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Authors: Kawada, Shin-ichiro, Harada, Masashi, Obara, Yoshitaka, Kobayashi, Shuji, Koyasu, Kazuhiro, et al.

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Karyosystematic Analysis of Japanese Talpine Moles in the Genera *Euroscaptor* and *Mogera* (Insectivora, Talpidae)

Shin-ichiro Kawada[1]*, Masashi Harada[2], Yoshitaka Obara[3], Shuji Kobayashi[4], Kazuhiro Koyasu[5,6] and Sen-ichi Oda[1]

[1]*Laboratory of Animal Management and Resources, Graduate School of Bio-Agricultural Sciences, Nagoya University, Nagoya, Aichi 464-8601, Japan*
[2]*Laboratory Animal Center, Osaka City University Medical School, Osaka, Osaka 545-8585, Japan*
[3]*Department of Biofunctional Science, Faculty of Agriculture and Life Science, Hirosaki University, Hirosaki, Aomori 036-8561, Japan*
[4]*Department of Asian Studies, Chukyo Women's University, Obu, Aichi 474-0011, Japan*
[5]*Research Institute of Advanced Oral Science, School of Dentistry, Aichi-Gakuin University, Nagoya, Aichi 464-8650, Japan*
[6]*The Second Department of Anatomy, School of Dentistry, Aichi-Gakuin University, Nagoya, Aichi 464-8650, Japan*

ABSTRACT—A detailed analysis was done on the karyotypes of four species of mole in the genera *Euroscaptor* and *Mogera* using a G-banding technique. All four species examined had a chromosome number of 2n = 36, as reported previously. *Euroscaptor mizura* and *M. wogura* from Aichi Prefecture had almost the same chromosome constitution and G-banding patterns. These common karyotypes were considered to be equivalent to the hypothetical ancestral karyotype of *Mogera*, since two distinct genera derived from an ancestor share G-banding homologies. According to this hypothesis, the karyotype of *M. imaizumii* might have been derived from the ancestral karyotype through pericentric inversion in one pair of acrocentrics, chromosome 11. Two geographically isolated populations of *M. tokudae* had different karyotypes: the Echigo population had the same karyotype as *M. imaizumii*, whereas the Sado population had a derivative karyotype that was able to be explained by pericentric inversions in three pairs of acrocentrics, remaining chromosome 11 as subtelocentric. *Mogera wogura* from South Korea differed considerably in terms of chromosome constitution from the Japanese population of *M. wogura*. However, we deduced that pericentric and paracentric inversions in four pairs of acrocentrics would explain the karyotype differences between the Japanese and Korean populations. Furthermore, from the morphology of chromosome 11, we can conclude that the Korean mole was derived from primitive *M. wogura* through four inversions, quite separately from the lineage of *M. imaizumii* and *M. tokudae*. Thus, inversion rearrangements appear to have played a major role in the chromosomal evolution of Japanese talpine moles.

Key words: talpine mole, *Mogera*, karyotype, G-banding, inversion

INTRODUCTION

Since the first description of the Japanese talpine mole *Talpa wogura* by Temminck (1842), Japanese moles that belong to the subfamily Talpinae have been variously classified by several Japanese and non-Japanese taxonomists. Such analysis has yielded two radically different classifications, namely, classification into one species *Talpa micrura* at one extreme (Schwartz, 1948) and classification into nearly ten species at the other (Imaizumi 1960, 1970; Hutterer, 1993). After repeated changes in the classification of talpine moles, Abe (1995) and Motokawa and Abe (1996) tried to bring some order to the inconsistent systems for classification of Japanese moles on the basis of morphological characteristics, while

* Corresponding author: Tel. +81-52-789-4179;
FAX. +81-52-789-4179.
E-mail: i981103d@mbox.media.nagoya-u.ac.jp

leaving some problems unresolved. In the new classification, the Japanese moles belonging to *Mogera* were classified into three species, *M. wogura*, *M. imaizumii* and *M. tokudae* (Motokawa and Abe, 1996). Moreover, Abe (1995) regarded the moles distributing from Korean Peninsula to Russian Far East as geographic variants of the large Japanese mole *M. wogura*, even though these moles had long been considered to represent a different species from *M. wogura* (Stroganov, 1948; Yudin, 1989; Gorman and Stone, 1990). The new classification system has recently been evaluated and found to be compatible with the molecular phylogenetic relationships inferred from analyses of sequences of mitochondrial genes for CO1 and cytochrome *b* (Okamoto, 1999; Tsuchiya *et al.*, 2000).

