Distribution of the molossinus allele of Sry, the testis-determining gene, in wild mice

Article *in* Molecular Biology and Evolution · December 1994

Impact Factor: 9.11 · Source: PubMed

10 authors, including:

Nobumoto Miyashita
Kagawa University
81 PUBLICATIONS **2,474** CITATIONS
SEE PROFILE

Alexey Kryukov
Institute of Biology and Soil Science, …
37 PUBLICATIONS **440** CITATIONS
SEE PROFILE

Distribution of the Molossinus Allele of *Sry*, the Testis-Determining Gene, in Wild Mice

Claude M. Nagamine, Toshihiko Shiroishi,† Nobumoto Miyashita,† Kimiyuki Tsuchiya,‡ Hidetoshi Ikeda,§ Namikawa Takao,‖ Xiang-Lin Wu,# Mei-Lei Jin,** Feng-Shan Wang,†† Alexei P. Kryukov,‡‡ J. Akbarzade,§§ and Kazuo Moriwaki†*

*Department of Cell Biology, Vanderbilt University School of Medicine; †National Institute of Genetics; ‡Miyazaki Medical College; §National Institute of Animal Health; ‖Nagoya University; #Lanzhou Institute of Biological Products; **Shanghai Laboratory Animal Center; ††Tianjin Institute of Industrial Hygiene and Occupational Diseases; ‡‡Institute of Biology and Pedology; and §§Razi State Institute

When the Y chromosome of the laboratory inbred mouse strain C57BL/6 (B6) is replaced by the Y of certain strains of *Mus musculus domesticus,* testis determination fails and all XY fetuses develop either as hermaphrodites or XY females (XY sex reversal). This suggests the presence of at least two alleles of *Sry,* the male-determining gene on the Y: *M. m. domesticus* and B6. The B6 Y chromosome is derived from the Japanese house mouse, *M. m. molossinus* and therefore carries a molossinus *Sry* allele. As a first step to determine how the molossinus *Sry* allele evolved, its distribution pattern was determined in wild mice. The cumulative data of 96 *M. musculus* samples obtained from 58 geographical locations in Europe, North Africa, and Asia show the molossinus *Sry* allele is restricted to Japan and the neighboring Asian mainland and confirm that Japanese *M. m. molossinus* mice were derived in part from a race of *M. m. musculus* from Korea or Manchuria. *Sry* polymorphisms, as illustrated by the molossinus *Sry* allele, can serve as molecular markers for studies on the evolution of wild *M. musculus* populations and can help determine the role sex determination plays in speciation.

Introduction

The laboratory mouse, one of the best-known genetic models, is derived from the house mouse, *Mus musculus. Mus musculus* is a polytypic species that diverged into four subspecies within the last 0.6×10^6 yr (fig. 1) (Bonhomme and Guénet 1989; Auffray et al. 1990; She et al. 1990). *Mus m. domesticus* is indigenous to western Europe and the Mediterranean basin but was transported by humans around the world. *Mus m. musculus* is found from northern and eastern Europe across Asia, north of the Himalayas, to the Pacific coast. *Mus m. bactrianus* is indigenous to the region between Iran and Burma. *Mus m. castaneus* occupies southeast Asia south of the Chang Jiang (Yangtze) River in China (Marshall 1986; Moriwaki et al. 1986; Bonhomme and Guénet 1989; Frisman et al. 1990; Moriwaki et al. 1990; Sage et al. 1993). In Japan, a distinct population, historically referred to as *M. m. molossinus,* arose from the hybridization of *M. m. musculus* and *M. m. castaneus* (Yonekawa et al. 1988; Bonhomme et al. 1989). The majority of the laboratory mouse strains are hybrids that were derived from, at minimum, *M. m. domesticus* and *M. m. molossinus* (Nagamine et al. 1992).

Key words: *Sry,* testis-determining gene, *Mus musculus, M. m. domesticus, M. m. molossinus,* XY sex reversal, Y chromosome.

Address for correspondence and reprints: C. M. Nagamine, Vanderbilt University School of Medicine, Department of Cell Biology, Nashville, Tennessee 37232-2175.

Mol. Biol. Evol. 11(6):864–874. 1994.

0737-4038/94/1106-0005$02.00

The *M. musculus* Y chromosome is polymorphic and can be divided into two types (domesticus and musculus) based on three molecular characteristics: restriction fragment length polymorphisms (RFLPs) for the Y-linked genes, zinc finger protein on the Y, *Zfy-1* and *Zfy-2* (Mardon et al. 1989; Nagamine et al. 1989*a*); the absence (domesticus-type) or presence (musculus-type) of an 18-bp deletion in the *Zfy-2* gene (Nagamine et al. 1990, 1992); and differences in hybridization patterns obtained with recombinant DNA probes specific to murine Y-repetitive sequences (Bishop et al. 1985; Nishioka and Lamothe 1987; Boursot et al. 1989; Tucker et al. 1989). The domesticus-type Y chromosome is found in *M. m. domesticus* and *M. m. bactrianus;* the musculus type is present in *M. m. musculus, M. m. castaneus,* and *M. m. molossinus.* Approximately 75% of classical laboratory mouse strains carry a musculus-type Y (Nishioka and Lamothe 1987). This molecular difference between the domesticus- and musculus-type Ys may have biological significance. The laboratory inbred mouse strain C57BL/6 (B6) has a musculus-type Y. However, when its Y chromosome is replaced by certain domesticus-type Y chromosomes, for example, a Y from the Posch-1 strain, testis determination fails and all XY fetuses develop either as hermaphrodites or XY females (XY sex reversal) (Eicher et al. 1982; Nagamine et al. 1987*b;* Biddle and Nishioka 1988). Placing the B6 musculus-type Y on a *M. m. domesticus* genomic background does not result in sex reversal. The B6 and Posch-1 strains

