukaido (20)	1	1	Liver	MU1[b]
Koajiro (21)	1	0	Liver	MU1[b]
Mishima[c] (22)	12	11	Liver	MU1[b]
Numazu (23)	1	0	Liver	MU1[b]
Shinanomachi (24)	3	0	Liver	MU1[b]
Iiyama (25)	1	0	Tail[c]	MU1
Wajima (26)	1	0	Liver	MU1[b]
Nishiho (27)	1	0	Liver	MU1[b]
Anjo (28)	1	0	Liver	MU1[b]
Mizuho (29)	1	0	Liver	MU1[b]
Gobo (30)	0	1	Liver	MU1[b]
Momoyama (31)	1	0	Liver	MU1
Osaka[c] (32)	2	1	Liver	MU1[b]
Takatsuki (33)	2	1	Liver	MU1
Mine (34)	1	0	Liver	MU1
Main island Shikoku:				
Kochi (35)	0	1	Liver	MU1
Marugame (36)	0	1	Liver	BA
Main island Kyushu:				
Hakozaki[d] (37)	4	4	Liver	MU1[b]
Sasaguri (38)	1	0	Liver	DO1
Izumi (39)	1	1	Liver	MU1[b]
Miyazaki[d] (40)	3	4	Liver	MU1[b]
Kagoshima (41)	4	0	Liver	MU1[b], CA3
Isolated islands:				
Tsushima (42)	1	0	Liver	MU1[b]
Ogasawara (43)	2	0	Liver	MU1[b], DO2
Nishinoshima (44)	0	1	Blood[c]	MU1
Yonaguni (45)	3	0	Liver	MU1[b]
Korea:				
Sueon (46)	2	1	Liver	MU1
Kojuri (47)	1	0	Liver	MU2

Table 1 (*Continued*)

Locality (No. in Fig. 1)	Male	Female	Organ	mtDNA Haplotype[a]
China:				
Changchun (48)	3	0	Liver	MU1
Beijing (49)	5	2	Liver	MU1, CA1, DO3
Taiyuan (50)		3?	Blood	MU1
Nanking (51)	2	0	Liver	MU3, CA1
Shanghai (52)	5	4	Liver	MU1, CA1
Chengtu (53)	1	1	Liver	MU1
Lanzhou (54)	4	0	Liver	MU1
Jiayguang (55)	2	0	Liver	MU1
Turufan (56)	1	0	Liver	MU4
Urumuchi (57)	2	0	Liver	MU1
Kunmin (58)	5	0	Liver[c]	CA1, BA
Taiwan:				
Taichun (59)	1	0	Liver	CA1
Philippines:				
Quezon City (60)	3	2	Liver	CA1
Indonesia				
Bogor (–)	1	0	Liver	CA1
Malaysia				
Kota Kinabalu (–)	0	2	Liver	CA4, BA

[a] MU, CA, DO, and BA represent the 6-base restriction-site haplotypes characteristic of *musculus, castaneus, domesticus,* and *bactrianus,* respectively. The subtypes within each haplotype are numbered.

[b] At least one mouse was also analyzed with 4-base restriction enzymes. See table 2 for the restriction haplotypes.

[c] The total DNA fraction isolated from the organ was used for restriction analysis of mtDNA by Southern blot hybridization.

[d] Mice trapped in these localities were examined with 12 additional restriction enzymes (see text for details).