Cytogenetic and chromosomal investigations, both in animals and in plants, have provided exceedingly valuable information for estimations of genetic and systematic relationships among closely related taxa since chromosomal changes often play an important role in the development of reproductive isolation (White, 1978; King, 1993). To date, conventional karyotypes have been reported for most of this taxon, both domestic and foreign, as reviewed by Tsuchiya (1985), but chromosome banding analysis has been applied to only a few talpid species (Yates *et al.*, 1976; Hamada and Yosida, 1980; Capanna, 1981; Yates and Pedersen, 1982; Zima, 1983; Jiménez *et al.*, 1984a, b, 1988; Yates and Moore, 1990; Kawada and Obara, 1999, Harada *et al.*, 2001). To our knowledge, no karyotypes of *Euroscaptor* have been published after either in conventional staining or differential staining. With respect to Japanese moles, Tsuchiya (1985, 1988) reported the chromosome numbers and conventional karyotypes of *Euroscaptor* and *Mogera*, including Korean samples. He classified the karyotypes of the Japanese moles into five groups based on the number of pairs of acrocentric chromosomes: the 8 acrocentric pair-type (8A-type) of *E. mizura* and *M. wogura* from Japan (cited as *M. kobeae* in Tsuchiya 1988); the 7A-type of *M. imaizumii* (cited as *M. wogura*, ibid.), the 6A-type of *M. imaizumii* from Wakayama Prefecture (cited as *M.* sp., ibid.), the 5A-type of *M. wogura* from Korea (cited as *M. coreana*, ibid.) and the 4A-type of *M. tokudae*. Tsuchiya (1988) proposed, referring to data from conventional staining exclusively, that mainly pericentric inversions might have been involved in the evolution of chromosomes in Japanese Talpinae. However, no objective confirmation of this hypothesis has been provided to date.

In this report, we describe the G-banded karyotypes of four species of moles in the genera *Euroscaptor* and *Mogera* and an examination of their karyological relationships, with special attention if rearrangements in acrocentric chromosomes provide evidence in support of Tsuchiya's proposal (Tsuchiya, 1988).

MATERIALS AND METHODS

A total of 36 specimens from four species of mole, namely, *Euroscaptor mizura*, *Mogera wogura*, *M. imaizumii* and *M. tokudae*, were captured in mole traps or pitfall traps at various localities in Honshu (Japan) and Korea (Fig. 1, Appendix 1). Species were morphologically identified on the basis of cranial characteristics and external body dimensions, according to Abe (1998). Species designations were also made according to Abe (1998) and a mole from Korea was designated *M. wogura* according to Abe (1995). Even in sympatric populations, such as those of *M. wogura* and *M. imaizumii*, species were correctly identified on the basis of a significant difference in body size. Details of the specimens examined are summarized in Table 1, together with abbreviations of the species and/or populations examined. For convenience, names of species were abbreviated as follows: *E. mizura*, EMI; *M. wogura* from Aichi Prefecture, MWO-A; *M. imaizumii*, MIM; *M. tokudae* from the Echigo Plain and Sado Island in Niigata Prefecture, MTO-E and MTO-S, respective-

