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Chromosomal evolution and zoogeographic origin of southeast Asian shrews (genus *Crocidura*)

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Abstract. To the origins and evolution of Indomalayan shrews, we investigated the chromosomal variations of 14 species of *Crocidura* from SE Asia. Intraspecific polymorphism was mainly due to variation in the number of short chromosomal arms but *C. lepidura* and *C. hutanis* showed a polymorphism due to a centric fusion. The undifferentially stained karyotypes were similar in 9 species, all possessing $2n = 38$ and $FN = 54-56$ (68); *C. fuliginosa* had $2n = 40$ and $FN = 54-58$. These karyotypes are close to the presumed ancestral state for the genus *Crocidura*. Four species from Sulawesi had a reduced diploid number ($2n = 30-34$), a trend not observed among other SE Asian species but present in few Palaearctic taxa. Compared to the apparent stasis of karyotypic evolution observed among other SE Asian species, the high degree of interspecific differences reported among Sulawesian shrews is unusual and needs further investigation.

Stasis and reduction in diploid number found in both Indomalayan and Palaearctic species suggest that these two groups share a common ancestry. This is in sharp contrast to most Afrotropical species which evolved towards higher diploid and fundamental numbers. The zoogeographical implications of these results are discussed.

Key words. Soricidae; Sunda Shelf; Sulawesi; chromosomes; zoogeography.

The Malay Archipelago consists of an array of islands spread between the Asian and Australian continents. The continental islands such as Borneo, Java or Sumatra are part of the Sunda Shelf[1] (fig. 1). During the low sea levels of the Pleistocene glaciations[2], they were connected by dry land. Faunal exchanges were thus much easier during these periods. However, this was not the case for islands of the eastern parts of the archipelago (e.g. Sulawesi, fig. 1), because these are true oceanic islands surrounded by deep waters[3]. For terrestrial animals, over-water colonization is the only means of reaching these oceanic islands. According to the classical model of allopatric speciation[4], the topographical configuration of the Malay Archipelago should promote the emergence of new species through peripheral isolation. Shrews of the genus *Crocidura* are small terrestrial mammals which constitute a strikingly speciose group with about 151 species recorded from the Palaearctic, Indomalayan and Afrotropical regions[5]. Within the Malay Archipelago, there are 27 species, most of which are restricted to single islands[6]. To obtain a better understanding of the causes of this diversification, it is important to reconstruct the evolutionary history of this group. Fossils of *Crocidura* are almost absent from the Malay Archipelago and palaenotological records in Africa and Europe are still too fragmentary to draw its zoogeographical history[7,8]. Other methods are required to reconstruct the evolution of the genus *Crocidura*.

In a survey of the karyotypes of 38 species sampled over the entire range of the genus, Maddalena and Ruedi[9] showed two contrasting evolutionary trends corresponding respectively to the African and to the Eurasiatic species of *Crocidura*. Starting from a hypothetical ancestral karyotype with about 38 chromosomes, the African clade evolved through an increase of both diploid (2n) and fundamental number (FN), up to $2n = 56$ and $FN = 86$ in *C. fuscomurina*. In contrast, W Palaearctic and the few known karyotypes of Asian species remained either close to the presumptive ancestral state or decreased in diploid number. The latter tendency is extreme in *C. serezkyensis*[10] which has $2n = 22$ and $FN = 34$. This overview included the karyotypes of only four Indomalayan species[9]. One of these species is *C. attenuata* from Thailand[11] with a chromosome formula typical of the African clade ($2n = 50$ and $FN = 66$). *C. attenuata* seems to be an exception to the otherwise congruent zoogeographical trends of chromosomal evolution in shrews. This raises the question whether the Indomalayan species really show the same evolutionary tendencies as the Palaearctic species or whether they share a common ancestor with the Afrotropical clade. Clearly, the karyotypes of more Indomalayan species are needed in order to answer this question. The primary goal of this paper is therefore to document the chromosomal variation of several SE Asian taxa of *Crocidura*. As the systematics of these taxa are very problematic[6,12], the karyotypic

