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Implications of the genetic divergence between European wild mice with Robertsonian translocations from the viewpoint of mitochondrial DNA

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SUMMARY

Genetic divergences between the wild mouse populations with various Robertsonian translocations from the Poschiavo Valley, Yugoslavia, Milan and the Apenninies, were estimated based on the mitochondrial (mt) DNAs. The mtDNAs isolated from the liver were analysed by agarose slab-gel electrophoresis after digestion with eight kinds of restriction endonucleases: BamHI, EcoRI, HindII, HindIII, PstI, HpaI, HpaII and BglI. These preparations were further used to make restriction maps, from which sequence divergence between each Rb variation was calculated to be 0·2–2·2%. These rather larger values appear to be in conflict with the present concept that the Rb variations occurred during the last several thousand years. Both, however, might be reconciled by assuming genetic introgression of the founder with a small number of Rb translocations into other subspecies populations genetically remote and the subsequent rapid accumulation of Rb translocations unique to each population due to an unknown mechanism occurring specifically in the intersubspecies hybrids between *M. m. domesticus* and the other *M. m.* subspecies. This was the case also in a new Rb (9.15) translocation obtained from Ogasawara Islands in Japan which was the intersubspecies hybrid between *M. m. molossinus* and *M. m. domesticus*.

1. INTRODUCTION

Since the first discovery of the wild mouse population with Robertsonian (Rb) karyotype variations consisting of seven pairs of metacentrics in the Poschiavo Valley (Gropp, Tettenborn & Lehmann, 1970), a number of Rb variations have been found in the Rhaetian Alps (Gropp *et al.* 1972; Winking *et al.* 1977), Lombardy (Capanna, Vittoria Civitelli & Cristaldi, 1977; Capanna & Riscassi, 1978), the

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Apennines (Capanna *et al.* 1976), Sicily (v. Lehman & Radbruch, 1979), Yugoslavia (Winking, Dulic & Gropp, 1979; Dulic & Dunderski, 1980) and southern Germany, Spain and Scotland (Adolph & Klein, 1981). Among them, the Rb system of Rhaeto-Lombardia is a continuum with a great diversity of gradations and transitions of karyotypes (Gropp *et al.* 1982). The Apennine Rb system seems to be another great continuum as possibly the Dalmatian (Zadar) and Sicilian populations. Recently, the whole picture of Rb systems in wild mice has been reviewed comprehensively by Sage (1981). For further understanding of the evolutionary process of such systems, more accurate evaluation of the genetic status of each Rb variation is desirable.

We have already demonstrated that the major *Mus musculus* subspecies in the Old World can be differentiated by the restriction patterns of their mitochondrial (mt) DNAs. Furthermore, the divergence times between them have been computed by restriction maps (Yonekawa *et al.* 1980, 1981, 1982). In the present study, we attempted to apply this type of approach to infer the genetic position of each Rb variation. The restriction analysis of their mtDNAs revealed genetic divergence to a considerable extent. This suggests the possibility that Rb rearrangement occurs in the intersubspecies hybrids. Finally we have introduced a new Rb translocation obtained from a Japanese wild population which is probably an intersubspecies hybrid and which allows us to consider the mechanism of Rb rearrangement somehow related to the occurrence of intersubspecies hybrids.

2. MATERIALS AND METHODS

(i) *Mice*

Five lines of Robertsonian variations abbreviated as Pos, Zadar, Mil-II, CD and CB, each of which have been maintained in the Medizinische Hochshule Lübeck as stocks homozygous for their characteristic Rb chromosomes, were used. Pos with seven Rb translocations, (1.3) (4.6) (5.15) (11.13) (8.12) (9.14) (16.17), originated from the Poschiavo Valley in southeastern Switzerland (Gropp *et al.* 1970). Zadar with six Rb chromosomes, (1.11) (5.15) (6.12) (10.14) (9.13) (8.17), was obtained from Dalmatia in Yugoslavia (Winking *et al.* 1979; Dulic & Dunderski, 1980). Mil-II with eight Rb chromosomes, (3.4) (2.8) (6.7) (5.15) (10.12) (11.13) (9.14) (16.17), was collected near Milan in northern Italy (Gropp *et al.* 1982). CD carrying nine Rb chromosomes, (1.7) (3.8) (6.13) (4.15) (10.11) (2.18) (5.17) (12.14) (9.16), came from Abruzzi near Ciffaducale in the Apennines of central Italy, and CB carrying nine Rb chromosomes, (1.18) (2.17) (4.11) (6.7) (3.13) (5.15) (8.14) (10.12) (9.16), came from Molise also in the Apennines (Capanna *et al.* 1976). Nineteen mice were collected by M.M. in 1977 on the Ogasawara Islands (Chichi Is.) located about 1000 km south of Tokyo. Among them we found a Rb variation.

