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Comparisons of the banded karyotypes between the small Japanese flying squirrel, *Pteromys momonga* and the Russian flying squirrel, P. *volans* (Rodentia, Sciuridae)

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Abstract — Two flying squirrel species, *Pteromys momonga* and *Pteromys volans* contain 2n=38 with the same fundamental number of 68, but differ in their karyotypes. Almost all the autosomes and the sex chromosomes have either complete or partial G-band correspondence, and thus provide definitive evidence for the taxonomic relationship of these *Pteromys* species. The karyotypic differences occurred as results of pericentric inversion, tandem fusion, and deletion of autosomes and the Y chromosome. No Robertsonian event occurred. Both species possess one pair of Ag-NORs in identical G-banded chromosomes but different morphology resulting from pericentric inversion. Despite the conservatism of G-band patterns, a striking accumulation of C- heterochromatin has occurred in the *P. momonga* karyotype. Flow-cytometric DNA determination demonstrated that P. *volans* genome contains approximately 15 % less DNA. These differences seem to be prima rily reflected by chromosomal deletions in the *P. volans* karyotype rather than ac cumulation of the C-heterochromatin in the *P. momonga* karyotype. The present findings are crucial for explaining the direction of karyotype evolution of these species, and suggest that the karyotype of *P. momonga* is ancestral and that *P. volans* is derived from it.

Key words: banded karyotype, flow cytometry, *Pteromys momonga, Pteromys volans*, Russian flying squirrel, small Japanese flying squirrel.

INTRODUCTION

In flying squirrels, the genus *Pteromys* is classified into only two species of *P. momonga* and *P. volans* (CoRBET and HILL 1991; NOWAK 1991). P. *volans* is widely distributed in the cen tral to northern parts of the Eurasian Conti nents, Sakhalin, and Hokkaido island of Japan, while *P. momonga* is endemic to Honshu, Shikoku, and Kyushu islands of Japan (N OWAK 1991; YANAGAWA 1996). Although these two *Pteromys* species have shared several morphological attributes such as pelage and body size, they are classified mostly by distinct characteristics of a number of nipples and the occurrence of a specialized penial structure (IMAIZUMI 1960). Recently a phylogenetic approach was undertaken by OSHIDA *et al.* (2000) who ana lyzed mitochondrial DNA in several flying squirrel genera including the two *Pteromys* species and found that *P. momonga* closely related to *P. volans.*

Chromosome examination of the genus *Pteromys* revealed that both *P. momonga* and *P. volans* show a diploid number of 38 chromo-

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somes with a fundamental number of 68 but different karyotypes (TSUCHIYA 1979; RAUSCH and RAUSCH 1982; YANAGAWA *et al* 1996). Although these reports have been valuable for morphometric characterization of the chromosome complement of the two species, these studies are based on conventionally stained chromosomes. Banding studies in *Pteromys*

