Establishment and Characterization of the MSKR Inbred Strain Originated from Japanese Wild Mice (*Mus musculus molossinus*)

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ABSTRACT. A new inbred strain, MSKR, originated from Japanese wild mice was established in April, 1998. The MSKR mice were 60% of the C57BL/6N inbred mice in the 60-day body weight. Tail length/head-body length and hind-foot length/head-body length of the MSKR mice were significantly smaller than those of the C57BL/6N mice (0.896 vs 1.061, 0.189 vs 0.204), but ear length/head-body length of the MSKR mice was significantly larger than that of the C57BL/6N mice (0.143 vs 0.137). The age of the first parturition and size of the first litter were 63.20 ± 2.71 days and 6.20 ± 0.37 , respectively, at the 20th and 22nd inbreeding generations. Genetic characterization of the MSKR strain was performed using 34 microsatellite markers, 29 biochemical markers, 9 immunogenetic markers, 3 coat color markers, and mitochondrial DNA RFLP-haplotypes. The result indicated that this newly established inbred strain has some different gene constitution from already known *molossinus* and common laboratory strains.—KEY WORDS: inbred strain, mouse, *Mus musculus molossinus*.

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Many investigators use a numerous number of mice as a material for biomedical research. However, many strains of common laboratory strains have similar genetic constitution, because almost all of them originated from a few ancestral stocks kept by Miss Lathrop in Granby [18]. The limited gene variation is undesirable for a laboratory animal that will be used in various research fields. One of the method to solve this problem is to introduce new genes from a wild genetic pool to a laboratory genetic pool.

In Japan, wild mice that are classified as *Mus musculus molossinus* are distributed. The *molossinus* mouse has been originated from a natural intersubspecific hybrid between *M. m. castaneus* inhabiting southwest Asia and *M. m. musculus* distributed in north Asia [36]. In the 1960s, Kondo and Moriwaki proposed that this subspecies is an excellent material to improve laboratory mice [15, 17], because this subspecies was suspected to be greatly different in gene constitution from common laboratory mice derived from *domesticus* subspecies [31, 32, 35]. During 1973–1993, several inbred strains were established from wild *molossinus* mice [1, 20, 21]. Genetic studies with these inbred strains and captive mice have confirmed that the genome of the Japanese wild mice differs from common laboratory mice, and many biochemical, mitochondrial DNA (mt DNA), and nucleic DNA polymorphisms have been found in *molossinus* mice [10, 30, 36]. However, none of the *molossinus* mice used in these studies originated in Kinki-area. Thus, establishment of new strains based on the *molossinus* mice from Kinki-area was desired to supply unique materials to research fields of life sciences.

Recently, we have established a new inbred strain MSKR based on *molossinus* mice captured in Kinki-area. This

strain is externally characterized by a new mutant coat color named “tawny” controlled by a mutant gene *Mc1r*^{taw} [29]. This paper describes the process of establishing the MSKR strain and the basic characteristics in body size, reproductive ability and genetic profiles.

MATERIALS AND METHODS

Mice: To capture original wild mice, Sherman Live Traps (160 × 50 × 65 mm, SFA, H.B. Sherman Traps, Inc., Florida, U.S.A.) were set within and around human habitation at Sakai-city, Osaka-Prefecture, Japan during January 1991 to December 1992. Captured animals were steeped into a medicated bath with Niguvon (Bayer Yakuhin, Ltd., Osaka, Japan) to remove external parasites.

Captive mice and their descendants were paired in a polycarbonate cage (227 × 329 × 125 mm, TM-PC-10-IV, Tokiwa Kagaku Kikai Co., Ltd., Tokyo, Japan) with a wire netting lid that was bedded with clean wood chips (CLEA Japan, Inc., Osaka, Japan). In a cage, a paper box (100 × 100 × 50 mm) was placed as a shelter. The cages were set in a temperature- and light-controlled room (21 ± 3°C, 14/10-hr light/dark cycle), and tap water and commercial diet (CE-2, CLEA Japan, Inc.) were supplied to the animals for *ad libitum* consumption. Pups were weaned at 25 days after birth and paired with their sisters or brothers according to the method of Kondo [15].

