

## Mapping Homology Between Human and Black and White Colobine Monkey Chromosomes by Fluorescent In Situ Hybridization

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We used in situ hybridization of chromosome specific DNA probes ("chromosome painting") of all human chromosomes to establish homologies between the human and the white and black colobus (*Colobus guereza* 2n = 44). The 24 human paints gave 31 signals on the autosomes (haploid male chromosome set). Robertsonian translocations between chromosomes homologous to human 14 and 15, 21 and 22, form colobine chromosomes 6 and 16, respectively. Reciprocal translocations were found between human chromosomes 1 and 10, 1 and 17, as well as 3 and 19. The alternating hybridization signals between human 3 and 19 on Colobus chromosome 12 show that in this case a reciprocal translocation was followed by a pericentric inversion. The hybridization data show that in spite of the same diploid number and similar Fundamental Numbers, the black and white colobine monkey differs from *Presbytis cristata*, an Asian colobine, by 6 reciprocal translocations. Comparisons with the hybridization patterns in other primates show that some Asian colobines have a more derived karyotype with respect to African colobines, macaques, great apes, and humans. Chromosome painting also clearly shows that similarities in diploid number and chromosome morphology both between colobines and gibbons are due to convergence. Am. J. Primatol. 42:289–298, 1997. © 1997 Wiley-Liss, Inc.

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### INTRODUCTION

*Colobus guereza* (black and white colobine monkey) is an African colobine distributed from Ethiopia to northern Tanzania and west to the Nigerian/Cameroon border [Oates, 1994]. It is found in a wide range of habitats: montane forests, savanna gallery forests, and from medium-altitude to lowland moist forests. Taxonomists [Napier & Napier, 1985; Vogel and Wrinkler, 1990; Groves,

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1993; Oates et al., 1994] agree in recognizing two genera of African colobines: *Procolobus* and *Colobus*, but there is no consensus on the number and phylogeny of the species. Initially all black and white colobus were included in a single species [Schwarz, 1929]. Vogel and Wrinkler [1990] divided them into two species, *Colobus guereza* (northern black and white colobus) and *Colobus polykomos* (southern black and white colobus). Other classifications recognize four [Groves, 1989] or five species in the genus *Colobus* [Oates et al., 1994]. Further, Napier and Napier [1985] included the red colobus in genus *Colobus*, but in the other classifications it is considered as part of genus *Procolobus*.

In many regards colobines can be considered as specialized primates. The name *Colobus* derives from the Greek word *Kolobos* meaning mutilated, because they have reduced thumbs, which in *Procolobus verus* are completely absent [Fleagle, 1988]. They have a remarkable digestive system with a chambered stomach and a particular lysozyme composition adapted to a diet rich in cellulose such as mature leaves. They are the only group of primates which can be considered as foregut fermenters [Hill, 1958; Chivers, 1994; Kay and Davies, 1994] and present a striking case of convergence with ruminants [Stewart et al., 1987; Stewart and Wilson, 1987]. The teeth, which have sharp high cusps on molars, also show a dietary adaptation [Oates & Davies, 1994]. Colobines also differ from Cercopithecinae in not having cheek pouches. Ethological studies suggested that some behavioral characteristics of the colobines such as female transfer, absence of female social ranks, and forms of collaboration among non-related mothers, could be considered as specializations associated with their folivorous diet [McKenna, 1979; Wrangham, 1980; Moore, 1984].

## KARYOLOGY OF AFRICAN COLOBINES

Reports concerning African colobine cytogenetics are rare. The studies with classical staining were sufficient only to establish that the karyotype was composed of 44 biarmed (submetacentric or metacentric) chromosomes and that one pair of "marked" chromosomes was present. The morphology of the Y was not clear. The karyotypes of the three species studied *Colobus polykomos*, *C. badius* and *C. kirkii* appeared similar if not identical [see Chiarelli, 1963]. Asian colobines (genus *Presbytis*) apparently differed only by the presence of a small pair of acrocentric chromosomes [Chiarelli, 1963; Ushima et al., 1964].