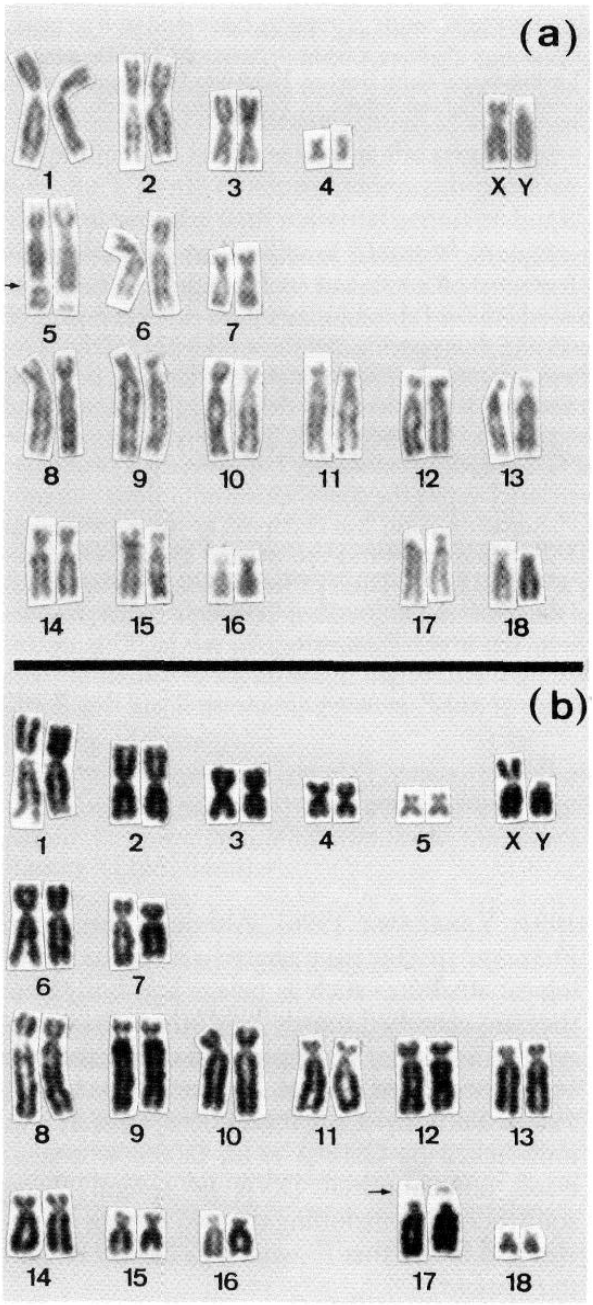

Fig. 1 — Conventional karyotypes of (a) *Pteromys momonga* and (b) *P. volans*. Arrow and arrowhead indicate secondary constriction (SC) and satellite, respectively.

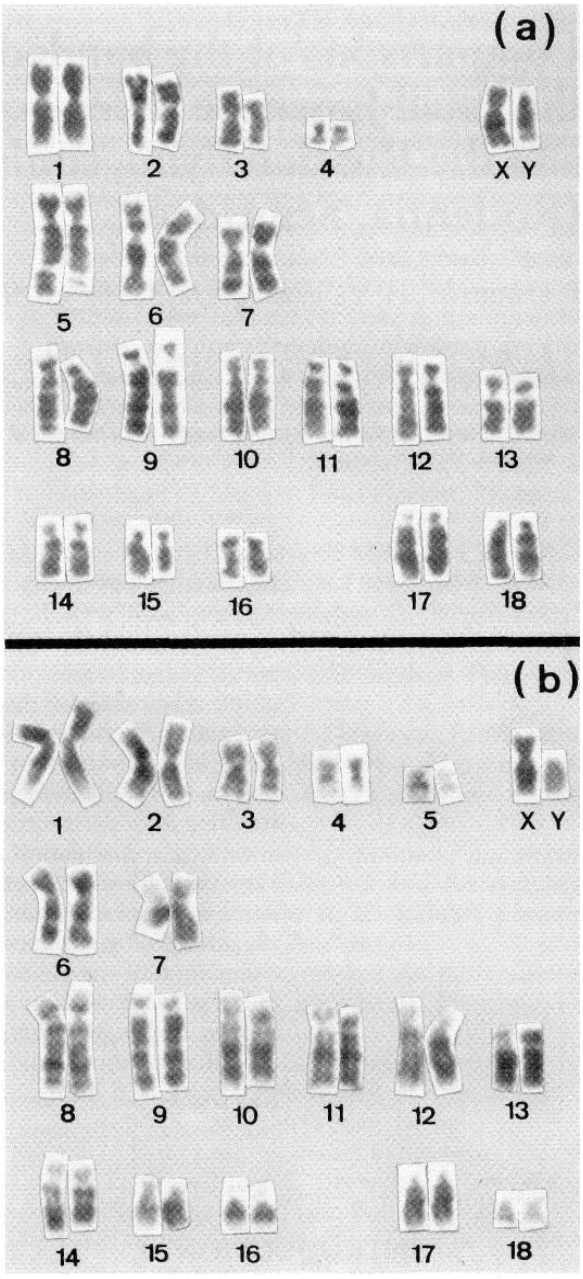

Fig. 2 — G-banded karyotypes of (a) *Pteromys momonga* and (b) *P. volans.*

species have been performed (OsmDA and YOSHIDA 1996; OSHIDA *et al.* 1996a), but a comparative cytogenetic analysis is not undertaken to determine the phylogenetic relationships within this genus. The same diploid number but the different karyotype in these *Pteromys* species suggests to us that Robertsonian mechanisms are responsible for some of the karyo-

