

Chromosomal Evolution in Three Species of Murid Rodents of Taiwan

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Hon-Tsen Yu, Yin-Ping Fang, Chih-Wen Chou, Shiao-Wei Huang and Fu-Hsie Yew (1996) Chromosomal evolution in three species of murid rodents of Taiwan. *Zoological Studies* 35(3): 195-199. Three species of Taiwanese murid rodents, *Rattus losea*, *Niviventer culturatus*, and *N. coxingi*, are karyotyped. Their diploid chromosome number ($2N$) and fundamental number (FN) are as follows: *R. losea*, $2N = 42$ and FN = 60; *N. culturatus* $2N = 46$ and FN = 52; and *N. coxingi* $2N = 46$ and FN = 54. *R. losea* has the same karyotypic complements as its conspecific population from Thailand except for the X chromosome. All the species of *Niviventer* share the same $2N = 46$, but vary in their FNs which range from 50 to 64. The karyotypic complements of *N. culturatus* and *N. coxingi* indicate that they are not sister taxa, supporting the hypothesis that their presence in Taiwan was derived from multiple incursions from the Asian continent.

Key words: Karyotype, *Rattus*, *Niviventer*, Speciation, Biogeography.

Chromosomal changes are often considered to be associated with population differentiation that initiates speciation and eventually leads to adaptive phyletic lineages (Patton and Sherwood 1983, King 1993). Therefore, karyotypic studies of natural populations provide the raw materials to investigate their evolutionary relationships and to infer the possible underlying events leading to their present relationships.

Rattus losea is a murid rat that is widely distributed in Indochina, Hainan Is., southeastern China, and Taiwan (Musser and Newcomb 1985). Musser and Newcomb (1985) defined the morphological boundary of the species and indicated that the population in Taiwan possesses the largest body size among all the conspecific populations. It is a common rat in the lowlands of Taiwan found around human dwellings and in agricultural lands. Two other murid rats, *Niviventer culturatus* and *N. coxingi* (Aoki and Tanaka 1941, Musser 1981), are native to Taiwan and have a parapatric elevational distribution: *N. coxingi* from sea level to ca. 2 000 m and *N. culturatus* from

2 000 to 3 600 m (Yu 1994 1995). Unlike *R. losea*, these 2 species live on forested mountain slopes. Standard karyotypes of these 3 species have never been published.

Here the karyotypes of the 3 native rats of Taiwan are presented and compared with the published karyotypes of their respective conspecific or congeneric populations. The differences in chromosomes are discussed in terms of possible evolutionary patterns.

MATERIALS AND METHODS

Animals were collected during several field trips from February through August 1995. Seven rats representing 3 species, *Rattus losea* (Pingtung 50 m, 1 male; Orchid Island 20 m, 1 male and 2 females), *Niviventer culturatus* (Da-Yu-Ling 2 560 m, 1 male and 1 female), and *N. coxingi* (Shi-Tou 1 140 m, 1 male) were karyotyped. Animals were treated with a subcutaneous injection of yeast approximately 24 h prior to karyotyping to stimulate

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mitotic activity. The rats were injected with 1 ml colchicine (0.025%) per 100 g body weight into the body cavity 20-40 min before sacrifice. Bone marrow cells were extracted from the femur and suspended in 0.075 M KCl for 20 min and fixed in glacial acetic acid and methanol (1:3). Metaphase cells were spread by air-drying and/or flame-drying methods and stained in 6% Giemsa (BDH 35086 4X) diluted with Gurr buffer (BDH 33193 2D). At least 30 bone marrow cells from each animal were examined to determine diploid chromosome number ($2N$). Fundamental numbers (FN) were counted including autosomes and 2 X chromosomes. For convenient comparisons, chromosomes were categorized into 4 groups according to their shapes: metacentric (M), submetacentric (SM), subtelocentric (ST), and telocentric (= acrocentric) (T) chromosomes (see Musser 1981 for verbal and visual definitions).