The most stunning interpretation of the classical staining results by some cytogeneticists was to propose a strict phylogenetic relationship between colobines and hylobatids [Chiarelli, 1963, 1972]. With classical staining the gibbon (lar species group) appeared to have a karyotype identical to African colobines: the same diploid and fundamental number (number of chromosome arms) and the presence of one pair of "marked chromosomes" [Stanyon et al., 1995].

## Banding

The reports on African colobines, genus *Colobus*, are limited to R-banding [Dutrillaux et al., 1982; Muleris et al., 1986]. Altogether, the R-banding patterns of three species of African colobines (*Colobus vellerosus*, *C. palliatus*, and *C. abyssinicus*) have been described. Following Groves [1993] *Colobus abyssinicus* can be considered as a synonym of *Colobus guereza*. They did not describe the Y chromosome of *C. guereza (abyssinicus)* apparently because only a single female was studied.

In contrast to the conclusions reached from classical staining they noted a

number of differences (pericentric inversions) between all African colobines and that *Presbytis cristata* differed from all African colobines by at least 7 pericentric inversions and 5 reciprocal translocations. However, these authors made no attempt to compare or consider the relationship between African colobines and gibbons [Dutrillaux et al., 1982; Muleris et al., 1986].

We hoped that more complete and reliable data on African colobines chromosomes by combining chromosome banding with chromosome painting could be used to better examine evolutionary hypothesis both among Asian and African colobines and between colobines and other primates especially gibbons. It is clear that chromosomes can be a useful tool in evolutionary studies only if homologous structures are compared. The hybridization of DNA probes derived from specific chromosomes (chromosome painting) can rapidly establish between species chromosomal homology and eliminate errors due to confusing convergence and homology, a problem which has up to now limited the usefulness of chromosomes for reconstructing phylogeny [Stanyon et al., 1995]. Chromosomal painting has been used to establish the homologies between the chromosomes of humans and great apes (chimpanzees, gorilla, orangutan), lesser apes (*H. lar*, *H. syndactylus*, and *H. concolor*), macaques [Wienberg et al., 1990, 1992; Jauch et al., 1992; Stanyon et al., 1992, 1995; Koehler et al., 1995a,b] and a species of Asian colobines, *Presbytis cristata* [Bigoni et al., 1997].

The "chromosome painting" of human chromosome-specific DNA probes allowed us to establish the homologies between the human and the black and white colobus monkey karyotypes. We then compared the colobine hybridization pattern with that of other primates recently studied by chromosome painting such as Asian colobines, hylobatids, great apes, and macaques. Further, no G-banded karyotypes and no standardized ideograms of *Colobus guereza* are available. There are not even any descriptions of the Y chromosome for this species. Here we report on the G-banding pattern of *Colobus guereza*, describe the Y-chromosome and provide a standardized ideogram.

## MATERIALS AND METHODS

Standard chromosome preparations were obtained from fibroblast cultures kindly provided by Dr. O. Ryder from the San Diego Zoological Society, San Diego, CA, of a male (third passage, sample 7911).

Culture techniques and chromosome preparation followed standard procedures. Briefly, the tissue culture medium was DMEM supplemented with 10% FCS, 100 µ/ml penicillin, 100 µg/ml streptomycin. Chromosome preparations were stored in fixative at -20°C until needed or on slides at -80°C.

G-banding was done according to Small et al. [1985]. The ideogram was prepared measuring ten metaphase spreads and calculating the relative length of chromosomes and chromosome arms. Chromosomes were numbered according to their relative length.

## Hybridizations

To insure correct chromosome identification and assignment of hybridization signals *C. guereza* chromosomes were trypsin G-banded and photographed before hybridization. Slides were then destained and postfixed with formaldehyde [Arnold et al., 1992]. The identification of chromosomes was also facilitated by DAPI staining performed concurrently with in situ hybridization.