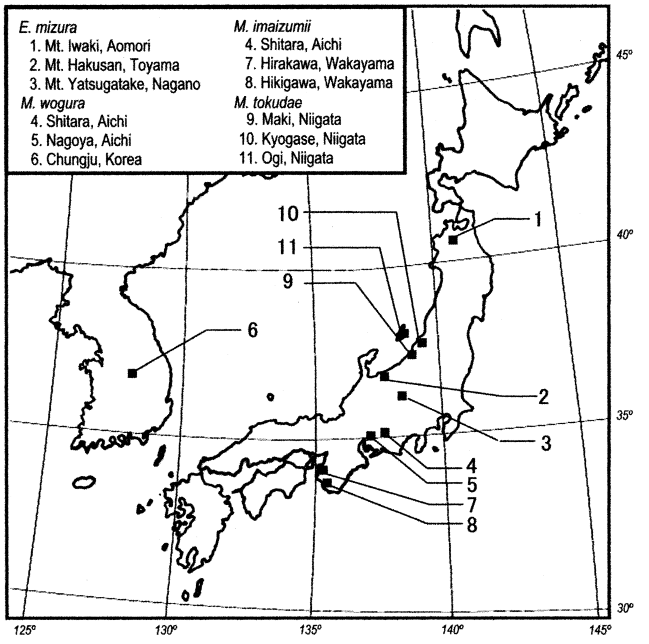

Fig. 1. Collection localities of talpine moles examined in this study.

Table 1. Species, abbreviations, specimens and sites of collection of the four species of mole examined.

Species	Abbreviation	Specimens examined	Site of collection
Euroscaptor mizura	EMI	1♀2♂	Mt. Iwaki, Aomori Pref.; Mt. Hakusan, Toyama Pref.; Mt. Yatsugatake, Nagano Pref.; Japan
Mogera wogura	MWO-A	9♀4♂	Shitara & Nagoya, Aichi Pref., Japan
M. wogura	MWO-K	1♂	Chungju, Chungchongbuk-Do, Korea
M. imaizumii	MIM	5♀3♂	Shitara, Aichi Pref., Japan
M. imaizumii	MIM	3♀2♂	Hirakawa & Hikigawa, Wakayama Pref., Japan (Kii Peninsula)
M. tokudae	MTO-E	3♀1♂	Maki & Kyogase, Niigata Pref., Japan (Echigo Plain)
M. tokudae	MTO-S	2♂	Ogi, Niigata Pref., Japan (Sado Island)

ly; and *M. wogura* from Korea, MWO-K (Table 1).

Chromosome preparations were made from cultured fibroblasts, bone marrow cells or splenocytes by the conventional air-drying method. Karyotypes were analyzed basically according to the arrangement of the chromosomes of the large Japanese mole *M. wogura* (formerly *M. kobeae*) described by Tsuchiya (1988). We used the ASG method of Sumner *et al.* (1971) for G-banding. Chromosome morphology was determined according to Levan *et al.* (1964) as following categories: metacentric, M; submetacentric, SM; subtelocentric, ST; and acrocentric, A. The constitution of autosomal pairs was expressed as follows (with the autosomal chromosomes of *E. mizura* as the example): 4M + 3SM + 2ST + 8A.

RESULTS

For each species or population, we analyzed the karyotype of at least seven metaphases with conventional staining and nine metaphases with differential staining. No significant variations were observed after both differential and conventional staining. Figures 2a through 2e show the conventional

Fig. 2. Conventional karyotypes of *Euroscaptor* and *Mogera*. (a) *E. mizura* (male), (b) *M. wogura* from Japan (female), (c) *M. imaizumii* (female), (d) *M. tokudae* from Sado Island (male), and (e) *M. wogura* from Korea (male). Arrowheads and asterisks indicate secondary constrictions and crossing of chromosomes, respectively.

Table 2. The autosomal constitutions and sex chromosomes of the talpine moles examined.

Species (population)	2n	Fn	Autosome constitution				Sex chromosomes	
			M	SM	ST	A	X	Y
E. mizura	36	52	4	3	2	8	SM	dot
M. wogura (Aichi)	36	52	4	3	2	8	SM	dot
M. wogura (Korea)	36	58	5	4	3	5	SM	dot
M. imaizumii (Aichi)	36	54	4	3	3	7	SM	dot
M. imaizumii (Kii)	36	54	4	3	3	7	SM	dot
M. tokudae (Echigo)	36	54	4	3	3	7	SM	dot
M. tokudae (Sado)	36	60	5	3	5	4	SM	dot

Fn, Fundamental number; M, metacentric; SM, submetacentric; ST, subtelocentric; A, acrocentric; X, X chromosome; Y, Y chromosome; dot, dot-like minute chromosome.