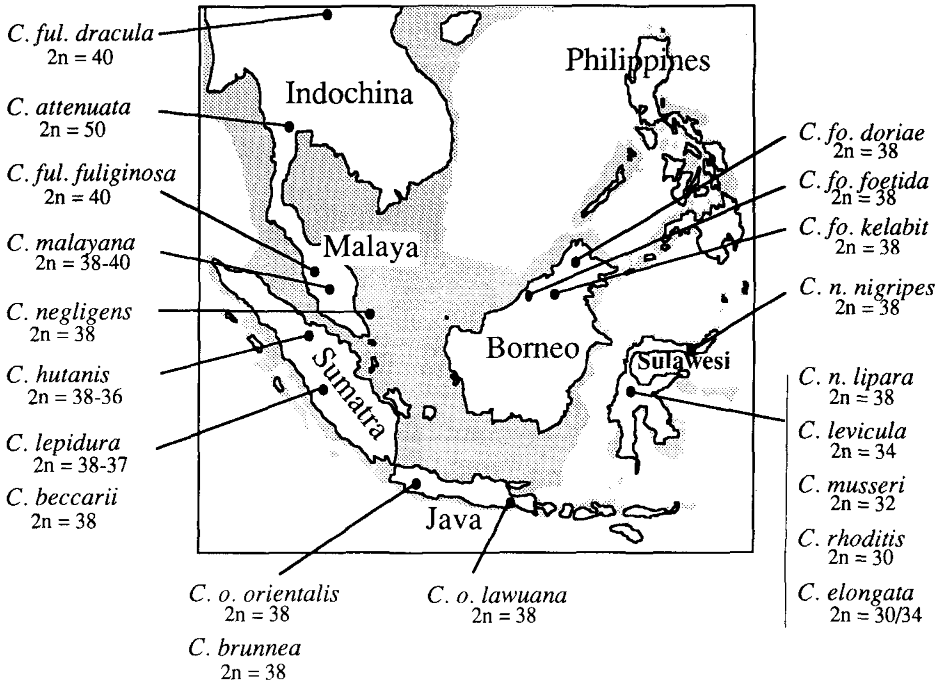

Figure 1. Geographical variation in diploid number in Southeast Asian taxa of *Crocidura*. Shaded areas are less than 120 meters deep at present and represent probable land connections during Pleistocene glaciations (modified from ref. 3).

variations may also help in their indentification, as has been the case for several European or African species (see e.g. ref. 13, 14 and 15).

Materials and methods

Forty-seven *Crocidura* representing 14 species and 4 subspecies from the Malay Archipelago have been karyotyped (fig. 1). They were sampled in Peninsular Malaysia (*C. malayana* and *C. fuliginosa*), in Tioman Island (*C. negligens*), in Borneo (*C. foetida*), in Sumatra (*C. lepidura*, *C. beccarii* and *C. hutanis*), in Java (*C. brunnea* and *C. orientalis*), and in Sulawesi (*C. nigripes*, *C. musseri*, *C. levicula*, *C. rhoditis* and *C. elongata*). The precise location of capture and the nomenclature used to designate these shrews are detailed in Ruedi[6,16]. Chromosomes were prepared from bone marrow cells according to Baker et al.[17] and stained with 4% Giemsa. Except for a few animals, preparations were processed directly in the field. The diploid number (2n) and total number of chromosomal arms (fundamental number, FN) were determined after examination of about 10 metaphases per animal. Chromosome morphology was described according to Zima and Kral[18]. Biarmed elements with subequal chromosomal arms were considered as metacentrics (M) and those with arms of unequal lengths as submetacentrics (SM). When the short arm represented less than half the size of the large one, the chromosome was regarded a subtelocentric (ST), and if only one pair of chromosomal arms was visible, an acrocentric (A).

