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Comparison of banded karyotypes between two subspecies of the red and white giant flying squirrel *Petaurista albomfus* (Mammalia, Rodentia)

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Abstract — Banded karyotypes of two related subspecies of *Petaurista alborufus castaneus* from southern China and *Petaurista alborufus lena* from Taiwan are presented and compared. Both subspecies had 2n=38 with a fundamental number of FN=72, and shared the gross chromosomal similarity and G-band homology. However, the size of their X chromosomes, C-band patterns and the number and sites of Ag-NORs revealed gross differences. These cytogenetic information suggest that each subspecies developed independently from the common ancestral form. Robertsonian mechanism was absent for their karyotype evolution. With regard to the number of NORs, the P. *alborufus lena* karyotype seemed to be primitive. The present findings suggest that *P. alborufus lena* could be categorized as a distinct species.

Key words: banded karyotype, constitutive heterochromatin, nucleolus organizer regions, *Petaurista alborufus*, red and white giant flying squirrel.

INTRODUCTION

Flying squirrels belonging to the genus *Petaurista* of the family Sciuridae were classified into five species: P. *alborufus, P. elegans, P. leucogenys, P. magnificus,* and *P. petaurista* (CoRBET and HILL 1980), each of which was intricately divided into various subspecies (L,E-KAGUL and McNEELY 1988). However, CORBET and HILL (1991, 1992) reclassified this genus into ten species: *P. alborufus, P. caniceps, P. elegans, P. leucogenys, P. magnificus, P. nobilis, P. petaurista, P. philippensis, P. sybitta,* and *P. xan-thotis.* Because of such complicated classification of *Petaurista,* it has been difficult to investigate their phylogenetic relationships.

In the genus *Petaurista,* the red and white giant flying squirrel *Petaurista alborufus* distributed in southern part of the Eurasian Continent and Taiwan was divided into seven subspecies (ELLERMAN and MORRISON-Scorr 1951; HILZHEIMER 1905; THOMAS 1923). However, this classification has been presently revised into four subspecies of P. *alborufus castaneus, P. alborufus lena, P. alborufus leucocephalus,* and *P. alborufus ochraspis* (CORBET and HILL 1992). As described by DARLINTON (1978), the formation of new species is determined by multiple factors including chromosomal changes which seem to be associated with the emergence of new species. Although it is not yet clear whether chromosomal changes are causally related or a consequence of evolution (FREDGA 1977), the analysis of differentially stained chromosomes is often employed in attempting to understand phylogenetic relationships among mammals (e.g., ELDER 1980; ROFE and HAYMAN 1985; OSHIDA *et al.* 1992; NAGAMACHI *et al.* 1996; Os-HIDA 1997). This approach is wholly independent of conventional characters and thus could be especially useful in such *Petaurista alborufus*

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group which had the intricate classification as above mentioned.

Several papers appeared dealing with karyotypes of Asian species of *Petaurista* groups in attempting to understand the nature and extent of chromosomal rearrangements among them (YoNG and DHALIWAL 1976; OSHIDA and OBARA 1991; OSHIDA *et al* 1992; OSHIDA 1997; OSHIDA and YOSHIDA 1999). In this group, *Petaurista alborufus lena* is found in only alpine regions of Taiwan (CHANG 1985), which was originally classified as a distinct species *Pteromys pectoralis* (SWINHOE 1870) and subsequently as *Petaurista lena* (THOMAS 1907). Further, CORBET and HILL (1992) described that *P. alborufus lena* may be categorized into a distinct species. This suggestive classification has been recently followed by OSHIDA *et al.* (2000) who demonstrated molecular phylogenetic relationships among these *Petaurista* species, on the basis of mitochondrial cytochrome *b* gene sequences.

In this paper, the chromosomes of two subspecies of *Petaurista alborufus castaneus* from southern China and *Petaurista alborufus lena* from Taiwan were examined. From a comparative analysis of the G-banding patterns, heterochromatin distributions and nucleolus organizer regions (NORs), the taxonomic relationship of these subspecies was proven.

MATERIAL AND METHODS

Two male specimens of *Petaurista alborufus castaneus* commercially imported from Hong-Kong to Japan in 1996 and a male of *Petaurista alborufus lena* from Taiwan were used. Their subspecies classification followed the description of CORBET and HILL (1992) and LECAGUL and McNEELY (1988). Air-dired chromosome preparations made from the primary fibroblast cultures of skin tissues derived from each specimen were stained for G- and C-bands and Ag-NORs according to methods reported previously (SUMNER *et al.*, 1971; SUMNER 1972; HOWELL and BLACK 1980).

