Caryologia
International Journal of Cytology, Cytosystematics and Cytogenetics

Firenze University Press
www.fupress.com/caryologia

OPEN ACCESS

Citation: H. Myoshu, M.A. Iwasa (2019) Differences in C-band patterns between the Japanese house mice (*Mus musculus*) in Hokkaido and eastern Honshu. *Caryologia* 72(2): 81-90. doi: 10.13128/cayologia-237

Published: December 5, 2019

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Differences in C-band patterns between the Japanese house mice (*Mus musculus*) in Hokkaido and eastern Honshu

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Abstract. We characterized and categorized the C-band patterns of the house mouse *Mus musculus* from four areas in Hokkaido and Honshu of the Japanese Islands as a biparental marker. The C-band patterns are categorized as polymorphic, monomorphic, or intermediate, corresponding to those of the Korean mice, C57BL/6, and F1 hybrid mice bred from the Japanese mice and a laboratory mouse, respectively. The C-band patterns mainly differ between mice from Hokkaido and Honshu. The polymorphic patterns are shown in mice in Honshu, while the intermediate patterns are shown in mice in Hokkaido, with an exceptional case of a monomorphic pattern found in one locality. In the other localities of Hokkaido and northeastern Honshu, the C-band patterns are not congruent with an estimation by maternal element in our previous study, whereas the congruence is observed in other localities. It is suggested that the characteristics of the Japanese house mice have been formed through complicated processes by different expansions between biparental and maternal elements.

Keywords. C-band pattern, *Mus musculus*, the Japanese house mouse, hybridization, replacement.

INTRODUCTION

The Japanese house mouse, *Mus musculus* (Mammalia, Rodentia), has been colonized in the Japanese Islands through artificially complicated processes by overseas mice based on the genetic and morphological analyses (Myoshu and Iwasa 2018). According to previous studies of intraspecific variations of mitochondrial DNA (mtDNA) haplotypes, the Japanese house mice have been derived from two lineages as the MUS and CAS types, corresponding to the subspecific *musculus* and *castaneus*, respectively, that currently occur in the Korean Peninsula and southern China (MUS-1c and CAS-1a groups in Suzuki et al. 2013). In addition, the colonization history of the Japanese house mice is estimated as following scenario: mice migrated primarily from southern China or southeastern Asia; secondarily, mice migrated from the Korean Peninsula and replaced the distributions of the former mice, considering the distribution patterns of mtDNA haplotypes

(Yonekawa et al. 1988; Terashima et al. 2006; Nunome et al. 2010, 2013; Suzuki et al. 2013; Kuwayama et al. 2017; Myoshu and Iwasa 2018). Nuclear genome analysis has shown evidence of the introgression and replacement (Kuwayama et al. 2017). Additionally, many studies suggest recent migrations by stowaway introduction in several areas, including non-port areas (Miyashita et al. 1985; Yonekawa et al. 2000; Tsuda et al. 2001, 2002; Terashima et al. 2006; Nunome et al. 2010; Kodama et al. 2015; Kuwayama et al. 2017; Myoshu and Iwasa 2018).

On the other hand, the distribution of nuclear DNA types is not always congruent with that of mtDNA haplotypes in the Japanese house mice. The sequence of the *musculus* lineage is observed in all targeted regions of the nuclear genome, or relatively short segments of the *castaneus* lineage are observed in some targeted regions, although its samples were obtained from a locality where the CAS type is exclusively observed (Kuwayama et al. 2017). In addition, our previous study (Myoshu and Iwasa 2018) shows that observed external characteristics sometimes do not coincide with the subspecific characteristics estimated by the mitochondrial haplotypes. Thus, these incongruences between nuclear traits and mtDNA traits suggest complicated hybridization and/or replacement process, regarding different progress between biparental and maternal elements. To elucidate the process of replacement by the Korean mice, it is necessary to comprehensively investigate the biparental element by a marker distinguishing the Korean mice.

Cytogenetically, variations in the C-band patterns have been well studied in wild house mice (Dev et al. 1973, 1975; Miller et al. 1976; Moriwaki and Minezawa 1976; Ikeuchi 1978; Moriwaki et al. 1985, 1986; Moriwaki 2010; Yonekawa et al. 2012; Myoshu and Iwasa 2016). By evaluating the C-band patterns, we can confirm whether the Japanese house mice have experienced hybridization and/or replacement with the mice that introduced from northern China and the Korean Peninsula, or not. According to these previous studies, the C-banding patterns of house mice can be roughly categorized into two patterns. The European, central and southern Asiatic, and laboratory mice show a monomorphism of C-band sizes in a homologue; almost all chromosomes carry smaller centromeric C-bands (hereinafter called a "monomorphic pattern"). On the other hand, northern Chinese and/or Korean mice show a polymorphism of C-band sizes in a homologue; a few chromosomes carry larger centromeric C-bands, and most of the residual chromosomes carry no C-band (hereinafter called a "polymorphic pattern"). The C-banding patterns of the Japanese house mice are visually categorized as the latter (Dev et al. 1973, 1975; Moriwaki and Minezawa 1976; Ikeuchi 1978; Moriwaki et al. 1985, 1986, 2009; Moriwaki 2010; Yonekawa et al. 2012), polymorphic states of C-bands (Myoshu and Iwasa 2016). The mice in southern China and/or southeastern Asia, which primarily migrated to the Japanese Islands (Suzuki et al. 2013; Kuwayama et al. 2017), shows the former type of C-band pattern (Moriwaki et al. 1986; Yonekawa et al. 2012). In addition, the C-band patterns of F1 offspring reveal the inheritance states of the C-band size, because the C-band size does not vary over a generation (Dev et al. 1973, 1975; Miller et al. 1976). Thus, the C-band pattern is a useful marker to comprehensively investigate the biparental element.

In this study, we statistically characterized the C-band patterns of house mice from four areas in Hokkaido and Honshu of the Japanese Islands that we previously analysed for mtDNA haplotypes and morphological characteristics (Myoshu and Iwasa 2018). According to results of previous mtDNA analysis and the present analysis, we elucidated the migration, hybridization, and replacement processes of maternal and biparental elements in the four areas.