Characteristics of the MSKR mice were compared with those of the MOM, MSM, NC/Nga, C57BL/6N and DBA/2 mice. MOM and NC/Nga were introduced from the Laboratory of Animal Genetics, Nagoya University (Chikusa, Nagoya, Japan). C57BL/6N and DBA/2 were

introduced from the Central Institute for Experimental Animals (Kawasaki, Kanagawa, Japan). MSM was obtained from National Institute of Genetics (Mishima, Shizuoka, Japan). The MOM and MSM are inbred strains originated from *molossinus* mice captured at Nagoya-city and Mishima-city, respectively.

Body size: Measurement was made based on the methods of Murakami [19] with the mice sufficiently anesthetized with ether. Body weight, head and body length (length from the tip of the nose to the anus), tail length (length from the anus to the tip of the tail excluding hairs), hind foot length (the length from the heel of the hind foot to the tip of the stretched finger without the claw), and ear length (the length from the lowest point of the basal notch to the extremity exclusive of hairs) were measured.

Reproductive ability: With females at the 0 to 19th generations, the age of the first parturition, intervals between each parturition, and the number of pups weaned were examined. The data were combined every 5 generations. The same investigations as the mentioned were carried out for a wild captive female and females at the 20th and 22nd generations.

Genetic profiles:

Detection of biochemical markers Plasma, erythrocyte, urine, kidney, liver, salivary gland, and pancreas were used as samples. Blood was collected from the retro-orbital plexus by heparinized capillary tubes and divided into plasma and erythrocytes by centrifugation (3,000 rpm for 15 min). The erythrocytes were washed three times in saline and lysed in three volumes of distilled water. The tissue samples were homogenized in five volumes of distilled water, and the supernatant was obtained by centrifugation (15,000 rpm for 30 min). The samples were stored at -80°C until use. The urine samples were collected immediately before electrophoresis and diluted in five volumes of distilled water. The methods and samples used in electrophoresis to detect each biochemical marker are summarized in Table 1.

Detection of immunogenetic markers The microcytotoxicity test using flat-type titration plates [26] was used to type 7 immunogenetic markers, i. e., complement component 3 (C3), CD5 antigen (Cd5), CD8 alpha chain (Cd8a), CD8 beta chain (Cd8b), histocompatibility 2 D (H2-D), histocompatibility 2 K (H2-K) and thymus antigen 1 (Thy1). Types of hemolytic complement (Hc) and immunoglobulin heavy chain constant region (IghC) were determined with agar diffusion methods described by Harada *et al.* [9]. All antisera used here were made in the Central Institute for Experimental Animals (Kawasaki, Kanagawa, Japan).

Detection of mt DNA restriction fragment length polymorphism Mt DNA was prepared from the liver and other soft tissue by an alkaline - SDS digestion simplified procedure [27]. The extracted mitochondrial DNA was digested with endonucleases, *Bam*HI, *Eco*RI, *Hind*II, *Hind*III, *Hpa*I, *Hae*I, *Pst*I, and/or *Bgl*II (Nippon Gene, Tokyo, Japan). The digested DNA was electrophoresed on 1.5% agarose gels and visualized by ethidium bromide staining.

Detection of the genotypes of microsatellite markers

Genotypes of microsatellite markers were determined by a PCR-SSLP (simple sequence length polymorphisms) method. Genomic DNA was prepared from the liver or earlobe by a simplified procedure using proteinase K- SDS digestion [16]. PCR primers were commercially obtained from Research Genetics (Huntsville, Alabama, U.S.A.). PCRs [25] were carried out in a total volume of 10 μl by a TAKARA thermal cycler (TAKARA, Shiga, Japan). The standard 10X PCR buffer (TAKARA, Shiga, Japan) with 1.5 mM MgCl_2 (final concentration) was used in reactions. PCR cycle was as follows: (1) first denature, 94°C for 3 min, (2) denature, 94°C for 30 seconds; annealing, 58°C for 1 min; and extension, 72°C for 1 min, 5 cycles, (3) denature, 94°C for 30 sec; annealing, 55°C for 1 min; and extension, 72°C for 1 min, 35 cycles, (4) final extension, 72°C for 10 min. SSLP phenotypes were visualized by PCR products electrophoresed on a 6% polyacrilamide gel system followed by ethidium bromide staining [24].

Detection of the genotypes of coat color markers To determine the basic coat color marker genes possessed by the MSKR mice, we mated the DBA/2 mice “*aa Tyrp1^bTyrp1^b Myo5a^dMyo5a^d*” (formerly “*aa bb dd*”) to the MSKR mice.

