

## Karyological study of the Malaysian mole, *Euroscaptor micrura malayana* (Insectivora, Talpidae) from Cameron Highlands, Peninsular Malaysia

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**Abstract.** We report the first karyological description of a southeast Asian mole, the Malaysian mole (*Euroscaptor micrura malayana*). The karyotype of the Malaysian mole includes 36 chromosomes, which consist of 18 biarmed and 16 acrocentric autosomes and the sex pair. The sex chromosomes are a small meta-submetacentric X chromosome and a minute dot-like Y chromosome, although the latter is somewhat larger than that of some talpid allies. Autosomal complements include one pair of NOR-bearing chromosomes. A comparative G-banding analysis with the Japanese congener *E. mizura* showed that these two species share high G-banding homology, and their differences on two pairs of chromosomes are explained by a single reciprocal translocation. The karyological similarity of these distant geographic species is discussed in a systematic and evolutionary context, based on comparisons to other species distributed between them.

**Key words:** *Euroscaptor micrura malayana*, evolution of Asian moles, G-band, Malaysian mole, reciprocal translocation.

The moles distributed in southeastern Asia had been classified in different genera, including *Talpa*, *Euroscaptor*, *Eoscalops*, and *Parascaptor*, and as different species (reviewed in Hutterer 1993). Schwartz (1948) stated that all of these moles were subspecies of a single species, *Talpa micrura*. However, subsequent taxonomic studies (Stroganov 1948; Ellerman and Morrison-Scott 1951; Corbet and Hill 1991) described distinct species from this area. These studies distinguished Asian moles from the European genus *Talpa* and classified them in the genus *Euroscaptor*.

Peninsular Malaysia represents the southern limits of talpid distribution (Corbet and Hill 1991). Chasen (1940) first described the moles living in the Malaysian Highlands. These specimens were collected in the Cameron Highlands of Pahang State. Based on its external mor-

phological characters, the Malaysian mole has been treated as a subspecies of the Himalayan mole, *Euroscaptor micrura* (Cranbrook 1962; Cranbrook and Medway 1962), or Kloss's mole, *E. klossi* (Chasen 1940; Yoshiyuki 1988; Hutterer 1993). Recently, Kawada et al. (2003) conducted a collection survey in the same locality and suggested that the preliminary morphological traits of the skull and external characters of the Malaysian mole were more similar to its Himalayan ally, supporting the opinion of Cranbrook (1962). To determine the systematic position of the Malaysian mole, a phylogenetic examination of southeast Asian moles is necessary (Kawada et al. 2003).

When considering the systematic and evolutionary relationships of mammals, karyological information is as informative as molecular data and can provide alterna-

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tive evolutionary hypotheses for mammalian taxa. It is considered that the change of chromosomal morphology can bring the hybrid sterility by the postmating isolation mechanism (White 1968; King 1991). However, karyological information on East Asian moles is restricted to Chinese (Kawada et al. 2002b) and Taiwanese (Lin et al. 2002) moles, for which karyotypes have been described using Giemsa staining. Kawada et al. (2001) summarized the karyosystematic relationships of four species from two genera, *Euroscaptor* and *Mogera*, from the Japanese islands and the Korean Peninsula based on the G-banded karyotypes. These studies showed that each Asian mole species had a distinct karyotype characterized by chromosome number (2n) and autosomal fundamental number (NFa). These traits are maintained in comparison with European and American mole species (summarized by Yates and Moore 1990; Kawada et al. 2002a, 2002b).