MATERIALS AND METHODS

Mouse samples

Wild-caught mice of the Japanese Islands (*Mus musculus*) were collected (n = 31; Table 1 and Figure 1) in the Sorachi and Iburi areas of Hokkaido (n = 3; HKD1, including BBI and HYK; Table 1, 1 and 2 in Figure 1(a)), the Hidaka area of Hokkaido (n = 8; HKD2, including MID, KB1, KB2, and NK2; Table 1, 3 to 6 in Figure 1(a)), Iwate and Miyagi Prefectures in Honshu (n = 3; HON1, including SYG, TNS, and FTK; Table 1, 7 to 9 in Figure 1(b)) and Kanagawa and Chiba Prefectures in Honshu (n = 11; HON2, including KZK, KMN, OHB, and CGS; Table 1, 10 to 12 in Figure 1(c)) using Sherman traps baited with oatmeal. We used the same division names and abbreviations of areas and localities in this study as in Myoshu and Iwasa (2018). In addition, a wild-caught mouse (*M. musculus*) collected in Seongmodo Island, neighboring the Korean Peninsula, was used (n = 1; SMD in Table 1; 14 in Figure 1(d)). A laboratory mouse (C57BL/6, Japan SLC Inc.) was also used for the analysis as a standard. Moreover, hybrid mice from a cross experiment using a female wild-caught mouse from Kanagawa Prefecture (specimen nos.: MAI-1239, 1306 and 1308) and a male C57BL/6N were analyzed (n = 3; Table 1) to confirm intermediate C-banding patterns from their parents.

Table 1. House mouse samples examined in this study.

Collecting locality (code*)	Specimen No. (sex)
Wild caught mice	
Sorachi and Iburi areas, Hokkaido, Japan (HKD1)	
Koshunai-cho, Bibai, Hokkaido (BBI, 1)	MAI-1919 (f)
Hayakita-tomioka, Abira-cho, Yufursu-gun, Hokkaido (HYK, 2)	MAI-1895 (f), 1915 (m)
Hidaka area, Hokkaido Japan (HKD2)	
Midorimachi, Hidaka-cho, Saru-gun, Hokkaido (MID,3)	MAI-1837 (m), 1840 (f)
Kabari, Hidaka-cho, Saru-gun, Hokkaido (KB1, 4)	MAI-1916 (m), 1917 (m)
Kabari, Hidaka-cho, Saru-gun, Hokkaido (KB2, 5)	MAI-1913 (m), 1918 (f)
Bansei, Niikappu-cho, Niikappu-gun, Hokaido (NK2, 6)	MAI-2004 (f), 2016 (m)
Northeastern Honshu area, Japan (HON1)	
Shimoyahagi, Rikuzentakata, Iwate Pref., Honshu (SYG, 7)	MAI-1289 (m)
Takinosato, Rikuzentakata, Iwate Pref., Honshu (TNS, 8)	MAI-1293 (m)
Futaki, Sendai, Miyagi Pref., Honshu (FTK, 9)	MAI-1843 (m)
Central Honshu area, Japan (HON2)	
Kohzaki, Katori-gun, Chiba Pref., Honshu (KZK, 10)	MAI-1991 (m), 1992 (f)
Kameino, Fujisawa, Kanagawa Pref., Honshu (KMN, 11)	MAI-1114 (f), 1120 (f), 1443 (m), 1444 (f)
Ohba, Fujisawa, Kanagawa Pref., Honshu (OHB, 12)	MAI-1239 (f), 1306 (f), 1308 (f), 1309 (m)
Akabane, Chigasaki, Kanagawa Pref., Honshu (CGS, 13)	MAI-1548 (f)
Korea	
Seongmodo Is., Ganghwa-gun, Incheon-gwangyeoksi, Korea (SMD, 14)	HEG007-97 (m)
Laboratory mouse C57BL/6N	MAI-1301 (m)
Hybrid mice (wild caught mice x Laboratory mouse)	
MAI-1239 x 1301	MAI-1450 (f), MAI-1452 (f)
MAI-1306 x 1301	MAI-1379 (f)
MAI-1308 x 1301	MAI-1563 (m)

*Code numbers are corresponding to those in Figure 1.

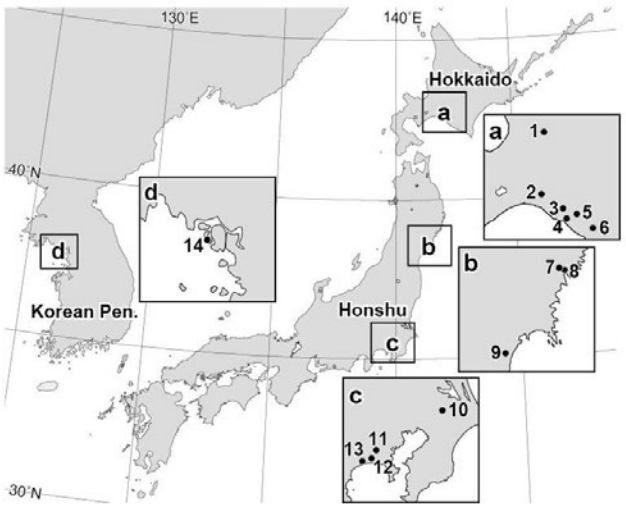

Fig. 1. Collection localities of the house mice examined in this study. Locality code numbers correspond to those in Table 1.

C-banding for somatic cells

Chromosome preparations were performed from bone marrow cells. Bone marrow cells were cultured in MEM including 15% calf serum containing colchicine (final concentration: 0.025 μg/ml) at 37 ˚C for 40 min. These cells were treated in 0.075 M KCl at 37 ˚C for 20 min as a hypotonic treatment. Subsequently, the cells were fixed with modified Carnoy's fixative (methanol : acetic acid = 3 : 1) three times. Then air-dried cells were primarily G-banded using the ASG technique (Sumner et al. 1971) to identify each chromosome by Committee of Standardized Genetic Nomenclature for Mice (1972) and Cowell (1984). After destaining using Carnoy's fixative, the cells were subsequently C-banded using the BSG technique by Summer (1972).