For comparison of mtDNAs, the cleavage maps of seven subspecies which we already reported (Yonekawa *et al.* 1981) were employed. These subspecies were as follows (abbreviations are shown in parentheses): *Mus musculus domesticus* (*dom*) from Skockholm, England and Windsor, Canada; *M. m. brevirostris* (*brv*) from Montpellier, France; *M. m. musculus* (*mus D*) from northern Jutland, Denmark;

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M. m. musculus (*mus B*) from Bulgaria; *M. m. bactrianus* (*bac*) from Afghanistan; *M. m. castaneus* (*cas*) from the Philippines; *M. m. molossinus* (*mol*) from 15 localities in Japan.

(ii) *Enzymes and chemicals*

Restriction endonucleases, BamHI, EcoRI, HindIII, and PstI were obtained from Boehringer Mannheim Yamanouchi; HindII, HpaI and HpaII from Bethesda Research Laboratories and BglI from Miles Laboratories. DNaseI was purchased from the Millipore Corp., RNase T_1 from Sankyo Co. Ltd and Agarose from Marine Colloids Inc.

(iii) *MtDNA preparation*

In each Rb line, pooled livers from five individuals, both males and females, were treated to isolate the liver mitochondria. This process was carried out in Lübeck and the mitochondrial fractions were sent to Saitama in a frozen state. The mtDNAs were prepared from frozen materials by the SDS-method. Subsequently they were purified by CsCl-ethidium bromide density-gradient centrifugation at 36000 rev./min for 40 h. Closed and open circular mtDNAs were separated and both were used for restriction analysis. For the electron microscopic analysis of the size of DNA fragments, open circular fractions were employed. The entire procedures of mtDNA preparation were previously described in detail by the authors (Yonekawa *et al.* 1978, 1980, 1981).

(iv) *Analysis of restriction patterns and restriction mapping*

Restriction endonuclease digestion of mtDNAs by eight kinds of enzymes and agarose slab-gel electrophoresis of the digests were performed as previously reported (Yonekawa *et al.* 1978). Mapping of cleavage sites on mtDNA by the partial digestion or double digestion technique was performed according to Brown & Vinograd (1974); Moore *et al.* (1977); Parker & Watson (1977) and Yonekawa *et al.* (1980, 1981).

(v) *Computation of sequence divergence from restriction maps*

The sequence divergence was calculated by the method of Gotoh *et al.* (1979). The phylogenetic diagram was made by the unweighted pair-group clustering method (Sokal & Michener, 1958).

(vi) *Analyses of chromosome C-bands and electrophoretic patterns of hemoglobins*

Chromosomal C-band patterns of mice from the Ogasawara Is. were analysed by Q.M-33258 Hoechst staining (Yoshida *et al.* 1975) of bone marrow preparations. The electrophoretic patterns of hemoglobins in the hemolysate were asseyed using Titan III cellulose acetate plates and 0·14 M Trisborate-E.D.T.A. buffer (pH 8·7).

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3. RESULTS

(i) *Restriction enzyme cleavage patterns*

Plate 1 shows agarose gel electrophoretic patterns of restriction enzyme digests of mtDNAs extracted from the five Rb variations and C57BL/6 strain as a reference for *M. m. domesticus*. Different patterns for each enzyme are designated by letters of the alphabet. In EcoRI, PstI and BglI treatments, no difference was observed among the six samples. Both HindIII and HaeII showed two different patterns in each run. BamHI, HindII and HpaI digestions exhibited three distinct patterns. Table 1 summarizes the cleavage patterns represented by letters.

Table 1. *Restriction enzyme cleavage patterns of mtDNAs obtained from five Rb variations*

	Restriction enzymes* & cleavage patterns							
	Bm	Ec	H2	H3	He2	Hp1	Ps	Bg
C57BL/6	A	A	A	A	A	A	A	A
Pos	E	A	A	B	A	A	A	A
Zadar	A	A	D	B	A	D	A	A
Mil-II	E	A	E	B	A	B	A	A
CD	F	A	E	B	D	A	A	A
CB	A	A	E	B	A	B	A	A

* Abbreviations. Bm: BamHI, Ec: EcoRI, H2: HindII, H3: HindIII, He2: HaeII, Hp1: HpaI, Ps: PstI, Bg: BglI.