RESULTS

Establishment of a new inbred strain: The original female mouse of the MSKR strain was captured on 26 May 1992 at Chayamadai, Sakai-city, Osaka-prefecture, Japan. A wild-type *molossinus* male was mated to this female. The male mouse was a son of the wild-type parents that were captured in November 1991 at Katakura, Sakai-city. Katakura is near to Chayamadai.

The original female showed a unique coat color on the back. Her back was light yellowish brown, although she had a white belly and black eyes as usually seen in wild-type *molossinus* mice. This trait was called “tawny” based on the dorsal coloration. Fifty-five F_1 offspring from the original pair showed wild-type coloration all over the body. Full-sib matings of these F_1 mice produced tawny colored progeny. Thus, based on these F_2 mutant mice, development of the mutant inbred strain was started. In April 1998, a pair of the mice at the 19th full-sib mating generation produced the 20th generation. At the point in time, the line became an inbred strain, and the strain name MSKR was assigned according to the international nomenclature rules [8]. Here, M and SK mean *molossinus* mice and Sakai-city where the original mice of this strain were captured, respectively. R is the arrangement number of the strain in the first author’s laboratory.

Body size: The body sizes of MSKR and C57BL/6N strain mice at 30 and 60 days old are shown in Tables 2 and 3. In both strains, males were significantly larger than females in both body weight and head-body length at both ages. In both sexes, body weight, head-body length, tail length, and hind-foot length of the C57BL/6N mice were significantly

Table 1. Summary of methods and samples to detect biochemical markers

Locus name	Locus symbol	Chromosome no.	Electrophoresis system	Sample organ	References
Alkaline phosphatase 1	<i>Akp1</i>	1	CA ^{a)}	kidney	[12]
Isocitrate dehydrogenase 1	<i>Idh1</i>		CA	kidney	[12]
Peptidase 3	<i>Pep3</i>		CA	kidney	[12]
Amylase 1	<i>Amy1</i>	3	CA	salivary gland	[11]
Amylase 2	<i>Amy2</i>		CA	pancreas	[33]
Carbonic anhydrase 1	<i>Car1</i>		CA	erythrocyte	[6]
Carbonic anhydrase 2	<i>Car2</i>		CA	erythrocyte	[12]
Alkaline phosphatase 2	<i>Akp2</i>	4	CA	kidney	[34]
Glucose-6-phosphate dehydrogenase 1	<i>G6pd1</i>		CA	kidney	[12]
Major urinary protein 1	<i>Mup1</i>		PAG ^{b)}	urine	[12]
Phosphoglucumutase 2	<i>Pgm2</i>		CA	erythrocyte	[3]
Phosphoglucumutase 1	<i>Pgm1</i>	5	CA	erythrocyte	[12]
Lactate dehydrogenase regulator 1	<i>Ldr1</i>	6	CA	erythrocyte	[12]
Glucose phosphate isomerase 1, structural	<i>Gpi1-s</i>	7	CA	erythrocyte	[12]
Hemoglobin beta chain complex	<i>Hbb</i>		CA	erythrocyte	[12]
Esterase 1	<i>Es1</i>	8	CA	plasma	[12]
Esterase 2	<i>Es2</i>		CA	kidney	[22]
Esterase 5	<i>Es5</i>		CA	plasma	[23]
Glutamate oxaloacetate transaminase 2, mitochondrial	<i>Got2</i>		CA	liver	[4]
Malic enzyme, supernatant	<i>Mod1</i>	9	CA	kidney	[12]
Transferrin	<i>Trf</i>		CA	plasma	[12]
Esterase 3	<i>Es3</i>	11	CA	kidney	[12]
Hemoglobin alpha chain complex	<i>Hba</i>		IE ^{c)}	erythrocyte	[12]
Esterase 10	<i>Es10</i>	14	CA	erythrocyte	[12]
Nucleoside phosphorylase	<i>Np1</i>		CA	erythrocyte	[12]
Glutamic pyruvic transaminase 1, soluble	<i>Gpt1</i>	15	CA	liver	[7]
Glyoxylase 1 complex	<i>Glo1</i>	17	CA	erythrocyte	[12]
Kidney catalase	<i>Ce2</i>		CA	kidney	[22]
Glutamate oxaloacetate transaminase 1, soluble	<i>Got1</i>	19	CA	liver	[2]

a) Cellulose acetate membrane system.

b) 15% polyacrylamide gel system.

c) Isoelectric focusing system.

larger than those of the MSKR mice at both ages. In tail length/head-body length and hind-foot length/head-body length also, the C57BL/6N mice were significantly larger than the MSKR mice in each sex. On the other hand, in ear length/head-body length, the MSKR mice were significantly larger than C57BL/6N mice in each sex.