Quantification of the C-band pattern

Photographs of somatic metaphases were obtained using a digital camera under a microscope (Olym-

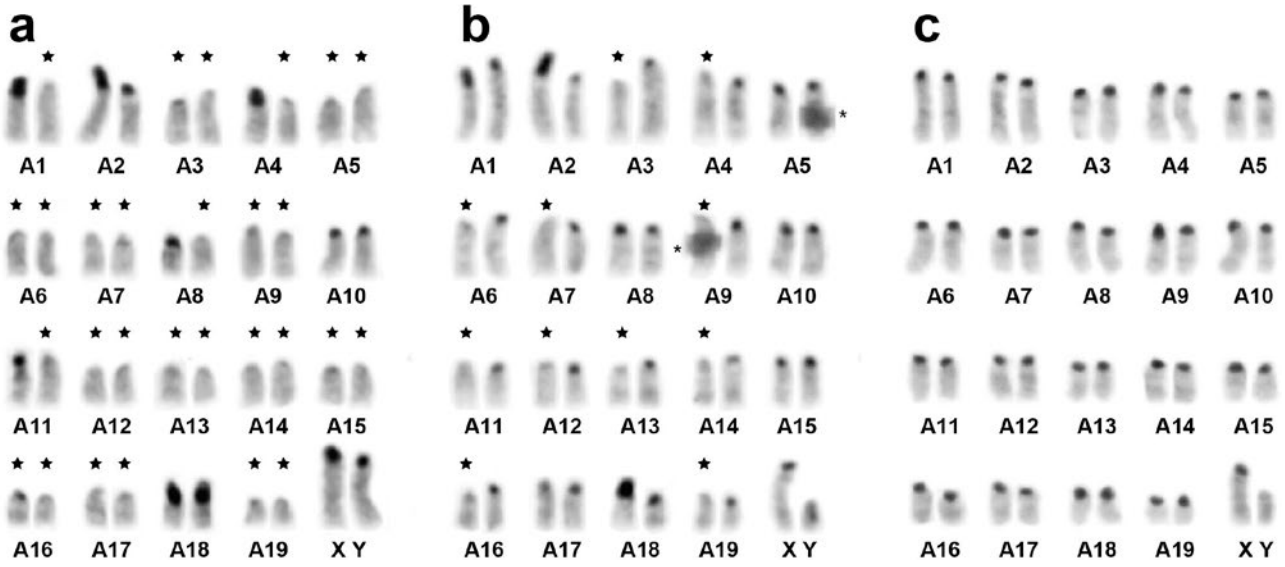

Fig. 2. Typical C-banded karyotypes of a wild-caught mouse (a, MAI-1239), a hybrid mouse (b, MAI-1452), and a C57BL/6N mouse (c, MAI-1301). Stars indicate chromosomes with null C-bands. Asterisks indicate crossing of chromosomes.

pus BX41). To correct errors caused by variations in the extension condition of chromosomes among metaphase plates, we calculated the relative lengths of the C-bands. Primarily, the boundary between negatively and positively stained regions was identified at the proximal region of the long arm in the No. 2 chromosomes according to Myoshu and Iwasa (2016). Subsequently, the distance from the distal end of the long arm to the proximal boundary of the negatively stained region was measured in one of the No. 2 chromosomes as a control length for all relative lengths on its metaphase using Adobe Illustrator CC. In addition, the lengths of all of positively C-banded regions on all chromosomes were measured by the same method. Finally, the relative lengths of the C-bands on each chromosome were calculated using following formula: the length of the positively stained C-band region of each chromosome / the control length of the No. 2 chromosome in its metaphase plate. When a positively stained C-band region was not observed in a chromosome or when the entire chromosome was stained lightly, as seen in the Y chromosome (Figure 2), we considered relative length of the chromosome to be zero. The relative lengths of C-bands for all chromosomes were calculated in five or more metaphase plates per individual.

All of the relative lengths of C-bands were categorized as classes by 0.1. The mean number of chromosomes of each class in an individual was calculated using following formula: the observed number of chromosomes included in each class in an individual / the number of observed metaphase plates in an individual. Regarding the set of all mean numbers in each class for an individual as the C-band pattern of the individual, we performed a clustering analysis for the C-band patterns of all individuals to estimate analogies among them. We first calculated Euclidian distances using all sets of the mean numbers for each class from all mouse samples. Then we performed a clustering analysis using the ward method based on these distances.

RESULTS

In the karyotypes of wild-caught mice, the hybrid individuals and C57BL/6, typical examples of the C-banded metaphases (samples prepared from wild-caught mice in SMD, HON1, HON2, NK2, and HKD1, and from C57BL/6N and the hybrid mice) were shown in Figure 3. These C-banding patterns showed the presence of chromosomes with null C-bands in the mouse samples without C57BL/6N carrying the Y chromosome negatively stained by C-banding (Figures 2 and 3). The C-band patterns of the hybrid individual seemed to be inherited from those of the parents (a wild-caught mouse and C57BL/6N) as their intermediate type (Figure 2). In addition, size variations of C-bands seemed to be confirmed in all of the mice excluding C57BL/6 (Figures 2 and 3).

The mean numbers of chromosomes with a C-band and with a null C-band were classified into classes based on the relative length; 0.01–0.10, 0.11–0.20, 0.21–0.30, 0.31–0.40, 0.41–0.50, and >0.51 as in Table 2, and the

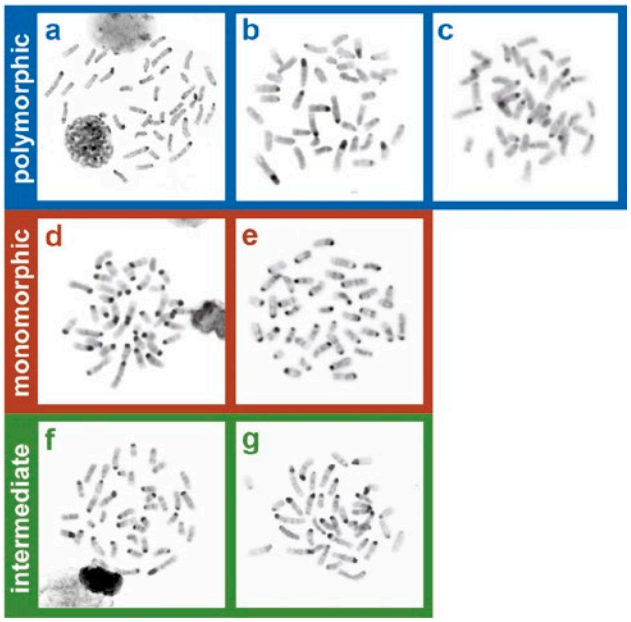

Fig. 3. Typical examples of C-banded metaphase plates of polymorphic patterns: Seongmodo Is. (a, HEG007-97), Ohba (b, MAI-1306), and Takinosato (c, MAI-1293); monomorphic patterns: C57BL/6N (d, MAI-1301) and Niikappu-2 (e, MAI-2004); and intermediate patterns: a hybrid mouse (f, MAI-1379) and Hayakita (g, MAI-1839).