Of the four species discussed by Kawada et al. (2001), the Japanese mountain mole, *E. mizura*, has many kin species in southeastern Asia (Ellerman and Morrison-Scott 1951; Corbet and Hill 1991). The distribution of *Euroscaptor* is centered in the Himalayas and southwestern China, and gaps separate this central area from the Malayan and Japanese mountains. In this sense, the Malaysian and Japanese mountain moles are two very isolated populations of *Euroscaptor*. A recent molecular phylogenetic study of these two species of *Euroscaptor* showed a relatively high genetic distance between them (Shinohara et al. 2004). This means that the two species of *Euroscaptor* have presumably been isolated for a long time. Furthermore, the distributions of *Euroscaptor* are patchy in this area, so the genus may be a relic species group. Comparative studies of representative *Euroscaptor* from both Japan and Malaysia will provide new insight into the evolution of this group. In this study, we determined karyotypes of the Malaysian mole by differential staining. We discuss the systematic relationships of Asian moles and speculate on their evolution based on a karyological study of *Euroscaptor* and *Mogera*.

## Materials and methods

We examined nine Malaysian moles (Specimens SIK0550 to SIK0558) collected in the BOH Estate, Cameron Highlands, Pahang, Malaysia, between 9 and 13 January 2002. The collecting locality and specimen data have been reported elsewhere (Kawada et al. 2003). Specific identification was made using the external mor-

phology, skull dimensions, and the shape of the auditory ossicles, as described in previous studies (Stroganov 1945; Cranbrook 1962; Kawada et al. 2003). The specimens were deposited in the Highland Animal Experimental Station (HAES), Graduate School of Bioagricultural Sciences, Nagoya University, Japan. Half of the specimens will be kept at the museum of the Department of Wildlife and National Parks, Kuala Lumpur, Malaysia.

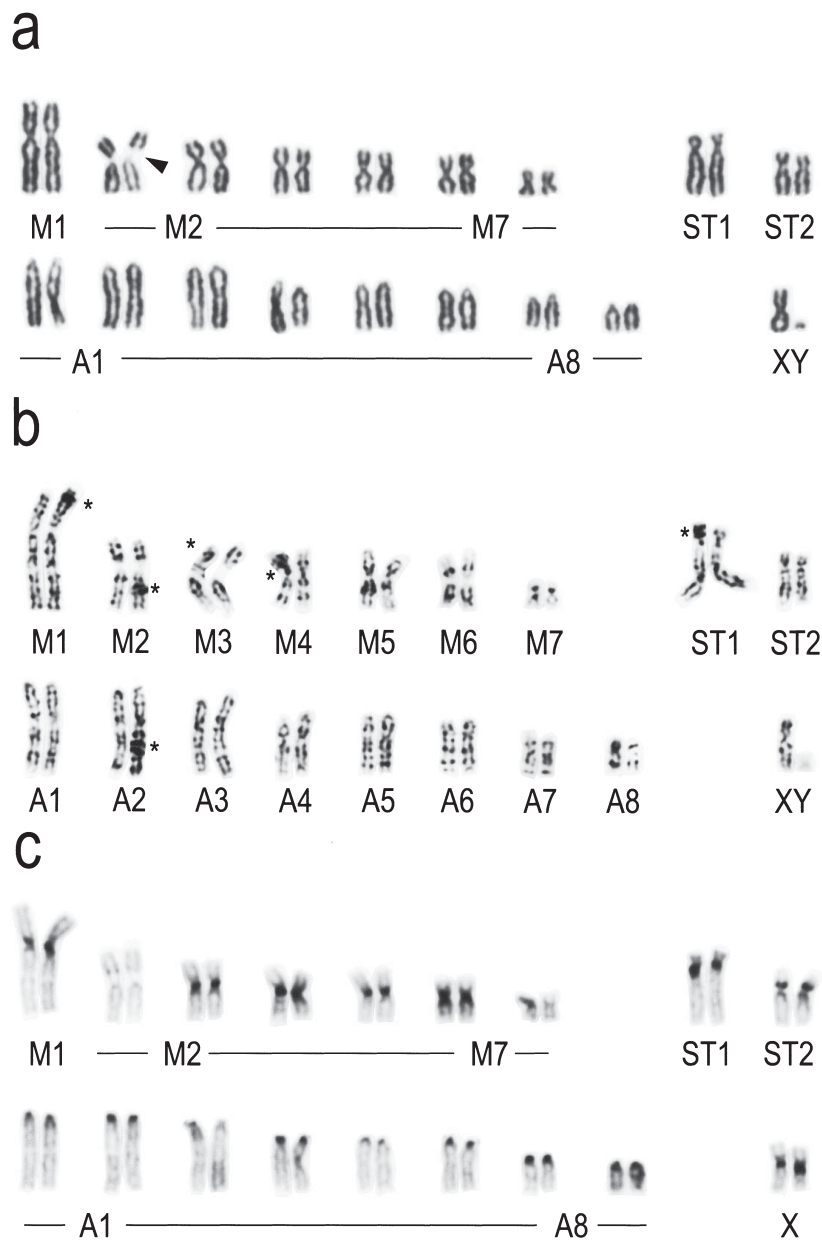
Bone marrow cells from the collected animals were cultured and fixed using standard procedures and a manual centrifugal manipulator at the collecting locality. Skin tissue samples were also collected, brought to HAES, and cultured. Giemsa-stained chromosomes were categorized as meta-submetacentric (M), subtelocentric (ST), and acrocentric (A) chromosomes based on Levan et al. (1963). Fixed chromosomes of four specimens were subjected to differential staining. G- and C-banding were performed according to the ASG method of Sumner et al. (1971) and the BSG method of Sumner (1972), respectively. For the comparative G-banding analysis, we referred to the G-banded karyotype of the Japanese mountain mole, *E. mizura* (Kawada et al. 2001).