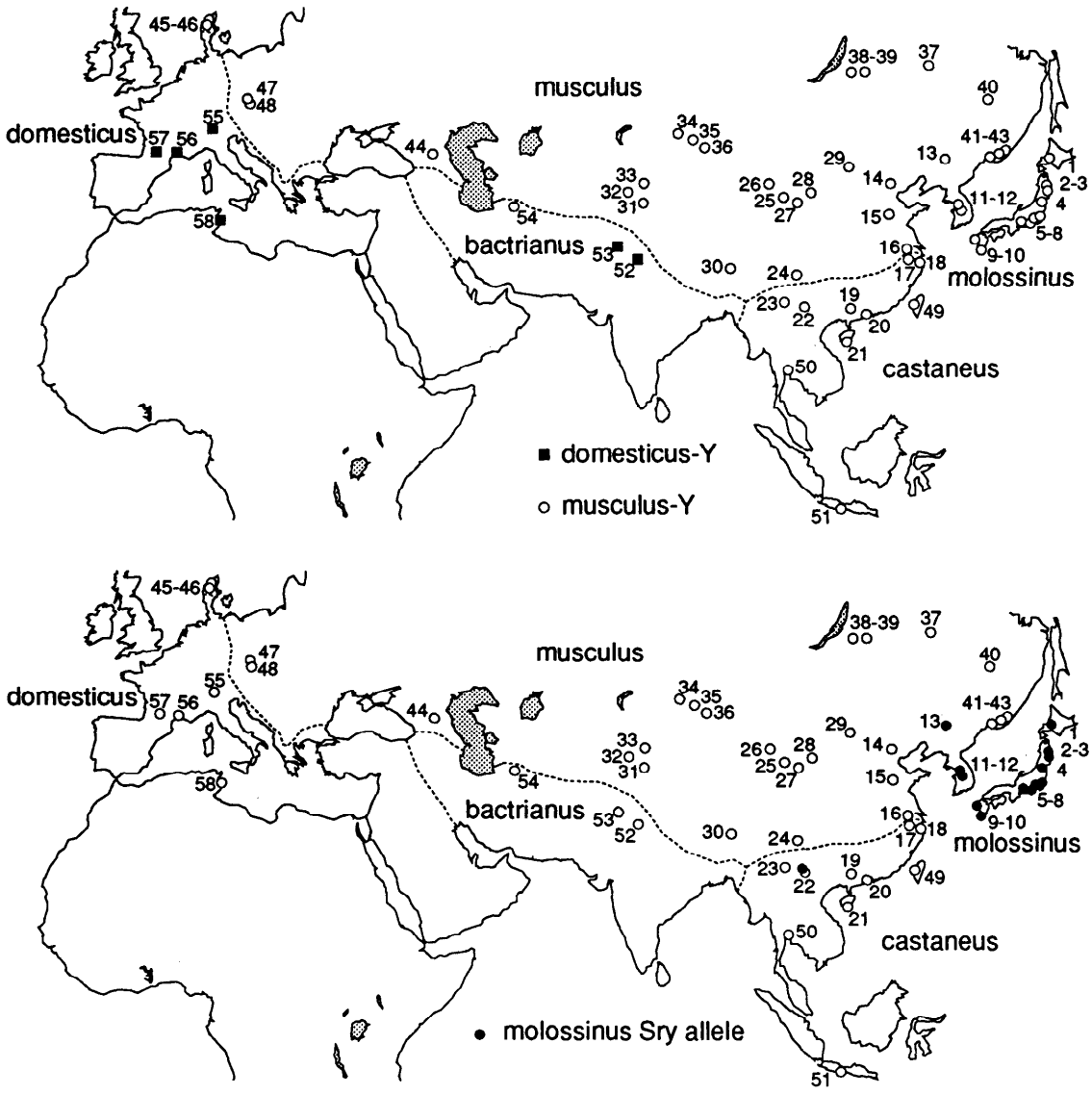

FIG. 1.—Map illustrating origin of samples. Numbers correspond to localities listed in table 1. Dotted lines depict the approximate boundaries of *Mus mus domesticus, M. m. bactrianus, M. m. musculus,* and *M. m. castaneus. Upper panel,* Distribution of musculus- and domesticus-type Y chromosomes as determined by the presence (○) or absence (■) of the *Zfy-2* deletion. *Lower panel,* Distribution of molossinus *Sry* allele (●) as determined by the *Sry TaqI* RFLP.

have normal testis determination. These genetic data suggest that the testis-determining gene on the Posch-1 (domesticus-type) and B6 (musculus-type) Y chromosomes differ because these two Ys have different testis-determining capabilities on the identical B6 genetic background. Eicher and colleagues (1982) hypothesized that at least two alleles of the Y-linked, testis-determining gene (*Tdy*) exist: Tdy^{dom} and Tdy^{B6}.

Convincing data suggest *Tdy* is allelic to the Y-linked gene, sex-determining region of the Y (*Sry*) (Gubbay et al. 1990; Sinclair et al. 1990; Koopman et al. 1991). *Sry* encodes a high mobility group–1 and –2 (HMG 1/2) protein, a protein family characterized by an evolutionarily conserved, approximately 85–amino acid, DNA-binding motif designated the HMG domain. The sequence encoding the HMG domain is called the HMG box. SRY is hypothesized to function as a transcription factor that positively or negatively regulates the transcription of other genes, triggering a cascade of gene interactions that ultimately transforms the mammalian gonad into a testis (Gubbay et al. 1990; Sinclair et al. 1990; Alexander-Bridges et al. 1992; Harley et al. 1992; Haqq et al. 1993; Giese et al. 1994; Grosschedl et al. 1994).

Defining the molecular differences between the *M. m. domesticus* and B6 *Sry* (=*Tdy*) alleles and how they

Table 1

Origin and Number of *Mus musculus* Specimens Studied and Results of Assays for Presence of the *Zfy-2* Deletion and Presence of Molossinus *Sry* Allele

Locality	Subspecies	*n*	*Zfy-2* Deletion	Molossinus *Sry*
Japan (city, island):				
1. Teine, Hokkaido	*M. m. molossinus*	1	+	+
2. Morioka, Honshu*	*M. m. molossinus*	1	+	+
3. Maesawa, Honshu	*M. m. molossinus*	1	+	+
4. Koriyama, Honshu	*M. m. molossinus*	1	+	+
5. Chiba, Honshu*	*M. m. molossinus*	1	+	+
6. Mishima, Honshu*	*M. m. molossinus*	1	+	+
7. Shizuoka, Honshu*	*M. m. molossinus*	1	+	+
8. Anjo, Honshu*	*M. m. molossinus*	1	+	+
9. Fukuoka, Kyushu*	*M. m. molossinus*	1	+	+
10. Izumi, Kyushu*	*M. m. molossinus*	1	+	+
Korea:				
11. Kojuri	*M. m. musculus*	1	+	+
12. Suwon	*M. m. musculus*	1	+	+
China (city, province):				
13. Changchun, Jilin*	*M. m. musculus*	1	+	+
14. Beijing	*M. m. musculus*	2	+	–
15. Jinan, Shandong	*M. m. musculus*	2	+	–
16. Yangzhou, Jiangsu	*M. m.musculus*	1	+	–
17. Zhenjiang, Jiangsu	*M. m. castaneus*	2	+	–
18. Shanghai	*M. m. musculus*	2	+	–
19. Guilin, Guangxi Zhuangzu	*M. m. castaneus*	2	+	–
20. Guangzhou, Guangdong	*M. m. castaneus*	1	+	–
21. Yaxian (Sanya), Guangdong	*M. m. castaneus*	1	+	–
22. Kunming, Yunnan	*M. m. castaneus*	1	+	–
		1	+	+
23. Dali, Yunnan	*M. musculus* sp.	2	+	–
24. Chengdu, Sichuan	*M. m. musculus*	2	+	–
25. Xining, Qinghai	*M. m. musculus*	1	+	–
26. Jiayuguan, Gansu	*M. m. musculus*	2	+	–
27. Lanzhou, Gansu	*M. m. musculus*	2	+	–
28. Yinchuan, Ningxia Huizu	*M. musculus* sp.	2	+	–
29. Wulate, Nei Mongol	*M. m. musculus*	2	+	–
30. Lhasa, Xizang (Tibet)	*M. m. musculus*	2	+	–
31. Hotan, Xinjiang Uygur	*M. m. musculus*	6	+	–
		(1)	+	–
32. Kashi, Xinjiang Uygur	*M. m. musculus*	1	+	–
33. Aksu, Xinjiang Uygur	*M. m. musculus*	2	+	–
34. Tacheng, Xinjiang Uygur	*M. m. musculus*	1	+	–
35. Manas, Xinjiang Uygur	*M. m. musculus*	2	+	–
36. Ürümqi, Xinjiang Uygur	*M. m. musculus*	2	+	–
37. Mohe, Heilongjiang	*M. m. musculus*	2	+	–
Russia:				
38. Teli settlement	*M. m. musculus*	1	+	–
39. Krementui	*M. m. musculus*	1	+	–
40. Birakan	*M. m. musculus*	4	+	–
41. Rudnaja-Ristan'	*M. m. castaneus*	1	+	–
42. Gorny	*M. m. musculus*	4	+	–
43. Vladivostok	*M. m. musculus*	4	+	–
44. Grozny	*M. m. musculus*	1	+	–
Denmark:				
45. Skive*	*M. m. musculus*	1	+	–
46. Vejrumbro*	*M. m. musculus*	1	+	–
Czechoslovakia (strain):				
47. Moravia (Czech I)*	*M. m. musculus*	1	+	–
48. Bratislava (Czech II)*	*M. m. musculus*	1	+	–

Table 1 (*Continued*)

Locality	Subspecies	*n*	*Zfy-2* Deletion	Molossinus *Sry*
Taiwan:				
49. Taichung*	*M. m. castaneus*	1	+	−
Thailand:				
50. Chonburi*	*M. m. castaneus*	1	+	−
Indonesia:				
51. Bandung*	*M. m. castaneus*	1	+	−
India:				
52. Delhi*	*M. m. bactrianus*	7	−	−
Pakistan				
53. Lahore*	*M. m. bactrianus*	1	−	
Iran:				
54. Mashhad*	*M. m. bactrianus*	1	+	−
Italy (strain):				
55. Tirano (Posch-1)*	*M. m domesticus*	1	−	−
France (strain):				
56. Toulouse (WLA76)*	*M. m. domesticus*	1	−	−
57. Montpellier (BFM/2)*	*M. m. domesticus*	1	−	−
Tunisia (strain):				
58. Monastir (WMP)*	*M. m. domesticus*	1	−	−