histograms of these mean numbers were indicated in Figure 4. On the basis of our observation and calculation of C-bands on a metaphase plate of C57BL/6 (Table 2 and Figure 3(d), upper histogram in Figure 4), we defined the C-bands (0.01–0.30 in relative length) as "smaller C-bands". In contrast, we defined the C-bands that is never observed in C57BL/6 (> 0.31 in relative length) as 'larger C-bands'. Furthermore, the result of clustering analysis showed clear discriminations with three major clades consisting of the HON1/HON2 mice and the SMD mouse, HKD1/HKD2 (without NK2) mice and the hybrid mice, and HKD2 (NK2) mice and C57BL/6 (Figure 5).

The HON1/HON2 mice and the SMD mouse in a major clade (Figure 5) showed high frequencies of null C-band chromosomes (the mean numbers ranged from 23.6 to 29.5 per metaphase plate, Table 2). In addition, there were residual chromosomes carrying positively stained C-bands showing variable sizes, including larger C-bands with relative lengths of not only 0.31–0.50 but also > 0.51 (sums of the mean numbers of chromosomes with relative lengths of > 0.31 ranged from 1.4 to 7.0 per metaphase). On the other hand, the mean numbers of smaller C-bands were lower (sums of the mean numbers of chromosomes with relative lengths of 0.01–0.30 ranged from 5.2 to 14.4 per metaphase). Of these, smaller C-bands with relative lengths of 0.21–0.30 were observed much more than those with relative lengths of 0.11–0.20 in HON2; however, individuals from HON1 showed the highest number of C-bands with relative lengths of 0.11–0.20 (Table 2 and Figure 4). Moreover, the larger C-bands on the No.2 chromosomes, which were usually observed in the HON1/HON2 mice (Figure 3(b) and 3(c), respectively) were not observed in the SMD mouse (Figure 3(a)).

Furthermore, hybrid mice and the HKD1/HKD2 (without NK2) mice belonged to the other major clade (Figure 5). The mean numbers of null C-bands were apparently lower (the mean numbers ranged from 14.2 to 19.4 per metaphase, Table 2) than those from HON1/HON2 and SMD (the mean numbers ranged from 23.6 to 29.5 per metaphase, Table 2) and higher than those of C57BL/6N and NK2 of HKD2 (the mean numbers ranged from 1.2 to 9.0 per metaphase, Table 2). Additionally, the mean numbers of the smaller C-bands (sums of the mean numbers with relative lengths of 0.01–0.30 ranged from 17.8 to 23.8 per metaphase, Table 2) was also intermediate between those from HON1/HON2 and SMD (5.2~14.4) and that from C57BL/6N (38.8). Moreover, there was lower number of larger C-bands, at least one chromosome per metaphase (sums of the mean numbers with relative lengths of > 0.31 ranged from 0.4 to 1.0 per metaphase, Table 2).

The third major clade consisted of C57BL/6 and mice from NK2 of HKD2 (Figure 5). C57BL/6 showed smaller C-bands in all of the chromosomes (sum of the mean numbers of chromosomes with relative lengths of 0.01–0.30 was 38.8 per metaphase, Table 2). In addition, C57BL/6 showed no larger C-band with relative lengths of > 0.31 and lower numbers of null C-bands (the mean number was 1.2 per metaphase, Table 2). Meanwhile, individuals from NK2 of HKD2 (specimen nos. MAI-2004 and MAI-2016) carried a pattern similar to that of C57BL/6, especially in terms of the higher number of appearances of smaller C-bands (sums of the mean numbers of chromosomes with relative lengths of 0.01–0.30 were 36.2 and 30.0 per metaphase in MAI-2004 and MAI-2016, respectively, Table 2). Moreover, they carried no more than a chromosome with a larger C-band (sums of the mean numbers of chromosomes with relative lengths of > 0.31 were 0.2 and 1.0 per metaphase in MAI-2004 and MAI-2016, respectively, Table 2) and several chromosomes with null C-bands (the mean numbers were 3.6 and 9.0 per cell in MAI-2004 and MAI-2016, respectively).

Table 2. Mean numbers of chromosomes with null C-band and C-band classified into each class of relative length.