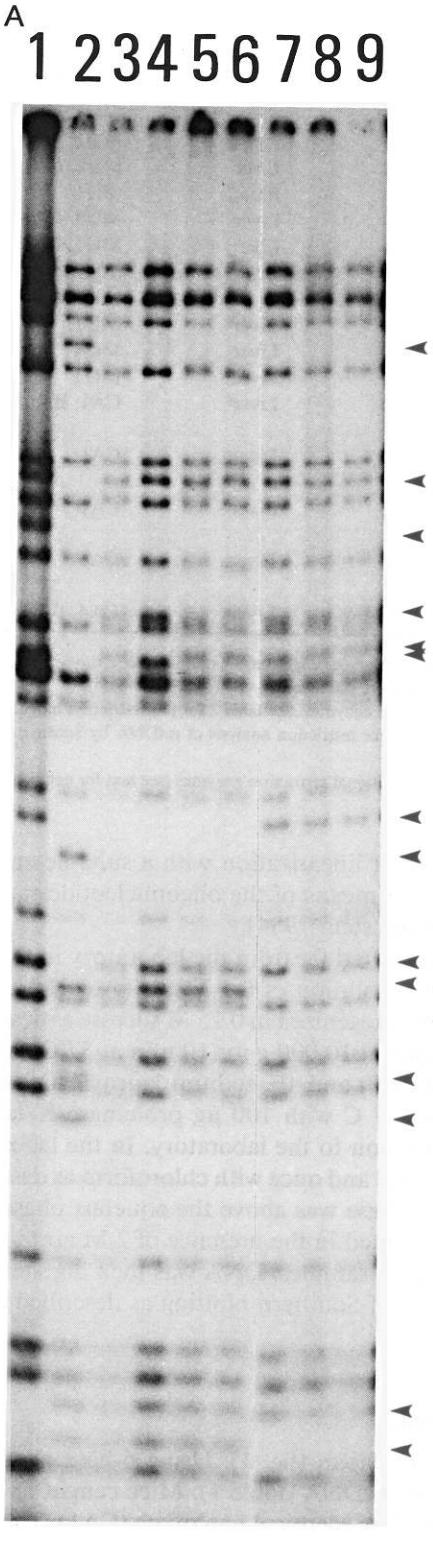
bromide density-gradient centrifugation. After linearization with a suitable enzyme, the mtDNA was labeled with ^{32}P-α-dCTP by means of the oligonucleotide random-primer extension method (Feinberg and Vogelstein 1984).

When blood and liver samples were prepared far from the laboratory in fields or in foreign countries, RSB was replaced by a solution of 0.5 M EDTA (pH 8.0) and 1% sodium lauroyl sarcosinate. Livers were homogenized in 0.25 M sucrose as described above. Then the homogenate was centrifuged at 10,000 *g* for 10 min at 37 C, and the pellet was suspended in 0.5 M EDTA (pH 8.0) and 1% sodium lauroyl sarcosinate. Samples were then incubated overnight at 37 C with 100 μg proteinase K/ml and kept at room temperature during transportation to the laboratory. In the laboratory samples were extracted three times with phenol and once with chloroform as described above. Under these conditions the phenol phase was above the aqueous phase. The three precipitations with ethanol were performed in the presence of 2 M ammonium acetate to remove the EDTA completely from samples. DNA was then digested with restriction enzymes and analyzed by means of Southern blotting as described above (fig. 2, panel B).

Results

Distribution of mtDNA Haplotypes in Japan

Mice were collected in 45 localities in Japan (fig. 1). Using 6-base restriction enzymes, we identified seven haplotypes of mtDNA (table 1). Mice caught at 11 localities north of or in Izumizaki (no. 13) had an identical haplotype (CA1). The mice



trapped in 30 localities south of Izumizaki (nos. 14–45) all shared another haplotype (MU1). The mice from Yahaba (no. 6), Marugame (no. 36), and Sasaguri (no. 38) had unique haplotypes, whereas mice caught in Kagoshima (no. 41) and Ogasawara (no. 43) were polymorphic and had either the MU1 or unique haplotypes of mtDNA (table 2).

The mtDNA haplotypes of the Japanese mice were compared with those that we had previously established, with 6-base restriction enzymes, for some authentic subspecies (Yonekawa et al. 1980, 1981, 1982). As already noted, the common haplotype in southern Japan (MU1) is unique to Japanese *Mus musculus molossinus,* but it differs by only one *Eco*RI site from the haplotype of Bulgarian *M. m. musculus* (Yonekawa et al. 1982; Boursot et al. 1984).