RESULTS

The shapes and sizes of the chromosomes from the 3 species are illustrated in Fig. 1.

R. losea (Fig. 1A): The diploid chromosomes are comprised of 20 pairs of autosomes and 1 pair of sex chromosomes, $2N = 42$. The autosomes include 2 pairs of subtelocentrics (Nos. 1-2), 7 pairs of metacentrics (Nos. 3-9), and 11 pairs of telocentrics (Nos. 10-20). The X chromosome is the 3rd smallest telocentric and the Y is the smallest telocentric chromosome. FN equals 60. The karyotypes of populations of the main island of Taiwan (in Pingtung) and Orchid Island (about 70 Km offshore of the southeast coast) show no notable differences.

N. culturatus (Fig. 1B): The diploid chromosome number is 46, with 22 pairs of autosomes and 1 pair of sex chromosomes. The autosomes include 3 pairs of small metacentrics (Nos. 1-3), and 19 pairs of telocentrics (Nos. 4-22). The X chromosome is about the 5th largest telocentric and the Y is the smallest telocentric chromosome. The FN is 52.

N. coxingi (Fig. 1C): The numbers of autosomes and sex chromosomes are the same as those of *N. culturatus*, $2N$ being 46. The autosomes include 3 pairs of small metacentrics (Nos. 1-3), and 19 pairs of telocentrics (Nos. 4-22). Interestingly, however, the X chromosome is a medium-sized submetacentric rather than the smallest chromosome as in *N. culturatus*. FN equals 54.