(ii) *Restriction enzyme cleavage maps*

Because the presence of considerable variations in the cleavage sites among mice with Rb translocations was suggested by the cleavage patterns on electrophoresis, we constructed cleavage maps of mtDNAs by partial digestion and double digestion techniques (Brown & Vinograd, 1974; Moore *et al.* 1977; Parker & Watson, 1977) to detect common and different sites in the restriction cleavages more precisely. Fig. 1 illustrates the cleavage maps of mtDNAs from five Rb variations. Sites less than 1 % apart in genome length were regarded as identical.

(iii) *Estimation of sequence divergence and time of divergence*

We estimated nucleotide sequence divergence between mtDNAs of the five Rb variations from their cleavage maps (Fig 1) using Gotoh's method (Gotoh *et al.* 1979). Sequence divergence between each Rb variation and each mouse subspecies was also computed. For this purpose, the cleavage maps of seven subspecies already reported by the authors (Yonekawa *et al.* 1981, 1982) are superimposed in Fig. 1. The numbers of common (*ci*) and different (*di*) sites were scored from the maps for every combination of Rb variations and subspecies as summarized in Table 2. By introducing the c_i and d_i values into Gotoh's equation (Gotoh *et al.* 1979), we can calculate the sequences divergence between various combinations of mice as shown in Table 3.

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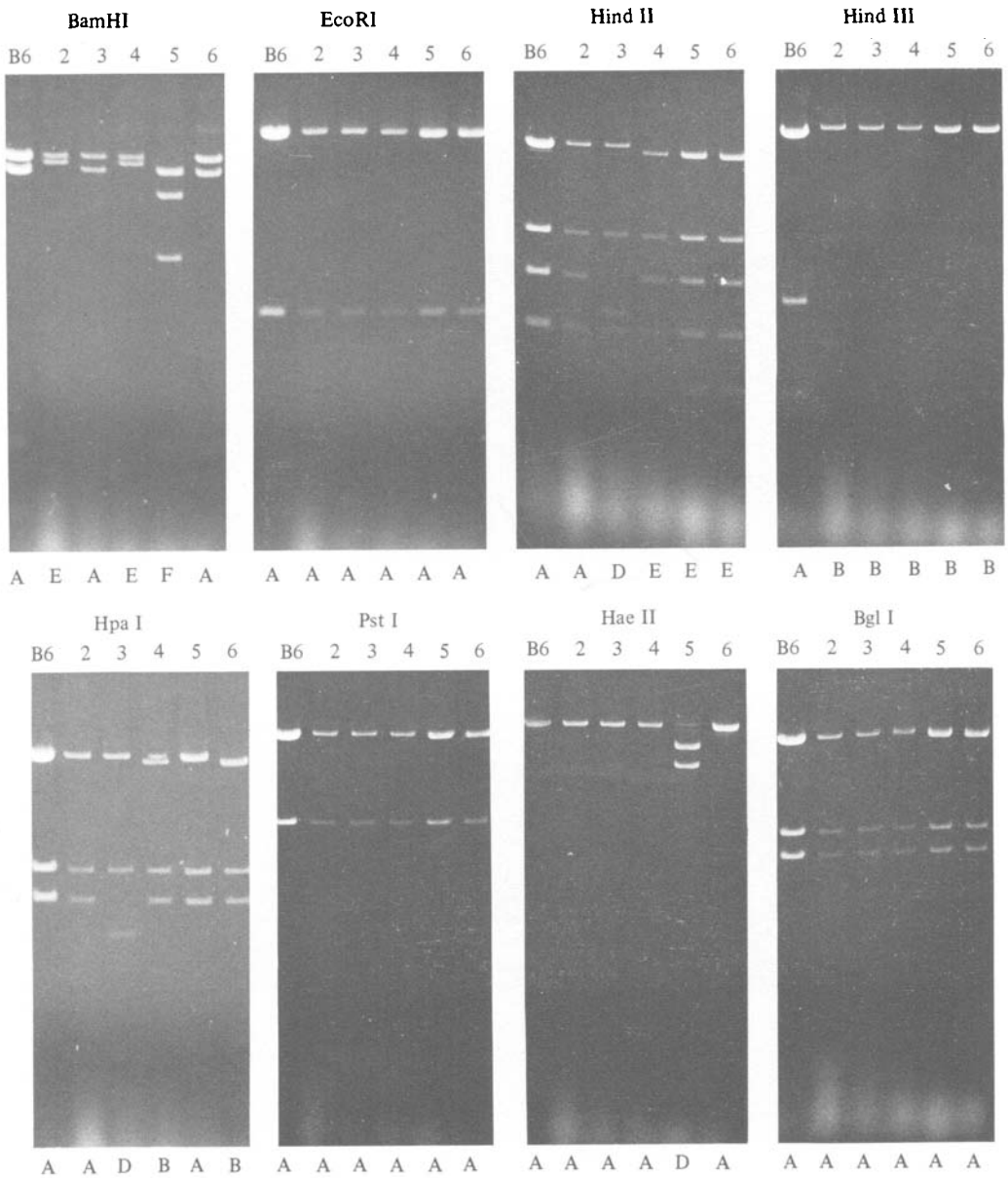

