

Chromosomes Homology Between Human (*Homo sapiens*) and Dusky Langur (*Trachypithecus obscurus*)

by Fluorescence *In Situ* Hybridization

ความเหมือนของโครโมโซมระหว่างมนุษย์ (*Homo sapiens*)

และค่างแว่นถิ่นใต้ (*Trachypithecus obscurus*)

โดยเทคนิคฟลูออเรสเซนซ์อินซิทูไฮบริไดเซชัน

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ABSTRACT

Human (*Homo sapiens* Linnaeus, 1758, 2n=46) and dusky langur (*Trachypithecus obscurus*, Ried, 1837, 2n=44) are classified in the same order which evolutionary close relatives. The chromosomal homology between both species was established by chromosome painting technique. The fluorescently labeled probes specific to human chromosomes 14, 15, 21 and 22 were developed. The probes were allowed to hybridize with dusky langur chromosome prepared from fibroblast culture of ear tissues. The results showed that the human chromosomes 14 and 15 probes hybridized to chromosome 5 of dusky langur while human chromosomes 21 and 22 probes hybridized to chromosome 19 of dusky langur. The hybridization patterns can be interpreted that dusky langur chromosomes 5 and 19 occurred by Robertsonian translocation from human chromosomes 14/15 and 21/22, respectively. Moreover, chromosome 19 of dusky langur bears the nucleolar organizer regions (NORs) which can be the marked chromosome of this species.

บทคัดย่อ

มนุษย์ และค่างแว่นถิ่นใต้ถูกจัดให้อยู่ในอันดับเดียวกันซึ่งมีความสัมพันธ์ทางวิวัฒนาการกันอย่างใกล้ชิด การศึกษาความเหมือนของโครโมโซมทั้งสองสปีชีส์ครั้งนี้ ใช้เทคนิคโครโมโซมเพ้นท์ โดยเตรียมโพรบจากโครโมโซมของมนุษย์คู่ที่ 14, 15, 21 และ 22 นำไปไฮบริไดซ์ร่วมกับโครโมโซมของค่างแว่นถิ่นใต้ที่เตรียมจากการเพาะเลี้ยงเซลล์ไฟโบรบลาสต์จากเนื้อเยื่อใบหู ผลการทดลองพบว่าโพรบของมนุษย์คู่ที่ 14 และ 15 จับกับโครโมโซมคู่ที่ 5 ของค่างแว่นถิ่นใต้ขณะที่โพรบของมนุษย์คู่ที่ 21 และ 22 จับกับโครโมโซมคู่ที่ 19 จากผลการทดลองครั้งนี้สามารถอธิบายเชิงวิวัฒนาการของโครโมโซมของทั้งสองสปีชีส์ได้

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ว่าโครโมโซมค่างแว่นถิ่นใต้คู่ที่ 5 และ 19 เกิดจากกระบวนการรวมตัวกันแบบโรเบิร์ตโซเนียนของโครโมโซมมนุษย์คู่ที่ 14 / 15 และคู่ที่ 21 / 22 ตามลำดับ นอกจากนี้ยังพบว่าโครโมโซมคู่ที่ 19 ของค่างแว่นถิ่นใต้มีบริเวณที่เรียกว่านิวคลีโอลาร์ออร์กาโนเซอรัเจียนส์ (นอร์) ซึ่งเป็นโครโมโซมเครื่องหมายของค่างสปีชีส์นี้

Key Words : Fluorescence *In Situ* Hybridization, Human (*Homo sapiens*) chromosomes, Dusky langur (*Trachypithecus obscurus*) chromosomes

คำสำคัญ : เทคนิคฟลูออเรสเซนซ์อินซิทูไฮบริไดเซชัน โครโมโซมมนุษย์ โครโมโซมค่างแว่นถิ่นใต้

Introduction

In the present, the population of wild animals has been decreased rapidly. Dusky langur (*Trachypithecus obscurus*) also called dusky leaf monkey is one of the species categorized in appendix II by CITES which is concerned to vulnerable species in the future. The dusky langur is evolutionarily close to human, therefore, it is interesting to understand the genetic similarities between these two mammals using molecular cytogenetics. In addition, there has not yet been reported on studying of dusky langur chromosomes comparing to human chromosomes by fluorescence *in situ* hybridization (FISH) technique.