NOTE.—Mice of undetermined subspecies status (nos. 23, 28) are listed as *M. musculus* sp. Results of samples marked by an asterisk (*) were previously reported (Nagamine et al. 1992). The number of each locality correspond to those in fig. 1. The presence of a musculus-type Y in *M. m. bactrianus* specimen 54 is hypothesized to be the introgression of a *M. m. musculus* Y into the *M. m. bactrianus* population of northeastern Iran (Nagamine et al. 1992). The presence of *M. m. castaneus* mtDNA in sample 41 corroborates allozyme data suggesting *M. m. castaneus* contributed to the genome of mice in the Primorye region of Russia (Frisman et al. 1990). *n* = number of specimens tested from each locality.

evolved may lead to a better understanding of the *M. m. domesticus* XY sex reversal phenomenon and the molecular genetics of mammalian sex determination. The B6 Y chromosome, and therefore its *Sry* allele, is derived from the Japanese wild house mouse, *M. m. molossinus* (Nagamine et al. 1992). As a first step to elucidate how the molossinus *Sry* allele evolved, its distribution pattern was determined in wild *M. musculus*. We studied two *Sry* mutations that have been identified in the molossinus *Sry* allele: a C-T transition in the HMG box at nucleotide (nt) 8491 and a *Taq*I RFLP that is due to a C-T transition at nt 8711 (Nagamine et al. 1992) (nucleotides numbered according to Gubbay et al. 1992). The first mutation results in the substitution of an isoleucine for threonine; the second mutation is silent and does not result in an amino acid change.

Our previous study suggested that the molossinus *Sry* allele is widespread in Japan (Nagamine et al. 1992). Its distribution pattern outside of Japan, however, was unclear. Of five east Asian samples studied (Changchun and Beijing, China; Taiwan; Thailand; Indonesia), the molossinus *Sry* allele was identified only from Changchun. We studied an additional 68 male *M. musculus* from Asia to determine the exact range of the molossinus *Sry* allele. We simultaneously typed the samples for the presence of a *Zfy-2* 18-bp deletion that is diagnostic for the musculus-type Y chromosome. The data document the distribution of the domesticus- and musculus-type Y chromosomes in the Old World and show that the molossinus *Sry* allele is restricted to Japan and the neighboring Asian mainland (Manchuria, Korea).

Material and Methods

Mus Systematics and Samples

The nomenclature used is based on Auffray et al. (1990) and Bonhomme and Guénet (1989) who classify *Mus musculus* as a polytypic species comprised of four subspecies: *M. m. domesticus, M. m. bactrianus, M. m. musculus,* and *M. m. castaneus*. Japanese mice, whose correct nomenclature is *M. m. musculus* × *castaneus,* are referred to as *M. m. molossinus,* to be consistent with the terminology of previous investigators (Yonekawa et al. 1988; Bonhomme et al. 1989; Moriwaki et al. 1990; She et al. 1990; Tucker et al. 1992*a*). The approximate boundaries of the subspecies' native ranges based on published reports are depicted in figure 1 (Marshall 1986; Moriwaki et al. 1986; Bonhomme and Guénet 1989; Frisman et al. 1990; Moriwaki et al. 1990; Sage et al. 1993).

Sixty-eight male *M. musculus* samples from 36 localities in China, Korea, Japan, and Russia (table 1; nos. 1, 3, 4, 11, 12, 14–44) were studied. Three (table

1, nos. 1, 3, 4) were Japanese wild mice and are listed as *M. m. molossinus*. Mitochondrial DNA (mtDNA) *Bam*H I RFLP analysis (Yonekawa et al. 1988) was used to determine the subspecies status of 61 of the remaining 65 samples. The subspecies designations correlated with the subspecies' native ranges except for sample 41 (Rudnaja-Pristan'), which had a *M. m. castaneus* mtDNA genome in the native range of *M. m. musculus*. This observation corroborates protein electrophoretic data identifying alleles characteristic of *M. m. castaneus* in the mice of this region of Russia (Frisman et al. 1990). Four samples representing two localities (table 1, nos. 23, 28) have not yet been typed and are listed as *M. musculus* sp. The mtDNA status of samples 41, 23, and 28 does not affect the conclusions of this report since all were subsequently typed as having a musculus-type Y (see below).

Twenty-eight specimens from an earlier study that were previously typed with regard to domesticus- or musculus-type Y and molossinus *Sry* allele (Nagamine et al. 1992) are also listed in table 1 (nos. 2, 5–10, 13, 45–58) and shown in figure 1 to give the reader a comprehensive view of the samples studied to date. Altogether, 96 samples representing 58 localities and including all recognized *M. musculus* subspecies were studied.

Genotyping for the Domesticus- or Musculus-type Y by *Zfy* PCR

The 68 *Mus musculus* samples were collected in the native ranges of *M. m. musculus, M. m. castaneus,* or *M. m. molossinus* and therefore should have a musculus-type Y (Nagamine et al. 1992). To confirm that the samples harbored a musculus-type Y, we analyzed all using the polymerase chain reaction (PCR) for the presence of a *Zfy-2* 18-bp deletion, a mutation diagnostic for musculus-type Ys (Nagamine et al. 1992).

The PCR for the *Zfy-2* deletion followed our published protocols (Nagamine et al. 1990). The *Zfy*-specific primers recognize the *Zfy-1* and *Zfy-2* genes equally and flank the *Zfy-2* deletion in musculus-type Ys. The predicted *Zfy-1* and *Zfy-2* amplified fragments, in the absence of the 18-bp mutation, are both 618 bp and therefore comigrate. One microliter of the specimen DNA (0.3–2.2 μg) was amplified in a final volume of 50 μl in a reaction mix containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.5 mM $MgCl_2$, 1.3 mM dNTP, 0.5 μM each primer (sense = 5′-AAG ATA AGC TTA CAT AAT CAC ATG GA-3′; antisense = 5′-CCT ATG AAA TCC TTT GCT GCA CAT GT-3′), and 1.0 U *Taq* DNA polymerase (Perkin-Elmer Cetus or Promega). Amplification was for 30 cycles in a Perkin-Elmer Cetus 480, 1 cycle = 45 s at 94°C, 25 s at 60°C, and 1 min at 72°C. The tubes were incubated for 10 min at 72°C after the last cycle. Five to 10 μl of the sample were size fractionated on 4% polyacrylamide gels, stained with ethidium bromide, then visualized with UV transillumination. Negative controls in which the DNA was omitted were run with each experiment to check for contamination by PCR-amplified products; none was found.

DNA samples from domesticus-type Ys give a single 618-bp band representing the comigration of the *Zfy-1* and *Zfy-2* amplified products (fig. 2*A*). Musculus-type Ys give four bands: a 600-bp *Zfy-2* band, a 618-bp *Zfy-1* band, and two bands representing *Zfy-1*/*Zfy-2* heteroduplexes (h1, h2) (Nagamine et al. 1989*b*, 1990). Heteroduplexes form because the sequences of the *Zfy-1* and *Zfy-2* amplified products are nearly identical. They are generated when the *Zfy-1* and *Zfy-2* PCR products reach a concentration level that favors a *Zfy-1* strand annealing to a complementary *Zfy-2* strand instead of to a *Zfy* primer or its complementary *Zfy-1* strand. The slower migration of the *Zfy-1*/*Zfy-2* heteroduplexes relative to the *Zfy-1*/*Zfy-1* and *Zfy-2*/*Zfy-2* homoduplexes and to each other is due to a combination of three factors: the mismatch between the *Zfy-1* and *Zfy-2* complementary strands resulting from the *Zfy-2* 18-bp deletion, the two heteroduplexes differing in their sequences in the mismatch region due to the presence of the sense or antisense *Zfy-2* strand, and to the capability of polyacrylamide gels to resolve these differences. The reader is referred to Nagamine et al. (1989*b*) for a detailed analysis of this phenomenon.