The common CA1 haplotype identified in northern Japan by means of 6-base restriction enzymes is identical to the common haplotype of *M. m. castaneus* in southeastern Asia. The CA3 haplotype from Kagoshima (no. 41) deviates from CA1 only by the *Hae*II sites, and the CA2 haplotype from Yahaba (no. 6) differs from CA1 by *Hin*dII and *Hpa*I sites (table 2). Despite the divergence from CA1, the authentic *M. m. castaneus* haplotype, the Yahaba mtDNA is still classified as *castaneus;* we have not found any other mice of this type, and they may represent a geographical contaminant of unknown origin or a new mutant haplotype.

The mice of Sasaguri (no. 38) and Ogasawara (no. 43) had an *M. m. domesticus* haplotype. The finding of mice with mtDNA identical to that of most laboratory mice could be explained by the proximity of Sasaguri to the site of an American army camp and a big seaport. Also, trading ships might have introduced to Ogasawara some mice that have a haplotype identical to that of *M. m. brevirostris* from Southern France and the laboratory strain NZB (table 1 of Yonekawa et al. 1982).

Although analysis with 6-base restriction enzymes is useful for identification of subspecies, the resolution is insufficient for detection of polymorphism within a population, because the number of restriction sites detected is too low. We therefore reexamined mtDNAs belonging to the two major types (six CA1 and 26 MU1 marked in table 1) with 4-base restriction enzymes, but we still did not uncover any polymorphism (table 2). We also selected MU1 mice collected from six well-separated localities and analyzed their mtDNAs by means of *Acc*I, *Ava*I, *Ava*II, *Bst*EI, *Bst*NI, *Hha*I, *Kpn*I, *Pvu*I, *Sst*I, and *Sst*II—as well as with *Sma*I, which did not cut any mtDNA. Even then we failed to detect any polymorphism within either of the two major maternal lineages of Japanese mice.

The northern limit of the *musculus*-type mtDNA is Ozuchi (no. 9), and the southern limit of the *castaneus*-type mtDNA is Izumizaki (no. 13). We therefore made an extensive survey at Koriyama (no. 11), a city just north of Sukagawa (no. 12) in the boundary zone between the two major populations (fig. 3). Most of the mice had

FIG. 2.—Panel A: Autoradiogram of mtDNA restriction fragments obtained by the 3′-end-labeling technique. The lane numbers correspond to the following localities (on fig. 1) where the mice were collected: 1, Urumuchi (57); 2, Turufan (56); 3, Jiayguang (55); 4, Lanzhou (54); 5, Chengtu (53); 6, Shanghai (52); 7, Changchun (48); 8, Hakozaki (37); and 9, Mishima (22). Arrows show the positions of variable fragments. The mtDNA was digested with *Mbo*I, and the film was exposed for 4 h. Panel B: Autoradiograms of mtDNA restriction fragments from whole DNA after Southern blot hybridization. Restriction fragments of five mice collected in Kunmin, China, are detectable, although the specimens were prepared 15 days before the analysis. The mtDNA was digested with *Eco*RI. Samples 1 and 2 show *bactrianus* pattern (C), and samples 3–5 show *musculus* pattern (B). Film was exposed for 15 h.

Table 2

Subspecies of *Mus musculus* Examined, Their Sources, and Composite Restriction-Fragment Patterns of mtDNAs Obtained from Each Sample