Hybridization of human DNA probes (chromosome paints) on *C. guereza* chro-

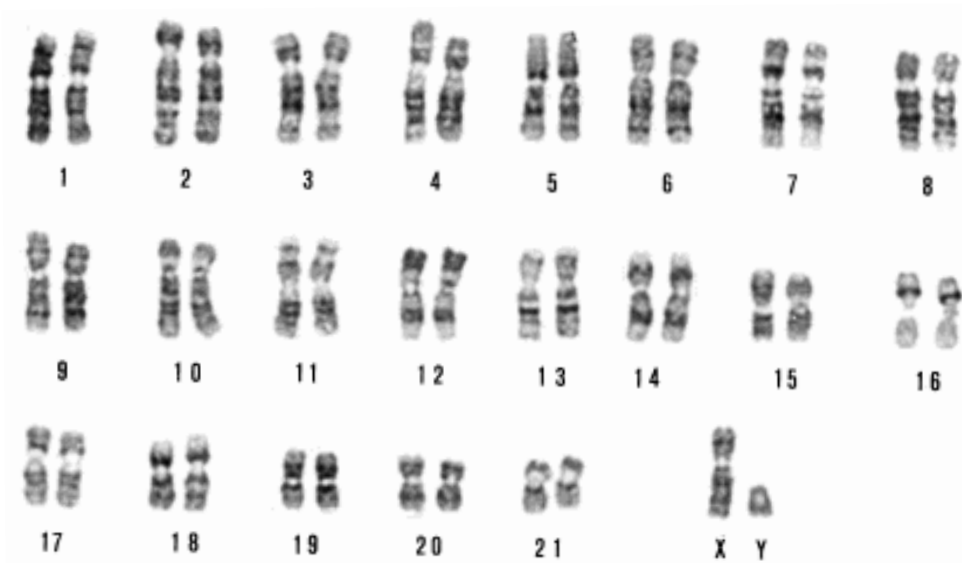


Fig. 1. A G-banded karyotype of *Colobus guereza*  $2n = 44$ .

mosomes was performed as previously reported [Wienberg et al., 1990, 1992; Jauch et al., 1992; Stanyon et al., 1992, 1995; Bigoni et al., 1997]. Biotinylated probes were detected with avidin conjugated to FITC. In a double hybridization experiment on the same metaphase, biotin-labeled probes were detected by avidin FITC, while digoxigenin-labeled probes were detected with TRITC-labeled antibodies.

## RESULTS

### G-Banding

We found that *Colobus guereza* has a diploid number of  $2n = 44$  composed of solely metacentric or submetacentric chromosomes (Fig. 1). One pair of submetacentric chromosomes (no. 16) was the "marked" chromosome or NOR (nucleolar organizer region) bearing chromosome. The Y chromosome was a fair-sized submetacentric Y with a relative length of 1.8, and a centromeric index of 0.6.

### In Situ Hybridization

All hybridizations of human DNA chromosome paints on colobus chromosomes provided bright signals with the exception of the human Y probe (Figs. 2,3). The 22 human autosomal probes were divided into 31 signals in the colobus

Fig. 2. Examples of conserved chromosome synteny between humans and black and white colobine monkeys. When chromosomal synteny is maintained two hybridization signals are seen. Examples of sequential G-banding and hybridization signals obtained with human chromosome specific DNA probes on metaphases of the black and white colobine monkey are as follows: (a,b) human chromosome 4 paint, (c,d) human chromosome 5 probe, (e,f) human chromosome 8 probe, (g,h) human chromosome 9 probe, (i,j) human chromosome 11 probe. Arrows in G-banded metaphases indicate colobine chromosomes hybridized by the respective human chromosome probes. Centromeric areas are often not hybridized (most evident in d and j) due to the suppression of repetitive sequences. All images were recorded directly on photographic film without computer image processing.