Southern Blot Analysis for the Molossinus *Sry* Allele

Genomic DNAs (10–15 μg) were digested with *Taq*I, size fractionated on 0.8% agarose gels, then Southern blotted to nylon membranes (Hybond N+, Amersham or MagnaCharge, Micron Separations, Inc.). The transferred DNAs were UV cross-linked to the filters, prehybridized at 65°C for 1–3 h in 0.25 M NaH_2PO_4 (pH 7.2), 7% sodium dodecyl sulfate (SDS), then hybridized at 65°C for 14–16 h with the denatured ^{32}P-*Sry* probe (2.0×10^6–3.0×10^6 cpm/ml) in 0.25 M NaH_2PO_4 (pH 7.2), 7% SDS, and 10% dextran sulfate. After hybridization, the filters were washed twice at low stringency (2 × SSC, 0.1% SDS, 55°C, 30 min/each; 1 × SSC = 0.15 M NaCl, 0.015 M sodium citrate) and once at high stringency (0.1 × SSC, 0.1% SDS, 55°C, 15 min) prior to autoradiography at −85°C using Kodak X-OMAT film and an intensifying screen. The *Sry* probe was generated by PCR (Nagamine et al. 1992). *Sry*-specific primers flanking the *Sry* HMG box (sense = 5′ GTG ACA ATT GTC TAG AGA GCA TGG A-3′, antisense = 5′ GCA GCT CTA CTC CAG TCT TGC C-3′) were used to generate a 382-bp *Sry* fragment from genomic DNA. The 382-bp fragment then served as a template to generate a 160-bp ^{32}P-labeled-*Sry* probe us-

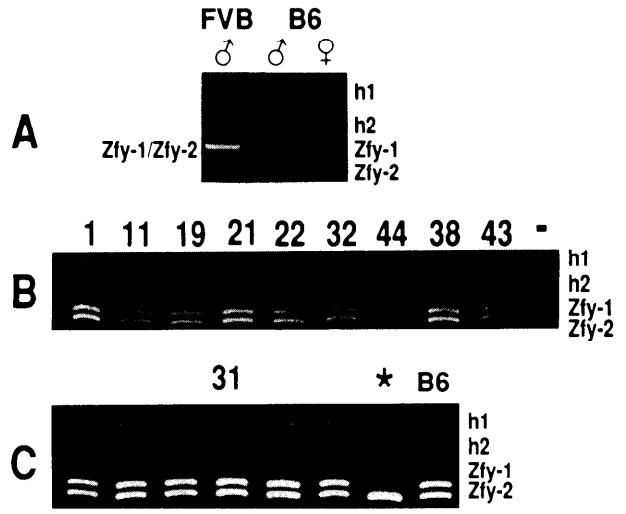

FIG. 2.—PCR amplification for *Zfy-2* deletion. *A*, *Zfy*-amplified products obtained from samples with domesticus- (FVB) or musculus-type Ys (B6). Absence of amplified products using B6 female DNA demonstrate the specificity of the *Zfy* primers. *B*, Example of *Zfy-2* genotyping. Numbers correspond to localities in table 1 and fig. 1. All have the *Zfy-2* deletion. A dash (-) indicates negative control in which DNA was omitted. *C*, *Zfy-2* genotyping of seven samples from Hotan, China (31) compared to B6 male. An asterisk (*) indicates exceptional *Zfy* genotype.

ing nested *Sry* primers (sense = GTC CCG TGG TGA GAG GCA CAA GT-3′, antisense = 5′-TTC CTC TCT GTG TAA GAT CTT CAA TC-3′).

DNA Sequence Analysis of the *Sry* HMG Box

The *Sry* HMG box was determined by direct sequencing of either single stranded DNA (ssDNA) or double stranded DNA (dsDNA) *Sry* templates. After amplification of the *Sry* HMG box from genomic DNA, the PCR amplified products were fractionated on 1% low-melting agarose gels and the *Sry* dsDNA isolated by melting the agarose block at 70°C then either freezing/thawing the molten agarose followed by filtration through a 0.45-μm Ultrafree-MC filter unit (Millipore Corp.) or digesting the agarose with Gelase (Epicentre Technologies). The *Sry* dsDNA was recovered by ethanol precipitation. *Sry* ssDNA was generated by linear amplification of the *Sry* dsDNA using the same PCR conditions but only a 5′ or 3′ *Sry* primer. The *Sry* ssDNA was purified by filtration through 30,000 NMWL Ultrafree-MC filter units. Sequencing was either by the dideoxy chain termination method using *Sry* dsDNA or ssDNA templates and Sequenase 2.0 (United States Biochemicals Corp.) or by the CircumVent Thermal Cycle kit (New England Biolabs, Inc.) using *Sry* dsDNA templates.

Results and Discussion

The 68 *Mus musculus* samples included 52 *M. m. musculus*, 9 *M. m. castaneus*, 3 *M. m. molossinus*, and 4 specimens of unknown subspecies status that were obtained from within the native ranges of *M. m. castaneus* (no. 23) or *M. m. musculus* (no. 28). Together with the samples from our previous study (table 1, nos. 2, 5–10, 13, 45–58), a total of 96 *M. musculus* samples from 58 geographical localities and including all recognized *M. musculus* subspecies (*M. m. musculus, M. m. castaneus, M. m. domesticus, M. m. bactrianus*) and the hybrid *M. m. molossinus* have been studied.

Distribution of *Mus musculus* Musculus- and Domesticus-Type Y Chromosomes

Mus m. musculus, M. m. castaneus, and *M. m. molossinus* have a musculus-type Y. Previous data suggest that a Y can introgress or enter across subspecies boundaries, which thus confounds the results (Boursot et al. 1989; Nagamine et al. 1992). Therefore, regardless of mtDNA subspecies designation or geographical origin of samples, all were typed for the presence of a *Zfy-2* 18-bp deletion, a mutation characteristic of musculus-type Ys, to confirm that a musculus-type Y chromosome was indeed present. PCR identified the *Zfy-2* deletion in all samples (fig. 2*B*). Together with our previous study (Nagamine et al. 1992), the *Zfy* data document the distribution of the musculus- and domesticus-type Ys in the Old World (fig. 1*A*).

One sample from Hotan, China (no. 31) gave only the 600-bp *Zfy-2* band, the *Zfy-1* and heteroduplex bands being absent (fig. 2*C*). Six additional samples from the same locality gave the expected *Zfy-2* deletion PCR pattern which suggests that the exceptional sample is either unique or rare (fig. 2*C*; table 1, no. 31). Southern blot analysis for *Sry* was consistent with other specimens from this locality (fig. 3*C*). This sample is placed in parentheses in table 1 until a more detailed analysis is completed. Our preliminary data suggest that the sample is a *M. m. musculus* with a partially deleted *Zfy-1* gene.