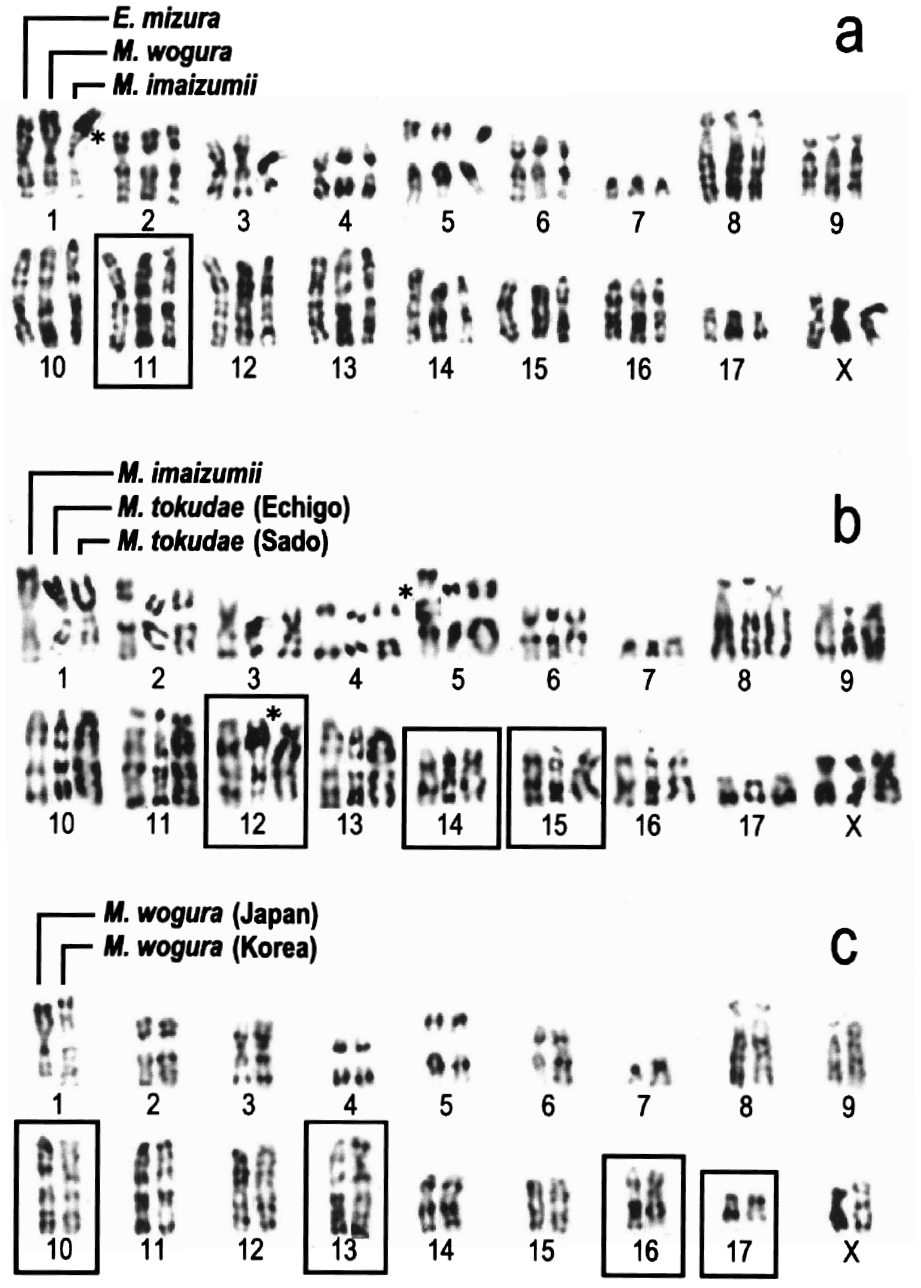

Fig. 3. Composite karyotypes after G-banding. (a) *E. mizura*, *M. wogura* and *M. imaizumii*, (left, center and right), (b) *M. imaizumii* and two local populations of *M. tokudae*, and (c) *M. wogura* from Japan and Korea. Boxes enclose chromosomes whose differences can be explained by inversion. Asterisks indicate crossing of chromosomes.

