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ORIGINAL ARTICLE

The chromosomal homology between dusky langur (*Trachypithecus obscurus* Ried, 1837) and human (*Homo sapiens*) revealed by chromosome painting

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Abstract
The presnt study provides illustated characterization of the chromosomal rearrangement of the male dusky langur (*Trachypithecus obscurus* Ried, 1837; TOB) using Chromosome Painting technique. Ear tissues specimen collection was kindly provided by Songkla Zoo, Songkla province, Thailand. The fibroblast cells were cultured and cryopreserved after that all 24 Human chromosome-specific probes (22 autosomes, X and Y) were hybridized in situ to the TOB mitotic cells and captured and analyzed by GENUS software. The results showed that the TOB chromosomes were highly rearranged compared to the 30 conserved regions of human chromosomes of different sizes. Majority, TOB chromosomes, such as TOB chromosomes 1, 2, 3, 4, 9, 11, 12, 13, 15, 17, 18, 20, 21, X and Y which were homologous to only one human chromosome, are conserved.. Some of those were constituted of regions being two TOB chromosomes that composed one of HSA homologous regions or one TOB chromosomes homologous to two human chromosomes. This is the first report of molecular cytogenetics on TOB using all 24 human chromosome-specific probes. The chromosome mappingis useful for a comparisonof Langur either in the same or other genus that had been reported to elucidate and clarify evolutionary relationship of primates shared by the same common ancestor.

Keywords Dusky langur · *Trachypithecus obscurus* Ried, 1837 · Chromosome painting

Introduction

Trachypithecus obscurus (TOB) [6] also called dusky leaf monkey, is a species classified in the family Cercopithecidae, subfamily Colobinae. The colobines divided during evolution into an African clade and an Asian clade [7]. *T. obscurus* is one species of Asian langurs distributed in tropical Asiai ncluding Thailand that can be found only in southern part of the country.

In the nineteenth century, classification and taxonomy of colobine monkeys have not been yet settled and are subject to continued revision [11, 12, 18, 19, 21, 22, 29]. For instance, there is no consensus even on the number of genera and species. Therefore, dusky langur is either classified in

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genus *Trachypithecus* [22] or genus *Presbytis* [19]. However, the scheme of Oates [22] was used as the classification system which put dusky langur into the genus *Trachypithecus*.

The phylogenetic tree of this group was disputed as many authors. For instance the protein-coding mt genes, fragment of X-chromosome and 54 nuclear genes, complete cytb gene and 7 mt genes, 15 mt genes and 43nuclear genes, 83 mobile elements and nuclear genes [30]. However, it was still difficult to resolve the clarity relationship among them.

In the previous cytological studies, the diploid numbers of colobines of both genera including African (*Colobus*) and Asian (*Presbytis*) colobines were $2n = 44$ [9, 28]. All chromosomes can be divided into two groups; metacentric and submetacentric according to their centromeric index, with the exceptions that the Asian langurs have a pair of small acrocentric chromosomes [20]. The chromosome banding in Asian colobines, i.e., genus *Presbytis* has also been reported [10, 17, 23, 26].

After the fluorescence in situ hybridization (FISH) technique was introduced, the following chromosomal homologies have been successfully established between karyotypes of humans and greatapes (chimpanzees, gorilla, and orangutan), lesser apes (gibbons and siamang) and macaques by using chromosome painting technique [13, 15, 16, 27, 31–33].

Later, there was a report of the chromosomal homologies between humans and two langurs (*Semnopithecus francoisi* and *S. phayrei*) usingchromosome painting through chromosome-specific probes from 23 human chromosomes (22 autosomes plus the X) [20]. The other related colobine species were also reported on chromosome relationship such as *Presbytis cristata*, *Nasalis larvatus*, *Colobus guereza*and *Pygathrix nemaeus* [1–4]. Subsequently, Sangpakdee et al. [25] reported some information regardingchromosomal homology and relationship between human and dusky langur chromosomes.

As the determination of complete chromosomal homologies among primate species is essential for the study of primate chromosome evolution and taxonomy, therefore the chromosomal homologies and the relationship between dusky langur and human chromosome by in situ hybridization of 24 human chromosome probes to dusky langur metaphase chromosomes were undertaken, and the same is reported in this communication.