Reproductive ability: Table 4 shows the age of the first parturition, parturition interval in the 1st to 4th parturitions, and the number of pups weaned. The first parturition was made at 56 to 117 days of age at each generation throughout the course of inbreeding, and there were no significant differences among generations. Parturition interval also was not significantly different in any generations. Furthermore,

the number of pups weaned was basically constant through the inbreeding, with one exception. The average number of pups in the 2nd and subsequent litters was significantly different only between F₀-F₄ (4.88 ± 0.75) and F₁₅-F₁₉ (7.27 ± 0.33) generations.

Genetic profiles:

Biochemical markers Table 5 shows genotypes at 29 biochemical loci in the MSKR strain. The genotypes at 27 biochemical loci, except for *Mup1* and *Pgm1*, were the same as those already reported in other inbred strain mice. The MSKR mice showed new electrophoretic phenotypes of the MUP1 and PGM1, which are shown in Figs. 1 and 2, respectively. MUP1 zymogram of the MSKR mice was

Table 2. Measurements (mean $\pm$ S.E.)¹⁾ of the body in MSKR and C57BL/6N males

	30 days old		60 days old	
	MSKR (10) ²⁾	C57BL/6N (8)	MSKR (8)	C57BL/6N (7)
Body weight (g)	9.75 $\pm$ 0.19 ^a	16.58 $\pm$ 0.34 ^b	14.60 $\pm$ 0.58 ^a	23.50 $\pm$ 0.63 ^b
Head-body length (cm)	6.72 $\pm$ 0.04 ^a	7.71 $\pm$ 0.07 ^b	7.88 $\pm$ 0.08 ^a	8.88 $\pm$ 0.07 ^b
Tail length (cm)	6.25 $\pm$ 0.04 ^a	7.91 $\pm$ 0.10 ^b	7.06 $\pm$ 0.10 ^a	9.42 $\pm$ 0.08 ^b
Hind foot length (cm)	1.42 $\pm$ 0.02 ^a	1.76 $\pm$ 0.02 ^a	1.49 $\pm$ 0.01 ^a	1.81 $\pm$ 0.02 ^a
Ear length (cm)	1.04 $\pm$ 0.02 ^a	1.02 $\pm$ 0.02 ^b	1.13 $\pm$ 0.03 ^a	1.21 $\pm$ 0.04 ^b
Tail length/Head-body length	0.931 $\pm$ 0.006 ^a	1.026 $\pm$ 0.009 ^b	0.896 $\pm$ 0.013 ^a	1.061 $\pm$ 0.012 ^b
Ear length/Head-body length	0.153 $\pm$ 0.004 ^a	0.132 $\pm$ 0.003 ^b	0.143 $\pm$ 0.003 ^a	0.137 $\pm$ 0.004 ^a
Hind foot length/Head-body length	0.212 $\pm$ 0.003 ^a	0.228 $\pm$ 0.002 ^b	0.189 $\pm$ 0.002 ^a	0.204 $\pm$ 0.002 ^b

1) Means having the different superscripts within the line significantly differ at $P < 0.05$.

2) Number of mice measured.

Table 3. Measurements (mean $\pm$ S.E.)¹⁾ of the body in MSKR and C57BL/6N females