Specimen	Null C-band	Classification							
		Smaller C-band Relative length of C-band				Larger C-band Relative length of C-band			
		0.01~0.10	0.11~0.20	0.21~0.30	Total	0.31~0.40	0.41~0.50	>0.51	Total
HKD1									
MAI-1919	19,0	0	10,4	7,6	**18,0**	2,8	0,2	0	**3,0**
MAI-1895	18,2	1,4	11,2	7,6	**20,2**	1,6	0	0	**1,6**
MAI-1915	18,2	0,2	10,6	7,8	**18,6**	3,0	0,2	0	**3,2**
HKD2									
MAI-1837	15,8	1,2	13,4	5,6	**20,2**	2,4	1,4	0	**3,8**
MAI-1840	17,4	0	9,0	8,8	**17,8**	2,6	1,4	0	**4,0**
MAI-1916	19,4	0,8	13,0	5,6	**19,4**	1,2	0	0	**1,2**
MAI-1917	18,2	0,4	10,4	8,2	**19,0**	2,6	0,2	0	**2,8**
MAI-1913	16,6	0	13,3	7,9	**21,2**	1,9	0,3	0	**2,2**
MAI-1918	18,4	0,8	15,6	4,8	**21,2**	0,2	0,2	0	**0,4**
MAI-2004	3,6	1,2	31,4	3,6	**36,2**	0,2	0	0	**0,2**
MAI-2016	9,0	3,2	23,4	3,4	**30,0**	0,6	0,4	0	**1,0**
HON1									
MAI-1289	25,0	0	7,4	5,2	**12,6**	2,0	0,4	0	**2,4**
MAI-1293	23,6	0,4	10,4	3,6	**14,4**	1,2	0,6	0,2	**2,0**
MAI-1843	27,6	0,6	2,8	4,4	**7,8**	2,6	2,0	0,0	**4,6**
HON2									
MAI-1991	28,0	0	1,0	4,6	**5,6**	3,4	2,6	0,4	**6,4**
MAI-1992	27,3	0	3,0	5,0	**8,0**	3,3	0,9	0,6	**4,7**
MAI-1114	29,5	0	1,2	4,0	**5,2**	2,0	1,8	1,5	**5,3**
MAI-1120	29,2	0,2	0,8	4,2	**5,2**	3,2	1,5	1,0	**5,7**
MAI-1443	27,8	0	5,2	5,6	**10,8**	0,8	0,6	0	**1,4**
MAI-1444	29,2	0	0	3,8	**3,8**	2,8	2,6	1,6	**7,0**
MAI-1239	28,2	0,2	1,8	5,4	**7,4**	3,0	1,0	0,4	**4,4**
MAI-1306	24,8	1,3	6,2	3,1	**10,7**	2,4	1,9	0,2	**4,6**
MAI-1308	28,0	0,2	3,8	4,2	**8,2**	2,7	1,0	0,2	**3,8**
MAI-1309	28,0	0	2,4	3,6	**6,0**	2,5	2,2	1,3	**6,0**
MAI-1548	29,2	0	3,6	3,8	**7,4**	2,8	0,6	0	**3,4**
Korea									
HEG007-97	25,2	0,2	9,6	3,4	**13,2**	1,0	0,6	0	**1,6**
Labolatory mouse									
MAI-1301	1,2	0,2	34,8	3,8	**38,8**	0	0	0	**0**
Hybrid mice									
MAI-1450	14,4	0	16,6	7,2	**23,8**	0,8	0,6	0,4	**1,8**
MAI-1452	14,4	0,2	13,2	9,0	**22,4**	1,4	1,0	0,8	**3,2**
MAI-1379	15,8	1,4	16,2	4,0	**21,6**	0,8	1,6	0,2	**2,6**
MAI-1563	14,2	1,6	16,2	4,4	**22,2**	2,8	0,6	0,2	**3,6**

DISCUSSION

The polymorphic pattern in two areas geographically isolated in the Japanese Islands, HKD1/HKD2 (without NK2) and HON1/HON2, are categorized into two groups (Figure 5), which include the SMD and hybrid mice, respectively. On the other hand, the monomorphic pattern consists of many smaller C-bands without a null C-band and a larger C-band (Table 2 and Figure 4) and belongs to the cluster including C57BL/6N (Fig-

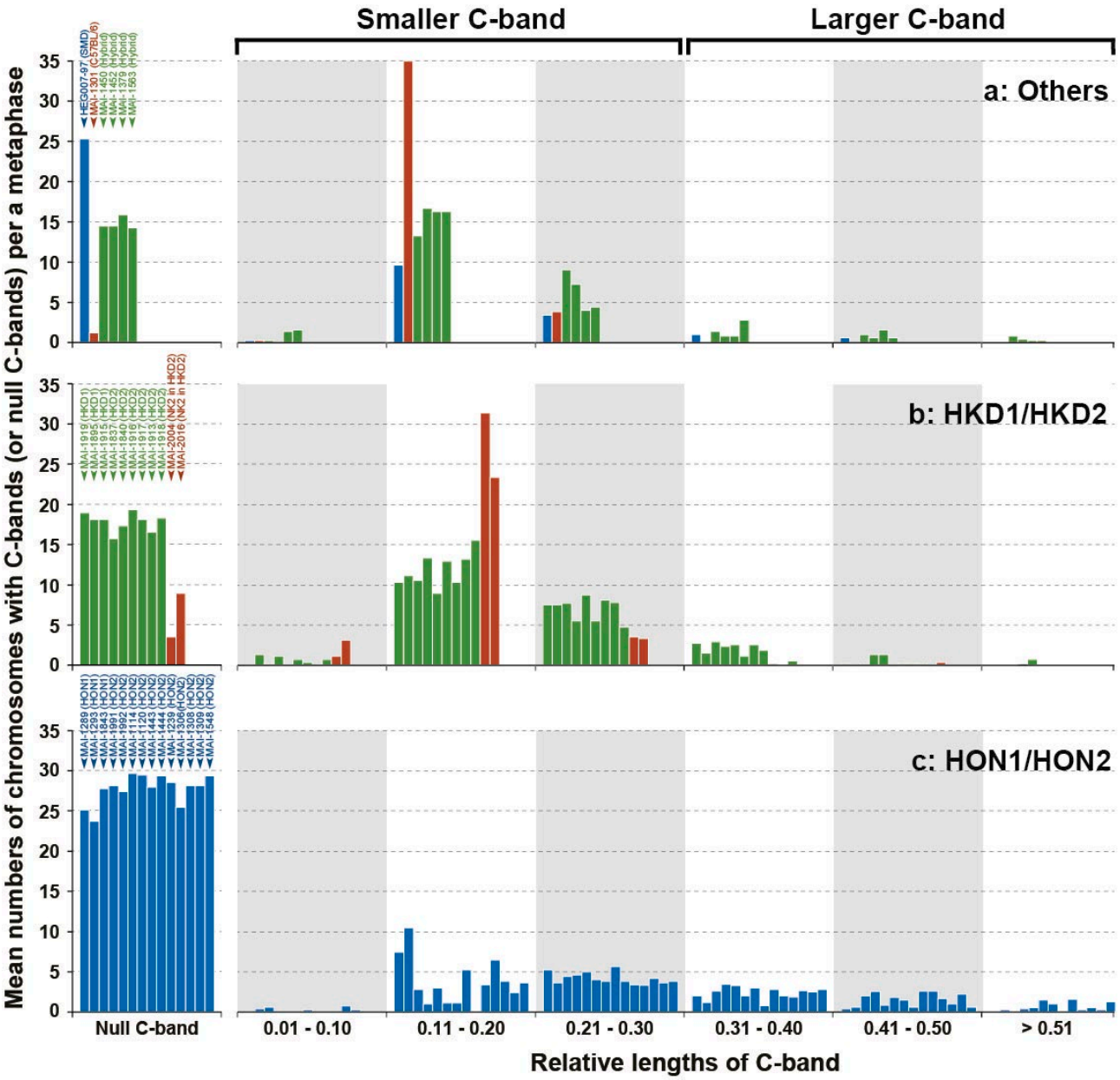

Fig. 4. Histograms showing mean numbers of chromosomes without C-bands or with C-bands per metaphase, classified into each class of relative length with 0.01–0.10, 0.11–0.20, 0.21–0.30, 0.31–0.40, 0.41–0.50, and >0.51.