Materials and Methods

The following techniques were performed at Key Laboratory of Cellular and Molecular Evolution, Kunming Institute of Zoology, the Chinese Academy of Sciences, Kunming, Yunnan, China as some parts of the M.Sc. thesis.

Chromosomes preparation from fibroblast culture

The male *Trachypithecus obscurus* ear tissue were collected at Songkhla Zoo, Songkhla province, Thailand and then transferred to Key

Laboratory of Cellular and Molecular Evolution for fibroblast cell culture and preparing the metaphase chromosomes. The cell line was grown at 37 °C in DMEM medium enriched with 20% newborn calf serum. Before chromosome harvesting, the cells were treated with 0.05 µg/ml colchicines (Sigma) for 1 hr. Chromosome preparation followed standard procedures, which include a 15–20 minute hypotonic treatment in 0.075 M KCl, four fixations in methanol–glacial acetic acid (4:1) and air drying. The fluorescence *in situ* hybridization using DAPI–banding concurrently with *in situ* hybridization was performed to facilitate the chromosome identification. G–banding was also established for karyotype numbering and arrangement following Kampiranont (2003).

FISH technique

Human chromosome–specific probes were kindly 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 with hybridization buffer (50% deionized formamide, 10% dextran sulphate, 2X 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 hr. Slides were denatured by incubation in 70% formamide/2X SSC solution at 65 °C for 2 min., quenched in ice-cold 70% ethanol and dehydrated through 70%, 90%, and 2 times 100% ethanol series. The preannealed probes were applied onto slides and allowed to hybridize 24 hr. at 37 °C. Post-hybridization washes were two 5 min. incubations in 50% formamide, 50% 2X SSC at 50 °C followed by two 5 min. incubation in 2X 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 drying and added 15 µl mounting solution then cover with a clean glass coverslip.

Results

G-banded karyotype of *T. obscurus*

T. obscurus were found to have the same diploid numbers of $2n = 44$ with the other colobine monkeys in the previous reports (Chen et al., 1981; Bigoni et al., 1997a, 1997b, 2004; Lui et al., 1997; Nie et al., 1998). According to the karyotype numbering and arrangement system as Kampiranont (2003), we found that *T. obscurus* karyotype composition is: 22(M) + 18(SM) + 2(A), X(M) and Y(SM). The metacentric chromosome 19, bears the NORs (nucleolar organizer regions) which implies to be marked chromome (The chromosomes are divided into three groups on the basis of centromeric index: metacentrics, submetacentrics and acrocentric chromosomes.) All of the chromosomes are ordered according to their relative length and sizes. Figure 1 shows the metaphase chromosome and karyotype by G-banding technique of *T. obscurus*.

We also found that *T. obscurus* have the similar G-banding patterns with other Asian colobines.

Hybridization of human chromosome-specific probes onto metaphases of *T. obscurus*

The hybridization signal of human chromosome-specific probes 14/15 and 21/22 gave bright painting on the dusky langur chromosomes 5 and 19, respectively. Human chromosome 14 and 15 probes hybridized to one region on dusky langur chromosome 5, where human chromosome 21 and 22 probes hybridized on dusky langur chromosome 19 (Figure 2).

Robertsonian translocation

The human chromosomes 14 and 15 probes hybridized to chromosome 5 of dusky langur while chromosome 21 and 22 probes hybridized to chromosome 19. The hybridization patterns can be interpreted that dusky langur chromosomes 5 and 19 occurred by Robertsonian translocation from human chromosomes 14/15 and 21/22, respectively (Figure 3).

Discussions

G-banded Karyotypes of dusky langur

We confirmed that the diploid karyotype number of *T. obscurus* is $2n = 44$, common to all the other species of the Colobinae (Bigoni et al., 1997a, 1997b, 2004; Chiarelli, 1963; Chen et al., 1981; Lui et al., 1997; Nie et al., 1998). G-banded Karyotypes of dusky langur gave similar but not identical karyotypes with other colobines (*Semnopithecus francoisi*, *S. phayrei*) (Nie et al., 1998). The difference may be caused from the numbering system does not follow published report.