	30 days old		60 days old	
	MSKR (6) ²⁾	C57BL/6N (6)	MSKR (7)	C57BL/6N (7)
Body weight (g)	8.88 $\pm$ 0.27 ^a	14.88 $\pm$ 0.50 ^b	12.71 $\pm$ 0.36 ^a	19.37 $\pm$ 0.65 ^b
Head-body length (cm)	6.56 $\pm$ 0.04 ^a	7.43 $\pm$ 0.11 ^b	7.72 $\pm$ 0.09 ^a	8.46 $\pm$ 0.01 ^b
Tail length (cm)	6.25 $\pm$ 0.05 ^a	7.79 $\pm$ 0.06 ^b	7.13 $\pm$ 0.10 ^a	9.14 $\pm$ 0.07 ^b
Hind foot length (cm)	1.43 $\pm$ 0.02 ^a	1.71 $\pm$ 0.01 ^b	1.47 $\pm$ 0.02 ^a	1.74 $\pm$ 0.02 ^b
Ear length (cm)	1.03 $\pm$ 0.02 ^a	1.02 $\pm$ 0.02 ^a	1.13 $\pm$ 0.02 ^a	1.17 $\pm$ 0.03 ^a
Tail length/Head-body length	0.954 $\pm$ 0.009 ^a	1.049 $\pm$ 0.009 ^b	0.924 $\pm$ 0.007 ^a	1.081 $\pm$ 0.010 ^b
Ear length/Head-body length	0.157 $\pm$ 0.003 ^a	0.138 $\pm$ 0.003 ^b	0.147 $\pm$ 0.002 ^a	0.139 $\pm$ 0.004 ^a
Hind foot length/Head-body length	0.217 $\pm$ 0.004 ^a	0.230 $\pm$ 0.003 ^b	0.191 $\pm$ 0.003 ^a	0.206 $\pm$ 0.003 ^b

1) Means having the different superscripts within the line significantly differ at $P < 0.05$.

2) Number of mice measured.

Table 4. Reproductive performance in the MSKR strain during the course of its development

Captured female				F ₀ –F ₄		F ₅ –F ₉	
		No. of mice observed	Mean $\pm$ S.E. ¹⁾	No. of females observed	Mean $\pm$ S.E.	No. of females observed	Mean $\pm$ S.E.
Age of the first parturition	–	–	–	5	64.80 $\pm$ 3.81 ^a	5	68.60 $\pm$ 2.66 ^a
Intervals between litters							
1–2	1	44	–	5	32.20 $\pm$ 4.69 ^a	5	25.00 $\pm$ 0.87 ^a
2–3	1	25	–	4	25.25 $\pm$ 1.11 ^a	3	25.00 $\pm$ 1.16 ^a
3–4	1	41	–	4	32.50 $\pm$ 5.55 ^a	3	25.67 $\pm$ 0.88 ^a
No. pups weaned							
first litter	–	–	–	5	4.60 $\pm$ 0.93 ^a	5	6.80 $\pm$ 0.21 ^a
Second and subsequent litters	1 (5) ²⁾	5.40 $\pm$ 2.61 ^{ab}		5 (16)	4.88 $\pm$ 0.75 ^a	5 (12)	5.92 $\pm$ 0.78 ^{ab}

F ₁₀ –F ₁₄				F ₁₅ –F ₁₉		F ₂₀ –F ₂₂	
		No. of females observed	Mean $\pm$ S.E. ¹⁾	No. of females observed	Mean $\pm$ S.E.	No. of females observed	Mean $\pm$ S.E.
Age of the first parturition	5	77.60 $\pm$ 10.45 ^a		5	65.60 $\pm$ 1.44 ^a	5	63.20 $\pm$ 2.71 ^a
Intervals between litters							
1–2	5	28.40 $\pm$ 2.46 ^a		5	29.20 $\pm$ 3.02 ^a	5	28.40 $\pm$ 3.19 ^a
2–3	5	30.20 $\pm$ 3.10 ^a		5	27.40 $\pm$ 2.54 ^a	3	29.00 $\pm$ 3.51 ^a
3–4	5	30.20 $\pm$ 4.93 ^a		3	29.33 $\pm$ 2.40 ^a	1	28.00 $\pm$ –
No. pups weaned							
first litter	5	6.20 $\pm$ 0.40 ^a		5	6.60 $\pm$ 0.40 ^a	5	6.20 $\pm$ 0.37 ^a
Second and subsequent litters	5 (19) ²⁾	6.21 $\pm$ 0.43 ^{ab}		5 (15)	7.27 $\pm$ 0.33 ^b	5 (9)	7.00 $\pm$ 0.69 ^{ab}

1) Means without the same superscripts within the line significantly differ at $P < 0.05$.

2) No. of litters.