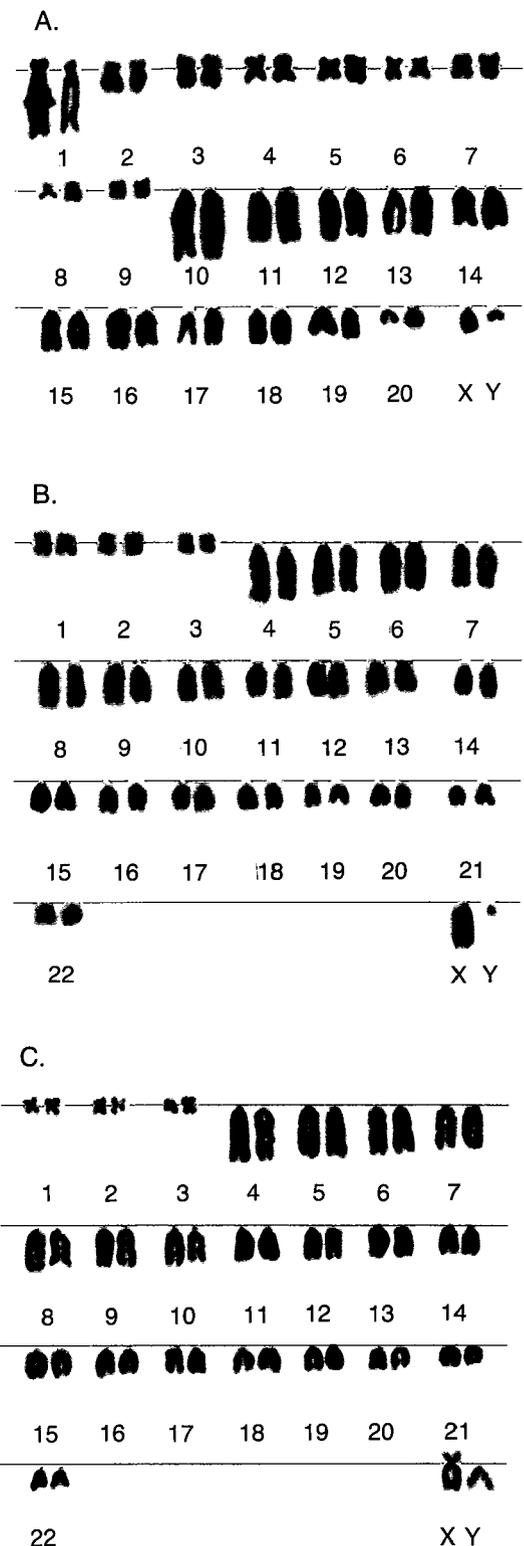


Fig. 1. Karyotypes of 3 species of murid rodents of Taiwan. (A) *Rattus losea*, (the long arm of chromosome no. 1 on the left is superimposed by a small metacentric); (B) *Niviventer culturatus*; and (C) *Niviventer coxingi*.

DISCUSSION

Table 1 summarizes the karyotypic complements of populations of *R. losea* and *Niviventer* from various geographical origins.

The karyotype of the Taiwanese *Rattus losea* is basically the same as that of its Thai counterpart (Markvong et al. 1973) except that the X chromosome is much smaller. Based primarily on size and pelage color, Musser and Newcomb (1985) assigned the Taiwanese *R. losea* to the status of a subspecies *R. losea losea*, whereas the rest of the populations from mainland China, Hainan Is., and Indochina another subspecies, *R. losea sakeratensis*. Our karyotypic result corroborates this designation of subspecies. As the X chromosomes are different between the 2 subspecies, we would further postulate that these 2 subspecies might prove to be separate species. Since X chromosomes often carry genes implicated in the causation of hybrid sterility (Wu and Palopoli 1994), the reproductive isolation between the 2 subspecies may have already been established.

The chromosomal and molecular evolution is well studied for *Rattus rattus* (Yoshida 1980, Baverstock et al. 1983a) and Australian *Rattus* species group (Baverstock et al. 1983b, Baverstock

et al. 1986). In particular, the *R. rattus* complex has 5 recognizable chromosomal races: (1) $2N = 42$ **SEA**, found throughout Southeast Asia; (2) $2N = 42$ **Jap**, occurring in Japan; (3) $2N = 42$ **Mau**, confined to the island of Mauritius; (4) $2N = 40$, restricted in the highlands of Sri Lanka; and (5) $2N = 38$, originally distributed from India west to the Middle East, but having expanded its range into Europe, Africa, North and South America, and Australia during the last few centuries by sailing on humans' ships. Molecular data (Baverstock et al. 1986) indicate the $2N = 40$ and $2N = 38$ races to be 2 separate lineages. However, the 3 races of $2N = 42$ do not cluster as a monophyletic group. The $2N = 42$ **SEA** and $2N = 42$ **Jap** races have fixed differences at 4 allozyme loci and are yet still more closely related to each other than to any other races. The $2N = 42$ **Mau** race is included in the $2N = 38$ group. It is proposed that the $2N = 42$ **Mau** race underwent a rapid chromosomal evolution by 2 pairs of fissions subsequent to the colonization of the island by the $2N = 38$ race. Similar studies with molecular markers are needed to elucidate the evolutionary history in *R. losea*.

The diploid chromosome numbers of *Niviventer* are invariable, being 46 in all species of which karyotypes are known (Table 1). Most karyotypic