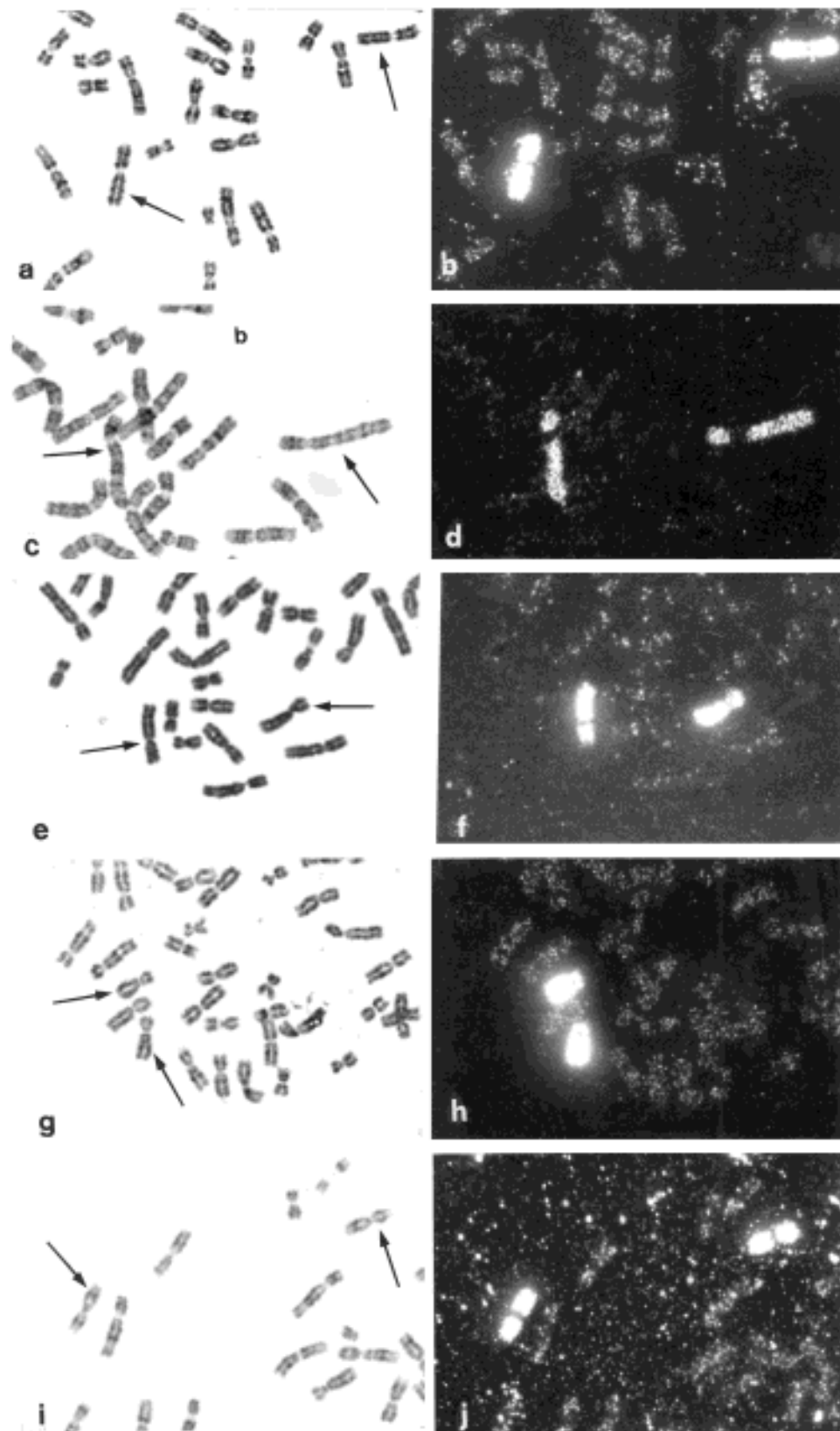


Figure 2.

karyotype (Fig. 4). Twelve human DNA paints hybridized to a single *Colobus guereza* chromosome: HSA4, HSA5, HSA6, HSA7, HSA8, HSA9, HSA11, HSA12, HSA13, HSA16, HSA18, and HSA20. Human paints HSA14 and HSA15 were found associated on one chromosome of *Colobus guereza* (CGU6) while paints for HSA21 and HSA22 hybridized to CGU16 (marked chromosome).