Subspecies and Collection Localities (No. in Fig. 1)	6-Base Haplotypes	6-Base Enzymes								4-Base Enzymes				
		*Bam*HI	*Hin*dII	*Hpa*I	*Eco*RI	*Hae*II	*Pst*I	*Hin*dIII	*Bgl*I	*Mbo*I	*Hinf*I	*Taq*I	*Hpa*II	*Hae*III
M. m. castaneus:														
Indonesia:														
Bogor (–)	CA1	C	C	C	B	C	B	B	A	L	K	...	...	...
Philippines:														
Quezon City (60)	CA1	C	C	C	B	C	B	B	A	J	L	...	D	...
Taiwan:														
Taichun (59)	CA1	C	C	C	B	C	B	B	A	I	L	...	E	...
Malaysia:														
Kota Kinabalu (–)	CA4	C	C	C	D	C	B	B	A	M	L	...	...	...
M. m. molossinus:														
castaneus type:														
Japan:														
10 Localities (1–5, 7, 8, 11–13)	CA1	C	C	C	B	C	B	B	A	I	J	G	D	F
Yahaba (6)	CA2	C	G	D	B	C	B	B	A	...	...	...	...	...
Kagoshima (41)	CA3	C	C	C	B	D	B	B	A	...	...	...	...	...
China:														
Four localities (49, 51, 52, 58)	CA1	C	C	C	B	C	B	B	A	...	...	...	...	...
musculus type:														
Japan:														
33 Localities (9–11, 14–35, 37, 39–45)	MU1	D	A	A	B	C	B	B	A	B	B	B	A	F

Korea:													
Sueon (46)	MU1	D	A	A	B	C	B	B	A	B	C	...	...
Kojuri (47)	MU2	D	A	A	B	E	B	B	A	B	B	...	...
China:													
Changchun (48)	MU1	D	A	A	B	C	B	B	A	B	D	B	A
Beijing (49)	MU1	D	A	A	B	C	B	B	A	...	...	...	...
Shanghai (52)	MU1	D	A	A	B	C	B	B	A	C	G	C	A
Lanzhou (54)	MU1	D	A	A	B	C	B	B	A	C	E	C	A
Chengthu (53)	MU1	D	A	A	B	C	B	B	A	C	E	C	G
Jiayuguang (55)	MU1	D	A	A	B	C	B	B	A	C	H	C	H
Urumuchi (57)	MU1	D	A	A	B	C	B	B	A	F	I	F	J
Nanking (51)	MU3	D	A	—	D	—	B	B	A	...	...	...	...
Turufan (56)	MU4	B	A	A	B	C	B	B	A	E	E	E	I
domesticus type:													
Japan:													
Sasaguri (38)	DO1	A	A	A	A	A	A	A	A	A	A	A	A
Ogasawara (43)	DO2	E	A	A	A	A	A	B	A	...	...	...	...
China:													
Beijing (49)	DO3	A	A	A	A	A	A	B	A	...	...	...	...
bactrianus type:													
Japan:													
Marugame (36)	...	C	...	...	C	...	...	...	...	...	...	...	...
China:													
Kunmin (58)	BA	C	B	B	C	B	B	B	B	...	...	...	...
Kota Kinabalu (–)	BA	C	B	B	C	B	B	B	B	...	...	...	...

NOTE.—Haplotypes are designated as in table 1 on the basis of 6-base restriction enzymes. Restriction patterns are lettered alphabetically by the order of their discovery; A is found in old inbred strains of laboratory mice. Ellipses = not determined. Photographs of all patterns can be found in our previous papers (Yonekawa et al. 1980, 1981, 1982; Boursot et al. 1984; Moriwaki et al. 1984).