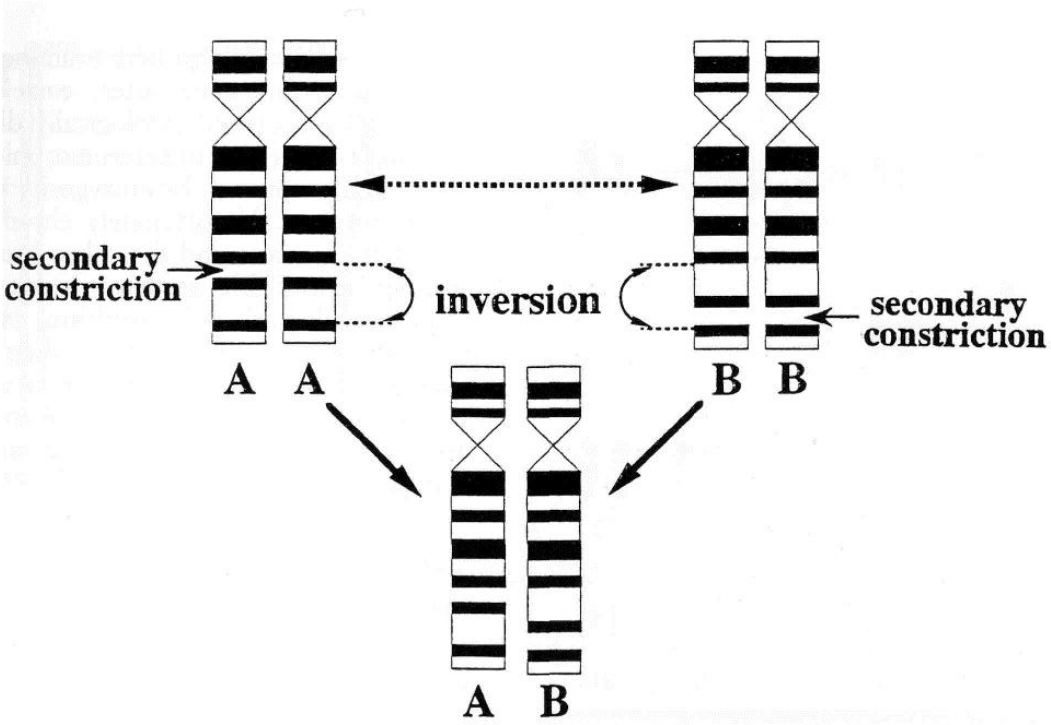

Fig. 3 — Ideograms representing paracentric inversion found in no. 5 pair of *Pteromys momonga.*

typic difference between the two species. To test this hypothesis and to critically evaluate the chromosomal similarities between the two *Pteromys* species, we made a comparison of G-bands, location of heterochromatin, number and location of nucleolus organizer regions (NORs), and amount of DNA content in the two species of *P. momonga* and *P. volans.*

MATERIALS AND METHODS

Skin biopsy explants were obtained from the specimens of a litter of one male and two females of *P. momonga* which were captured in Fukui Prefecture, and three males and three females of *P. volans orii* were captured in Hokkaido island. Chromosome preparations were obtained from each primary fi - broblast culture by our routine method (O SHIDA and YOSHIDA 1994). G- and C-bandings (SEABRIGHT 1971; SUMNER 1972), and silver staining for nucleo lus organizer regions (Ag-NORs) (HOWELL and BLACK 1980) were performed. Fluorescence *in situ* hybridization (FISH) using human 18S and 28S ribosomal RNA (rRNA) genes as a probe was applied for NORs (OSHIDA and YOSHIDA 1996).

In each species, the DNA amount of fibroblast from female specimen was measured by flow cytometry according to NICOLETTI *et al.* (1991). Each fibroblast by growing confluently contact inhibition, which was supposed to be mostly in the Gl phase, was used. Cell suspensions after trypsinization were washed with phosphate buffered saline (PBS), treated with 0.1 mg/ml RNase for 5 min at room temperature, and stained with 100 µg/ml propidium iodide (PI; Sigma) for 30 min at room temperature. After washing with PBS, PI fluorescence of individual cells was measured using a flow cytometer (Epics Elite, Miami, FL) with excitation by a 488-nm argon ion laser and emission collected through a 530/30-nm band-pass filter and a 630-nm long pass filter. As control, human peripheral lymphocytes separated by density gradient using Ficoll-Paque so lution (Phamacia, LKB) were used.