Distribution of Molossinus *Sry* Allele

The molossinus *Sry* allele can be distinguished from the *Sry* alleles of *Mus m. domesticus, M. m. bactrianus, M. m. musculus,* and *M. m. castaneus* by a *Taq*I RFLP (molossinus = 4.5-kb fragment, nonmolossinus = 2.1 kb) (Nagamine et al. 1992). Among the 68 samples tested, Southern blots identified the molossinus *Sry* allele in six samples: three *M. m. molossinus* samples from northern Japan, two *M. m. musculus* samples from Korea, and one of two *M. m. castaneus* from Kunming, China (table 1; fig. 3*A*–3*C*). The molossinus *Sry* allele was previously identified in seven different localities in Japan and in Changchun, China (table 1, nos. 2, 5–10, 13) (Nagamine et al. 1992). The cumulative data reveal a clustering of samples with molossinus *Sry* alleles in

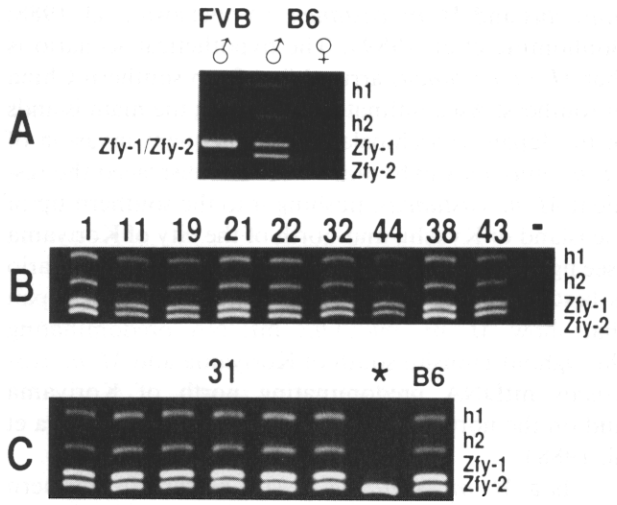

FIG. 2.—PCR amplification for *Zfy-2* deletion. *A, Zfy*-amplified products obtained from samples with domesticus- (FVB) or musculus-type Ys (B6). Absence of amplified products using B6 female DNA demonstrate the specificity of the *Zfy* primers. *B,* Example of *Zfy-2* genotyping. Numbers correspond to localities in table 1 and fig. 1. All have the *Zfy-2* deletion. A dash (-) indicates negative control in which DNA was omitted. *C, Zfy-2* genotyping of seven samples from Hotan, China (31) compared to B6 male. An asterisk (*) indicates exceptional *Zfy* genotype.

ing nested *Sry* primers (sense = GTC CCG TGG TGA GAG GCA CAA GT-3′, antisense = 5′-TTC CTC TCT GTG TAA GAT CTT CAA TC-3′).

DNA Sequence Analysis of the *Sry* HMG Box

The *Sry* HMG box was determined by direct sequencing of either single stranded DNA (ssDNA) or double stranded DNA (dsDNA) *Sry* templates. After amplification of the *Sry* HMG box from genomic DNA, the PCR amplified products were fractionated on 1% low-melting agarose gels and the *Sry* dsDNA isolated by melting the agarose block at 70°C then either freezing/thawing the molten agarose followed by filtration through a 0.45-μm Ultrafree-MC filter unit (Millipore Corp.) or digesting the agarose with Gelase (Epicentre Technologies). The *Sry* dsDNA was recovered by ethanol precipitation. *Sry* ssDNA was generated by linear amplification of the *Sry* dsDNA using the same PCR conditions but only a 5′ or 3′ *Sry* primer. The *Sry* ssDNA was purified by filtration through 30,000 NMWL Ultrafree-MC filter units. Sequencing was either by the dideoxy chain termination method using *Sry* dsDNA or ssDNA templates and Sequenase 2.0 (United States Biochemicals Corp.) or by the CircumVent Thermal Cycle kit (New England Biolabs, Inc.) using *Sry* dsDNA templates.

Results and Discussion

The 68 *Mus musculus* samples included 52 *M. m. musculus,* 9 *M. m. castaneus,* 3 *M. m. molossinus,* and 4 specimens of unknown subspecies status that were obtained from within the native ranges of *M. m. castaneus* (no. 23) or *M. m. musculus* (no. 28). Together with the samples from our previous study (table 1, nos. 2, 5–10, 13, 45–58), a total of 96 *M. musculus* samples from 58 geographical localities and including all recognized *M. musculus* subspecies (*M. m. musculus, M. m. castaneus, M. m. domesticus, M. m. bactrianus*) and the hybrid *M. m. molossinus* have been studied.

Distribution of *Mus musculus* Musculus- and Domesticus-Type Y Chromosomes

Mus m. musculus, M. m. castaneus, and *M. m. molossinus* have a musculus-type Y. Previous data suggest that a Y can introgress or enter across subspecies boundaries, which thus confounds the results (Boursot et al. 1989; Nagamine et al. 1992). Therefore, regardless of mtDNA subspecies designation or geographical origin of samples, all were typed for the presence of a *Zfy-2* 18-bp deletion, a mutation characteristic of musculus-type Ys, to confirm that a musculus-type Y chromosome was indeed present. PCR identified the *Zfy-2* deletion in all samples (fig. 2*B*). Together with our previous study (Nagamine et al. 1992), the *Zfy* data document the distribution of the musculus- and domesticus-type Ys in the Old World (fig. 1*A*).

One sample from Hotan, China (no. 31) gave only the 600-bp *Zfy-2* band, the *Zfy-1* and heteroduplex bands being absent (fig. 2*C*). Six additional samples from the same locality gave the expected *Zfy-2* deletion PCR pattern which suggests that the exceptional sample is either unique or rare (fig. 2*C;* table 1, no. 31). Southern blot analysis for *Sry* was consistent with other specimens from this locality (fig. 3*C*). This sample is placed in parentheses in table 1 until a more detailed analysis is completed. Our preliminary data suggest that the sample is a *M. m. musculus* with a partially deleted *Zfy-1* gene.

Distribution of Molossinus *Sry* Allele

The molossinus *Sry* allele can be distinguished from the *Sry* alleles of *Mus m. domesticus, M. m. bactrianus, M. m. musculus,* and *M. m. castaneus* by a *Taq*I RFLP (molossinus = 4.5-kb fragment, nonmolossinus = 2.1 kb) (Nagamine et al. 1992). Among the 68 samples tested, Southern blots identified the molossinus *Sry* allele in six samples: three *M. m. molossinus* samples from northern Japan, two *M. m. musculus* samples from Korea, and one of two *M. m. castaneus* from Kunming, China (table 1; fig. 3*A*–3*C*). The molossinus *Sry* allele was previously identified in seven different localities in Japan and in Changchun, China (table 1, nos. 2, 5–10, 13) (Nagamine et al. 1992). The cumulative data reveal a clustering of samples with molossinus *Sry* alleles in

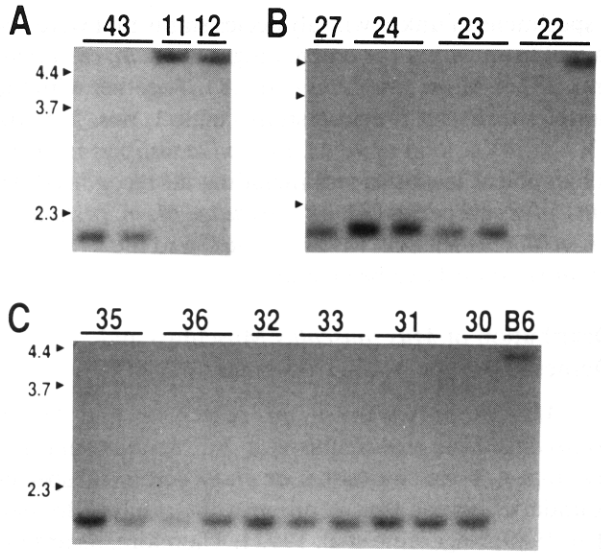

FIG. 3.—Southern blot analysis for the *Sry TaqI* RFLP. Photos show representative results. Numbers correspond to localities in table 1 and fig. 1. Arrowheads indicate 4.4-, 3.7-, and 2.3-kb markers. *A,* Molossinus *Sry* allele in Korean (nos. 11, 12) but not Vladivostok, Russia samples (no. 43). *B,* Molossinus *Sry* allele in one of two Kunming samples (no. 22). The 2.1-kb *Sry* band in the first Kunming sample is not obvious in the photo but was visible on the autoradiograph. Other samples represent neighboring Chinese localities. *C,* Absence of molossinus *Sry* allele in central Asia. Note that the specimen with the exceptional *Zfy* genotype in fig. 2 (no. 31, first sample) is indistinguishable from other samples of the same region.

Japan and the neighboring Asian mainland (Manchuria, Korea) (fig. 1*B*).

We previously hypothesized that the molossinus *Sry* allele identifies a race of *M. m. musculus* that was present in China and Korea and that this race was distinct from *M. m. musculus* of Europe and Central Asia. Furthermore, it was this race of *M. m. musculus* that invaded Japan, giving rise to *M. m. molossinus* (Nagamine et al. 1992). The present data are in keeping with this hypothesis.