## Results

### *Karyological description of the Malaysian mole*

All nine specimens of Malaysian mole had 36 chromosome components. A conventional Giemsa-stained karyotype is shown in Fig. 1a. The karyotype consisted of 18 banded and 16 acrocentric autosomes and sex pair. Autosome number M1 was the largest meta-submetacentric chromosome pair, and number M2 had a secondary constriction on the proximal short arm, which appeared as a gap with Giemsa staining. The next five pairs (M3–M7) ranged from large to small metacentrics. Chromosomes ST1 and ST2 were subtelocentric pairs, distinguished from each other by their size. The subsequent autosomes (A1–A8) were acrocentric pairs of gradually decreasing size, although the first three pairs were similar large acrocentrics. In male specimens, the sex chromosome pair consisted of a metacentric X chromosome, which was similar to chromosomes M4 to M6, while the Y chromosome was a minute dot, although it was somewhat larger than that of other talpid species described previously.

The G- and C-banded karyotypes are shown in Fig. 1b and 1c, respectively. Each chromosome pair was distinguished by the peculiar arrangement of the G-bands and



**Fig. 1.** Conventional (a), G-banded (b) and C-banded (c) karyotypes of the Malaysian mole, *Euroscaptor micrura malayana*. Asterisks and arrowhead mean crossings of chromosomes and secondary constriction, respectively. In the conventional and C-banded karyotypes, chromosome number M2 to M7 and A1 to A8 were not identifiable, thus unnumbered.

numbered. C-bands were localized in the centromeric position of each chromosome, although the centromeric to proximal short arm of chromosome M2 was not stained.

*Comparative G-banding analysis of the Malaysian and Japanese mountain moles*

The G-banded karyotypes of the Malaysian mole and Japanese mountain mole were compared. The composite G-band karyotype of the two species is shown in Fig. 2.

Most of the autosome pairs and the sex chromosomes of the two species shared identical G-banding patterns throughout the lengths of the chromosomes. Chromosomes M1 and A7 in the Malaysian mole had homologs in the Japanese mountain mole chromosomes 1 and 13. Each arm of M1 and A7 of the Malaysian mole matched the chromosome of the other species. The arm combinations of the two chromosomes in the two species are shown in Fig. 3. The short (p) and long (q) arms of the Malaysian mole M1 shared the same G-banding pattern