Materials and methods

Cell cultureand chromosomal preparation

Ear tissues of the male *T. obscurus* were collected at Songkla Zoo, Songkla province, Thailand. The fibroblast cells were cultured and cryopreserved in laboratory of the Kunming Cell Bank of the Chinese Academy of Sciences. Thereafter, the cells line were grown at 37 °C in DMEM medium enriched with 20% newborn calf serum. After a week, the growing cells were treated with 0.05 mg/ml colchicine (Sigma) for 1 h to harvest metaphase chromosomes. Chromosome preparations were then operated following standard protocol.

Classical banded staining of chromosomes

After spreading the cells on slides, G-banding metaphases were performed by trypsin using 10% Giemsa's. The DAPI-banding was applied concurrently with in situ hybridization to facilitate chromosome identification.

Fluorescence in situ *hybridization*

The 24 Human chromosome-specific probes (22 autosomes, X and Y) were hybridized in situ to the mitotic cells in accordance with the following. The biotin and cy3 labeled human probes were resuspended in 10 ml of hybridization buffer (50% deionized formamide, 10% dextran sulphate, 2X SSC, 0.5M phosphate buffer, pH 7.3), then denatured at 65 °C for 10 min and incubated (pre-annealing) at 37 °C for 1 h. The mitotic chromosomes were denatured in 70% formamide/2X SSC solution at 65 °C for 2 min, quenched in ice-cold 70% ethanol and passed throughthe series of 70%, 90% and twice of 100% ethanol. The pre-annealed probes were then applied on the slides to hybridize for 24 h at 37 °C.

Post-hybridization washing was done by incubating the slides twice in 50% formamide: 50% 2XSSC at 50 °C for 5 min each, then incubation in 2X SSC at 50 °C for 5 min. The biotin-labeled probes were detected with fluorescein isothyocyanate (FITC) anti-avidin. The slides were counterstained with DAPI, air-dried and mounted with coverslip. The hybridized mitotic metaphases were visualized under fluorescence microscope with a specific filter set for FITC and cy3. Hybridization signals and G-bands were captured and analyzed by GENUS software.

Results and discussions

G-banded karyotype of dusky langur

Dusky langur (*T. obscurus*) has the same diploid numbers of $2n = 44$ as in other colobine monkeys [1–4, 8, 20]. According to the karyotype numbering and arrangement system followed by Kampiranont [14], all chromosomes are ordered according to their relative length and size. The karyotype of T. obscurusconsists of 22 metacentric, 18 submetacentric, 2 acrocentricautosomes, sex-X metacentric and sex-Y submetacentric chromosomes (Fig. 1). This individual male