Five human DNA probes were divided into segments localized on two or three different colobus chromosomes. The DNA probe specific to human chromosome 2 hybridized to two pairs of colobine chromosomes (nos. 8 and 17). Hybridization signals from four human paints were fragmented and found hybridized in various combinations on individual colobine chromosomes: CGU chromosome 5 was composed of segments homologous to human chromosome 1 and 10; CGU 11 by segments homologous to human 1, 10 17; CGU 12 and 18 by segments to human 3 and 19; CGU 13 by segments of human 1 and 17. Further, the colobus chromo-

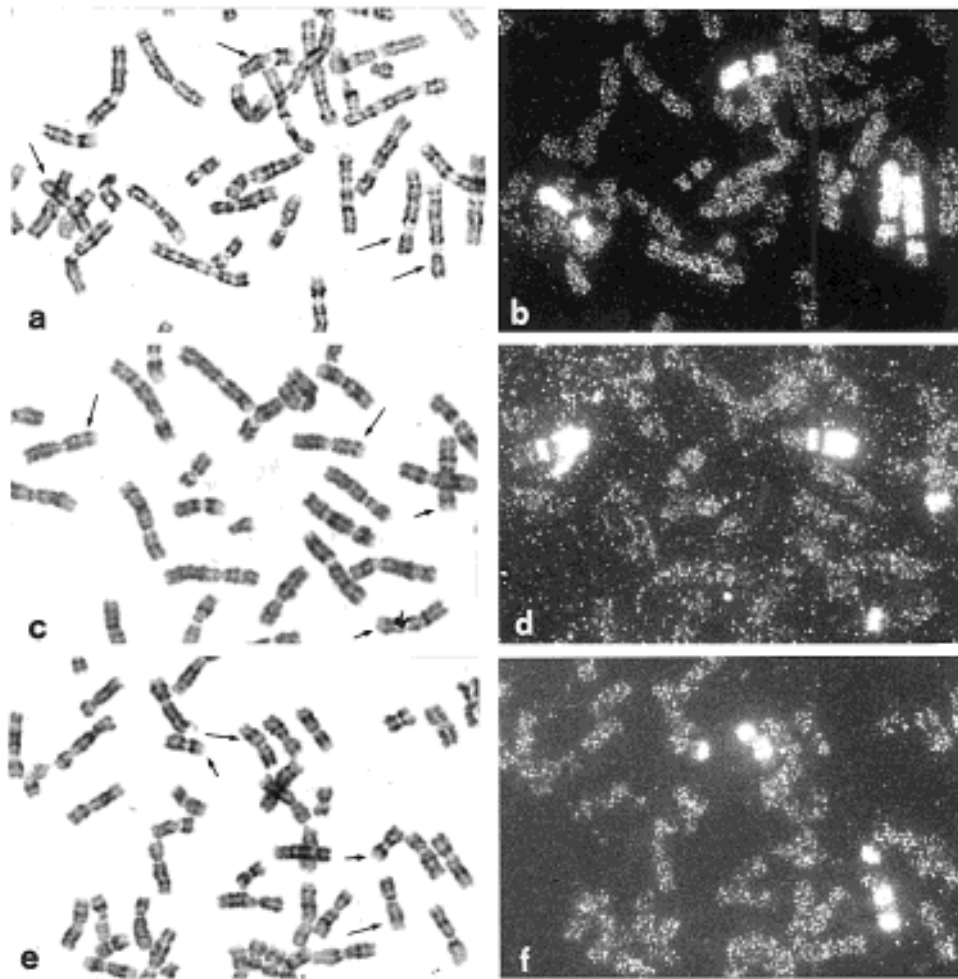


Fig. 3. Examples of disrupted chromosomal synteny in black and white colobines compared to the human condition. Disrupted chromosomal synteny is shown by the presence of more than two signals per metaphase. Examples of the sequential G-banding and hybridization signals are as follows: (a,b) human chromosome 2 probe, (c,d) human chromosome 17 probe, (e,f) human chromosome 19 library.