The only outlier is a sample from Kunming, China (fig. 1, *lower panel,* no. 22). We surmise this sample is either a recent introduction or sampling error for two reasons: a second sample from this city (table 1, no. 22) and all samples from neighboring localities had non-molossinus *Sry* alleles, and this sample is present in the *M. m. castaneus* range, whereas all other samples with the molossinus *Sry* allele were obtained from the *M. m. musculus* range (fig. 1, *lower panel*). Additional samples are required to determine whether a stable population with a molossinus *Sry* allele is present in Kunming.

Introgression of Molossinus Y into Northern Japan

Mitochondrial DNA and biochemical data suggest that Japanese *Mus m. molossinus* is a hybrid of *M. m. musculus* and *M. m. castaneus* (Yonekawa et al. 1988; Bonhomme et al. 1989). The hypothetical scenario is that *M. m. castaneus* arrived first from southern China or southeast Asia, ultimately occupying the main islands in the Japanese archipelago. A subsequent invasion of *M. m. musculus* hybridized with and displaced the resident *M. m. castaneus,* pushing it to the southern tip of the island of Kyushu and north of the city of Koriyama (see fig. 1, site 4) on the island of Honshu. This scenario is best illustrated by mtDNA data of northern Japan that show *M. m. musculus* mtDNA predominating throughout Honshu south of Koriyama and *M. m. castaneus* mtDNA predominating north of Koriyama and on the northern island of Hokkaido (Yonekawa et al. 1988).

Is a *M. m. castaneus* Y still present in northern Japan? The *Sry* RFLP discriminates between *M. m. castaneus* and molossinus Y chromosomes. Three *M. m. molossinus* samples (table 1, nos. 1–3) were obtained north of Koriyama (fig. 1, nos. 1–3). All had the molossinus *Sry* allele (table 1). The data suggest that the molossinus Y introgressed into the *M. m. castaneus* mtDNA population of northern Japan faster than the maternally inherited mtDNA and that male dispersal is playing an important role in the invasion. These results corroborate biochemical data showing the presence of *M. m. musculus* nuclear genes in the *M. m. castaneus* mtDNA population of northern Japan (Bonhomme et al. 1989), data suggesting that the introgression of a Y chromosome from one *M. musculus* subspecies into another is not always inhibited (Boursot et al. 1989; Nagamine et al. 1992), and data suggesting that the Y chromosome can introgress as much as three times faster than mtDNA (Berry et al. 1990). Additional samples are needed to determine whether a *M. m. castaneus* Y remains in northern Japan or is extinct.

DNA Sequence Analysis of the Molossinus *Sry* HMG Box

The HMG domain is the only domain of SRY that is evolutionarily conserved. A C-T transition in the *Sry* HMG box was identified in a single *Mus m. molossinus* from southern Japan (Kyushu); this mutation was absent in samples of *M. m. domesticus* (n=6), *M. m. bactrianus* (2), *M. m. musculus* (2), and *M. m. castaneus* (2) as well as the related *Mus* species, *M. spretus* (3), *M. macedonicus* (2), *M. spicilegus* (1), and *M. caroli* (1) (Graves and Erickson 1992; Tucker et al. 1992*a;* C. M. Nagamine, unpublished data). It was possible that this mutation was recently derived and therefore restricted to southern Japan. We checked whether this HMG box mutation was present in molossinus *Sry* alleles outside southern Japan. The *Sry* HMG box from 6 specimens representing molossinus Ys from northern

and central Japan (fig. 1, *lower panel,* nos. 1, 7) and the Asian mainland (fig. 1, *lower panel,* nos. 11–13, 22) were PCR amplified and directly sequenced. The C-T transition was identified in all six molossinus *Sry* alleles (fig. 4). We conclude that the molossinus *Sry* allele carries at least two mutations: one identified by a *Taq*I RFLP and the other being a C-T transition in its HMG box.

Additional mutations unique to the molossinus *Sry* allele may be present. DNA sequencing of the *Sry* open-reading frames (ORF) of *M. m. domesticus* and *M. m. molossinus* revealed an additional 12 polymorphic sites 3′ of the HMG box (Coward et al. 1994). However, until the *M. m. musculus, M. m. castaneus,* and *M. m. bactrianus Sry* genes are also sequenced, we cannot assign these characteristics to either the *M. m. domesticus* or *M. m. molossinus Sry* alleles.

Sry Alleles and XY Sex Reversal

Testis determination is triggered by the *Sry* locus on the Y chromosome (Koopman et al. 1991). The XY sex reversal that occurs when a *Mus m. domesticus* Y is introduced into the B6 strain suggests *Sry* alleles exist. It has been hypothesized that XY sex reversal is due in part to the *M. m. domesticus Sry* allele acting at a later stage of fetal development than the molossinus *Sry* allele, thus allowing ovarian tissues to develop in XY fetal gonads (Eicher and Washburn 1986; Palmer and Burgoyne 1991).

XY sex reversal does not occur with all *M. m. domesticus* Y chromosomes (Nagamine et al. 1987*a,b;* Biddle et al. 1991). Three types of *M. m. domesticus* Y chromosomes have been identified based on their ability to induce testes when introduced into the B6 strain (reviewed in Nagamine 1993). The first Y, which is found in the FVB/N and SJL strains, is capable of normal testis determination when introduced into B6. The second is found in the AKR strain. Introducing the AKR Y into B6 results in testis differentiation proceeding at a slower pace and the presence of ovotestes at 14–16 d of gestation. (Testis differentiation is initiated at 12 d postcoitus, and the gestation period is 19 d in these strains). The criterion for designating the gonads as ovotestes is based on the presence of meiotic germ cells in the affected tissues at the poles of the gonads in histological sections (Nagamine et al. 1987*b*). In the mouse, oocytes initiate meiosis at 14–15 d of gestation; spermatocytes enter meiosis only after birth. The presence of meiotic germ cells, albeit rare, suggests that the affected tissues are following the ovarian pathway and that the gonads are, by definition, ovotestes. However, the sex reversal is transient. The gonads continue to masculinize, the ovarian tissues become difficult to identify because of atresia of the oocytes, and no evidence of hermaphroditism is seen at birth (Nagamine et al. 1987*b*). The third Y is found in the Posch-1 strain. Introducing the Posch-1 Y into B6 results in testis differentiation proceeding at an even slower pace and resulting in the presence of ovotestes or XY ovaries at birth. One can predict three *M. m. domesticus Sry* alleles if these genetic effects are due to *Sry* polymorphisms.

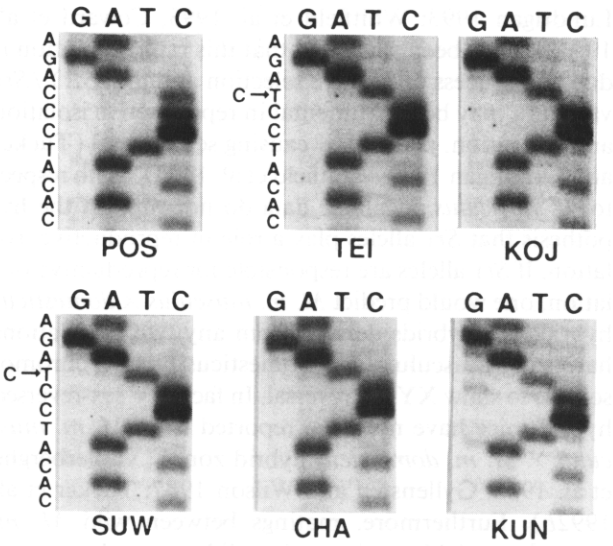

FIG. 4.—DNA sequence analysis for the *Sry* HMG box mutation. DNA sequence for a normal *Mus musculus Sry* allele is represented by the Posch-1 strain (POS). The C-T transition is found in the molossinus *Sry* allele from northern Japan (TEI = Teine; fig. 1, no. 1), Korea (KOJ = Kojuri, SUW = Suwon; fig. 1, nos. 11, 12), and China (CHA = Changchun, KUN = Kunming; fig. 1, nos. 13, 22).