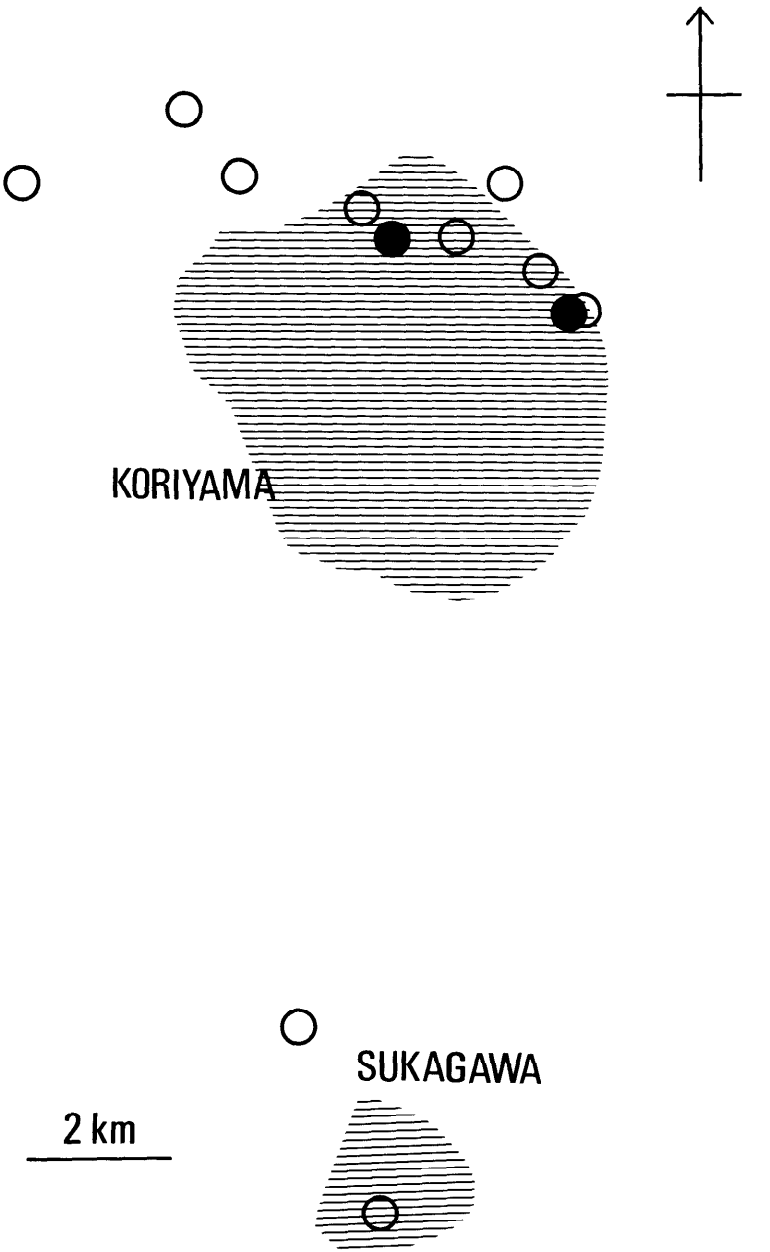


FIG. 3.—Localities in and near Koriyama where wild mice were collected at the boundary of the mtDNA populations. Circles (○) show mice with *castaneus* mtDNA, and black dots (●) show mice with *musculus* mtDNA. Dotted areas are mountains, and hatched areas are towns.

castaneus-type mtDNA, but two mice trapped in different places had *musculus*-type mtDNA. At one of these places we also caught mice with *castaneus* mtDNA, confirming that the boundary is near Koriyama.

Mice in Neighboring Countries

Mice were also collected from countries neighboring Japan (fig. 1), and their mtDNAs were examined by means of 6-base restriction enzymes. Like Japanese mice, the Chinese mice had two major mtDNA classes; mice with *musculus* type mtDNA were found north of the Yang-tzu River, and mice with *castaneus* type mtDNA were found in the southern part of China (table 1). Little polymorphism was detected with the 6-base enzymes, but 4-base enzymes revealed extensive diversity in the *musculus* class, in that mice from different localities showed distinct haplotypes (table 2). Korean mice all had *musculus*-type mtDNA, and some were identical to the Japanese *musculus* type. *Castaneus* mice of southeastern Asia also showed extensive polymorphism when examined with 4-base enzymes. We could not do a similar analysis of the *castaneus* mice from southern China because the amounts of material available were too small. At any rate, it appears that the monomorphism of the two major mtDNA types is peculiar to Japanese mice.