Table 1. Karyotypes of populations of *Niviventer* spp.^a and *Rattus losea*^b

Species	2N	Autosomes				Sex		FN
		M	SM	ST	T	X	Y	
<i>Rattus</i>								
<i>losea</i> (Taiwan)	42	7	0	2	11	T ^c	T	60
<i>losea</i> (Thailand)	42	7	0	2	11	T	T	60
<i>Niviventer</i>								
<i>culturatus</i> (Taiwan)	46	3	0	0	19	T	T	52
<i>coxingi</i> (Taiwan)	46	3	0	0	19	SM	T	54
<i>bukit</i> (Con Son Is., Vietnam)	46	3	0	1	18	T	T	54
<i>bukit</i> (Thailand)	46	3	0	4	15	T	T	60
<i>bukit</i> (Thailand)	46	3	0	1	18	T	T	54
<i>bukit</i> (Malaya)	46	3	0	1	18	T	T	54
<i>bukit</i> (Java)	46	3	0	4	15	T	T	60
<i>huangi</i> (Hong Kong)	46	3	0	4	15	ST	T	62
<i>cremoriventer</i> (Malaya)	46	3	0	1	18	T	T	54
<i>cremoriventer</i> (Java)	46	3	0	4	15	T	T	60
<i>confucianus</i> (North Thailand)	46	3	3	1	15	T	T	60
<i>rapit</i> (Malaya)	46	3	0	4	15	T	T	60
<i>lepturus</i> (Java)	46	3	0	0	19	T	T	52
<i>hinpoon</i> (Thailand)	46	3	0	1	18	T	T	54

^aData of non-Taiwanese species are summarized by Musser (1981).

^bData of Thai population are taken from Markvong et al. (1973).

^cThe X chromosome is smaller in the Taiwanese than in the Thai population.

^d $2N$ = diploid chromosome number, M = metacentric, SM = submetacentric, ST = subtelocentric, T = telocentric, FN = fundamental number.

variation is contained in the fundamental numbers, which range from 52 to 60. The differences result from the variability of numbers of 3 shape classes in the autosomes (SM, ST, and T), while 3 pairs of metacentrics (M) are consistently present in all *Niviventer* populations. The shape changes seem to be the outcomes of pericentric inversion which is one of the major sources of chromosomal variation in rodents (Yoshida 1973, Patton and Sherwood 1983).

Sex chromosomes in *Niviventer* offer an interesting case. The X chromosome is telocentric in all populations except in *N. coxingi* (Taiwan; submetacentric) and *N. huangi* (Hong Kong; subtelocentric). Y chromosomes are invariably the smallest telocentrics except in *N. coxingi* (a medium-sized telocentric). In a closely related genus *Rattus* (i.e., an outgroup), all X chromosomes are telocentrics and all Y chromosomes are the smallest telocentrics (Yong 1969, Markvong et al. 1973, Yoshida 1973). By either outgroup comparison or the in-group assumption that "common is primitive", it is clear that the sex chromosomes of *N. coxingi* and *N. huangi* are the derived states in the genus.

Using allozyme data, Yu (1995) proposed a secondary incursion hypothesis to explain the parapatric elevational distribution of *N. coxingi* and *N. culturatus* because they are not sister taxa of each other. The secondary-incursion hypothesis is further supported by karyotypic data. It is noteworthy that *N. lepturus* (Java) possesses identical karyotypic complements as *N. culturatus* (Taiwan); therefore they are sister taxa of each other. Both species are soft-furred animals and confined to highlands in their respective distributional islands where at least 1 congener also occurs. It is likely that a parallel secondary incursion of *Niviventer* also occurred in Java, although this hypothesis needs to be tested with more data.

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臺灣產三種鼠類之染色體進化

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臺灣三種鼠類：小黃腹鼠(*Rattus losea*)、高山白腹鼠(*Niviventer culturatus*)及刺鼠(*N. coxingi*)的核型已被定出，其染色體套數($2N$)與染色體臂數(FN)分別如下：小黃腹鼠(42, 60)、高山白腹鼠(46, 52)及刺鼠(46, 54)。臺灣產小黃腹鼠之核型除X染色體以外，其餘皆與泰國產相同。所有的 *Niviventer* 種類之雙套染色體數皆為 46，而染色體臂數則為 50 至 64 不等。由高山白腹鼠及刺鼠的核型可看出，彼此並非最接近的姐妹種(sister taxa)，此一結果支持了「此兩鼠種在臺灣的分布乃多次由亞洲大陸遷入的結果」之假說。

關鍵詞：核型, *Rattus*, *Niviventer*, 種化現象, 生物地理學。

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