Results

The chromosome formulas of the 47 shrews analysed are given in the table. Intraspecific variations are mostly due to non Robertsonian processes, e.g. addition of short heterochromatic arms of B-chromosomes[21]. Two species from Sumatra, *C. lepidura* and *C. hutanis*, showed a polymorphic translocation of two acrocentrics; for *Crocidura* standards, this is a rather unusual event found previously only in *C. suaveolens*[22] and *C. russula*[23]. In the absence of differential staining, it is impossible to know if it is always the same pair of acrocentrics which is involved in these rearrangements. Heterozygote individuals of *C. lepidura* and of *C. hutanis* showing such fusion are illustrated in figure 2A and 2B. Most of the species presented in the table possess 38 chromosomes and 54 to 58 chromosomal arms. Representatives of this karyotype are illustrated in figure 2D and 2E by *C. foetida* from Borneo and *C. orientalis* from Java respectively. Although no banding has been performed we assume these karyotypes to be homologous. This is not an unrealistic assumption as other studies involving G-banded chromosomes of Palaearctic *Crocidura* showed high percentage of homology of the various chromosomal forms[10,24], even when *Crocidura* are compared to *Suncus*[25].

The apparent intraspecific polymorphism exhibited by *C. elongata* (table) is more difficult to interpret as the two individuals involved have a very different chromosome complement. No simple rearrangement could account for this apparent polymorphism. Both individuals

Table 1. Chromosomal variations of 47 SE Asian shrews sampled in Malaysia and Indonesia.

Species (Reference number)	Sex chrom.		Autosomes			**2n**	**FN**
	X	Y	M/SM	ST	A		
Crocidura fuliginosa							
3752	SM	—	6	10	22	**40**	**58**
3610, 3747, 3753	SM	A	6	8	24	**40**	**56**
3621	SM	—	6	6	24	**40**	54
Crocidura malayana							
3611	SM	SM	10	16	12	**40**	**68**
3550	SM	M	9	18	10	**39**	**68**
3551	SM	M	8	19	9	**38**	**67**
3977-9	ST	M	8	18	10	**38**	**66**
3991-4, 3620	ST	SM	8	14	14	**38**	**62**
Crocidura negligens							
3564	ST	SM	8	14	14	**38**	**62**
Crocidura foetida ssp							
3980 (*foetida*)	ST	—	8	10	18	**38**	**58**
3981 (*kelabit*)	ST	SM	8	8	20	**38**	**56**
3950, 3986-8 (*doriae*)	ST	SM	8	8	20	**38**	**56**
Crocidura nigripes ssp							
4366-7 (*lipara*)	SM	ST	8	8	20	**38**	**56**
4417 (*nigripes*)	SM	—	8	8	20	**38**	**56**
Crocidura beccarii							
4475, 4506	ST	SM	8	8	20	**38**	**56**
Crocidura lepidura							
4444-5, 4458, 4505	A	SM	8	8	20	**38**	**54**
4443	A	—	9	8	18	**37**	**54**
Crocidura brunnea							
4545, 4547	SM	A	8	8	20	**38**	**56**
Crocidura orientalis ssp							
4511, 4517 (*lawuana*)	ST	SM	8	8	22	**38**	**56**
4557-8 (*orientalis*)	ST	M	8	8	22	**38**	**56**
Crocidura hutanis							
4426	ST	ST	8	6	22	**38**	**54**
4432	ST	—	9	6	20	**37**	**54**
4431	ST	—	10	6	18	**36**	**54**
Crocidura levicula							
4380	SM	SM	6	10	16	**34**	**52**
Crocidura musseri							
4397	—*	—	8	12	10	**32**	**54**
Crocidura rhoditis							
4401	SM	ST	8	10	10	**30**	**50**
Crocidura elongata							
4396	SM	—	10	16	6	**34**	**62**
4365	SM	A	10	14	4	**30**	**56**
Croc. fuliginosa dracula[20]	SM	SM	6	8	24	**40**	**56**
Crocidura horsfieldii[19]	M	SM	4	4	28	**38**	**48**
Crocidura attenuata[11]	—*	—	6	8	34	**50**	**66**

For comparative purpose, the karyotypes of three other known Indomalayan taxa are also included in this table. Species nomenclature follows our recent revision of the genus in the Malay Archipelago[6]. The autosomes are separated into three groups: metacentric or submetacentric (M/SM), subtelocentric (ST) and acrocentric elements (A). Diploid number (2n) and fundamental number (FN) are also given for each species.

*Sex chromosomes were not identified.

have been sampled in C Sulawesi: the male 4365 (fig. 2C) was caught in the lowlands, and the female 4396 in montane forest. These two different karyotypes as well as some morphological evidence[6] suggest that these animals may represent two cryptic species; however, such a hypothesis needs to be supported by the analysis of further specimens.