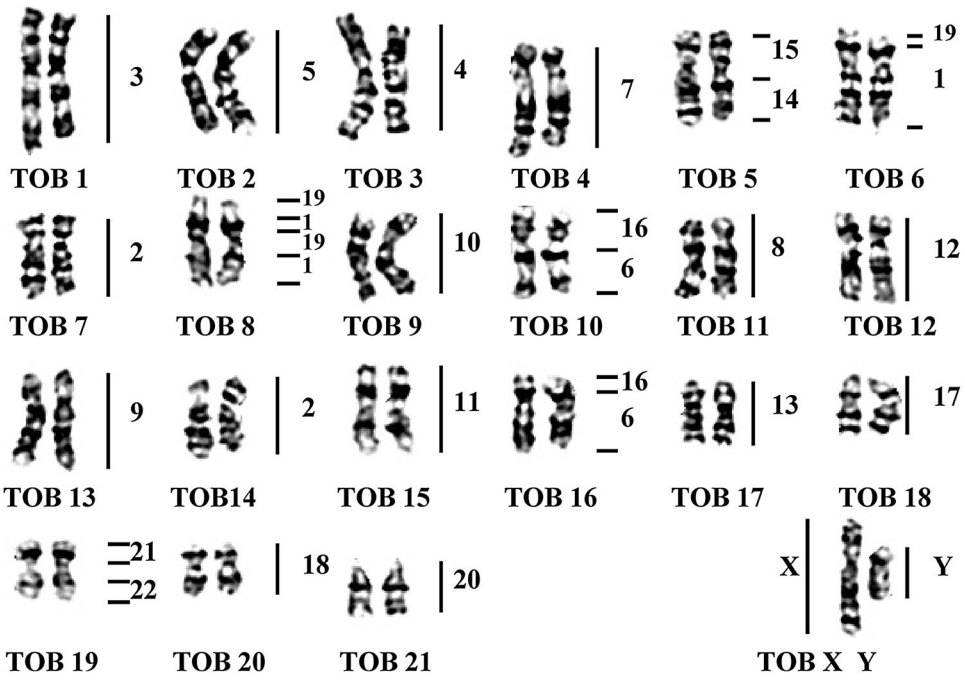

Fig. 1 G-banded karyotype of male Dusky langur (*Trachypithecus obscurus*) $2n = 44$. Chromosomes are numbered below and the homology to human chromosome is indicated to the right. The karyotype numbering and arrangement are per Sangpakdee et al. [25]. Chromosome 19 is the NOR-bearing marker chromosome

dusky langur has revealed the same karyotype as described by Sangpakdee et al. [25]. In addition, the G-banding pattern of this speciesis also similar to other Asian colobines [1–4, 8, 20].

FISH revealed the homology between T. obscurus (TOB) and H. sapiens (HSA)

The karyotype of TOB had undergone extensive rearrangements, with 30 conserved regions of different sizes. Majority of TOB chromosomes are conserved. For instance, TOB chromosomes 1, 2, 3, 4, 9, 11, 12, 13, 15, 17, 18, 20, 21, X and Y which were homologous to only one HSA chromosome. Additionally, some of those were constituted of regions being homologous to HSA chromosomes. For examples, (1) two TOB chromosomes compose of HSA homologous regions such as TOB chromosome 7 and 14, and (2) TOB chromosomes that consist of two HSA homologous regions such as TOB chromosome 5, 6, 8, 10, 16 and 19 (Fig. 1).

The hybridization signal of whole HSA chromosome-specific probes 1 and 19 demonstrated bright signals on TOB chromosomes 6 and 8. The HSA 1 probe hybridized to one region on TOB 6 and two regions on TOB 8, and HSA 19 hybridized to the other region left on TOB 6 and 8 (Fig. 2). The comparison of G-banding patterns of TOB and HSAwere analyzed using the terminology of Rooney and Czepulkowski [24] and found that the alternating hybridization pattern of the conserved segments homologous toHSA 1 and 19 on TOB 6 and 8 resulted from reciprocal translocation followed by pericentric inversion as described by Sangpakdee et al. [25]. These chromosomal phenomena patterns were also discovered in *P. cristata* [2], *Semnopithecus francoisi*, *S. phayrei* [20] and *N. larvatus* [4]

The other two HSA probes hybridized on TOB chromosomes showing the homology presented as reciprocal translocation of HSA 6, 16 on TOB 10, 16 which is different from the other colobine monkey species (e.g., the HSA 6, 16 presented the reciprocal homology on chromosomes 9, 16 of *P. cristata* [2] while on chromosomes 15, 18 of *S. francoisi* and *S. phayrei* [13]).

The hybridization pattern of HSA 14, 15 fusions showed chromosomal homology relationship on TOB 5, while HSA 21, 22 fusions were homology on TOB 19, except only HSA 2 which demonstrated the chromosomal homology separatingin TOB 7 and 14, respectively (Fig. 2).

The hybridization patterns of HSA 14, 15 and 21, 22 probes resulted that *T. obscurus* chromosomes are conserved syntenic homologies to those human chromosomes which concerning occurred by Robertsonian translocation. In agreement with Bigoni et al. [2] reported that the hybridization pattern of *P. cristata* demonstrates the existence of the following associations of HSA 14/15, 21/22 owing to simple Robertsonian changes. This chromosomal fusion were also revealed in *C. guereza* on chromosomes 6 and 16 [3], *S. francoisi* and *S. phayrei* on chromosomes 12 and 21 [20], *N. larvatus*on chromosomes 17 and 14 [4] and *P. nemaeus* on chromosomes 4 and 21 [1]. Out of these associations, HSA 14/15 pattern was also found in macaque species [32]. In addition, the association of human chromosomes 21 and 22 were formed the marker