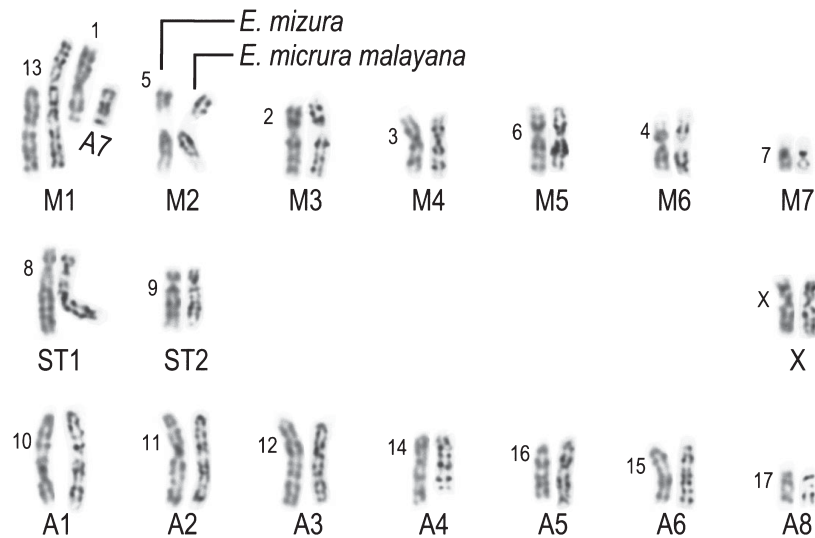


Fig. 2. Composite G-banded karyotype of the Malaysian mole and the Japanese mountain mole, *E. mizura*.

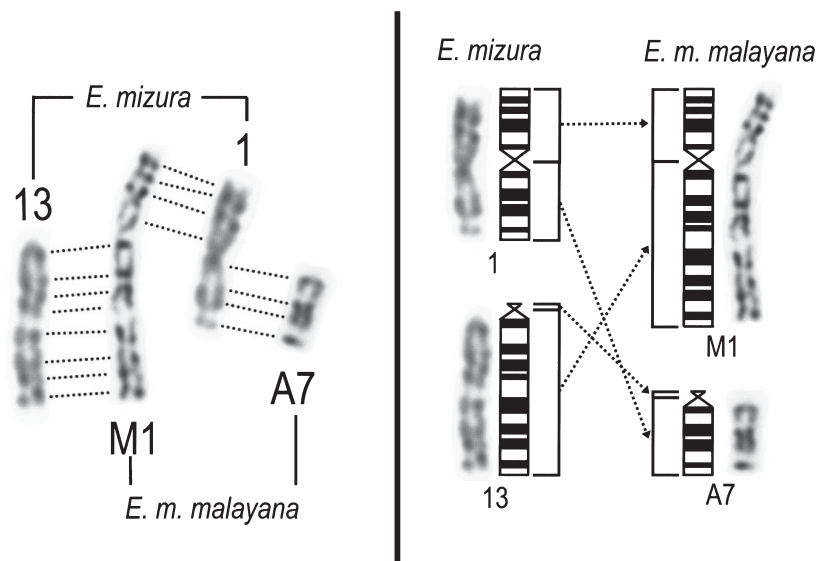


Fig. 3. Summary of the G-band homology of chromosomes M1 and A7 of the Malaysian mole and chromosomes 1 and 13 of the Japanese mountain mole. Dot lines and arrows mean identical bands and chromosomal arms between species, respectively.