RESULTS AND DISCUSSION

Pteromys momonga

The karyotype of P. *momonga,* 2n=38, consisted of 4 pairs of metacentrics, 3 pairs of submetacentrics, 9 pairs of subtelocentrics, 2 pairs of acrocentrics, a submetacentric X chromosome and a large acrocentric Y chromosome similar to the size of the X (Figs, la and 2a) in conformity with those reported previously (TSUCHIYA 1979; YANAGAWA *et al,* 1996). We found an interstitial secondary constriction (SC) in long arms of a pair no. 5 in all three

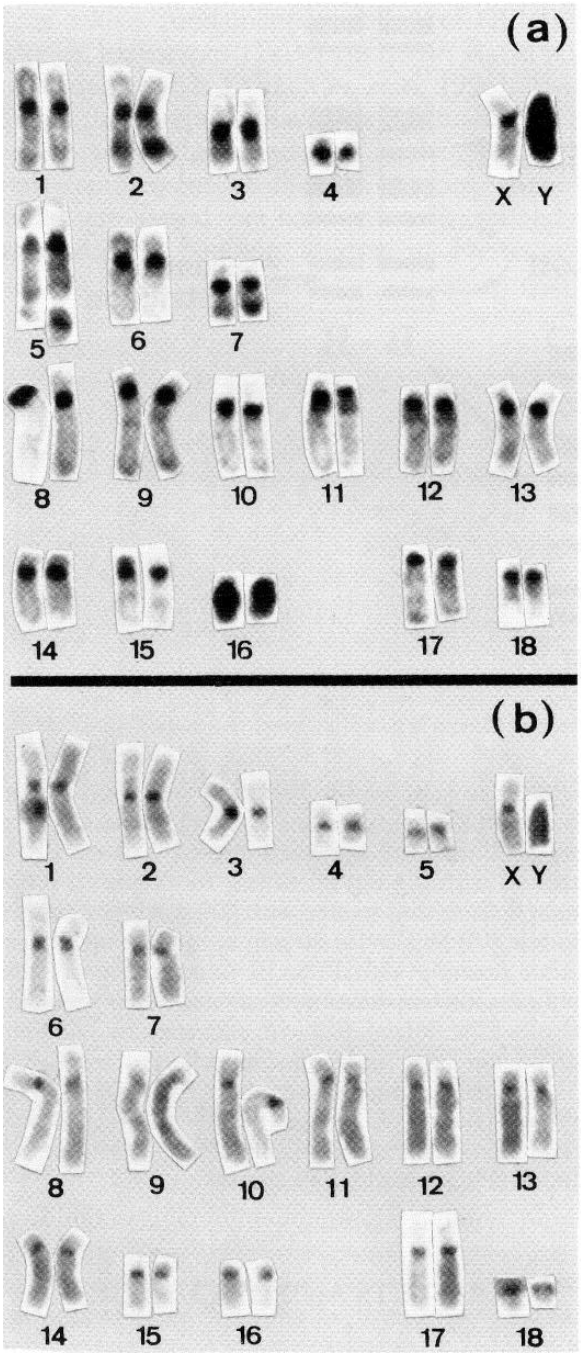

Fig. 4 — C-banded male karyotypes of (a) *Pteromys momonga* and (b) *P. volans.*

specimens. However, the location of SC appeared differently in each homologous chromosome 5, showing heteromorphic feature. This hetromorphic SC pattern was consistently present in all three specimens. G-banding demonstrated that the heteromorphism has been arisen by a paracentric inversion in the long arm involving the SC site (Fig. 3). Since all the three specimens of *P. momonga* here examined were derived from the same litter, each parent seemed to have carried cytologically different no. 5 pair of either type of heteromorphic no. 5, and the chromosome heterozygosity of the progeny resulted. Unfortunately chromosome study of the parents and the other specimens could not be available, so that it is not known whether cytological polymorphism exists in population of *P. momonga*. C-banding exhib ited large C-blocks near centromeres of all auto- somes and the X chromosome. No. 16 and the Y chromosome was entirely heterochromatic (Fig. 4a), in agreement with OSHIDA *et al.* (1996a).

