The Homology and Relationship of Human (*Homo sapiens*) Chromosomes 1, 19 and Dusky Langur (*Trachypithecus obscurus*) Chromosomes 6, 8 Demonstrated with Chromosome Painting

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**Summary**  Chromosomal relationship between humans and dusky langurs (*Trachypithecus obscurus*, 2n=44) was established by chromosome painting using chromosome specific DNA probes of the human chromosome 1 and 19 which each gave hybridization signals on two non-homologous dusky langur chromosomes. The results show that the human chromosome 1 and 19 probes hybridized to three regions of dusky langur on the autosomes 6 and 8. The human chromosome 1 probe hybridized to one region on the dusky langur chromosome 6 and two regions on the dusky langur chromosome 8, where the human chromosome 19 probe hybridized with the same pattern but on different regions. Hybridization patterns of human painting probes on dusky langur, when compared with the data of other species in the same genus suggest that the alternating hybridization pattern of the conserved segments homologous to human chromosomes 1 and 19 on dusky langur chromosomes 6 and 8 is the result of the translocation followed by the pericentric inversion. Moreover, the present research also indicates that the dusky langur’s chromosomes 6 and 8 have the same hybridization patterns as other Asian colobines.

**Key words**  *Trachypithecus obscurus*, Chromosome painting, Reciprocal translocations.

The dusky langur (*Trachypithecus obscurus*) is also called the dusky leaf monkey belongs to the family Cercopithecidae, subfamily Colobinae. There are three groups of colobine monkeys: the colobus monkeys of Africa, the langurs and the odd-nosed monkeys of Asia. *T. obscurus* is a species of Asian langurs distributed throughout South-east Asia. However, it can be found only in the southern part of Thailand. The classification and taxonomy of colobines has not been yet settled and is subject to continued revisions (Napier and Napier 1967, 1985, Groves 1970, Oates *et al.* 1984, Vogel and Winkler 1990). For instance, there is no consensus even on the number of genera and species. The dusky langur is either classified in the genus *Trachypithecus* (*Oates *et al.* 1984) or the genus *Presbytis* (Napier and Napier 1985). The scheme of Oates *et al.* (1984) was used as the classification system which put dusky langur into the genus *Trachypithecus*.

In the previous studies using the classical staining, the diploid numbers of both African (genus *Colobus*) and Asian (genus *Presbytis*) colobines were demonstrated that they are to be 2n=44 (Chiarelli 1963, Ushima *et al.* 1964). All chromosomes can be divided into two groups; the metacentric and the submetacentric according to their centromeric index, with the exceptions that the Asian langurs have a pair of small acrocentric chromosomes while the African colobines have

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Fig. 1. Metaphase chromosome plate (top) and karyotype (bottom) of male Dusky langur (*Trachypithecus obscurus*) 2n (diploid)=44 by G-banding technique.
metacentric chromosomes (Nie et al. 1998).

The determination of complete chromosomal homologies among primate species is essential for the study of the primate chromosome evolution and taxonomy. With the introduction of fluorescence in situ hybridization (FISH) using whole chromosome-specific probes (chromosome painting) (Nie et al. 1998), it is now possible to establish the chromosomal homology and to study the relationship between humans and dusky langurs on the basis of DNA sequence homologies. There are many reports on chromosome banding in Asian colobines, i.e., the genus Presbytis (Sharma et al. 1972, Krishna-Murthy et al. 1979, Ponsá et al. 1983, Dutrillaux et al. 1984). The following chromosomal homologies have been successfully established: between karyotypes of humans and great apes (chimpanzees, gorilla, and orangutan), lesser apes (gibbons and siamang), and macaques by using the chromosome painting technique (Wienberg et al. 1990, 1992, Stanyon et al. 1992, 1995, Jauch et al. 1992, Koehler et al. 1995a, b, Yu et al. 1997). Nie et al. (1998) reported the chromosomal homologies between humans and Francois monkeys and Phayre’s leaf monkeys established by chromosome painting using chromosome-specific probes from 23 human chromosomes (22 autosomes plus the X). Here we report the chromosomal homologies and the relationship between human chromosomes 1 and 19 on dusky langur chromosome by chromosome painting.