Table 5. Genotypes at biochemical loci of the MSKR strain

Chromosome No.	1			3				4				5	6	7	
Locus symbol	<i>Akp1</i>	<i>Idh1</i>	<i>Pep3</i>	<i>Amy1</i>	<i>Amy2</i>	<i>Car1</i>	<i>Car2</i>	<i>Akp2</i>	<i>G6pd1</i>	<i>Mup1</i>	<i>Pgm2</i>	<i>Pgm1</i>	<i>Ldr1</i>	<i>Gpi1-s</i>	<i>Hbb</i>
Genotype	<i>b</i>	<i>b</i>	<i>b</i>	<i>b</i>	<i>b</i>	<i>b</i>	<i>a</i>	<i>b</i>	<i>c</i>	*	<i>a</i>	**	<i>a</i>	<i>a</i>	<i>d</i>

Chromosome No.	8				9		11		14		15	17		19
Locus name	<i>Es1</i>	<i>Es2</i>	<i>Es5</i>	<i>Got2</i>	<i>Mod1</i>	<i>Trf</i>	<i>Es3</i>	<i>Hba</i>	<i>Es10</i>	<i>Np1</i>	<i>Gpt1</i>	<i>Ce2</i>	<i>Glo1</i>	<i>Got1</i>
Genotype	<i>a</i>	<i>c</i>	<i>a</i>	<i>b</i>	<i>a</i>	<i>b</i>	<i>b</i>	<i>g</i>	<i>c</i>	<i>b</i>	<i>c</i>	<i>b</i>	<i>a</i>	<i>a</i>

* Different from *a*- or *b*-type electrophoretic pattern shown in Fig. 1.** Different from *a*-, *b*-, *c*-, and *d*-type electrophoretic patterns shown in Fig. 2.

Table 6. Genotypes at immunogenetic loci of the MSKR strain

Chromosome No.	3	6		9	12	17			19
Locus symbol	<i>Hc</i>	<i>Cd8a</i>	<i>Cd8b</i>	<i>Thy1</i>	<i>IghC</i>	<i>C3</i>	<i>H2D</i>	<i>H2K</i>	<i>Cd5</i>
Genotype	<i>l</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>b</i>	<i>c</i>	***	***	<i>b</i>

*** Not reacted to *b*, *d*, *k*, *q* and *s* haplotype antisera.

Table 7. Mitochondrial DNA RFLP haplotypes in the MSKR strain

Restriction enzyme		<i>Bam</i> H I	<i>Eco</i> R I	<i>Hind</i> II	<i>Hind</i> III	<i>Hpa</i> I	<i>Hae</i> II	<i>Pst</i> I	<i>Bgl</i> I
Cleavage pattern	MSKR	D	B	A	B	A	C	B	A
	MOM ^{a)}	D	B	A	B	A	C	B	A
	C57BL/6N ^{a)}	A	A	A	A	A	A	A	A

a) From Yonekawa [35].

different from already known types controlled by *Mup1^a* and *Mup1^b*. PGM1 zymogram showed a faster band than the already known fast-type band controlled by the *Pgm1^a* allele.

Immunogenetic markers Table 6 shows genotypes at immunogenetic loci in the MSKR strain. The genotypes at the *Hc*, *Cd8a*, *Cd8b*, *Thy1*, *IghC*, *C3*, and *Cd5* were the same as those reported in other inbred strain mice. Whereas, all kinds of antisera for *H2-D^b*, *H2-D^d*, *H2-D^k*, *H2-D^q*, and *H2-D^s*, and *H2-K^b*, *H2-K^d*, *H2-K^k*, *H2-K^q*, and *H2-K^s* did not react with lymphocytes of the MSKR mice.

Mt DNA RFLP-haplotype Table 7 shows RFLP-haplotypes of mt DNA extracted from the MSKR mice. Cleavage pattern of the MSKR was the same as that of the MOM which was derived from *M. m. molossinus*, but different from that of the C57BL/6N.

Microsatellite markers Table 8 shows size polymorphisms of 34 microsatellite markers detected by the PCR-SSLP method among 5 inbred strains, C57BL/6N, NC/Nga, MOM, MSM and MSKR. The C57BL/6N and MSM strains were used as representation of common laboratory strains (*Mus musculus domesticus*) and *molossinus* inbred strains, respectively. The NC/Nga and MOM strains were investigated as strains which are somewhat genetically distant from C57BL/6N, and MSM strains, respectively [8, 14]. Figure 3 shows inter-strain polymorphism frequency based on a total of 34 microsatellite markers. Polymorphism frequency was 41.2% and 44.1% between the MSKR to

MSM and MSKR to MOM, respectively, similar to the value obtained between the C57BL/6N and NC/Nga (38.2%). Polymorphism frequency was over 80% between laboratory strains and *molossinus* strains.