karyotypes of *Euroscaptor mizura* (EMI), *Mogera wogura* from Aichi Prefecture (MWO-A), *M. imaizumii* (MIM), *M. tokudae* from Sado Island (MTO-S) and *M. wogura* from Korea (MWO-K), respectively. The chromosome number was 2n=36 in all four species examined, as reported previously (Tsuchiya, 1985, 1988), and the sex chromosomes, a medium-sized submetacentric X chromosome and a dot-like minute Y chromosome, were also similar in size and morphology in all four species. However, the patterns of autosomal chromosomes were rather complex, as summarized in Table 2: EMI and MWO-A had the same autosomal constitution, namely, 4M + 3SM + 2ST + 8A, despite the fact that they were two different species belonging to distinct genera. Furthermore, *M. wogura* exhibited chromosomal polymorphism, which was characterized by two cytotypes: 4M + 3SM + 2ST + 8A in MWO-A and 5M + 4SM + 3ST + 5A in MWO-K. Similarly, *M. tokudae* also had two cytotypes: 4M + 3SM + 3ST + 7A in MTO from the Echigo Plain in Niigata Prefecture (MTO-E) and 5M + 3SM + 5ST + 4A in MTO-S. The populations of MIM from Aichi Prefecture and the Kii Peninsula had the same autosomal constitution, 4M + 3SM + 3ST + 7A, and they exhibited no chromosomal polymorphism even though the two geographic locations are apparently isolated from each other (Abe, 1995). Thus, MIM and MTO-E also had the same autosomal constitution, 4M + 3SM + 3ST + 7A, despite the fact that the species were distinct.

In all species examined, the SM1 chromosome had a typical secondary constriction on the proximal region of its short arm (Fig. 2, arrowheads), as has been noted in all other species of Talpidae described to date (Tsuchiya, 1985). The secondary constriction was intensely stained by Ag-NOR staining, which is known to reveal the nucleolar organizing region (data not shown). Our data are generally in accord with those in the previous report of Tsuchiya (1988) except in the case of MTO-E and MIM from the Kii Peninsula, in which Tsuchiya found the autosomes 5M + 3SM + 5ST + 4A and 4M + 4SM + 3ST + 6A, respectively. We have no persuasive explanation for these discrepancies.

Figure 3a shows pair-matching analysis, by side-by-side arrangement, of the EMI, MWO-A and MIM chromosomes, which was performed by reference to G-band homologies. All chromosomes apart from chromosome 11 clearly revealed a high degree of G-band homology among the three species. In chromosome 11, the morphological discrepancy, namely an acrocentric 11 in EMI and MWO-A and a subtelocentric 11 in MIM, can be explained by pericentric inversion (Fig. 4a). As clearly shown in the pair-matching analysis in Fig. 3b, MIM and MTO-E exhibited almost perfect G-band homology for all chromosomes, whereas the G-banding patterns of chromosomes 12, 14 and 15 of MTO-S differed from those of MIM and MTO-E. These differences in chromosome morphology can also be explained by pericentric inversion (Figs. 3b and 4b). Chromosome 11 was subtelocentric in MTO-E and MTO-S, as well as in MIM. Fig. 3c shows the pair-matching of G-banded chromosomes of MWO-A and MWO-K, which are the same species but inhabit geographically isolated areas, namely, Japan and Korea. The pair-matching analysis revealed that the karyotypes of MWO-A and MWO-K would be identical to each other if the chromosomes were rearranged via three pericentric inversions (chromosomes 10, 13 and 16) and one