Pteromys volans

The karyotype of *P. volans* (2n=38) con sisted of 5 pairs of metacentrics, 11 pairs of submetacentrics and/or subtelocentrics, 2 pairs of acrocentrics, a submetacentric X chromosome, and an acrocentric Y chromosome similar to the long arm of the X chromosome (Figs. 1b and 2b), in conformity with a previous report (OSHIDA and YOSHIDA 1996). One pair of no. 17 had satellite on its short arm in all six specimens here examined. C-banded chromosomes of *P. volans* presented in Fig. 4b clearly showed rela tively small amounts of constitutive heterochro- matin present in all chromosomes including the X. Only the Y chromosome was entirely heterochromatic.

DNA content

Flow cytometric measurement of nuclear DNA content in *P. momonga* and *P. volans* showed each peak value of 380.0±16.8 in *P. momonga*, 331.4+15.5 in *P. volans*, and 447.5±13.2 in human lymphocytes (Fig. 5). The mode of the DNA content was significantly different between *P. momonga* and *P. volans* ($P<0.01$), and also demonstrated that *P. volans* contains approximately 15 % less DNA than *P. momonga.*

Chromosome comparison

A comparison of G-banded chromosomes of *P. momonga* and *P. volans* is shown in Fig. 6. Almost all the chromosomes of *P. volans* had banding homology entirely or segmentally in the karyotype of *P. momonga*. The X chromo-

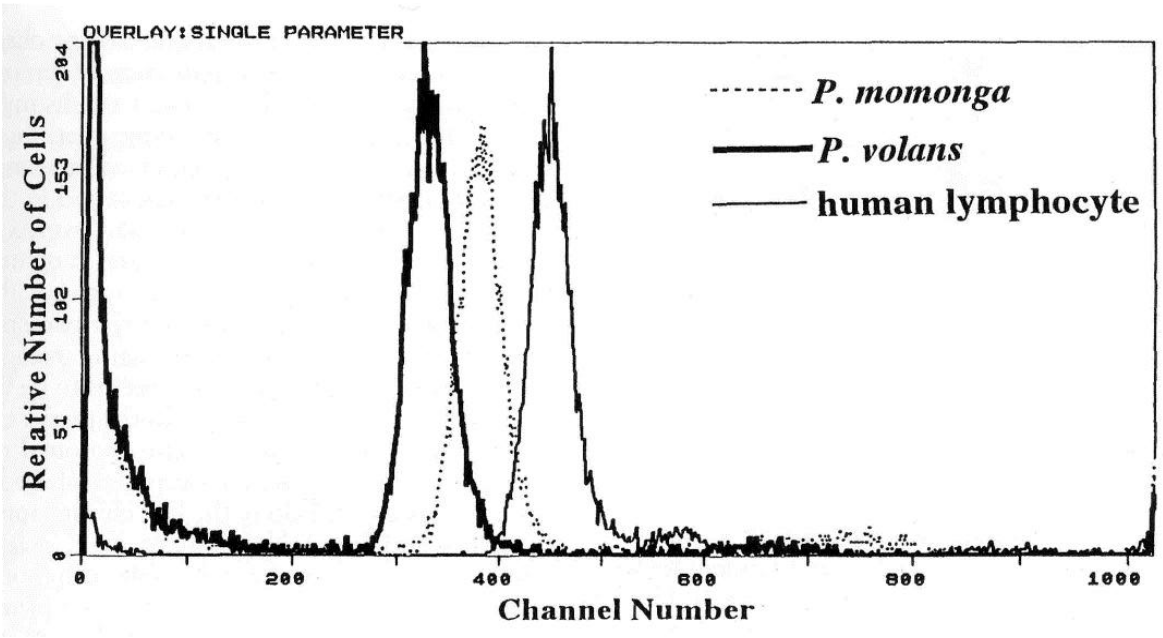

Fig. 5 — Flow cytometric spectra of P. *momonga* (dotted line), *P. volans* (thick line) and human (thin line).

some in both species was clearly homologous. Similarly, the Y chromosome appeared homologous, but the length of the *P. momonga* Y was larger than the *P. volans* one. Although the two species showed the same diploid number of 38 with a fundamental number of 68, the detailed comparison of the karyotypes of *P. momonga* and *P. volans* strongly exhibited nu-