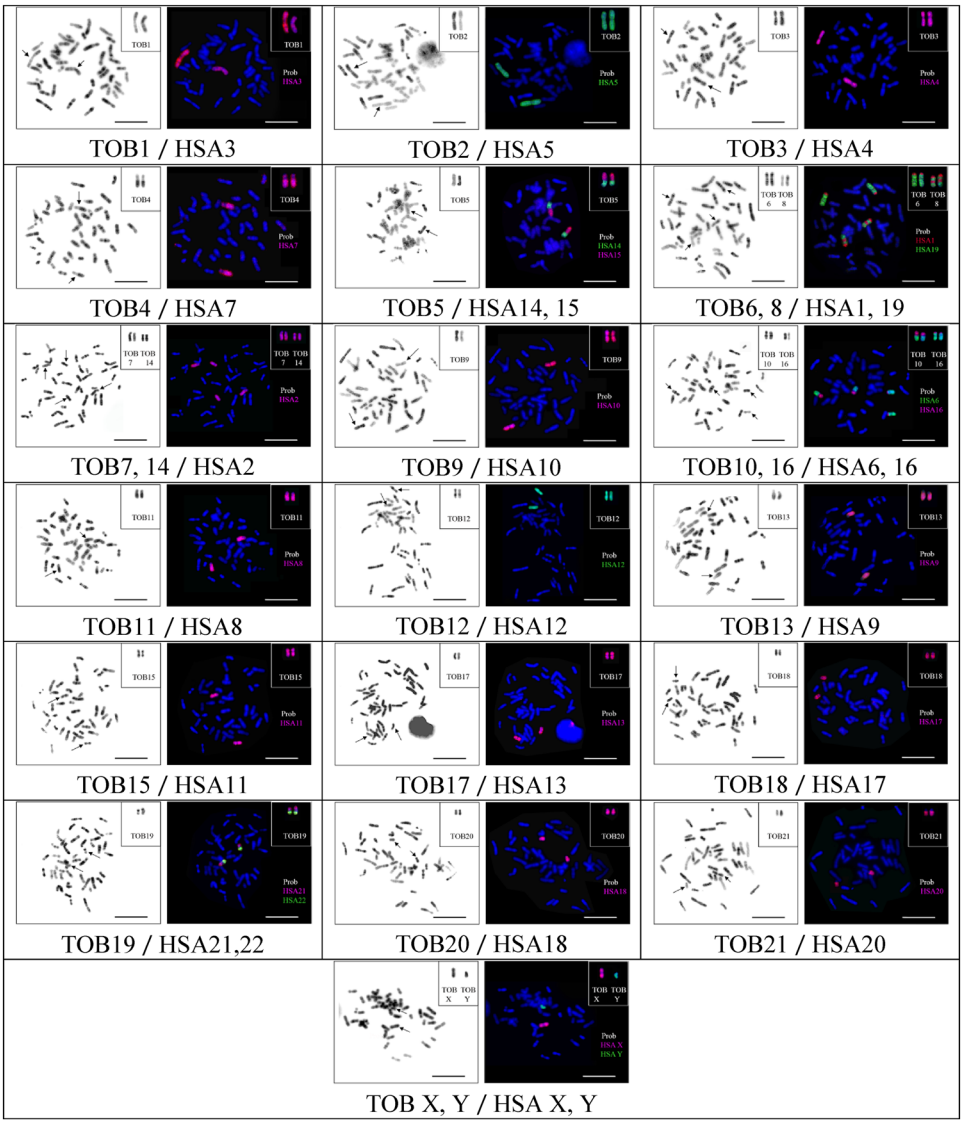

Fig. 2 The hybridization patterns and homology between dusky langur (*Trachypithecus obscurus*) and human (*Homo sapiens*) by chromosome painting technique

chromosome which is found in all documents of Asian colobines [1, 2, 4, 5, 20] and also *C. guereza*, the African species [3].