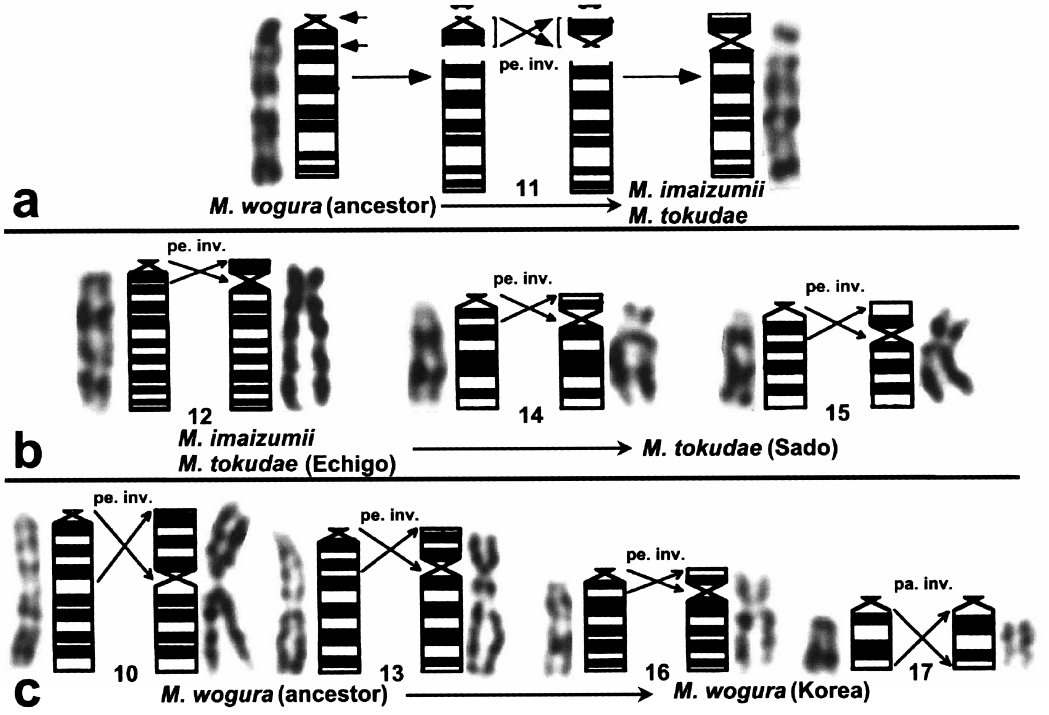

Fig. 4. Schematic representation of evolutionary processes by inversion rearrangements in chromosome 11 (a), in chromosomes 12, 14 and 15 (b) and in chromosomes 10, 13, 16 and 17 (c). pe., pericentric; pa., paracentric; inv., inversion.

paracentric inversion (chromosome 17). Chromosome 11 of MWO-K was acrocentric, having the same morphology as chromosome 11 of EMI and MWO-A but not of MIM and MTO. In MIM and MTO, chromosome 11 was subtelocentric.

DISCUSSION

Morphological and molecular analyses have firmly established that *Euroscaptor* is the most primitive genus of Japanese talpines (Imaizumi, 1955, 1960; Abe, 1967; Okamoto, 1999; Tsuchiya *et al.*, 2000). Thus, *E. mizura* can be regarded as a reasonable outgroup in this study. In addition to this recognition, since the respective chromosomes of EMI and MWO-A had identical G-banding patterns (Fig. 3a), it is considered that these two species derived from an ancestor without any chromosomal modification. Therefore, the karyotype shared by EMI and MWO-A, represented by the autosomal constitution 4M + 3SM + 2ST + 8A, can be cladistically regarded as equivalent to the hypothetical ancestral karyotype of Japanese talpine moles. It might also be reasonable to suggest, in view of the nature of chromosome 11, that MIM and MTO-E are derivatives of this ancestral karyotype. Furthermore, it is very likely that MIM was derived first from MWO-A via pericentric inversion of chromosome 11, with rearrangement from acrocentric to subtelocentric and subsequently MIM differentiated to MTO-E. Where this event occurred remained in conjecture, but it was most likely around Echigo Plain, supposed from the restricted distribution of MTO-E (Abe, 1998). However, Abe (1967) proposed that MTO might have speciated from the primitive *M. imaizumii* (cited as *M. wogura* in Abe, 1967) on Sado Island, and that the Echigo population was considered as a secondary invader from Sado Island. Then, maintaining the subtelocentric chromosome 11 in its present configuration, MTO-S might have experienced pericentric inversions in chromosomes 12, 14 and 15, wherefrom new autosomal constitution, 5M + 3SM + 5ST + 4A, was achieved. Thus, MIM, MTO-E and MTO-S can be regarded as representatives of a lineage that is characterized by a subtelocentric chromosome 11. By contrast, MWO-K carries the ancestral acrocentric type of chromosome 11. If we accept that MWO-A is derived from EMI, it then appears that MWO-K has maintained an acrocentric chromosome 11 in contrast to MIM-MTO lineage, and additionally experienced three pericentric inversions in chromosomes 10, 13 and 16 and one paracentric inversion in chromosome 17 (Figs. 3c and 4c). Summarizing these findings, we propose the cladistic relationship among talpine moles that is shown in Fig. 5, which includes a schematic representation of G-banded acrocentric autosomes. The karyological kinship between MIM and MTO is consistent with the molecular phylogenetic relationships derived from restriction fragment length polymorphism (RFLP) analysis of nuclear ribosomal and mitochon-