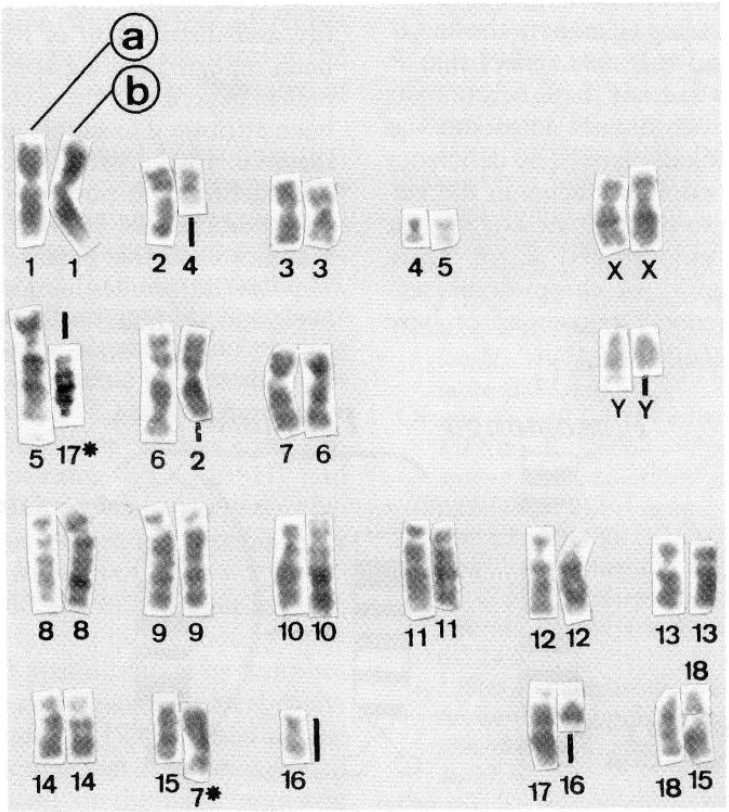

Fig. 6 — Comparison of chromosomes of (a) P. *momonqa* and (b) *P. volans.* Numbers correspond to those in the respective species Solid lines indicate the absence of matching segments *volans* chromosome.. Asterisks indicate inversion.

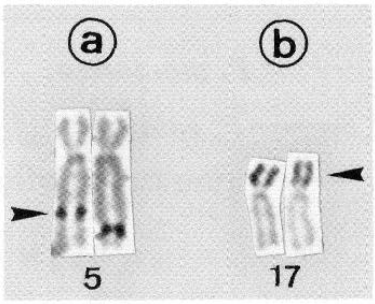

Fig. 7 — NORs-bearing chromosomes of (a) P. *momonga* and (b) P. *volans*. Arrowheads indicate Ag-NORs.

merous chromosomal rearrangements between the two species. The features of the chromosome rearrangements were pericentric inversions (P. *momonga* 5 — *P. volans* 17 and P. *momonga* 15 — P. *volans* 7), and tandem fusion (P. *momonga* 18 — P. *volans* 15 and 18). However, no Robertsonian relationship was observed. In addition to these rearrangements, chromosomes 2q, 5p, 6q, 16, 17q and Yq of P. *momonga* did not have any matching segments in the karyotype of P. *volans* because of the apparent lack of, or the diminished amount of, chromosome segments in P. *volans* karyotype. The entire long arm of P. *momonga* 16 had no apparently corresponding element in the karyotype of P. *volans* and this may reflect that P. *momonga* 16q was entirely heterochromatic, since no such heterochromatic autosome was seen in the P. *volans* karyotype. The deficiency in matching chromosome segments in the *volans* karyotype was also substantiated by nuclear DNA content, as demonstrated above. Thus, changes in the quantity of chromosome seg ments in *P. volans* genome on comparison have been mostly attributed to simple loss of chromosome segments without reduction of diploid chromosome number. The present results indicate that the degree of karyotypic rearrangements between these two species is more extensive and complex rather than that found in the other squirrel species of *Callosciurus* (OSHIDA *et al.* 1996b) and *Sciums* (OSHIDA and YOSHIDA 1997). Our initial hypothesis that some of the chromosome differences between the two species were the result of Robertsonian rearrangement is clearly disproved. There seems to be lit tle doubt that one and perhaps both species ex hibit the results of numerous chromosomal rearrangements since the two species last shared a common ancestor. Taking the lost chromosome segments and less DNA content in *P. volans* into consideration, we may be able to hypothesize that the P. *momonga* form is more primitive and that *P. volans* is the most derived cytogenetically.