Coat color markers The F₁ progeny between the DBA/2 and MSKR mice showed a coat color of white bellied agouti. This result apparently indicated that the MSKR mice have a genotype of “A^wA^w Tyrp1Tyrp1 Myo5aMyo5a” (formerly “A^wA^w BB DD”) for 3 coat color genes.

DISCUSSION

Body size: Body weight, head and body length tail length/head-body length, hind-foot length/head-body length, and ear length/head-body length of the MSKR (Tables 2 and 3) correspond with the report of Tsuchiya *et al.* [28], in which the body shape of *molossinus* mice is described. Thus, the MSKR strain mice are thought to have typical body shape of *M. m. molossinus*.

Reproductive ability: The reproductive ability of the MSKR strain was basically constant through the inbreeding process. In other words, the increased inbreeding did not decrease the reproductive ability. This seems to be due to the precise selection for sires and dams with high ability at each generation. Generally, inbred strains from *molossinus* subspecies have lower reproductive ability than common laboratory strains. For example, in the females of the MOA strain, which is also derived from *molossinus* subspecies,

Table 8. Polymorphisms of microsatellite markers by SSLP analysis

Marker	Strain polymorphisms ^{a)}	Marker	Strain polymorphisms
<i>D1Mit9</i>	MOM=MSM=R>B6=NC ^{b)}	<i>D8Mit13</i>	B6=NC>MOM=MSM>R
<i>D1Mit14</i>	NC>B6>MOM=MSM=R	<i>D9Mit6</i>	B6>NC>MSM=R>MOM
<i>D2Mit7</i>	NC=MOM=MSM=R-B6	<i>D9Mit16</i>	B6=NC>MSM=R>MOM
<i>D2Mit25</i>	B6=NC>MOM=MSM=R	<i>D10Mit15</i>	MOM>>R>>MSM>B6-NC
<i>D2Nds2</i>	MOM=MSM=R>>B6=NC	<i>D11Mit10</i>	B6=NC>MSM=R>MOM
<i>D3Mit11</i>	B6=NC>MOM=MSM=R	<i>D11Mit15</i>	MOM=MSM=R>B6=NC
<i>D3Mit17</i>	R>NC-MOM=MSM>B6	<i>D12Mit8</i>	MSM>B6=MOM=R>NC
<i>D4Mit16</i>	B6>NC=MOM=MSM=R	<i>D13Mit9</i>	B6=NC>MOM=MSM=R
<i>D4Mit17</i>	MOM=MSM=R-B6=NC	<i>D13Mit14</i>	B6=NC>MSM>MOM-R
<i>D5Mit51</i>	B6=NC>MOM=R-MSM	<i>D14Mit28</i>	MOM-MSM=R>NC-B6
<i>D6Nds2</i>	B6=NC>MOM=MSM=R	<i>D14Mit102</i>	MOM=MSM=R>B6>NC
<i>D6Mit13</i>	B6=NC=MSM-MOM-R	<i>D15Mit15</i>	B6=NC-R-MOM=MSM
<i>D6Mit16</i>	R>MOM=MSM>B6=NC	<i>D16Mit4</i>	NC-MSM=R-B6>MOM
<i>D7Mit9</i>	B6=NC-MOM=MSM-R	<i>D17Mit20</i>	B6=NC=MOM>MSM=R
<i>D7Mit18</i>	MOM=MSM=R>B6=NC	<i>D18Mit9</i>	MOM=MSM-B6=NC-R
<i>D7Nds6</i>	B6>NC-MSM>>MOM	<i>D19Mit11</i>	B6>NC=MOM=MSM=R
<i>D8Mit3</i>	MOM=MSM=R>B6-NC	<i>DXMit3</i>	MSM>B6=NC-MOM=R

a) Relative size of alleles: =, There is no difference between the two. -, There is a small size variation between the two. >, The left side is larger than the right. >>, The left side is larger than the right, and the size difference is extremely large.

b) B6=C57BL/6N, R=MSKR.