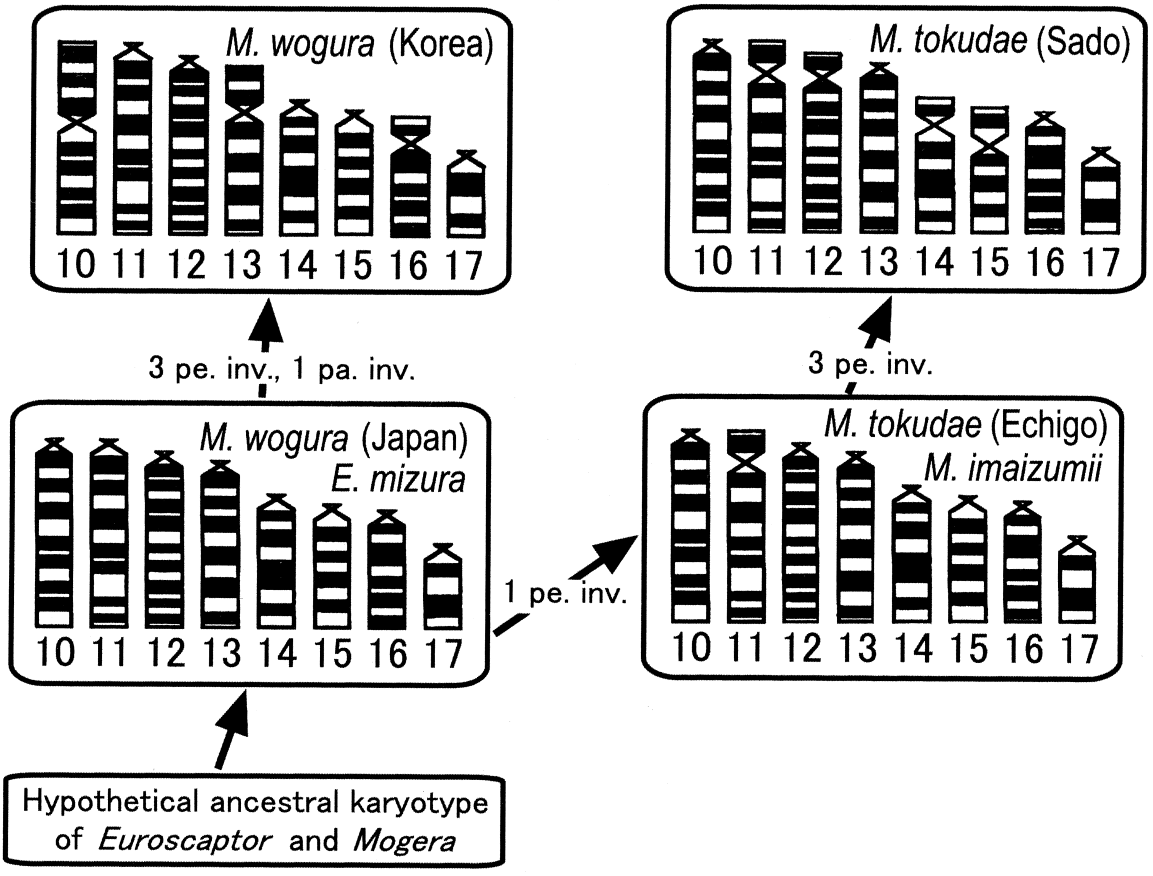

Fig. 5. Karyosystematic relationships among the talpine moles examined in this study. pe., pericentric; pa., paracentric; inv., inversion.