RESULTS

Petaurista alborufus castanetus

This subspecies had a $2n = 38$ with a fundamental number of autosomal arm (FN) of 72 and its karyotype containing 12 pairs of submetacentrics (nos. 1-12) and 6 pairs of metacentrics (nos. 13-18), the submetacentric X and the acrocentric Y chromosomes (Fig. la). Nos. 8 and 13 had a secondary constriction in their long arms. G-banded chromosomes of this species were shown in Fig. 2a. All chromosomes were distinctive, but the short arm of the X stained uniformly.

Petaurista alborufus castanetus had constitutive heterochromatin in centromeric regions in all autosomes, and both the X and Y chromosomes also possessed only centromeric heterochromatin and their arms were not stained intensely (Fig. 3a).

By use of silver staining, chromosomes 8, 12 and 13 had Ag-NORs, which appeared, in two different sites in each long arm (Fig. 4a). Although sites of Ag-NORs in nos. 8 and 13 can be discerned before silver staining as a secondary constriction, Ag-NORs were found at the telomeric regions (nos. 12 and 13) and the long arm of nos. 8 and 12. Two sites of Ag-NORs in no. 12 were often found as fused ones because of their close location. The present two male specimens showed Ag-NORs in each homologous chromosome of nos. 8 and 13 pairs. However, Ag-NORs were found mostly in one homology in the pair no. 12, suggesting variable NOR-activity in this site.

Petaurista alborufus lena

This red and white giant flying squirrel was characterized by 2n=38 with an FN of 72, and the karyotype exhibited 7 pairs of submetacentrics (nos. 1-12) and 11 pairs of metacentrics (nos. 13-18). The X was the largest metacentric and the Y was acrocentric (Fig. 1b). The short arm of no. 16 had a satellite. The karyotype was essentially identical to the previous report (OSHIDA *etal.* 1992).

Petaurista alborufus lena had two categories of constitutive heterochromatin, centromeric and noncentromeric as shown in Fig. 3b. All autosomes and both the sex chromosomes possessed centromeric heterochromatin. The large blocks of C-heterochromatin (C-blocks) were found on terminal region of the short arms of nos. 1-7, 9, 10, 13, and 15-18, and the long arms of nos. 15, 16, and 18. Each arm of the X and Y