ure 5). Our previous study (Myoshu and Iwasa 2018) indicates the occurrences of three *Cytb* haplotypes that are confirmed in the same areas of the Japanese Islands, as shown in Table 1. In the areas showing the polymorphic pattern similar to SMD, only the subspecific *castaneus* (CAS) type and only the subspecific *musculus* (MUS) type occur in HON1 and HON2, respectively. Additionally, only the CAS type and multiple haplotypes including the CAS, MUS, and the subspecific *domesticus* (DOM) types occur in the area showing the polymorphic pattern similar to that of hybrid mice, HKD1, and HKD2 without NK2, respectively. On the other hand, not the MUS type but rather the CAS and DOM types occur in NK2 showing the monomorphic pattern. According to these results, a concordant combination between biparental C-band pattern and maternal *Cytb* haplotype is shown in HON2 and HKD2 but is not shown in HON1 and HKD1.

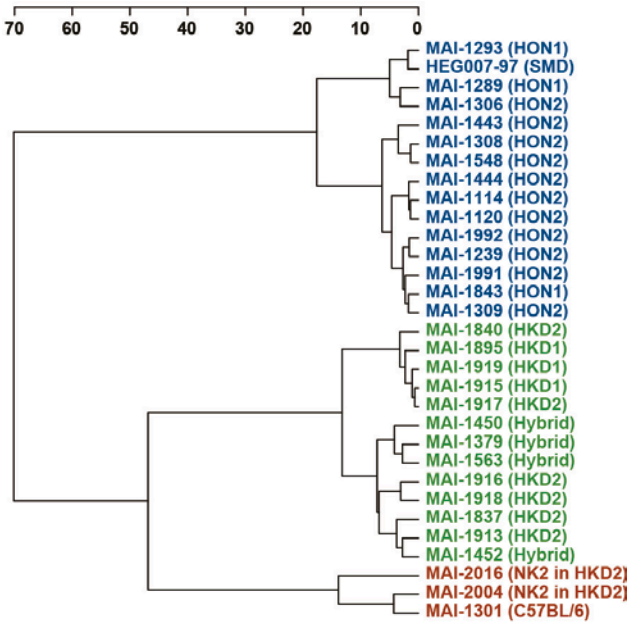

Fig. 5. Cluster analysis considering the mean numbers of chromosomes without C-bands or with C-bands in each class. Top values indicate Euclidian distances.

The present C-band results can agree with the finding of *Cytb* properties in HON2/HKD2 and the potential founders, mice in northern China and/or the Korean peninsula (Yonekawa et al. 1988; Terashima et al. 2006; Nunome et al. 2010, 2013; Suzuki et al. 2013), carry the polymorphic C-bands and the MUS type as in the HON2 mice (Myoshu and Iwasa 2018; Figures 3, 4, 5). On the basis of these results, it is a feasible explanation from both viewpoints by chromosome and mtDNA traits (Myoshu and Iwasa 2018; Figures 3, 4, 5) that the HON2 mice have maintained the traits of potential founders. In HKD2, on the basis of a simple inheritance of the C-band size (Dev et al. 1975; Figure 2), the hybridization between mice carrying a combination of the monomorphic pattern and the CAS or DOM type, and mice carrying a combination of the polymorphic pattern and the MUS type from northern China and/or the Korean peninsula is concordantly estimated by both viewpoints from these analyses (Myoshu and Iwasa 2018; Figures 3, 4, 5). The monomorphic pattern of NK2 is in accordance with our previous results showing not only the occurrence of *Cytb* haplotypes (the CAS and DOM types) but also their morphological characteristics (external body dimensions and coat coloration) (Myoshu and Iwasa 2018). There is a possibility that the distribution in the Japanese Islands of the predominant mice which were introduced from northern China and/or the Korean Peninsula (Yonekawa et al. 1988; Terashima et al. 2006; Nunome et al. 2010, 2013; Suzuki et al. 2013) has not yet expanded into NK2. However, putting emphasis on their larger head and body length as the subspecies *M. musculus domesticus* (Myoshu and Iwasa 2018) and the occurrence of the "intact" stowaway haplotype of nuclear DNA (Nunome et al. 2010; Kodama et al. 2015) and mtDNA (Yonekawa et al. 2000; Tsuda et al. 2001, 2002) in the Japan Islands, a more feasible explanation is that a relatively recent introduction(s) has led stowaway mice to this locality.

In the areas with exclusive occurrence of the CAS type from southern China and/or southeastern Asia (Suzuki et al. 2013; Kuwayama et al. 2017), HKD1 and HON1, there are discordances between the both viewpoints mentioned above (Myoshu and Iwasa 2018; Table 2; Figures 3, 4, 5). In contrast to the *Cytb* finding, the C-band patterns of the HKD1 mice and the HON1 mice are estimated to have been affected by the introgression from northern Chinese and/or Korean mice carrying the polymorphic patterns (Yoshida and Kodama 1983; Moriwaki et al. 1985, 1986; Moriwaki 2010; Yonekawa et al. 2012). In addition, although the CAS type occurs exclusively in both areas, the C-band patterns are not categorized into the same groups (Figure 5). Specifically, the numbers of null C-bands (23.6–27.6) and smaller C-bands (7.8-14.4) of the HON1 mice are especially more similar to those in the HON2 mice (24.8-29.5 null C-bands; 3.8-10.8 smaller C-bands) than those in the HKD1 mice (18.0-20.2 null C-bands; 18.6-20.2 smaller C-bands). Moreover, the sizes of the smaller C-bands differ between HON1 (more frequent relative length: 0.11-0.20, Table 2 and Figure 4) and HON2 (more frequent relative length: 0.21-0.30, Table 2 and Figure 4). These results suggest that the C-band patterns of HON1 and HKD1 are not genetically identical.