The rest of HSA probes demonstrated a whole single signal on entire TOB chromosomes including HSA 3/TOB 1, HSA 4/TOB 3, HSA 5/TOB 2, HSA 7/TOB 4, HSA 8/TOB 11, HSA 9/TOB 13, HSA 10/TOB 9, HSA 10/TOB 9, HSA 11/TOB15, HSA 12/TOB 12, HSA 13/TOB 17, HSA 17/TOB 18, HSA 18/TOB 20, HSA 20/TOB 21, HSA X/TOB X and HSA Y/TOB Y, respectively.

According to the chromosomal phenomena occurred by Robertsonian fusion of chromosomes 14/15 and 21/22, and reciprocal translocation followed by pericentric inversion of chromosome 1/19 and including 6/16 reciprocal translocation based on human chromosome painted probes that shared the same synthenic segment, our results suggest that *T. obscurus* is closely related to the colobine monkeys genera *Presbytis*, *Semnophithecus*, *Nasalis*, *Pygathrix* and *Colobus*, respectively.

More information of cytogenetic data would be desirable to clarify the phylogenetic position of those leaf monkeys and in particular their relationship to *T. obscurus*. Use of reciprocal chromosome painting and of either subchromosomal or some specific probes of decreasing size such as BACS, multicolor banding probe sets and recitative DNA probes would effectively contribute to the study of colobines and help to define breakpoints, which may have phylogenetic significance.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethics approval Under the permission of animal for scientific purposes code U1-05662-2559.

Consent to participate Yes.

Consent for publication Yes.