Comparison of mtDNA cleavage patterns in the five lines of Rb variations. B6: C57BL/6J as a control *Mus musculus domesticus*, 2: Pos, 3: Zadar, 4: Mil-II, 5: CD, 6: CB. The mtDNAs after digestion with a restriction enzyme were electrophoresed in 1 % agarose slab gels. Letters under the photographs refer to the type of cleavage patterns. The same letters are used in Table 1.

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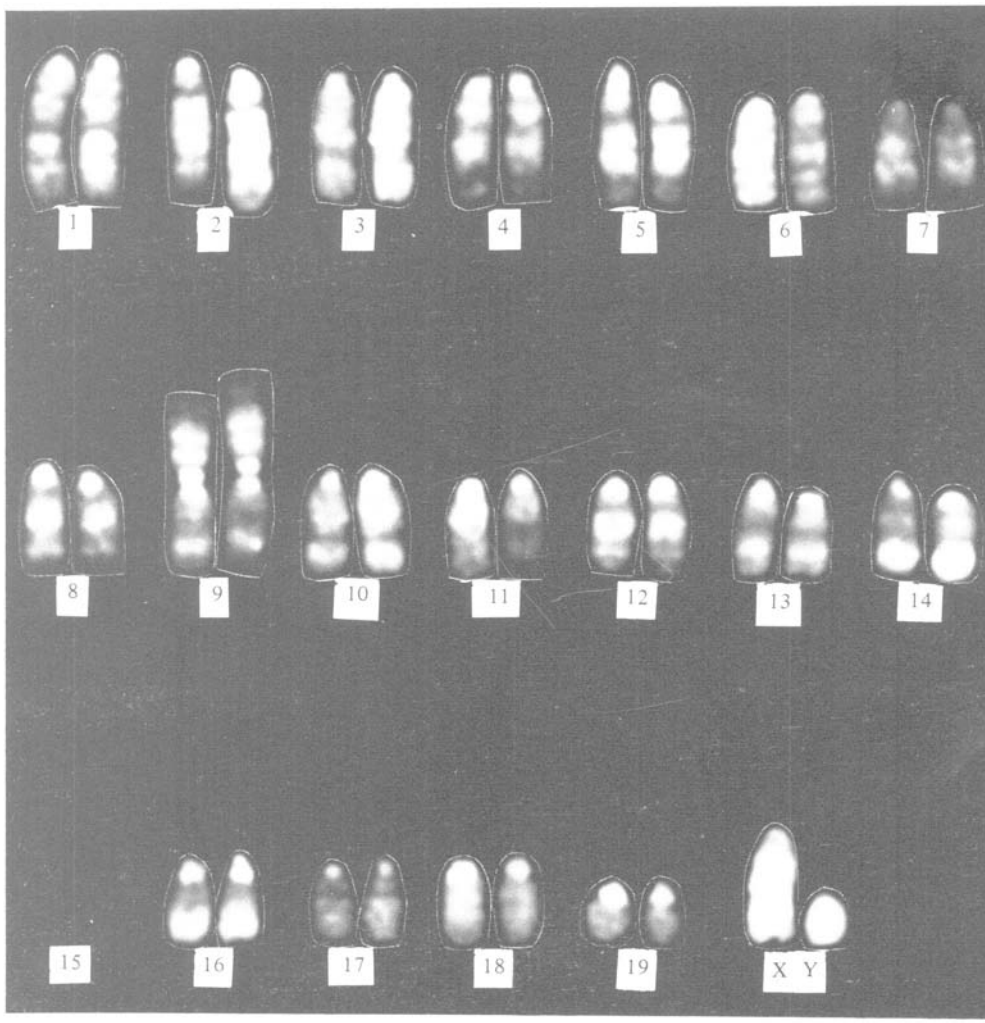