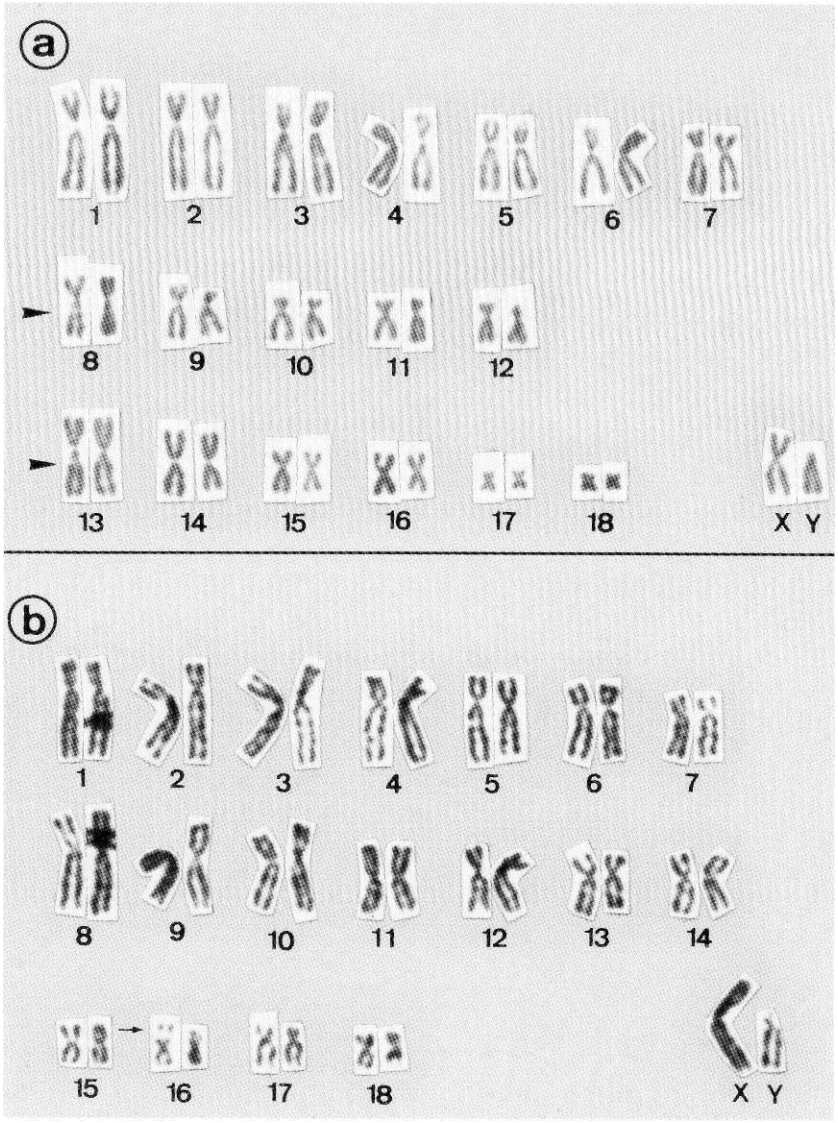

Fig. 1 — Karyotypes after Giemsa-staining. (a). *Petaurista alborufus castaneus,* male, 2n=38. (b). *Petaurista alborufus lena,* male, 2n=38. Arrow heads and arrow indicate secondary constrictions and satellite, respectively.

chromosomes were not stained intensely by C-banding. As shown in Fig. 3, the gross difference was apparently observed in the distribution and the amount of constitutive heterochromatin between *P. alborufus lena* and *P. alborufus castanetus.*

Silver staining occurred at satellite region in the short arm of no. 16 and the distal end of the short arm of no. 17 (Fig. 4b). Appearance of Ag-NORs was consistently in each of four chromosomes of these pairs, indicating no variation for NOR activity in each homology.

Comparisons

Karyotypes of both subspecies differed in the numbers of metacentrics and submetacen-trics each other (Fig. 1). In addition, as shown in Fig. 1, the size of the X chromosome appeared differently in the two species. The difference was confirmed by measurements for relative length taken from each conventional karyotype, which revealed *7.22+0.22* in the *lena* X chromosome and 6.31 ±0.27 in the *castaneus* X. This indicated that the *lena* X chromosome

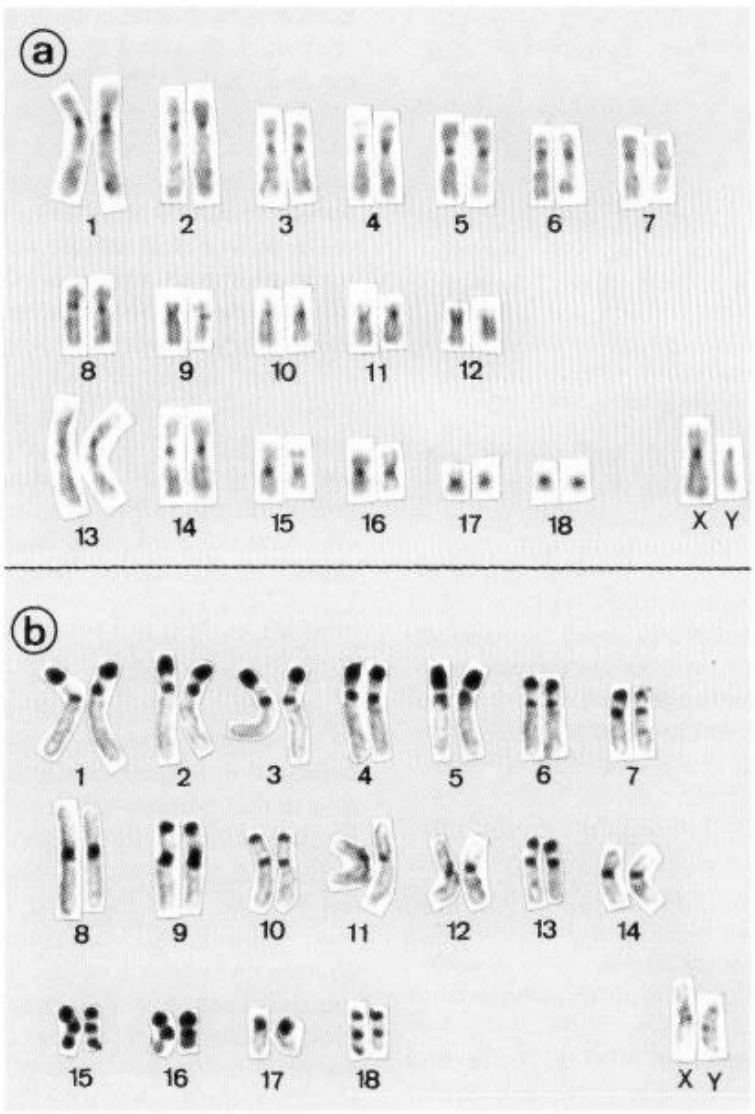

Fig. 3 — C-banded karyotypes. (a). *Petaurista alborufus castaneus.* (b). *Petaurista alborufus lena.*

The C-banded chromosomes of *P. alborufus lena* possess apparently greater amounts of heterochromatin than does *P. alborufus castaneus.* NORs appeared also differently in the numbers and sites in these subspecies. *Petaurista alborufus castaneus* appeared to have six NOR sites in three pairs (nos. 8, 12 and 13) because Ag-NORs appeared in two sites in the long arm of each NOR-carrying chromosome, while *P. alborufus lena* possessed two NORs in two pairs (nos. 16 and 17), being only one NOR site in each chromosome.