Materials and methods

Cell culture and chromosomal preparation

Ear tissues were collected from male T. obscurus at Songkla Zoo, Songkla province, Thailand. Metaphase chromosomes were prepared from fibroblast cells of male T. obscurus, which were provided by the Kunming Cell Bank of the Chinese Academy of Sciences. The cell line was grown at 37°C in DMEM medium enriched with 20% newborn calf serum. Before harvesting for chromosome analysis, the cells were treated with 0.05 μg/ml colchicine (Sigma) for 1 hr. Chromosome preparation followed standard procedures, which included a 15–20 min hypotonic treatment in 0.075 M KCl, four fixations in methanol–glacial acetic acid (4:1) and air drying. G-band metaphase separated from separation with fluorescence in situ hybridization was performed. The use of DAPI-banding concurrently with in situ hybridization also facilitated chromosome identification. The karyotype numbering and arrangement were identified following the method of Kampirananont (2003).

Fluorescence in situ hybridization

Human chromosome-specific probes (1 and 19) were provided by Dr. Wenhui Nie, Key Laboratory of Cellular and Molecular Evolution, Kunming Institute of Zoology, the Chinese Academy of Sciences, Kunming, Yunnan, China. Human chromosome-specific probes were resuspended in 10 μl hybridization buffer (50% deionized formamide, 10% dextran sulphate, 2× SSC, 0.5 M phosphate buffer, pH 7.3), denatured at 65°C for 10 min and preannealed by incubation at 37°C for 1 h. Samples on slides were denatured by incubation in 70% formamid/2× SSC solution at 65°C for 2 min, quenched in ice-cold 70% ethanol and dehydrated through with an ethanol series (70%, 90%, and 2 times 100%). The preannealed probes were applied onto slides and allowed to hybridize for 24 h at 37°C. Post-hybridization washes were two 5 min incubations in 50% formamide, 50% 2× SSC at 50°C followed by two 5 min. incubation in 2× SSC at 50°C. Biotin-labeled probes were visualized by fluorescein isothiocyanate (FITC)-avidin and cy3 labeled probes. The slides were counterstained with DAPI, air dried 15 μl mounting solution was added and then cover with a clean glass cover slip.

Microscopy

FISH prepared slides were visualized on a fluorescent microscope combined with a video cam-
Fig. 2. Metaphase chromosomes of male Dusky langur (*Trachypithecus obscurus*) 2n=44 showing the hybridization patterns obtained with human chromosome-specific probes 1 and 19 bound to dusky langur chromosomes 6 and 8 by using human chromosome 19 cy3-labelled probes, and chromosome 1 biotin-labeled probes detected with avidin-FITC. Chromosomes were counter stained with DAPI, bars=10 µm.

Fig. 3. The relationship between human chromosomes (*Homo sapiens*, (HSA)) 1, 19 and dusky langur (*Trachypithecus obscurus*, (TOB)) chromosomes 6, 8 are the result of a reciprocal translocation followed by pericentric inversion * fis. (fission) and fus. (fusion).
era equipped with a specific filter set for FITC and cy3. Hybridization signals and G-bands were captured by GENUS software on an Apple computer which was provided by Key Laboratory of Cellular and Molecular Evolution, Kunming Institute of Zoology, the Chinese Academy of Sciences, Kunming, Yunnan, China.