Several studies using biparental markers, which are the haplotypes on the haemoglobin bate chain (*Hbb*) locus (Minezawa et al. 1979; Miyashita et al. 1985; Kawashima et al. 1991, 1995; Ueda et al. 1999; Sato et al. 2006, 2008; Yonekawa et al. 2012), eight (Nunome et al. 2010) and seven (Kodama et al. 2015) linked nuclear genes, would provide a suggestion for why the difference in the C-band pattern has been caused in the same mtDNA occurrence areas. According to these previous studies, the distributions of the polymorphic C-band patterns (Moriwaki and Minezawa 1976; Moriwaki et al. 1985, 1986; Yonekawa et al. 2012; Myoshu and Iwasa 2016; Table 1 and Figures 4, 5) overlap the distributions of the *p* (Minezawa et al. 1979; Miyashita et al. 1985; Kawashima et al. 1995; Ueda et al. 1999; Yonekawa et al. 2012) and the MUS-II haplotype groups (Nunome et al. 2010; Kodama et al. 2015) which are derived from the

musculus lineage. The other hapolotype groups, which are recognized as the *d* (Minezawa et al. 1979; Miyashita et al. 1985; Kawashima et al. 1995; Yonekawa et al. 2012) and the recombinant haplotype groups (Nunome et al. 2010; Kodama et al. 2015) mainly derived from the *castaneus* lineage, have been observed in northern Honshu and Hokkaido localities. However, a survey of the *Hbb* allele reveals the difference in *p* frequency between the northeastern area of Honshu (37.0% in Minezawa et al. 1979) and Hokkaido (7.9% in Minezawa et al. 1979). Thus, the introgression of the lineage with MUS-II and *p* haplotypes, which has been derived from Eurasian mice carrying the polymorphic C-band patterns, has strongly affected the HON1 mice more than the HKD1 mice. In addition, since male mice have larger dispersal areas than female mice (Pocock et al. 2005), expansions of maternal genetic traits would be later than those of the paternal and biparental genetic traits. On the basis of this dispersal pattern, it is estimated that the polymorphic patterns as a biparental trait primarily expand into populations including both male and female mice with monomorphic patterns and the CAS type, by male mice considering the larger dispersal potential. Therefore, the polymorphic patterns from male mice have less affected mice in HKD1 than in HON1, based on the sign of hybridization in its C-band patterns is significantly observed in HKD1. In HON1, where the C-band patterns are similar to those in HON2 and SMD, the mice may have been replaced completely by the male mice carrying the polymorphic patterns. Otherwise, the HON1 mice have hybridized and/or maintained the traits of mice carrying a discordant combination, for example the polymorphic pattern and the CAS type, which may have been caused in other places as suggested in Searle et al. (2009), Nunome et al. (2010), Kodama et al. (2015) and Kuwayama et al. (2017).

ACKNOWLEDGEMENTS

We thank Dr. Keisuke Nakata for cooperation in current sampling of wild mice.

REFERENCES

Committee of Standardized Genetic Nomenclature for Mice. 1972. Standard karyotype of the mouse, Mus musculus. J Hered. 63: 69–72.

Cowell JK. 1984. A photographic representation of the variability in the G-banded structure of the chromosomes in the mouse karyotype: A guide to the identification of the individual chromosomes. Chromosoma. 89: 294–320.

Dev VG, Miller DA, Miller OJ. 1973. Chromosome Markers in *Mus musculus*: Strain Differences in C-banding. Genetics. 75: 663–670.

Dev VG, Miller DA, Tantravahi R, Sehreck RR, Roderiek TK, Erlanger BF, Miller OJ. 1975. Chromosome markers in *Mus musculus*: Differences in C-banding between the subspecies *M. m. musculus* and *M. m. molossinus*. Chromosoma. 53: 335–344.

Ikeuchi T. 1978. Notes on the centromeric heterochromatin of the Japanese wild mouse (*Mus musculus molossinus*). Chrom Inf Serv. 25: 24–26.

Kawashima R, Miyashita N, Wang C, He X, Jin M, Wu Z, Moriwaki K. 1991. A new haplotype of β-globin gene complex, Hbb^{w1}, in Chinese wild mouse. Jpn J Genet. 66: 491–500.

Kawashima T, Miyashita N, Tsuchiya K, Li H, Wang F, Wang CH, Wu XL, Wang C, Jin ML, He XQ, et al. 1995. Geographical distribution of the *Hbb* haplotypes in the *Mus musculus* subspecies in Eastern Asia. Jpn J Genet. 70: 17–23.

Kodama S, Nunome M, Moriwaki K, Suzuki H. 2015. Ancient onset of geographical divergence, interpopulation genetic exchange, and natural selection on the *Mc1r* coat-colour gene in the house mouse (*Mus musculus*). Biol J Linn Soc. 114: 778–794.

Kuwayama T, Nunome M, Kinoshita G, Abe K, Suzuki H. 2017. Heterogeneous genetic make-up of Japanese house mice (*Mus musculus*) created by multiple independent introductions and spatio-temporally diverse hybridization processes. Biol J Linn Soc. 122: 661–674.

Miller DA, Tantravahi R, Dev VG, Miller OJ. 1976. Q-and C-band chromosome markers in inbred strains of *Mus musculus*. Genetics. 84: 67–75.

Minezawa M, Moriwaki K, Kondo K. 1979. Geographical distribution of Hbb^{p} allele in the Japanese wild mouse, *Mus musculus molossinus*. Jpn J Genet. 54: 165–173.

Miyashita N, Moriwaki K, Minezawa M, Yonekawa H, Bonhomme F, Migita S, Yu ZC, Lu DY, Cho WS, Thohari M. 1985. Allelic constitution of the hemoglobin beta chain in wild populations of the house mouse, *Mus musculus*. Biochem Genet. 23: 975–986.