Dendrogram of *M. musculus* mtDNA

To determine the phylogenetic relationships of Japanese mice, we compared their haplotypes with those of other subspecies previously reported elsewhere (Yonekawa et al. 1980, 1981, 1982; Ferris et al. 1982, 1983*a*, 1983*b*; Boursot et al. 1984). Sequence divergences between pairs of mtDNA were estimated on the basis of the number of changed and total restriction sites (Gotoh et al. 1979), and the phylogenetic dendrogram shown in figure 4 was constructed by means of the unweighted-pair-group clustering method (Sokal and Michener 1958). The dendrogram has two main branches, one with only *M. m. domesticus* and one with all Asian subspecies and *M. m. musculus,* and we found the following four major clusters: *domesticus, musculus, castaneus,* and *bactrianus.* We did not detect a cluster of mice from Japan, Korea, and China separated from the other clusters to the extent that one would expect for an authentic subspecies. Instead, the mtDNA of *M. m. molossinus* belongs to either of two lineages—*M. m. castaneus* or a local race of *M. m. musculus.*

Discussion

Mitochondrial Genome of Japanese Mice

The extensive geographical survey of Japanese mice in this paper revealed two major types of mtDNA and hence two maternal lineages. Each lineage was essentially monomorphic, suggesting that they may have passed through severe bottlenecks. The mtDNA of the northern major lineage is identical to that of *Mus musculus castaneus* trapped in Southeast Asia and differs from that of the southern major lineage by an estimated 2.6% sequence divergence. We confirmed our previous observation that the southern haplotype, albeit unique to *M. m. molossinus,* is very closely related to the mtDNA of Bulgarian *M. m. musculus* (Yonekawa et al. 1981, 1982). The same type of mtDNA was also found in Korea, and closely related variants were collected in northern China. Although these mtDNAs formed a cluster in our phylogenetic dendrogram, this cluster was separated from Bulgarian *M. m. musculus* by no more than 0.4% sequence divergence, less than the distance between many of the European *M. m. domesticus* variants. We clearly did not find the kind of ancient, independent branch that one would have expected if *M. m. molossinus* is an authentic subspecies.