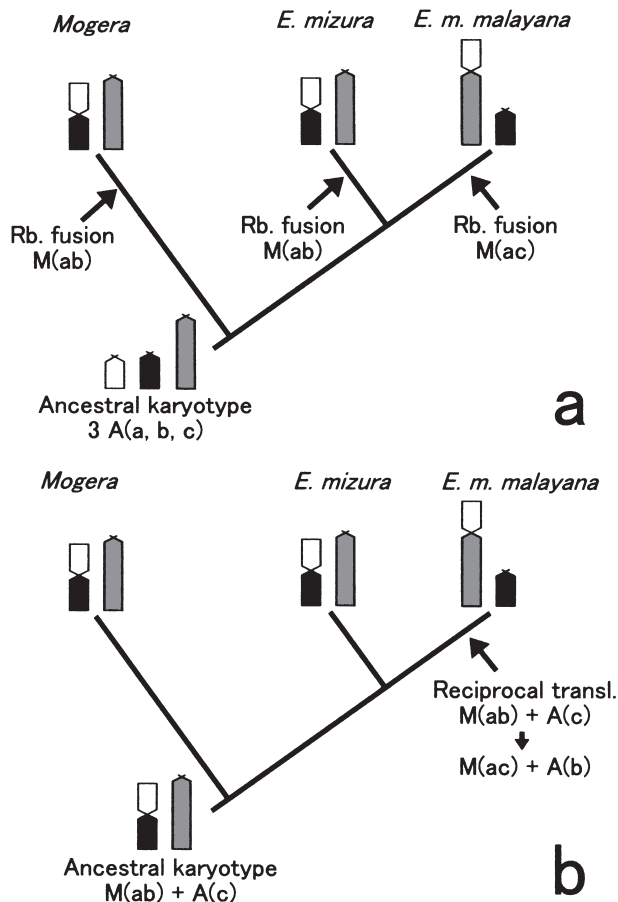
as the Japanese mountain mole 1p and 13q, respectively. Conversely, the G-banding pattern of the Malaysian mole A7q matched the Japanese mountain mole 1q (Fig. 3).

**Discussion**

*Karyological relationship between the Malaysian and Japanese mountain moles*

The chromosome number in the Malaysian mole was  $2n = 36$ , which is the same as in Japanese mole species belonging to the genera *Euroscaptor* and *Mogera* (Tsuchiya 1988; Kawada et al. 2001). Examining the

composite karyotype, two pairs of chromosomes had different banding patterns, although they showed monobrachial homology (Fig. 2). In general acceptance as a most common chromosomal rearrangement (King 1993), such monobrachial homology can be the result of different Robertsonian fusions becoming fixed in each species from an ancestor with three acrocentrics (Baker and Bickham 1986). According to Kawada et al. (2001), the Japanese mountain mole has the ancestral karyotype of Japanese mole species, including *Mogera*. The large Japanese mole *M. wogura*, has a G-banding pattern identical to that of the Japanese mountain mole. Given a



**Fig. 4.** Alternative hypotheses explaining the G-band homologies observed in the two species of *Euroscaptor*. Robertsonian (Rb.) rearrangement (a) explains the kinship of the genera *Euroscaptor* and *Mogera* less parsimoniously than does the reciprocal translocation (Recip. transl.) hypothesis (b).

Robertsonian fusion in this situation, it is difficult to determine the phylogenetic relationships of these two species in different genera, *Euroscaptor* and *Mogera* (Fig. 4a).

As another clue to their karyological relationships, the one-to-one correspondences of arm combinations are well explained by a reciprocal translocation (Fig. 4b). The short and long arms of the two respective chromosomes in the karyotype of the Japanese mountain mole were exchanged to make the arm combinations in the Malaysian mole. According to this explanation, the break points of each chromosome are positioned very near the centromeric region. Since the A chromosomes have a small short arm, this chromosomal change involved four chromosome arms (Fig. 3).

Although we observed some minor differences between the two *Euroscaptor* species from Malaysia and Japan, they were similar in that the chromosome number

of both was  $2n = 36$  and both karyotypes included eight A chromosome pairs. The G-band patterns of *E. micrura malayana* had extensive homology to *E. mizura*. It seems odd that these two congeneric mole species from Malaysia and Japan share a similar karyological profile, considering their disjunct distributions. In a recent molecular phylogenetic study, Shinohara et al. (2004) found that a large genetic distance separated Malaysian and Japanese *Euroscaptor*. Moreover, Imaizumi (1998) proposed that the Japanese mountain mole should not be included in the genus *Euroscaptor* because of its unique skull shape. We think that the genetic and morphological differences between the two species reflect their long geographic isolation, while the karyological similarity between them implies that the Malaysian and Japanese *Euroscaptor* diverged without undergoing drastic chromosomal changes.