Comparison of C-banded chromosomes revealed that the two species differed greatly in the amount of constitutive heterochromatin, because of large blocks of centromeric heterochromatin and the entirely heterochromatic no. 16 in P. *momonga* (Fig. 4). Changes in the quan tity and distribution of heterochromatin have been reported among species in several mam malian genera, and these changes have generally been attributed to simple gain or loss of C-band material. In the present study, it seemed that the euchromatic portion of one *P. momonga* chromosome (no. 16) has become heterochromatinized and that heterochromatic segment in *P. volans* autosomes has lost through karyotype evolution. Although such variability in hetero-

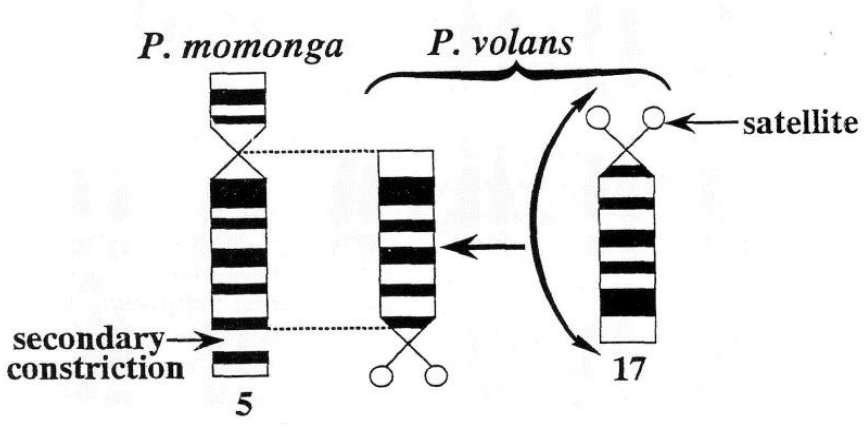

Fig. 8 — Diagrammatic presentation for inversion of P. *momonga* no. 5 and P. *volans* no. 17.

chromatin content may alter the total DNA content for *P. momonga* and *P. volans*, it seems to provide minor contribution between these species, and chromosome deletions found in *P. volans* genome appear to be the major contributors to alteration of the total DNA content and also karyotype diversity within the genus.

In both species, Ag-NOR-bearing chromosomes can be easily identified and appeared in the SC of a pair of *P. momonga* no. 5 and in the satellite region of *P. volans* no. 17 pair, respectively (Fig. 7). The location of Ag-NORs differed in each homologous chromosome of *P. momonga* no. 5 pair, reflecting heteromorphism in the location of SC. The precise correlation between silver stained NORs and those stained by *in situ* hybridization (data not shown) was obtained in both species in agreement with OSHIDA and YOSHIDA (1996, 1999). Both species have an equivalent number of NOR sites, and the G-band homology is present in each NOR-carrying chromosome, suggesting that NORs in the two *Pteromys* species may be originally identical in their chromosomal sites. The *P. volans* acrocentric no. 17 can be derived from its counterpart of biarmed *P. momonga* no. 5 by a pericentric inversion without loss of NORs and with loss of chromosomal element of the short arm of *P. momonga* no. 5, as illustrated in Fig. 8.

The present comparison of G-banding patterns in the two species clearly demonstrates that most chromosomal segments are conserved except for *P. momonga* chromosomes 2q, 5p, 6q, 16, and 17q to which their corresponding *P. volans* chromosomes are absent. This speculates that these chromosome segments can provide little contribution to syntenic conservation between *P. momonga* and *P. volans* genomes. A more detailed analysis using such as comparative chromosome painting (ZOO-FISH) and gene mapping technology will be needed to determine the patterns of genome exchange or conservation in the *Pteromys*.

IMAIZUMI (1960, 1985) regarded *P. momonga* as a relic species indigenous to Honshu, Shikoku, and Kyushu islands by morphological, anatomical, and biogeographical analyses, whereas KAWAMURA (1990), based on the fossil records, suggested that *P. momonga* had derived from *P. volans* or the other ancestral form, and that *P. volans* is more primitive than *P. momonga*. As already mentioned above, the present cytogenetic findings are crucial for explaining the direction of karyotype evolution of these species, and suggest that the karyotype of *P. momonga* may be ancestral and that *P. volans* may be derived from it, although caution must be exercised in arriving at evolutionary judgments based on karyology alone.