The *Sry* loci of these three *M. m. domesticus* Ys are grossly intact based on RFLP analysis. We have been unable to identify *Sry* RFLPs among the *M. m. domesticus* Y chromosomes of FVB/N or SJL, AKR, and Posch-1 using the restriction enzymes *Eco*RI, *Sst*I, *Taq*I, *Bam*HI, *Hin*dIII, and *Msp*I (C. M. Nagamine, unpublished observations). A similar conclusion was reached by other investigators (Gubbay et al. 1992).

More recently, DNA sequence analysis identified a site in the *Sry* ORF of *M. m. domesticus* mice that correlates with degree of sex reversal: a polymorphic CAG (glutamine) repeat 3′ of the HMG box. FVB/N and SJL *Sry* loci have 12, AKR has 13, and Posch-1 has 11 CAG repeats (Coward et al. 1994). The CAG polymorphisms confirm the hypothesis that *Sry* alleles exist in *M. m. domesticus.* Whether the CAG polymorphism is the direct cause of the XY sex reversal phenomenon or works in concert with additional *Sry* or Y chromosomal mutations remains to be clarified.

The *SRY/Sry* locus 3′ of the HMG box is highly variable among primate and rodent species, which suggests that *SRY/Sry* is rapidly evolving (Tucker and

Lundrigan 1993; Whitfield et al. 1993; Coward et al. 1994). It has been suggested that this rapid evolution is due to a process of positive selection and that *SRY/Sry* variation may be instrumental in reproductive isolation and speciation, perhaps by causing sex reversal (Tucker and Lundrigan 1993; Whitfield et al. 1993). With respect to *M. musculus,* available data do not support the hypothesis that *Sry* alleles play a role in reproductive isolation. If *Sry* alleles are responsible for reproductive isolation, one would predict *M. m. musculus* × *domesticus* hybrids (or hybrids derived from any two populations harboring musculus- and domesticus-type Y chromosomes) to show XY sex reversal. In fact, XY sex-reversed hybrid mice have not been reported from *M. m. musculus* × *M. m. domesticus* hybrid zones (Vanlerberghe et al. 1986; Gyllensten and Wilson 1987; Tucker et al. 1992*b*). Furthermore, matings between wild *M. m. musculus* and *M. m. domesticus* did not result in either abnormal sex ratios (suggestive of XY sex reversal) or hybrid sterility (Vanlerberghe et al. 1986). In Japan, the presence of *M. m. domesticus* mtDNA and nuclear genes in the southern population of *M. m. molossinus* suggest that hybridization between *M. m. domesticus* and *M. m. molossinus* is also possible (Yonekawa et al. 1988; Bonhomme et al. 1989). We surmise that if *Sry* alleles play a role in speciation, its effects are more subtle and indirect. For example, *Sry* alleles may correlate with different levels of aggressive/territorial behavior based on when the fetal testes differentiated and the length of time and/or concentration of testosterone a fetus is exposed to in utero. In any case, the identification of *Sry* alleles makes it now possible to perform laboratory and field studies on wild *M. musculus* populations to directly test how populations harboring different *Sry* alleles interact in the wild and, what role, if any, these alleles play in reproductive fitness.

Y Polymorphisms and Systematics of *Mus musculus*

The mammalian Y chromosome is normally transmitted only from father to son. In the absence of germline mutations, polymorphic genes located on the nonrecombinant region of the Y are clonally inherited and can be used for evolutionary or population genetic studies to delineate patrilineal inheritance. These data complement those based on maternally inherited mtDNA. For example, the *Sry* and *Zfy-2* polymorphisms in this report delineate populations of Y chromosomes that, for the most part, correlate with and expand on data obtained by other investigators using a variety of methods (Marshall 1986; Frisman et al. 1990; Moriwaki et al. 1990; She et al. 1990). Furthermore, the molossinus *Sry* allele has been instrumental in confirming historic records suggesting that Asian mice were used in the generation of the laboratory strains (Nagamine et al. 1992).

Given the close commensal relationship of house mice and humans, mouse *Sry* polymorphisms may prove useful to trace, indirectly, the origins of indigenous peoples. In the case of Japan, it has been suggested that the Japanese islands were initially populated by humans from southeast Asia or southern China and that a second human invasion from central China pushed the original occupants to the extreme northern and southern regions of the Japanese archipelago. The distribution of *M. m. castaneus* and *M. m. musculus* mtDNA support the anthropological data (Yonekawa et al. 1988). The present molossinus *Sry* allele data suggest that the second invasion of humans came from Korea or Manchuria.

Acknowledgments

This work was supported by the National Institutes of Health Grant HD28976 to C.M.N. We thank Drs. Y. Nishioka (McGill University) and M. Mitchell (University of Tennessee, Memphis) for their comments on this manuscript, Dr. H. Yonekawa for sharing mtDNA data, and J. Hannel and H. D. Thomas for their technical assistance.

LITERATURE CITED

ALEXANDER-BRIDGES, M., L. ERCOLANI, X. F. KONG, and N. NASRIN. 1992. Identification of a core motif that is recognized by three members of the HMG class of transcriptional regulators: IRE-ABP, SRY, and TCF-1α. J. Cell. Biochem. **48**:129–135.

AUFFRAY, J.-C., J. T. MARSHALL, L. THALER, and F. BONHOMME. 1990. Focus on the nomenclature of European species of *Mus.* Mouse Genome **88**:7–8.

BERRY, R. J., G. S. TRIGGS, V. BAUCHAU, C. S. JONES, and P. SCRIVEN. 1990. Gene flow and hybridization following introduction of *Mus domesticus* into an established population. Biol. J. Linn. Soc. **41**:279–283.

BIDDLE, F. G., B. A. EALES, and Y. NISHIOKA. 1991. A DNA polymorphism from five inbred strains of the mouse identifies a functional class of *domesticus*-type Y chromosome that produces the same phenotypic distribution of gonadal hermaphrodites. Genome **34**:96–104.

BIDDLE, F. G., and Y. NISHIOKA. 1988. Assays of testis development in the mouse distinguish three classes of *domesticus*-type Y chromosome. Genome **30**:870–878.

BISHOP, C. E., P. BOURSOT, B. BARON, F. BONHOMME, and D. HATAT. 1985. Most classical *Mus musculus domesticus* laboratory mouse strains carry a *Mus musculus musculus* Y chromosome. Nature **315**:70–72.

BONHOMME, F., and J.-L. GUÉNET. 1989. The wild house mouse and its relatives. Pp. 649–662 *in* M. F. LYON and A. G. SEARLE, eds. Genetic variants and strains of the laboratory mouse. Oxford University Press, Oxford.

BONHOMME, F., N. MIYASHITA, P. BOURSOT, J. CATALAN, and K. MORIWAKI. 1989. Genetical variation and polyphyletic origin in Japanese *Mus musculus.* Heredity **63**: 299–308.

BOURSOT, P., F. BONHOMME, J. CATALAN, and K. MORIWAKI. 1989. Variations of a Y chromosome repeated sequence across subspecies of *Mus musculus*. Heredity **63**:289–297.

COWARD, P., K. NAGAI, D. CHEN, H. D. THOMAS, C. M. NAGAMINE, and Y.-F. C. LAU. 1994. Polymorphism of a CAG trinuceotide repeat within *Sry* correlates with B6.Y^{Dom} sex reversal. Nature Genet. **6**:245–250.

EICHER, E. M., and L. L. WASHBURN. 1986. Genetic control of primary sex determination in mice. Annu. Rev. Genet. **20**:327–360.

EICHER, E. M., L. L. WASHBURN, J. B. I. WHITNEY, and K. E. MORROW. 1982. *Mus poschiavinus* Y chromosome in the C57BL/6J murine genome causes sex reversal. Science **217**:535–537.

FRISMAN, L. V., K. V. KOROBITSINA, L. V. YAKIMENKO, F. M. BOKSHTEIN, and A. I. MUNTYANU. 1990. Genetic differentiation of U.S.S.R. house mice: electrophoretic study of proteins. Biol. J. Linn. Soc. **41**:65–72.