DISCUSSION

The present study demonstrated that the karyotype of *P. alborufus castanetus* and *P. alborufus lena* apparently shares the gross chromosomal similarity and degree of G-band homology, indicating that two are closely related. Despite extensive G-band pattern similarities between these subspecies, substantial differences exist in chromosomal constitution, constitutive heterochromatin, Ag-NORs sites and the size of X chromosomes. Since G-band ho-

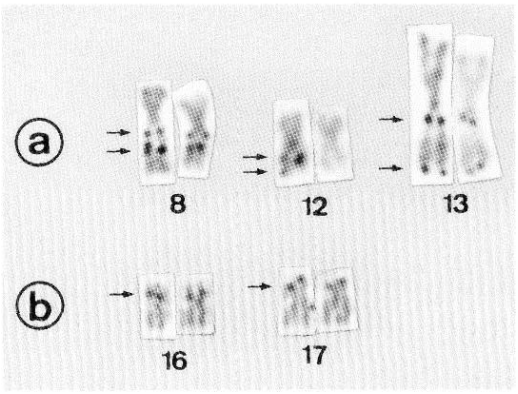

Fig. 4 — Localization of Ag-NORs. (a). *Petaurista alborufus castaneus.* (b). *Petaurista alborufus lena.* Arrows indicate Ag-NORs.

mology was present in the C-band and NOR-carrying chromosomes, the only repetitive units and NOR sites themselves seem to have appeared or been lost through karyotypic evolution. Discordance in the amount of heterochromatin and number and location of NORs is not uncommon in each genus of the family Sciuridae (OSHIDA *et al.* 1992; OSHIDA 1997; OSHIDA and YOSHIDA 1999). Although the evolutionary significance of these aspects to the karyotype is poorly understood, the process that resulted in alteration of both constitutive heterochromatin and NORs patterns seemed to have occurred independently in *P. alborufus castanetus* and in *P. alborufus lena.*

Knowing the ancestral sites of NORs and constitutive heterochromatin would help to elucidate phylogenetic relationships in the family Sciuridae. Although this question has not been answered yet, the present finding in NOR sites would provide the course of NOR evolution in *P. alborufus.* Assuming that the ancestor in the family Sciuridae had one site of NORs and then led to numerous NORs, the process that resulted in numerous NOR sites in different chromosomes occurred independently at least once in *P. alborufus lena* and four times in *P. alborufus castaneus.* Further, if the more ancestral condition is represented by the presence of one NOR at a secondary constriction, as previously described (Hsu *et al.* 1975; SCHMID 1978; MAHONY and ROBINSON 1986), *P. alborufus lena* conditions are probable primitive. On the other hand, in C-banded karyotypes of the giant flying squirrels including *P. alborufus lena,* it has been reported that C-blocks exist commonly in the terminal regions of some autosomes (OSHIDA and OBARA 1991; OSHIDA *et al.* 1992; OSHIDA 1997). However, there were not terminal C-blocks in C-banded karyotype of P. *alborufus castaneus* at all (Fig. 3a), suggesting that *P. alborufus castaneus* may be more distantly related to the other *Petaurista* species.

In addition, OSHIDA and OBARA (1991) and OSHIDA *et al.* (1992) described that Y chromosomes of *Petaurista* are euchromatic along entire arms, although mammalian Y chromosomes have usually a large quantity of heterochromatin (e.g., GAMPERL *et al.* 1982; GRAVES and WATSON 1991). As Y chromosomes of *lena* and *castaneus* were not stained positively, euchromatic Y chromosome may have been shared in this genus through the process of karyotypic evolution.

Recently OSHIDA *et al.* (2000), on the basis of molecular phylogenetic analysis, suggested that *P. alborufus castaneus* is most distantly related to the other *Petaurista* species and that *P. alborufus lena* is more closely related to *P. petaurista* and *P. philippensis.* Judging from this molecular phylogenetic results and the present findings, *P. alborufus castaneus* might have earlier evolved from the other *Petaurista* species with chromosomal rearrangements such as alteration of C-heterochromatin and NORs, and then, in Taiwan, *P. alborufus lena* might have evolved independently from the group consisting of *P. petaurista* and *P. philippensis.* Therefore, *P. alborufus lena* could be categorized as a distinct species from *P. alborufus castaneus.*

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