Moriwaki K. 2010. Award Recipient (Evolutionary history of muroid rodents inscribed in the genome). Mamm Sci. 50: 67–80. Japanese.

Moriwaki K, Minezawa M. 1976. Geographical distribution of No. 18 chromosome polymorphism. Ann Rep Natl Inst Genet. 27: 46–47.

Moriwaki K, Miyashita N, Yonekawa H. 1985. Genetic survey of the origin of laboratory mice and its implica-

tion in genetic monitoring. In: Archibald J, Ditchfield J, Rowsell HC, editors. The Contribution of Laboratory Animal Science to the Welfare of Man and Animals. Stuttgart (BW): Gustav Fischer Verlag; p. 237–247.

Moriwaki K, Miyashita N, Suzuki H, Kurihara Y, Yonekawa H. 1986. Genetic features of major geographical isolates of *Mus musculus*. Curr Top Microbiol Immunol 127: 55–61.

Moriwaki K, Myashita N, Mita A, Gotoh H, Tsuchiya K, Kato H, Mekada K, Noro C, Oota S, Yoshiki A, et al. 2009. Unique inbred strain MSM/Ms established from the Japanese wild mouse. Exp Anim. 58: 123–134.

Myoshu H, Iwasa MA. 2016. Polymorphic state of C-bands in the Japanese house mice, *Mus musculus*. Cytologia. 81: 459–463.

Myoshu H, Iwasa MA. 2018. Colonization and differentiation traits of the Japanese house mouse, *Mus musculus* (Rodentia, Muridae), inferred from mitochondrial haplotypes and external body characteristics. Zool Sci. 35: 222–232.

Nunome M, Ishimori C, Aplin KP, Tsuchiya K, Yonekawa H, Moriwaki K, Suzuki H. 2010. Detection of recombinant haplotypes in wild mice (*Mus musculus*) provides new insights into the origin of Japanese mice. Mol Ecol. 19: 2474–2489.

Nunome M, Suzuki H, Moriwaki K. 2013. Historical introduction of Japanese wild mice, *Mus musculus*, from South China and the Korean Peninsula. Anim Syst Evol Divers. 29: 267–271.

Pocock MJO, Hauffe HC, Searle JB. 2005. Dispersal in house mice. Biol J Linn Soc. 84: 565–583.

Sato JJ, Tsuru Y, Hirai K, Yamaguchi Y, Mekada K, Takahata N, Moriwaki K. 2006. Further evidence for recombination between mouse hemoglobin beta b1 and b2 genes based on the nucleotide sequences of intron, UTR, and intergenic spacer regions. Genes Genet Syst. 81: 201–209.

Sato JJ, Shinohara A, Miyashita N, Koshimoto C, Tsuchiya K, Nakahara I, Morita T, Yonekawa H, Moriwaki K, Yamaguchi Y. 2008. Discovery of a new HBB haplotype w2 wild-derived house mouse, *Mus musculus*. Mamm Genome. 19: 155–162.

Searle JB, Jamieson PM, Gündüz İ, Stevens MI, Jones EP, Gemmill CE, King CM. 2009. The diverse origins of New Zealand house mice. Proc R Soc Lond B Biol Sci. 276: 209–217.

Sumner AT. 1972. A simple technique for demonstrating centromeric heterochromatin. Exp Cell Res. 75: 304–306.

Sumner AT, Evans HJ, Buckland RA. 1971. New technique for distinguishing between human chromosomes. Nat New Biol. 232: 31–32.

Suzuki H, Nunome M, Kinoshita G, Aplin KP, Vogel P, Kryukov AP, Jin ML, Han SH, Maryanto I, Tsuchiya K, et al. 2013. Evolutionary and dispersal history of Eurasian house mice *Mus musculus* clarified by more extensive geographic sampling of mitochondrial DNA. Heredity. 111: 375–390.

Terashima M, Furusawa S, Hanazawa N, Tsuchiya K, Suyanto A, Moriwaki K, Yonekawa H, Suzuki H. 2006. Phylogeographic origin of Hokkaido house mice (*Mus musculus*) as indicated by genetic marker with maternal, parental and bilateral inheritance. Heredity. 96: 128–138.

Tsuda K, Tsuchiya K, Aoki E, Iizuka S, Suzuki S, Uchida Y, Yonekawa H. 2001. Distributions of *Mus musculus* subspecies at coastal areas in Japan (2). J Jpn Quarant Med Assoc. 3: 150–160. Japanese with English abstract.

Tsuda K, Tsuchiya K, Aoki E, Iizuka S, Suzuki S, Uchida Y, Yonekawa H. 2002. Distributions of *Mus musculus* subspecies at coastal areas in Japan (3). J Jpn Quarant Med Assoc. 4: 136–147. Japanese with English abstract.

Ueda Y, Miyashita N, Imai K, Yamaguchi Y, Takamura K, Notohara M, Shiroishi T, Kawashima T, Ning L, Wang C, et al. 1999. Nucleotide sequences of the mouse globin beta gene cDNAs in a wild derived new haplotype Hbbw1. Mamm Genome. 10: 879–882.

Yonekawa H, Moriwaki K, Gotoh O, Miyashita N, Matsushima Y, Shi LI, Cho WS, Zhen XL, Tagashira Y. 1988. A hybrid origin of Japanese mice '*Mus musculus molossinus*': evidence from restriction analysis of mitochondrial DNA. Mol Biol Evol. 5: 63–78.

Yonekawa H, Tsuda K, Tsuchiya K, Aoki E, Iizuka S, Suzuki S, Uchida Y. 2000. Distributions of *Mus musculus* subspecies at coastal areas in Japan. J Jpn Quarant Med Assoc. 2: 51–58. Japanese with English abstract.

Yonekawa H, Sato JJ, Suzuki H, Moriwaki K. 2012. Origin and genetic status of *Mus musculus molossinus*: a typical example of reticulate evolution in the genus Mus. In: Macholán M, Baird SJE, Munclinger P, Piálek J, editors. Evolution of the House Mouse. Cambridge (UK): Cambridge University Press; p. 94–113.

Yoshida MC, Kodama Y. 1983. C-band patterns of chromosomes in 17 strains of mice. Cytogenet Cell Genet. 35: 51–56.