References

1. Bigoni F, Houck ML, Ryder OA, Wienberg J, Stanyon R. Chromosome painting shows that *Pygathrix nemaeus* has the most basal karyotype among Asian Colobinae. Int J Primatol. 2004;25(3):679–88.
2. Bigoni F, Koehler U, Stanyon R, Ishida T, Wienberg J. Fluorescence *in situ* hybridization establishes homology between human and silvered leaf monkey chromosomes, reveals reciprocal translocations between chromosomes homologous to human Y/5, 1/9, and 6/16, and delineates an X1X2Y1Y2/X1X1X2X2 sex-chromosome system. Am J Phys Anthropol. 1997;102(3):315–27.
3. Bigoni F, Stanyon R, Koehler U, Morescalchi AM, Wienberg J. Mapping homology between human and black and white colobine monkey chromosomes by fluorescent *in situ* hybridization. Am J Primatol. 1997;42(4):289–98.
4. Bigoni F, Stanyon R, Wimmer R, Schempp W. Chromosome painting shows that the proboscis monkey (*Nasalis larvatus*) has a derived karyotype and is phylogenetically nested within Asian Colobines. Am J Primatol. 2003;60(3):85–93.
5. Bigoni F. Omologia cromosomica e filogenesi nei primati: analisi molecolare e citogenetica. Genoa: University of Genoa; 1995.
6. Boonratana R, Traeholt CBW, HtunS. Trachypithecus obscurus. IUCN Red List Threat Species eT2203 9A9349397, 2008.
7. Brandon-Jones D, Eudey AA, Geissmann T, Groves CP, Melnick DJ, Morales JC, Shekelle M, Stewart CB. Asian primate classification. Int J Primatol. 2004;25(1):97–164.
8. Chen Y, Luo L, Shan X, Cao X. No TitlePrimates chromosome in China. Beijing: Science Publishing House; 1981.
9. Chiarelli B. Comparative morphometric analysis of primate chromosomes. III. The chromosomes of the genera *Hylobates Colobus* and *Presbytis*. Caryologia. 1963;16(3):637–48.
10. Dutrillaux B, Webb G, Muleris M, Couturier J, Butler R. Chromosome study of *Presbytis cristatus*: presence of a complex Y-autosome rearrangement in the male. InAnnales de genetique. 1984;27(1):148–53.
11. Groves CP. Primate taxonomy. Washington, DC: Smithsonian Institution; 2001.
12. Groves CP. The forgotten leaf-eaters, and phylogeny of the colobinae. Old World Monkeys, 1970.
13. Jauch A, Wienberg J, Stanyon R, Arnold N, Tofanelli S, Ishida T, Cremer T. Reconstruction of genomic rearrangements in great apes and gibbons by chromosome painting. Proc Natl Acad Sci. 1992;89(18):8611–5.
14. Kampiranont A. Cytogenetics. Department of Genetics, Faculty of Science, Kasetsart University, Bangkok, Thailand.; 2003
15. Koehler U, Arnold N, Wienberg J, Tofanelli S, Stanyon R. Genomic reorganization and disrupted chromosomal synteny in the siamang (*Hylobates syndactylus*) revealed by fluorescence *in situ* hybridization. Am J Phys Anthropol. 1995;97(1):37–47.
16. Koehler U, Bigoni F, Wienberg J, Stanyon R. Genomic reorganization in the concolor gibbon (*Hylobates concolor*) revealed by chromosome painting. Genomics. 1995;30(2):287–92.
17. Krishna-Murthy DS. Giemsa banding pattern in the langur monkey *Presbytis entellus* entellus (Dufresne). Curr Sci. 1979;48:180–1.
18. Napier JR, Napier PH, Napier JR. The natural history of the primates. Cambridge: MIT Press; 1985.
19. Napier JR, Napier PH. Handbook of living primates. New York: Academic Press; 1967.
20. Nie W, Liu R, Chen Y, Wang J, Yang F. Mapping chromosomal homologies between humans and two langurs (*Semnopithecus francoisi* and *S phayrei*) by chromosome painting. Chromosome Res: Int J Mol Supramol Evol Aspects Chromosome Biol. 1998;6(6):447–53.
21. Oates JF, Davies AG. Colobine monkeys: their ecology, behaviour, and evolution. Cambridge: Cambridge University Press; 1994.
22. Oates JF. The diversity of living colobines. Colobine Monkeys Their Ecol Behav Evol. 1994;45:45–73.
23. Ponsa M, De Boer LE, Egozcue J. Banding patterns of the chromosomes of *Presbytis cristatus pyrrhus* and *P. obscurus*. Am J Primatol. 1983;4(2):165–9.
24. Rooney DE, Czepulkowski B. Human cytogenetics: constitutional analysis: a practical approach, vol. 1. New York: Oxford University Press; 2001.
25. Sangpakdee W, Tanomtong A, Monthatong M, Pinthong K, Gomontean B, Nie W. The homology and relationship of human (*Homo sapiens*) chromosomes 1, 19 and dusky langur (*Trachypithacus obscurus*) chromosomes 6, 8 demonstrated with chromosome painting. Cytologia. 2008;73(4):349–55.
26. Sharma GP, Sobti RC, Gupta CM. An analysis of chromosomes in three primates from India. J Hum Evol. 1973;2(4):283–7.
27. Stanyon R, Wienberg J, Romagno D, Bigoni F, Jauch A, Cremer T. Molecular and classical cytogenetic analyses demonstrate an apomorphic reciprocal chromosomal translocation in *Gorilla gorilla*. Am J Phys Anthropol. 1992;88(2):245–50.
28. Ushijima RN, Shininger FS, Grand TI. Chromosome complements of two species of primates: *Cynopithecus niger* and *Presbytis entellus*. Science. 1964;146(3640):78–9.
29. Vogel C, Winkler P. NoLangurs and Colobi. In Grzimek's encyclopedia of mammals title. vol. 2. McGraw-Hill, London; 1990.
30. Wang XP, Yu L, Roos C, Ting N, Chen CP, Wang J, Zhang YP. Phylogenetic relationships among the colobine monkeys revisited: new insights from analyses of complete mt genomes and 44 nuclear non-coding markers. PLoS ONE. 2012;7(4):e36274.
31. Wienberg J, Jauch A, Stanyon R, Cremer T. Molecular cytotaxonomy of primates by chromosomal *in situ* suppression hybridization. Genomics. 1990;8(2):347–50.
32. Wienberg J, Stanyon R, Jauch A, Cremer T. Homologies in human and *Macasa fuscata* chromosomes revealed by *in situ* suppression hybridization with human chromosome specific DNA libraries. Chromosoma. 1992;101(5–6):265–70.
33. Yu D, Yang F, Liu R. A comparative chromosome map between human and *Hylobates hoolock* built by chromosome painting. Yi chuan xue bao = Acta genetica Sinica. 1997;24(5):417–23.