Mus molossinus OGS. Karyotype of a new RB variation (9.15) collected from the Ogasawara Islands in Japan, stained with Q.M. 33258 Hoechst.

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Sequence divergence between each Rb variant population ranged from 0·4 % to 2·2 %. Those between Rb variations and their neighbouring subspecies, *M. m. domesticus*, *M. m. brevirostris* and *M. m. musculus*, in Denmark were also in a similar range, 0·0 %–2·4 %. Nevertheless, the divergence between Rb variant populations and mouse subspecies from geographically remote such as *M. m. musculus* from Bulgaria, *M. m. bactrianus* from Pakistan and Afghanistan, *M. m. castaneus* from the Philippines and Taiwan and *M. m. molossinus* from Japan, were clearly greater than those mentioned above, ranging from 3·4 % to 7·5 %. As already reported by Yonekawa *et al.* (1981), the sequence divergence between *M. m. domesticus* and a number of the Asian subspecies mentioned above were in a similar range. Among the Rb variations, the Zadar population from Dalmatia (Yugoslavia) seems to be genetically remote to a certain extent from the others.

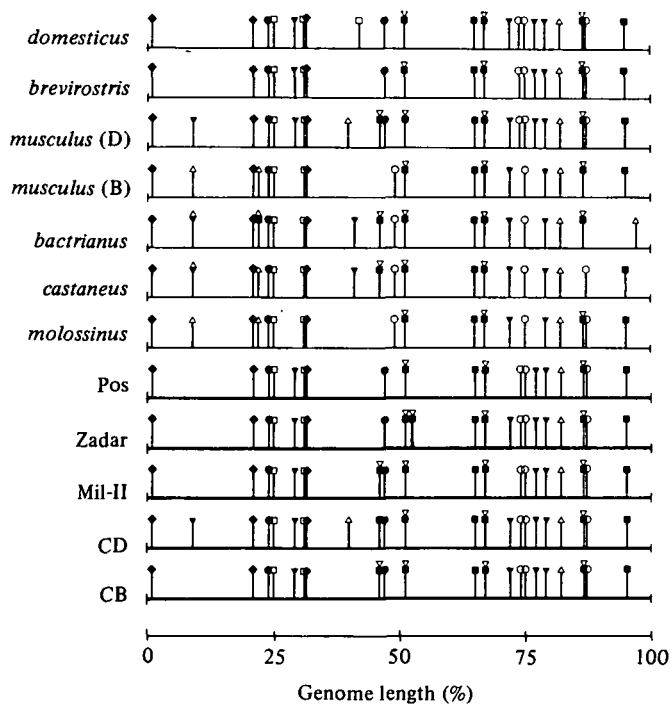

Fig. 1. Cleavage maps of mtDNAs from seven subspecies of mice (*Mus musculus*) and five lines of Rb variations. The former seven maps are from our previous data (Yonekawa *et al.* 1981). Cleavage sites for individual restriction enzymes are identified by the following symbols: ▼ BamHI, ○ EcoRI, ■ HindII, □ HindIII, ▽ HpaI, △ HaeII, ◆ BglI and ● PstI. The linear map is arranged by assuming that the origin of DNA replication is at position 0. Length is given as percent of the total genome.