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REFERENCES

CORBET G.B. and HILL J.E., 1991 — *A world list of mammalian species*. Oxford Univ. Press, Oxford.

HOWELL W.M. and BLACK D.A., 1980 — *Controlled silver-staining of nucleolus organizer regions with a protective colloidal developer: a 1-step method*. Experientia, 36: 1014-1015.

IMAIZUMI Y., 1960 — *Colored illustration of the mammals of Japan*. Hoiku-sha, Osaka (in Japanese).

—, 1985 — *Animal geography of Japan*. Anima: Mag. Natl. Hist., 145: 28-34 (in Japanese).

KAWAMURA Y., 1990 — *The origin of rodents in Japan, based on fossil records*. Abst. Ann. Meet. Mam. Soc. Jpn.: 70 (in Japanese).

NICOLETTI I., MIGLIORATI G., PAGLIACCI M.C., GRIGNANI F. and RICCARDI C, 1991 — *A rapid and simple method for measuring thymocyte apoptosis by propidium iodide staining and flow cytometry*. J. Immunol. Methods, 139: 271-279.

NOWAK R.M., 1991 — *Walker's mammals of the world*. The Johns Hopkins Univ. Press, Baltimore and London.

OSHIDA T., LIN L-K, YANAGAWA H., ENDO H. and MASUDA R., 2000 — *Phylogenetic relationships among six flying squirrel genera, inferred from mitochondrial cytochrome b gene sequences*. Zool. Sci., 17: 485-480.

OSHIDA T., YANAGAWA H. and YOSHIDA M.C., 1996a — *C-banded karyotype of the Japanese flying squirrel, Pteromys momonga*. Chrom. Inf. Serv., 61:24-25.

—, 1996b — *Comparison of G-banded karyotypes between two beautiful squirrel species, Callosciurus prevostii and C. erythraeus*. Chrom. Inf. Serv., 61: 26-27.

OSHIDA T. and YOSHIDA M.C., 1994 — *Banded karyotypes of Asiatic chipmunk, Tamias sihiricus lineatus Siebold*. Chrom. Inf. Serv., 57: 27-28.

—, 1996 — *Banded karyotype and the localization of ribosomal RNA genes of Eurasian flying squirrel, Pteromys volans orii (Mammalia, Rodentia).* Caryologia,49:219-225.

—, 1997 — *Comparison of banded karyotypes between the Eurasian red squirrel Sciurus vulgaris and the Japanese squirrel Sciurus Us.* Chrom. ScL, 1: 17-20.

—, 1999 — *Chromosomal localization ofnucleolus organizer regions in eight Asian squirrel species.* Chrom. Sci., 3: 55-58.

RAUSCH V.R.and RAUSCH R.L., 1982 — *The karyotype of the Eurasian flying squirrel, Pteromys volans (L.), with a consideration of karyotypic and other distinctions in Glaucomys spp. (Rodentia, Sciuridae).* Proc. Biol. Soc. Wash., 95: 58-66.

SEABRIGHT M., 1971 —*A rapid banding technique for human chromosomes.* Lancet, 2: 971.

SUMNER A.T., 1972 —*A simple technique for demonstrating centromeric heterochromatin.* Exp. Cell Res., 75: 304-306.

TSUCHIYA K., 1979 —*A contribution to the chromosome study in Japanese mammals.* Proc. Jpn. Acad., 55 Ser B: 191-195.

YANAGAWA H., 1996 — *The Japanese flying squirrel Pteromys momonga and the Russian flying squirrel Pteromys volans orii.* In: T. Kawamichi (Ed), "The Encyclopaedia of Animals in Japan", vol. 1, pp. 84-87. Heibonsha, Tokyo (in Japanese).

YANAGAWA H., OSHIDA T., TANIGUCHI M. and TAKETAZU K., 1996 — *Notes on the Japanese flying squirrel, Pteromys momonga captured in Fukui Prefecture.* J. Jpn. Wild. Res. Soc., 22: 8-16 (in Japanese with English abstract).

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