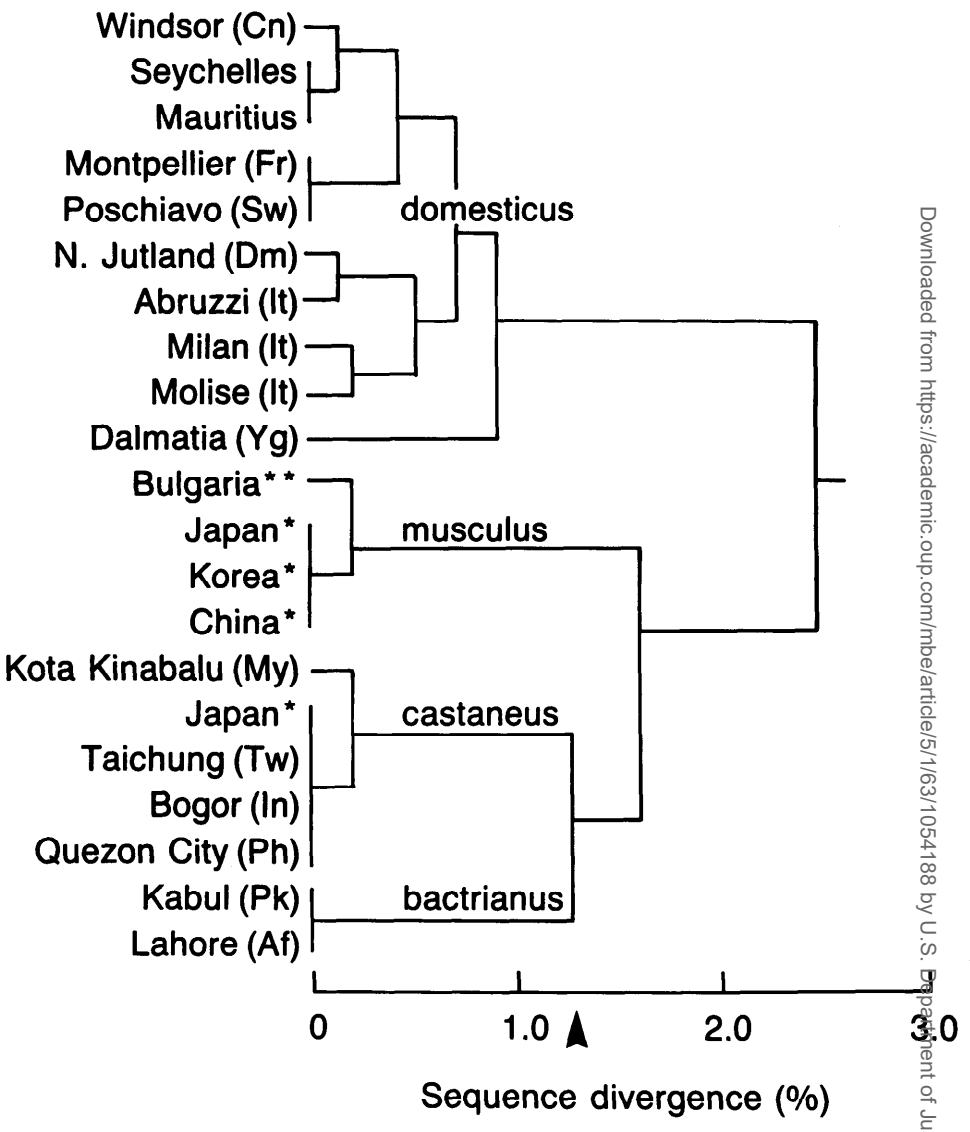


FIG. 4.—Dendrogram of mtDNA haplotypes from wild mice defined by the use of 6-base restriction enzymes. The arrow points to a sequence divergence value of 1.3% for the split between *castaneus* and *bactrianus*. Sequence divergence values are based on Yonekawa et al. (1980, 1981, 1982) and Moriwaki et al. (1984). Cn = Canada; Fr = France; Sw = Switzerland; Dm = Denmark; It = Italy; Yg = Yugoslavia; My = Malaysia; Tw = Taiwan; In = Indonesia; Ph = Philippines; Pk = Pakistan; and Af = Afghanistan.

*Localities where mice were collected are shown in table 1.

**Inbred line MBT (see Bishop et al. 1985) was used.

An Introgression Model

One possible explanation for our results would be to assume that *M. m. molossinus* is indeed an independent subspecies but that its original mtDNA has been completely supplanted by introgression of mtDNA of *castaneus* and *musculus* origin. Unidirectional introgression by mitochondrial genomes has been found repeatedly along the hybrid zone in Europe that separates *M. m. musculus* from *M. m. domesticus*, and the *domesticus* mtDNA introduced into Danish and Swedish populations of *M. m.*

musculus consists of a monomorphic maternal lineage (Ferris et al. 1983*a;* Boursot et al. 1984; Boursot 1985; Gyllensten and Wilson 1987). The southern or *musculus* haplotype of mtDNA is part of a continuous geographical distribution stretching from central and southern Japan over Korea into northern China, where is blends into other *musculus* lineages spread over the entire Eurasian continent.

On the other hand, this band of *musculus* origin separates the northern or *castaneus* haplotype from what is usually considered the native habitat in Southeast Asia. One might propose that *castaneus* mice migrated on an ocean current from the south, reached the northern part of Japan by chance, and established small colonies. From here the mtDNA must have spread very rapidly and efficiently to become uniformly distributed in the native *M. m. molossinus* population of the entire northern third of Japan.

Nuclear Genome of Japanese Mice

The mitochondrial genome is but a small piece of DNA, and its divergence between populations need not be correlated with morphological and biochemical divergence. Any conclusions about the origin and subspecies status of Japanese mice must also consider the nuclear genome. The existence of a *musculus* and a *castaneus* maternal lineage in Japan suggests that these species have contributed to the gene pool of *M. m. molossinus,* and, indeed, there is plenty of data compatible with the hypothesis of a hybrid origin.