(iv) *Phylogenetic diagram of Rb variations and the other mouse subspecies*

To demonstrate the phylogenetic relationship among these Rb-variant populations and the major subspecies of mice, a phylogenetic diagram was constructed using the unweighted pair-group clustering method (Sokal & Michener, 1958) with the kind help of Dr Y. Tateno. Fig. 2 clearly indicates that each one of the

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Table 2. *Numbers of cleavage sites which are common (c_i) or different (d_i) between pairs of Robertsonian variations and* Mus musculus *subspecies*

	Pos* *v.*						Zadar *v.*								Mil-II *v.*				CD *v.*	
	Zadar		Mil-II		CD		CB		Mil-II		CD		CB		CD		CB		CB	
Enzymes	c_i	d_i	c_i	d_i	c_i	d_i	c_i	d_i	c_i	d_i	c_i	d_i	c_i	d_i	c_i	d_i	c_i	d_i	c_i	d_i
BamHI	3	1	3	0	3	2	3	1	3	1	4	1	4	0	3	2	3	1	4	1
EcoRI	3	0	3	0	3	0	3	0	3	0	3	0	3	0	3	0	3	0	3	0
HindII	5	1	5	1	5	1	5	1	5	2	5	2	5	2	6	0	6	0	6	0
HindIII	2	0	2	0	2	0	2	0	2	0	2	0	2	0	2	0	2	0	2	0
HpaI	3	2	3	1	3	0	3	1	3	3	3	2	3	3	3	1	4	0	3	1
HaeII	1	0	1	0	1	1	1	0	1	0	1	1	1	0	1	1	1	0	1	1
PstI	2	0	2	0	2	0	2	0	2	0	2	0	2	0	2	0	2	0	2	0
BglI	3	0	3	0	3	0	3	0	3	0	3	0	3	0	3	0	3	0	3	0

	M. m. molossinus v.								*M. m. castaneus v.*								*M. m. bactrianus v.*							
	Zadar		Mil-II		CD		CB		Zadar		Mil-II		CD		CB		Zadar		Mil-II		CD		CB	
Enzymes	c_i	d_i	c_i	d_i	c_i	d_i	c_i	d_i	c_i	d_i	c_i	d_i	c_i	d_i	c_i	d_i	c_i	d_i	c_i	d_i	c_i	d_i	c_i	d_i
BamHI	2	2	1	3	2	3	2	2	2	4	1	5	3	3	2	4	2	4	1	5	3	3	2	3
EcoRI	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	1	3	1	3	1	3	1	3
HindII	5	1	5	1	5	1	5	1	4	2	5	1	5	1	5	1	4	3	5	2	5	2	5	2
HindIII	2	0	2	0	2	0	2	0	2	0	2	0	2	0	2	0	2	0	2	0	2	0	2	0
HpaI	3	2	3	1	3	0	3	1	2	4	3	1	2	2	3	1	3	3	4	0	3	1	4	0
HaeII	1	2	1	2	1	3	1	2	1	2	1	2	1	2	1	2	1	3	1	3	1	4	1	3
PstI	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
BglI	3	0	3	0	3	0	3	0	3	0	3	0	3	0	3	0	2	1	2	1	2	1	2	1

	M. m. domesticus v.								*M. m. musculus* (D) *v.*								*M. m. musculus* (B) *v.*							
	Zadar		Mil-II		CD		CB		Zadar		Mil-II		CD		CB		Zadar		Mil-II		CD		CB	
Enzymes	c_i	d_i	c_i	d_i	c_i	d_i	c_i	d_i	c_i	d_i	c_i	d_i	c_i	d_i	c_i	d_i	c_i	d_i	c_i	d_i	c_i	d_i	c_i	d_i
BamHI	4	0	3	1	4	1	4	0	4	1	3	2	5	0	4	1	2	2	1	3	2	3	2	2
EcoRI	3	0	3	0	3	0	3	0	3	0	3	0	3	0	3	0	1	3	1	3	1	3	1	3
HindII	5	1	5	1	5	1	5	1	5	2	6	0	6	0	6	0	5	1	5	1	5	1	5	1
HindIII	2	1	2	1	2	1	2	1	2	0	2	0	2	0	2	0	2	0	2	0	2	0	2	0
HpaI	3	2	3	1	3	0	3	1	3	3	4	0	3	1	4	0	3	2	3	1	3	0	3	1
HaeII	1	0	1	0	1	1	1	0	1	1	1	1	2	0	1	1	1	2	1	2	1	3	1	2
PstI	2	0	2	0	2	0	2	0	2	0	2	0	2	0	2	0	1	1	1	1	1	1	1	1
BglI	3	0	3	0	3	0	3	0	3	0	3	0	3	0	3	0	3	0	3	0	3	0	3	0

* Cleavage map of Pos is the same as that of *M. m. brevirostris*.