#### Systematic implications for Asian mole species

The chromosome numbers of Asian mole species vary with their distribution. The Siberian mole, *Talpa altaica*, found throughout Siberia, has  $2n = 34$  comprised by all biarmed autosomes and X chromosome (Kawada et al. 2002a), whereas the karyotypes of Far Eastern species of *Euroscaptor* and *Mogera* include several pairs of A chromosomes. The Japanese mountain mole (*E. mizura*) and most of the genus *Mogera* from Japan and Korea (*M. wogura*, *M. imaizumii*, and *M. tokudae*) have a diploid number of  $2n = 36$ , with different NFA, suggesting that the interspecific variation in NFA was caused by sequential pericentric inversions (Kawada et al. 2001). Within the genus *Mogera*, the Taiwanese mole, *M. insularis*, has a unique diploid number of  $2n = 32$ , and the karyosystematic positioning of this species awaits further chromosome banding studies (Lin et al. 2002). The greatest chromosome number was reported for the short-faced mole, *Scaptochirus moschatus*, which has 48 chromosomes, including many A chromosomes (Kawada et al. 2002b). Kawada et al. (2002b) postulated that the high diploid number of *S. moschatus* was derived from a *Mogera*-like ancestor with 36 chromosomes that experienced repeated Robertsonian fissions, as is also seen in the Indian muntjak, *Muntiacus muntjak* (Shi et al. 1980). The known karyotypes of Asian mole species are confined to specimens from the northern to eastern edge of their distribution.

The genus *Euroscaptor* (type species, *Talpa klossi* Thomas, 1929), a taxon in the center of the mole distribution in Asia, is defined by its dental formula of I3/3,

C1/1, P4/4, M3/3 = 44 (Miller 1940). Although some taxonomic reviews have considered this genus as synonymous to the European genus *Talpa*, which has the same dental formula (Schwartz 1948; Ellerman and Morrison-Scott 1951; Lekagul and McNeely 1988; Corbet and Hill 1991), a recent molecular phylogenetic study (Shinohara et al. 2004) separated the genus *Euroscaptor* from the genus *Talpa*. Since the karyotype of the genus *Talpa* consists of biarmed chromosomes (Kawada et al. 2002a), the fact that the Malaysian mole has a karyotype consisting of eight A chromosome pairs further supports the validity of the independent genus *Euroscaptor*. According to Kawada et al. (2001), the Japanese species of moles also have many A chromosomes in their karyotypes. Therefore, it is possible that the genera *Euroscaptor* and *Mogera*, defined by their reduced dental number (I3/2, C1/1, P4/4, M3/3 = 42), are a closely related group from a karyological perspective.

The taxonomy of moles from southeastern Asia is confused not only at the generic level, but also at the species level. This is in part because there are very few specimens collected from any one locality and deposited in museums, and most of the descriptions are based on qualitative morphological characters. Most of the species were described between the mid-1800s and early 1900s. It is therefore essential to revise these descriptions using objective tools for reconstructing the phylogenetic relationships of Asian moles. The distribution of *Euroscaptor* is centered on the Himalayas and southwestern China (Ellerman and Morrison-Scott 1951; Corbet and Hill 1991). Consequently, the Malaysian and Japanese populations are very isolated members of this genus to the south and east, respectively. The core area of *Euroscaptor* contains several kin species, including *E. grandis*, *E. klossi*, *E. longirostris*, *E. parvidens*, and *Parascaptor leucura*. Their karyotypes are unknown and require further study. Our results for the Malaysian mole suggest that the Malaysian and Japanese moles are closely related in terms of diploid number and G-banding pattern, and these patterns probably arose in their center of origin.

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