The Chinese races of *M. m. musculus* have many chromosomes with faintly stained C-bands. Such chromosomes are rarely found in other subspecies, including European *M. m. musculus* and *M. m. castaneus.* The frequency of these faintly stained C-bands in Japanese mice is intermediate between those of Chinese *M. m. musculus* and of *M. m. castaneus* (Moriwaki et al. 1982). Similar results were obtained for the *b*-allele of the modification enzyme(s) of liver gangliosides, Ggm^{b} (Hashimoto et al. 1984). The frequency of Hbb^{p} (the *p*-allele of the hemoglobin beta chain) is 100% in Chinese *musculus* mice and 40%–50% in Japanese mice, whereas it is only 20% in *M. m. castaneus* (Miyashita et al. 1985).

Certain nuclear markers characteristic of *M. m. castaneus* but rare in other subspecies, including *M. m. musculus,* were commonly found among Japanese mice. These markers included some haplotypes of rDNA spacer regions (Suzuki et al. 1985); an allele of a lymphocyte surface antigen, $Lyt\text{-}2.1^{w}$ (Kurihara et al. 1985); and $Fv\text{-}4^{r}$, an allele that confers resistance to Friend leukemia virus infection (Odaka et al. 1981). The fact that these markers are found also in the southern part of Japan, where they are associated with *musculus* mtDNA, is not explained well by our introgression model, which proposed that the northern mtDNA variant originated from the invasion of northern Japan by a small colony of *castaneus* mice.

Marshall (1981) reported that the skull features of *M. m. molossinus* are very similar to those of *M. m. musculus.* Minezawa et al. (1981) were the first to suggest that, from the viewpoint of biochemical genetics, *M. m. molossinus* is closely associated with *M. m. musculus,* and Sage (1981) and Bonhomme et al. (1984) found, on the basis of extensive studies of these two subspecies, that *M. m. molossinus* was contained in the phylogenetic cluster of *M. m. musculus.*

Hybrid Origin of Japanese Mice

Taking all the data together, we now propose that the Japanese *M. m. molossinus* originated from the hybridization of a Chinese race of *M. m. musculus* and the South-

east-Asian subspecies *M. m. castaneus*. This hypothesis explains how the heterozygosity of Japanese mice can be as high as that of other subspecies (Moriwaki et al. 1979), even though the lack of variation of the mtDNA suggests that they have passed through severe bottlenecks. Since the diversity is expected to be greater between than within subspecies, the genomes derived from two subspecies by means of hybridization and mixing may have much higher heterozygosity than those derived from a single subspecies, even if the founding populations were small.

We think that it is unlikely that the two subspecies invaded Japan simultaneously. If they had mixed while colonizing, one would expect to find the mtDNA variants to be scattered rather than clearly demarcated into a northern and southern type. At present, no selective advantage is known to be associated with any particular allelic forms of mtDNA in mice, and the only differential effect described is a cell-surface antigen. Mta, which does not differ between *musculus* and *castaneus* (Fischer Lindahl and Hausmann 1983; K. Fischer Lindahl, H. Yonekawa, and K. Moriwaki, in preparation). More likely, *M. m. castaneus* migrated first from southern China or Southeast Asia to Japan and established colonies all over. Later, Chinese *M. m. musculus* invaded Japan and displaced the previous occupants, which were driven to the distal northern regions of Japan, while the Chinese *M. m. musculus* settled with the present distribution. During this process, extensive hybridization took place, leading to the mixed nuclear genotype of present-day *M. m. molossinus*. It is for taxonomists to decide whether to grant subspecies status to Japanese mice.

This scenario is consistent with one of the leading hypotheses about the "origins of the Japanese human race" presented by Egami et al. (1981) on the basis of cultural and physical anthropology. They proposed that the Japanese people are descended from two ancestral lineages—that one came from Southeast Asia or southern China and the other invaded Japan from central China and that the second invaders expelled the previous occupants from the central to the distal regions of Japan. The strongest biological evidence supporting Egami et al.'s hypothesis is the finding in the northern and southern ends of Japan of (1) alleles of human hepatitis-B-virus antigens commonly found in Southeast Asia (Nishioka 1984) and (2) patients with HTLV-I, the human T-cell leukemia virus (Ishida and Hinuma 1986). Considering their commensalism, it seems very likely that mice invaded Japan from their native habitat together with humans.

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