Table 3. *Sequence divergence estimated from mtDNA among five variant Robertsonian populations and six subspecies of* Mus musculus

	Robertsonian variations				M. m. subspecies						
	Zadar	Mil-II	CD	CB	dom	brv	mus(D)	mus(B)	mol	cas	bac
					Sequence divergence (%)						
Pos	1·5	0·8	1·5	1·1	0·8	0·0	1·8	4·0	3·4	6·1	7·5
Zadar	—	2·2	2·1	1·8	1·4	1·5	2·4	4·9	4·0	6·1	7·4
Mil-II	—	—	1·4	0·4	1·5	0·8	1·0	4·8	4·2	4·9	6·1
CD	—	—	—	1·0	1·4	1·5	0·3	4·5	4·0	4·6	5·9
CB	—	—	—	—	1·1	1·1	0·7	4·2	3·6	4·3	5·5

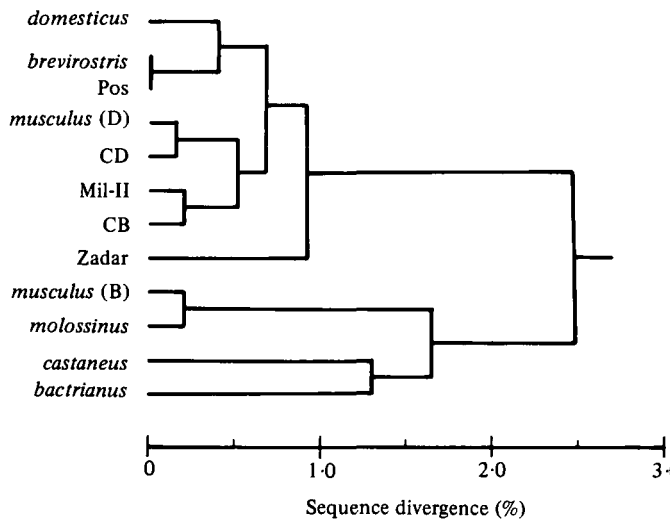

Fig. 2. Dendrogram of seven subspecies of mice and five lines of Rb variations obtained by the unweighted pair-group clustering method. The length of each branch is proportional to the value of the sequence divergence (%).

populations with Rb variations as well as *domesticus*, *brevirostris* and *musculus* (*D*), are closely related compared with the other subspecies: *bactrianus*, *castaneus*, *molossinus* and *musculus* (*B*). Among the Rb variants, the Zadar population is rather distant from the others, Pos belongs to the *domesticus-brevirostris* group, and CD, CB and Mil-II are grouped with *musculus* (*D*), as far as the mtDNAs are concerned.

(v) *A new Rb translocation obtained from the Ogasawara Islands*

We found a new Rb chromosome homozygous for the (9, 15) translocations on the Ogasawara Islands. The female mouse had a Hbb^s allele as already shown by Minezawa, Moriwaki & Kondo, (1979) and its chromosome C-bands were almost positive except for the no. 7 pairs (Plate 2). The other three individuals from the same locality exhibited positive C-bands on all autosomes. Mitochondrial DNAs, however, were completely of the *molossinus* type (Yonekawa *et al.* 1982).

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DISCUSSION

As far as our restriction analyses of mtDNAs are concerned, the various Rb stocks and populations appear to have considerable sequence divergences up to 2·2%. For instance, the value between 'Poschiavo' and 'Milan II' was computed as 0·8% (Table 3). The restriction pattern of the former was the same as that of *M. m. brevirostris*, and the latter has some similarity to that of *M. m. musculus* (Denmark) as well, whereas these two variants shared the four Rb translocations (5 . 15), (9 . 14), (11 . 13) and (16 . 17). In the same way, most of the Rb populations share some of the translocations such as (16 . 17), (9 . 14) and (5 . 15). (See the table summarized by Sage, 1981). Does this mean that they occurred prior to the subspecies differentiation, for instance, *M. m. brevirostris* and *M. m. musculus*? If so, these common Rb rearrangements should have arisen in Asia before the hypothetical migration of the *musculus* and the *domesticus-brevirostris* groups through different route to Europe (Schwarz & Schwarz, 1943; Brothwell, 1981; Thalar, Bonhomme & Britton-Davidian, 1981). However, the geographical distribution of variant Rb populations strongly suggests the spreading of Rb's from Rhaeto-Lombardia and the Apennines to the surrounding regions as expressed also in a hypothesis of the multiple single mutation events (Capanna *et al.* 1977). A typical 'stasipatric' distribution (White, 1968) of the common Rb's (8 . $\bar{1}$7) and (10 . 14) can be seen in the peripheral zones of distribution such as in the Zadar, Sicily (v. Lehman & Radbruch, 1977) and Tubingen (Adolph & Klein, 1981) populations.