The disagreement of karyotype composition may due to the different karyotype numbering system using in our report. We also found that *T. obscurus* chromosome 19 contains the nucleolar organizer regions (NORs) with the same banding pattern found on other species chromosomes which are *presbytis cristata*, *Semnopithecus francoisi*, *S. phayrei* and *Nasalis larvatus*.

Human Syntenic Groups Fragmented in *Trachypithecus obscurus*

The results from hybridization patterns of human chromosome-specific probes demonstrated that the dusky langur chromosomes showed conserved syntenic homologies to human chromosomes. Human chromosome probes 14/15 and 21/22 showed hybridization patterns on dusky langur chromosomes 5 and 19, respectively concerning that it was occurred from Robertsonian translocation. The results agree with Bigoni et al. (1997a) who reported that the hybridization pattern of *P. cristata* demonstrates the existence of the following associations 14/15, 21/22, due to simple Robertsonian changes. Out of these associations, only 14/15 was also found in macaques (Wienberg et al., 1992). Bigoni et al. (1977b) reported that there are two Robertsonian translocations : homologs 14/15 form *Colobus guereza* chromosome 6; 21/22 form CGU 16. Moreover, the same hybridization pattern by using human chromosomes-specific probes 14/15 and 21/22 were also found in *Semnopithecus francoisi* and *S. phayrei* on chromosomes 12 and 21 (Nie et al., 1998), Proboscis Monkey (*Nasalis larvatus*) on chromosomes 17 and 14 (Bigoni et al., 2003) and *Pygathrix nemaeus* on

chromosomes 4 and 21 (Bigoni 2004), respectively. We also found that The association of human chromosomes 21 and 22 forming the marker chromosome occurs in all documented Asian colobines (Bigoni, 1995; Bigoni et al., 1997a, 2003, 2004; Nie et al., 1998), and also in the African species *Colobus guereza* (Bigoni et al., 1997b).

Our results suggest that *T. obscurus* have the same conservative karyotypes and same hybridization patterns with the other colobines when using human chromosome-specific paint probes 14/15 and 21/22.

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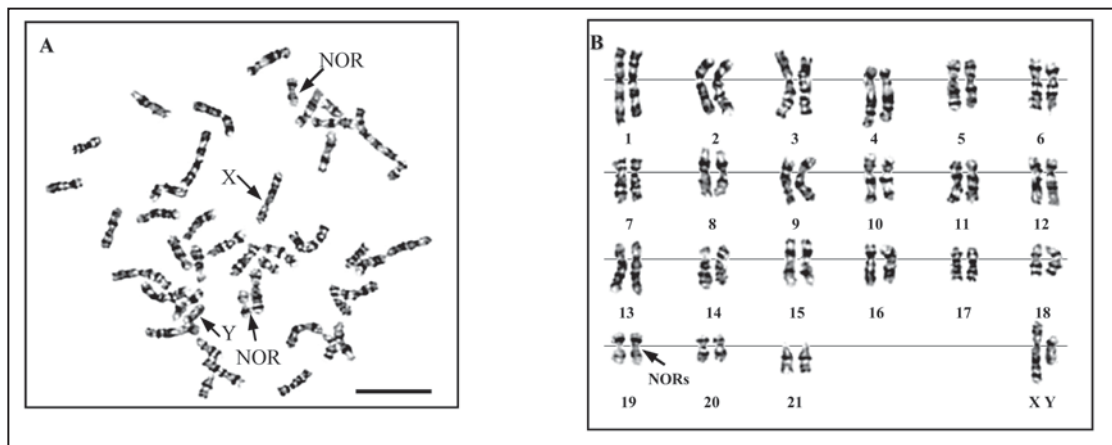


Figure 1 (A) Metaphase chromosome of male dusky langur (*Trachypithecus obscurus*)

(B) Karyotype by G-banding technique * NORs (nucleolar organizer regions), scale bar represent 10 μ m, satellite chromosomes arrows.