Results

**G-banded karyotype of dusky langur (T. obscurus)**

Dusky langur (T. obscurus) had the same diploid numbers of chromosomes (2n=44) as the other colobine monkeys in the previous reports (Chen et al. 1981, Bigoni et al. 1997ab, 2003, Nie et al. 1998). According to our scheme, the karyotype numbering and arrangement system of Kampranont (2003), we found that T. obscurus karyotype composition is: 22 (M) + 18 (SM) + 2 (A), X (M) and Y (SM). One pair of metacentric chromosome 19, the marked chromosome, bears the NOR (nucleolar organizer region) at the secondary constriction near the centromere of the long arm. The chromosomes are divided into three groups on the basis of the centromeric index: metacentrics, submetacentrics and acrocentric chromosomes. All of the chromosomes are ordered according to their relative length and size. Fig. 1 shows the metaphase chromosome and karyotype by G-banding technique of T. obscurus. We also found that T. obscurus has G-banding patterns that are similar to other Asian colobines.

**Hybridization of human chromosome-specific probes onto the metaphases of dusty langur (T. obscurus)**

The hybridization signal of human chromosome-specific probes 1 and 19 gave bright painting on the dusky langur chromosomes 6 and 8. The human chromosome 1 and 19 probes hybridized to three regions of dusky langur autosome 6 and 8, respectively. The human chromosome 1 probe hybridized to one region on dusky langur chromosome 6 and two regions on dusky langur chromosome 8, while the human chromosome 19 probe hybridized with the same pattern to different regions on the homologous chromosome (Fig. 2). We compared dusky langur chromosome banding patterns with the human chromosome banding pattern in term using the terminology of Roony (2001). The result shows that the alternating hybridization pattern of the conserved segments homologous to human chromosomes 1 and 19 on dusky langur chromosome 6 and 8 has resulted from reciprocal translocation followed by the pericentric inversion (Fig. 3).

Discussion

**G-banded Karyotypes of dusky langur (T. obscurus)**

We confirm that the 2n=44 of T. obscurus’s diploid karyotype number is common to all other species of the Colobinae (Chiarelli 1963, Chen et al. 1981, Bigoni et al. 1997ab, 2003, Nie et al. 1998). G-banded karyotypes of dusky langur show a similarity but are not identical with karyotypes of other colobines (Semnopithecus francoisi and S. phayrei) (Nie et al. 1998). The difference has been caused due to discrepancies between the numbering systems used. Chromosome 2 of T. obscurus has the same banding patterns as S. francoisi which is chromosome 2 type (Nie et al. 1998). We can also confirm that T. obscurus has the same marked chromosome with Presbytis cristata, S. francoisi, S. phayrei and Nasalis larvatus after compared in term of banding pattern.

**Human Syntenic Groups Fragmented in Trachypithecus obscurus**

The results from hybridization patterns of human chromosome-specific probes demonstrate that the dusky langur chromosomes have conserved syntenic homologies to entire human chromosomes. Human chromosome probes 1 and 19 showed hybridization patterns on dusky langur chro-
mosomes 6 and 8, which were resulted from the reciprocal translocation, then followed by the pericentric inversion. The result is in agreement with Bigoni et al. (1997a) who reported that P. cristata chromosome 6 and 8 may occurred by the reciprocal translocation between human chromosomes 1 and 19. The alternating pattern between chromosome segments homologous to human chromosomes 1 and 19 on the silvered leaf monkey chromosome 8 indicates a pericentric inversion followed with the translocation. T. obscurus also has the same hybridization pattern with human chromosome-specific probe 1 and 19 as do S. francoisi, S. phayrei (Nie et al. 1998) and N. larvatus (Bigoni et al. 2003). The fragmentations and associations of human chromosomes 1 and 19 can be explained with a reciprocal translocation. The associations occur in all Asian Colobinae documented but do not appear in the African species Colobus guereza, which has different translocations (Bigoni et al. 1997b). The most parsimonious explanation is that the reciprocal translocation occurred in the lineage of the Asian colobines can be used to distinguished them from the African colobines (Bigoni et al. 2004). Our results suggest that T. obscurus has the same conservative karyotypes and same hybridization patterns as other Asian colobinae when using human chromosome-specific paint probes.

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