Recently, Britton-Davidian and her colleagues (1980) estimated the genetic distances (D) between the Rb populations and neighbouring non-Rb populations in northern Italy by Nei's method (1972). The small D values seem to imply that most of the Rb rearrangements commonly observed in these populations should have occurred and spread in this area within a relatively shorter period. Usually, the spreading of chromosomal changes, even a single one, over Mendelian population seems to need a longer time, such as the order of 10^5 years as estimated in the case of Spalax (Nevo & Cleve, 1978). Recently, Ferris and his colleagues (1983) also stated that each Rb population may be rather young, based on the low degree of mtDNA divergence between some Rb population and karyotypically normal mice.

From a stochastic viewpoint, Birsky and his colleagues (1983) argued that if the migration rate of males is significantly higher than that of females, the fixation time for a new mutation is considerably longer in nuclear genomes than in mitochondrial genomes. In the demes of wild mice, the migration radius of a male is apparently greater than that of a female (Berry, 1970). Therefore, a genetic variation in the nuclear genome like a Rb variation might still be polymorphic in a larger area, even if mtDNA is well fixed. However, this is not the case. Rb variations are distributed in a limited area in the whole distribution of a given subspecies with a fixed mtDNA type such as the *brevirostris* type. This situation is rather unfavourable for the assumption of possible development of some Rb translocations in a common ancestor of *M. m. musculus* and *M. m. domesticus-brevirostris*.

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Considering such circumstantial evidence, it seems to be more plausible at this moment that a number of common Rb translocations now shared by many wild populations emerged in northern and central Italy probably in the order of 10^3 years as previously suggested by Thaler *et al.* (1981) and spread over the surrounding regions by accompanying human movement. Later, new and unique translocations might have been developed in special populations. This concept is supported by the cytogenetical survey of rare variants with NOR on the distal end of no. 4 chromosomes (Winking, Nielsen & Gropp, 1980). These variants were found in the Milan, Ancarano and Zadar populations which are geographically separated. In each population, moreover, the characteristic no. 4 chromosomes are involved in different combinations of Rb translocation, indicating the independent occurrence of Rb rearrangements in each population.

The greater genetic divergence between Rb variations demonstrated in the present study presumably indicates that spreading of the Rb's sometimes occurred beyond the subspecies barrier. It is noteworthy that a new Rb variant found on the Ogasawara Islands was definitely an intersubspecies hybrid which had a *molossinus*-like mtDNA and *domesticus*-like chromosome C-band pattern and Hbb^2. Recently, the 'hybrid dysgenesis' phenomena accompanied by the transposable genetic element have been investigated in Drosophila including augmented chromosome rearrangements (Kidwell, Kidwell & Sved, 1977; Thompson & Woodruff, 1978; Engels & Preston, 1981). Could not the emergence of intersubspecies hybrids be relevant to the extraordinarily rapid appearance of Rb translocations in each population?

Futhermore, almost all Rb variations so far reported seem to be related to the European wild mouse subspecies. Rb findings in India (Chakrabarti & Chakrabarti, 1977) and Marion Island (Robinson, 1978) are possibly explained by spread of European mice through commercial traffic, in particular the observation in the latter. Even in laboratory mice, a number of Rb translocations have occurred spontaneously. It is highly suggestive evidence that the majority of them are found in strains carrying another Rb metacentric originating from European wild mice (Gropp & Winking, 1981). Many intersubspecies hybrids have been observed by us in the northern part of Japan. Although the biochemical markers controlled by the nuclear genes are more similar to those of *M. m. molossinus* (Minezawa, Moriwaki & Kondo, 1980) and the chromosome C-band also showed *molossinus*-like patterns (Moriwaki *et al.* 1982), the restriction patterns of mtDNAs were the same as those of *M. m. castaneus* from Taiwan and the Philippines (Yonekawa *et al.* unpublished). No Rb translocation, however, has been observed in these hybrid populations in our survey on several hundred of individuals (Imai, Matsuda and Moriwaki, unpublished). We could not deny entirely the possibility that part of European wild mice carry a certain heritable or infectious factor intimately related to the emergence of Rb rearrangements in intersubspecies hybrids.

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