The newly described *C. musseri* from C Sulawesi[6] has a unique chromosome formula (fig. 2F) among Indomalayan species (table) showing that karyotype is an important additional character for its identification. The other sympatric species found in the mountains of C Sulawesi (*C. levicula*, *C. rhoditis*, *C. elongata* and *C. nigripes lipara*) also posses different chromosome formulae and may thus be readily identified by their karyotypes. The specimens of *C. fuliginosa* sampled in Peninsular Malaysia[21] with $2n = 40$ and $FN = 54-58$ (table 1) have the same karyotype as the Indochinese subspecies *C. fuliginosa dracula*[20]. As both taxa are the only Indomalayan shrews with this chromosome formula, they are probably conspecific[26].

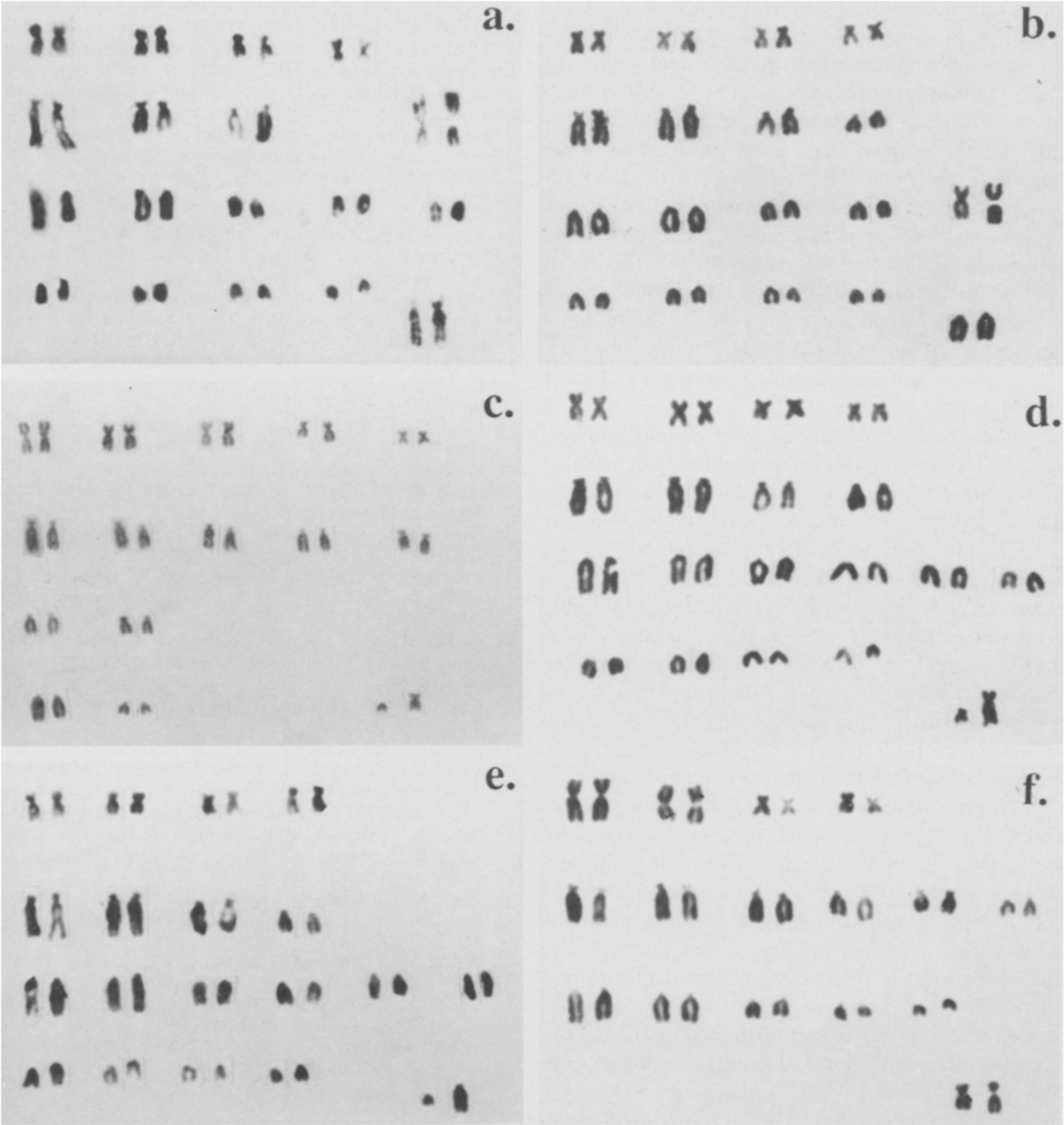

Figure 2. Karyotypes of representative shrews from the Malay Archipelago. All are illustrated for the first time: *a* female 4432 of *Crocidura hutanis*, Ketambe, N Sumatra; *b* female 4443 of *C. lepidura* from Mt Kerinci, C Sumatra; *c* male 4365 of *C. elongata* from Kamarora, C Sulawesi; *d* male 3981 of *C. foetida kelabit* from Bareo, W Borneo; *e* male 4517 of *C. orientalis lawuana* from E Java; *f* female 4397 of *C. musseri* from Mt Rorekatimbo, C Sulawesi. Other unpublished karyotypes of Indomalayan taxa may be found in Ruedi[16].

Discussion

The karyotypic trends observed in 14 species of SE Asian *Crocidura* (table) clearly support a common origin for Indomalyan and Palaearctic shrews. Indeed, both groups share either a conservative formula with a karyotype close to the presumed ancestral formula[9], or possess a reduced diploid number. The processes involved in such a reduction are probably mostly due to translocations, as exemplified by comparative banding studies[9,10,24]. The polymorphism observed in the karyotypes of several species such as *C. lepidura* or *C. hutanis* (figs. 2A and 2B) also constitute a good argument that centric fusions occur naturally in wild *Crocidura*. *C. attenuata* is the only known example of an Eurasiatic species evolving towards an increased chromosome formula.