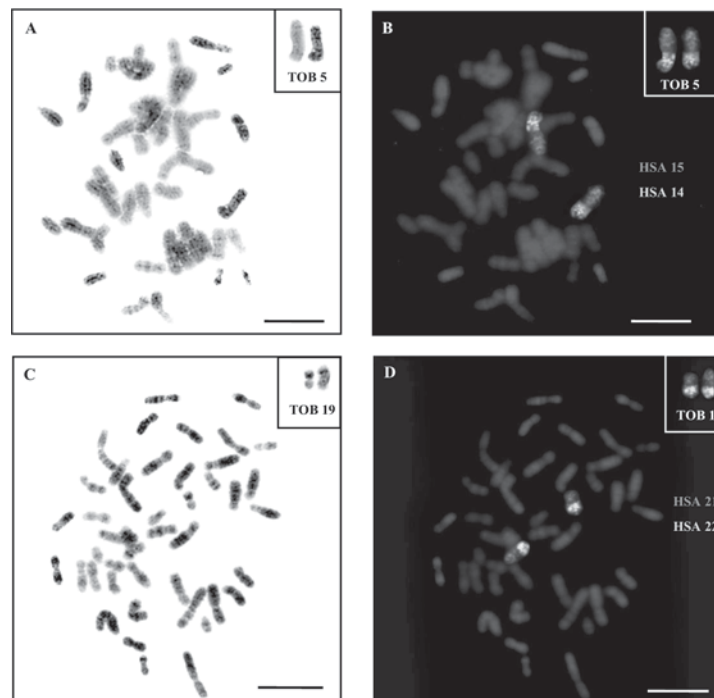


Figure 2 (A) DAPI-banding metaphase chromosomes of male dusky langur.

(B) Hybridization pattern of human probe 14 (HSA 14, biotin labeled) and 15 (HSA 15, Cy3 labeled) on dusky langur chromosomes 5 (TOB 5)

(C) DAPI-banding metaphase chromosomes of male dusky langur.

(D) Hybridization pattern of human probe 21 (HSA 21, Cy3 labeled) and 22 (HSA 22, biotin labeled) on dusky langur chromosomes 19 (TOB 19)

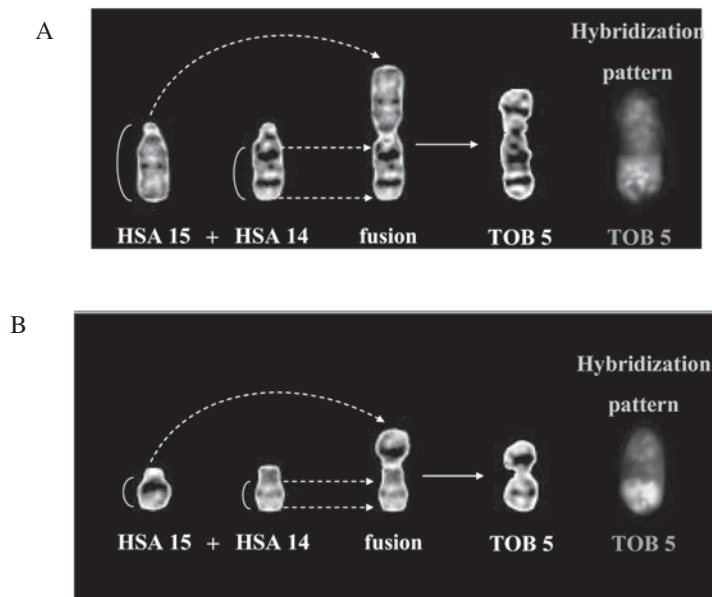


Figure 3 (A) Robertsonian translocation between human chromosome 15 fusion with human Chromosome 14 from to dusky langur chromosomes

(B) Robertsonian translocation between human chromosome 21 fusion with human chromosome 22 from to dusky langur chromosome 19

* HSA (*Homo sapiens*), TOB (*Trachypithecus obscurus*), human chromosomes use in term of Rooney (2001).