GIESE, K., J. PAGEL, and R. GROSSCHEDL. 1994. Distinct DNA-binding properties of the high mobility group domain of murine and human SRY sex-determining factors. Proc. Natl. Acad. Sci. USA **91**:3368–3372.

GRAVES, P. E., and R. P. ERICKSON. 1992. An amino acid change in the DNA-binding region of *Sry*, found in *Mus musculus domesticus* and other species, does not explain C57BL/6J-Y^{Dom} sex reversal. Biochem. Biophys. Res. Commun. **185**:310–316.

GROSSCHEDL, R., K. GIESE, and J. PAGEL. 1994. HMG domain proteins: architectural elements in the assembly of nucleoprotein structures. Trends Genet. **10**:94–100.

GUBBAY, J., J. COLLIGNON, P. KOOPMAN, B. CAPEL, A. ECONOMOU, A. MÜNSTERBERG, N. VIVIAN, P. GOODFELLOW, and R. LOVELL-BADGE. 1990. A gene mapping to the sex-determining region of the mouse Y chromosome is a member of a novel family of embryonically expressed genes. Nature **346**:245–250.

GUBBAY, J., N. VIVIAN, A. ECONOMOU, D. JACKSON, P. GOODFELLOW, and R. LOVELL-BADGE. 1992. Inverted repeat structure of the *Sry* locus in mice. Proc. Natl. Acad. Sci. USA **89**:7953–7957.

GYLLENSTEN U., and A. C. WILSON. 1987. Interspecific mitochondrial DNA transfer and the colonization of Scandinavia by mice. Genet. Res. **49**:25–29.

HAQQ, C. M., C.-Y. KING, P. K. DONAHOE, and M. A. WEISS. 1993. *SRY* recognizes conserved DNA sites in sex-specific promoters. Proc. Natl. Acad. Sci. USA **90**:1097–1101.

HARLEY, V. R., D. I. JACKSON, P. J. HEXTALL, J. R. HAWKINS, G. D. BERKOVITZ, S. SOCKANATHAN, R. LOVELL-BADGE, and P. N. GOODFELLOW. 1992. DNA binding activity of recombinant SRY from normal males and XY females. Science **255**:453–456.

KOOPMAN, P., J. GUBBAY, N. VIVIAN, P. GOODFELLOW, and R. LOVELL-BADGE. 1991. Male development of chromosomally female mice transgenic for *Sry*. Nature **351**:117–121.

MARDON, G., R. MOSHER, C. M. DISTECHE, Y. NISHIOKA, A. MCLAREN, and D. C. PAGE. 1989. Duplication, deletion, and polymorphism in the sex-determining region of the mouse Y chromosome. Science **243**:78–80.

MARSHALL, J. T. 1986. Systematics of the genus *Mus*. Pp. 12–18 *in* M. POTTER, J. H. NADEAU, and M. P. CANCRO, eds. The wild mouse in immunology. Springer, New York.

MORIWAKI, K., N. MIYASHITA, H. SUZUKI, Y. KURIHARA, and H. YONEKAWA. 1986. Genetic features of major geographical isolates of *Mus musculus*. Pp. 55–61 *in* M. POTTER, J. H. NADEAU, and M. P. CANCRO, eds. The wild mouse in immunology. Springer, New York.

MORIWAKI, K., T. SAGAI, T. SHIROISHI, F. BONHOMME, W. CHENGHUAI, H. XINQUAO, J. MEILEI, and W. ZHENGAN. 1990. Mouse subspecies differentiation and H-2 polymorphism. Biol. J. Linn. Soc. **41**:125–139.

NAGAMINE, C. M. 1993. XY sex reversal in the mouse. Pp. 83–105 *in* S. WACHTEL, ed. Molecular genetics of sex determination. Academic Press, San Diego.

NAGAMINE, C. M., K. CHAN, L. E. HAKE, and Y.-F. C. LAU. 1990. The two candidate testis-determining Y genes (*Zfy-1* and *Zfy-2*) are differentially expressed in fetal and adult mouse tissues. Genes Dev. **4**:63–74.

NAGAMINE, C. M., K. CHAN, C. A. KOZAK, and Y.-F. LAU. 1989*a*. Chromosome mapping and expression of a putative testis-determining gene in mouse. Science **243**:80–83.

NAGAMINE, C. M., K. CHAN, and Y.-F. C. LAU. 1989*b*. A PCR artifact: generation of heteroduplexes. Am. J. Hum. Genet. **45**:337–339.

NAGAMINE, C. M., Y. NISHIOKA, K. MORIWAKI, P. BOURSOT, F. BONHOMME, and Y.-F. C. LAU. 1992. The musculus-type Y chromosome of the laboratory mouse is of Asian origin. Mamm. Genome **3**:84–91.

NAGAMINE, C. M., T. TAKETO, and G. C. KOO. 1987*a*. Morphological development of the mouse gonad in *tda-1* XY sex reversal. Differentiation **33**:214–222.

———. 1987*b*. Studies on the genetics of *tda-1* XY sex reversal in the mouse. Differentiation **33**:223–231.

NISHIOKA, Y., and E. LAMOTHE. 1987. The *Mus musculus musculus* type Y chromosome predominates in Asian house mice. Genet. Res. **50**:195–198.

PALMER, S. J., and P. S. BURGOYNE. 1991. The *Mus musculus domesticus Tdy* allele acts later than the *Mus musculus musculus Tdy* allele: a basis for XY sex-reversal in C57BL/6-YPos mice. Development **113**:709–714.

SAGE, R. D., W. R. ATCHLEY, and E. CAPANNA. 1993. House mice as models in systematic biology. Syst. Biol. **42**:523–561.

SHE, J. X., F. BONHOMME, P. BOURSOT, L. THALER, and F. CATZEFLIS. 1990. Molecular phylogenies in the genus *Mus*: comparative analysis of electrophoretic, scnDNA hybridization, and mtDNA RFLP data. Biol. J. Linn. Soc. **41**:83–103.

SINCLAIR, A. H., P. BERTA, M. S. PALMER, J. R. HAWKINS, B. L. GRIFFITHS, M. J. SMITH, J. W. FOSTER, A.-M. FRISCHAUF, R. LOVELL-BADGE, and P. N. GOODFELLOW. 1990. A gene from the human sex-determining region encodes a protein with homology to a conserved DNA-binding motif. Nature **346**:240–244.

TUCKER, P. K., B. K. LEE, and E. M. EICHER. 1989. Y chromosome evolution in the subgenus *Mus* (Genus *Mus*). Genetics **122**:169–179.

TUCKER, P. K., B. K. LEE, B. L. LUNDRIGAN, and E. M. EICHER. 1992*a*. Geographic origin of the Y chromosomes in "old" inbred strains of mice. Mamm. Genome **3**:254–261.

TUCKER, P. K., and B. L. LUNDRIGAN. 1993. Rapid evolution of the sex determining locus in Old World mice and rats. Nature **364**:715–717.

TUCKER, P. K., R. D. SAGE, J. WARNER, A. WILSON, and E. M. EICHER. 1992*b*. Abrupt cline for sex chromosomes in a hybrid zone between two species of mice. Evolution **46**:1146–1163.

VANLERBERGHE, F., B. DOD, P. BOURSOT, M. BELLIS, and F. BONHOMME. 1986. Absence of Y-chromosome introgression across the hybrid zone between *Mus musculus domesticus* and *Mus musculus musculus*. Genet. Res. **48**:191–197.

WHITFIELD, L. S., R. LOVELL-BADGE, and P. N. GOODFELLOW. 1993. Rapid sequence evolution of the mammalian sex-determining gene SRY. Nature **364**:713–715.

YONEKAWA, H., K. MORIWAKI, O. GOTOH, N. MIYASHITA, Y. MATSUSHIMA, L. SHI, W. SU CHO, X.-L. ZHEN, and Y. TAGASHIRA. 1988. Hybrid origin of Japanese mice "*Mus musculus molossinus*": evidence from restriction analysis of mitochondrial DNA. Mol. Biol. Evol. **5**:63–78.

FREDERICK ALLENDORF, reviewing editor

Received March 2, 1994

Accepted July 12, 1994