Allozyme analyses of some Palaearctic and Indomalayan *Crocidura*[27] also confirm their common ancestry, as no conspicuous genetic differentiation separates these two groups. In contrast, when Eurasiatic and Afrotropical species are compared, both genetic[28] and karyological[9] data indicate that they constitute two very distinct, monophyletic clades. Moreover, these data also demonstrate that the African clade is phylogenetically more diverse than the others. It is also in Africa that two-thirds of all extant species of *Crocidura* are found[5]. For these reasons, and in absence of a convincing fossil record, we postulate that Africa is probably the site of origin for that genus, while *Crocidura* taxa subsequently invaded the Palaearctic and, more recently, the Indomalayan regions. They radiated into SE Asia, but conserved common features (such as karyotype) with their close Palaearctic relatives.

On a more local scale, variations in diploid numbers among SE Asian shrews are not randomly distributed over the Malay Archipelago (fig. 1). All those species which conserved a plesiomorphic state (table) are living on the Sunda Shelf while taxa with reduced diploid number represent four of the six species living on Sulawesi. According to allozyme data[16], the only species not conforming to this trend, *C. nigripes*, is a recent arrival to Sulawesi originating from the Sunda Shelf, while the other Sulawesian taxa are of more ancient origin. The reasons why the old Sulawesian species evolved towards a reduction in chromosome number while Sunda species remained stable are yet unknown. As Sulawesi is the only true oceanic island sampled in this report (fig. 1), most natural colonizations occured through scattered founder animals. The particular chromosomal evolution expected to occur during such demographic hazards[29] and the fact that early colonists probably faced new selective pressures on Sulawesi, may explain in part this intriguing pattern.

The karyotype appeared useful for differentiating sibling species such as *C. fuliginosa* and *C. malayana* from Malaysia[21], but not for other Sunda species which have a conservative formula (table). Again, it is for Sulawesian taxa that cytological characters are most pertinent as each species has a unique karyotype; this is particularly important as all species may occur in sympatry and are otherwise difficult to identify[6].

In order to understand the role of karyotypic evolution in the process of species differentiation among SE Asian shrews, it is essential to get a more precise picture of their phylogenetic and zoogeographic origins.

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