drial DNA (Suzuki, 1994, personal communication), but it is inconsistent with the cladistic relationship based on the nucleotide sequences of mitochondrial genes for CO1 and cytochrome *b* (Okamoto, 1999; Tsuchiya *et al*., 2000). It remains to be determined why these two types of molecular analysis lead to inconsistent conclusions. The chromosomes of MWO-K probably differentiated, separately from those in the MIM-MTO lineage, from those in the ancestral population of MWO-A. Such a possibility for the chromosomal derivation of MWO-K is compatible with the molecular findings reported by Tsuchiya *et al*. (2000), who found that the moles from Korea and Russian Far East are included in the same cluster as MWO-A. Moreover, according to Kryukov (unpublished data), the talpine mole from Russian Far East had almost the same karyotype as the Korean moles. Some authors have assumed that continental *Mogera* consists of two distinct species, namely, *M. wogura* (or *M. coreana*) and *M. robusta* (Stroganov, 1948; Tsuchiya, 1988; Yudin, 1989; Hutterer, 1993) from morphological standpoint, but these taxonomic dichotomy of two continental species might merely reflect local variations in body size, as pointed out by Abe (1995).

From a morphological perspective, Yoshiyuki and Imaizumi (1991) regarded MTO-E as a distinct species, *M. etigo*, and recent statistical analysis of skull measurements supports this designation (Yokohata, personal communication). Our chromosomal analysis also revealed extensive chromosomal variations between MTO-E and MTO-S, namely, 4M + 3SM + 3ST + 7A in MTO-E and 5M + 3SM + 5ST + 4A in MTO-S, suggesting the possibility of reproductive isolation between MTO-E and MTO-S as validities of both species. However, the most recent analysis of variations in the nucleotide sequences of the mitochondrial genes (Okamoto, 1999; Tsuchiya *et al*., 2000) revealed that MTO-E and MTO-S are closely related to each other with minor variations at an interpopulation level. Thus, there are, at least at present, certain inconsistencies between molecular data and chromosomal and/or morphological data with respect to the taxonomy of MTO-E and MTO-S. There is no question, however, in view of the molecular findings that the karyological differentiation of MTO-S from MTO-E occurred rather recently and reflects the reproductive isolation of the two populations.

It is well known that Robertsonian fusion/fission, reciprocal translocation, inversion and quantitative variations in C-heterochromatin have played major roles in the chromosomal evolution of mammals. For example, major rearrangements in the genus *Mus* and the common shrew *Sorex araneus* represent Robertosonian events (e. g., Capanna *et al*., 1976; Searle, 1986) and in mustelid carnivores they represent Robertosonian events and quantitative variations in C-heterochromatin (e. g., Obara, 1982, 1985a, b, 1987a, b). Gropp (1969) suggested that pericentric inversions, as well as translocations, were probably important in the karyotypic evolution of Talpidae, and Kratochvíl and Král (1972) proposed that decreases in numbers of acrocentric chromosomes in the genus *Talpa* are probably due to complicated rearrangements that include Robertsonian fusion/fission and inversions. Yates and Moore (1990) examined North American species of moles and their chromosomal analysis supported Gropp's suggestion. In an examination of the chromosomal relationship between the Japanese species of shrew-mole *Dymecodon pilirostris* and *Urotrichus talpoides*, Kawada and Obara (1999) found the evidence of pericentric inversion and duplications of heterochromatin. In the present study of Japanese talpine moles, we confirmed, from an analysis of G-banding, the exclusive involvement of inversions in interpopulational and interspecific chromosomal differentiation, which calls to mind the principle of "karyotypic orthoselection" proposed by White (1975). Therefore, we can infer that inversion rearrangements have played a major role in the chromosomal evolution of Japanese talpine moles.

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APPENDIX 1.

The code numbers of used materials.

Euroscaptor mizura: no numbered specimen of Shuji Kobayashi's collection , MH7100, 96Em-1.

Mogera wogura: SIK0001, SIK0002, SIK0003, SIK0004, SIK0008, SIK0063, SIK0064, SIK0066, SIK0129, SIK0130, SIK0140, SIK0148, SIK0338, MH7086.

M. imaizumii: SIK0017, SIK0019, SIK0027, SIK0036, SIK0097, SIK0132, SIK0145, SIK0146, SIK0157, SIK0168, SIK0219, SIK0220, SIK0221.

M. tokudae: SIK0195, SIK0196, SIK0308, SIK0309, SIK0202, SIK0203.