Fig. 1. The zymogram of major urinary protein (*Mup1*) with a polyacrilamide gel electrophoretic system. Lane 1: B type (*Mup1^b/Mup1^b*), from a C57BL/6N female, Lane 2: A type (*Mup1^a/Mup1^a*), from a DBA/2 female, Lane 3: unknown type, from a MOM female, Lane 4: unknown type, from a MSKR female, Lane 5: B type (*Mup1^b/Mup1^b*), from a C57BL/6N male, Lane 6: A type (*Mup1^a/Mup1^a*), from a DBA/2 male, Lane 7: unknown type, from a MOM male, Lane 8: unknown type, from a MSKR male. MOM and MSKR mice show different bands from both A and B types.

Fig. 2. The zymogram of phosphoglucosylase 1 (*Pgm1*) with a cellulose acetate electrophoretic system. Lanes 1: A type (*Pgm1^a/Pgm1^a*), from C57BL/6N mice, Lanes 2: unknown type, from MSKR mice, Lanes 3: B type (*Pgm1^b/Pgm1^b*), from DBA/2 mice, Lanes 4: B type (*Pgm1^b/Pgm1^b*), from MOM mice. *Pgm1* zymogram of the MSKR shows a faster band than A type which has so far been known as a fast type.

the age of the first parturition and litter size are 173.8 days and 4.9 pups, respectively [20]. Also, MUA and MUG strains show mean litter size 5.0 and 4.9, respectively [21]. When compared with other inbred strains of *molossinus* subspecies, the MSKR strain has excellent reproductive ability. The MSKR females are delivered of their first litter at 63.2 days of age with litter size of 6.2.

Genetic profiles: In this study, we investigated 29 biochemical and 9 immunogenetic polymorphic loci. Almost all loci showed already reported alleles or variants [5], except for *Mup1* and *Pgm1* (Figs. 1 and 2). With regard to *Mup1*, the MSKR showed a different electrophoretic band from known *a* and *b* types (Fig. 1). A similar phenotype has also been found in *molossinus*, *bactrianus*, and untaxonomical Asian mice by Kato [13]. Accordingly, the

new variant found in the MSKR strain is thought to be wide distributed in Asian mice.

At the *Pgm1* locus, there have been reported 4 variants, *a*, *b*, *c*, and *d* [5]. In the present study, the MSKR mice showed a different variant from all of *a*, *b*, *c*, and *d* types. Kato [13] also reported a different variant from all of the *a*, *b*, *c*, and *d*. The two new variants found by us and Kato [13] are thought to be different each other based on the following reason. Our variant showed a faster electrophoretic band than that of type *a* (Fig. 2), whereas the variant of Kato exhibited a slower band than that of type *b*.

In the present study, the MSKR showed the same mtDNA RFLP haplotype as the MOM strain of *molossinus* mice (Table 8). It has been revealed that the mtDNA RFLP

P/T	%	MSKR	MOM	MSM	C57BL/6	NC
MSKR			44.1	41.2	97.1	88.2
MOM	15/34			38.2	94.1	85.3
MSM	14/34	13/34			97.1	85.3
C57BL/6	33/34	32/34	33/34			38.2
NC	30/34	29/34	29/34	13/34		

Fig. 3. A matrix diagram showing degree of polymorphisms for microsatellite markers between inbred strains. The left lower half of the matrix is indicated by P/T, in which P=number of microsatellite markers showing polymorphisms between the two inbred strains, and T=total number of microsatellite markers examined. The right upper half represents the calculated frequency of polymorphisms.

haplotype of the MOM strain is the same as those of the MSM, MOA, and MOT strains, all of which are also based on *molossinus* subspecies [35]. Namely, it is concluded that the MSKR strain has the typical mtDNA RFLP haplotype of *M. m. molossinus*.

Over 80% of microsatellite DNA markers showed polymorphisms between MSKR and C57BL/6N mice and between MSKR and NC/Nga mice. Both C57BL/6N and NC/Nga are the strains which were derived from *M. m. domesticus*. Thus, the MSKR will be a useful mating partner with common laboratory strains in genetic linkage analysis. We have reported here only essential characteristics of the MSKR strain. Further studies from different points of view will discover new features of the MSKR strain in the future.

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