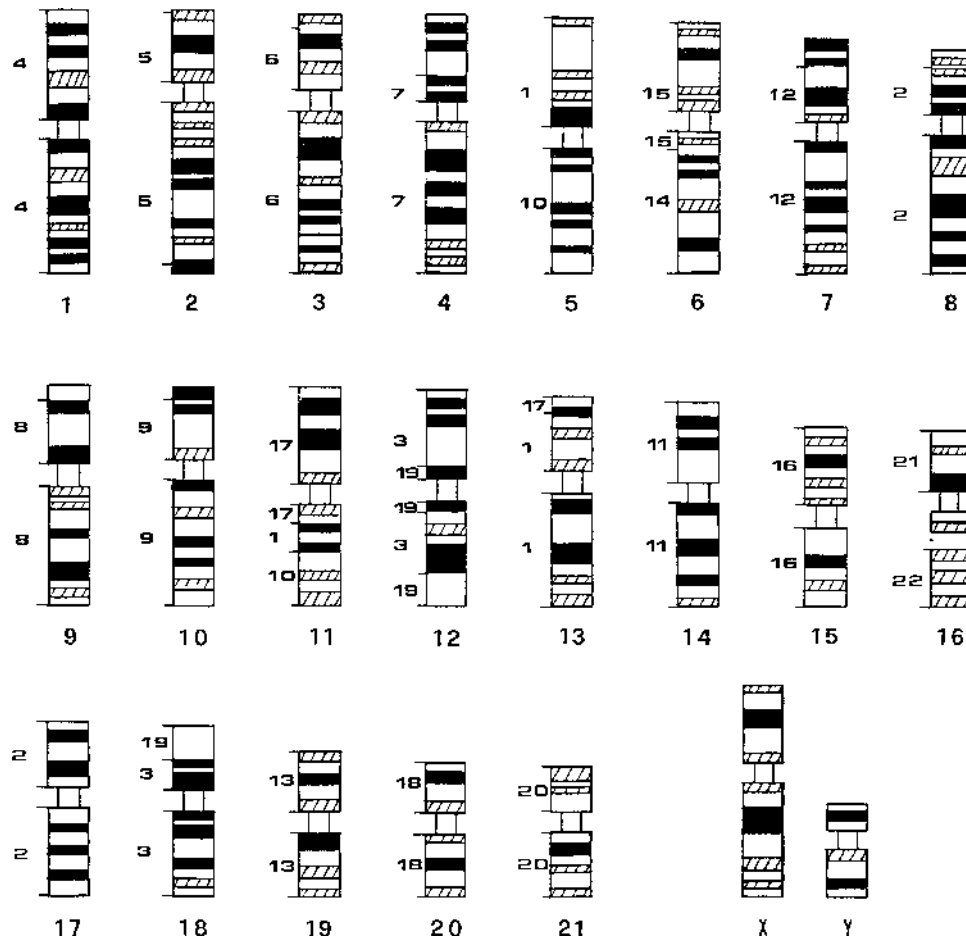


Fig. 4. Summary of the in situ hybridization pattern of DNA probes specific to whole human chromosomes on a G-banded ideogram of *Colobus guereza*. The colobine chromosomes are numbered below and the homology with human chromosomes or chromosome segments is on the left.

somes 12 were divided in four hybridization signals because of the alternate signals of paints homologous to segments of human chromosomes 3 and 19.

## DISCUSSION

We confirmed the results from previous reports including classical staining that the karyotype is composed solely of 44 metacentric/submetacentric chromosomes. However, the Y-chromosome, a fair-sized submetacentric, was described here for the first time.

Three species of African colobus have already been studied with R-banding technique by Muleris et al. [1986]. We compared our karyotype of *C. guereza* with the species they called *Colobus abyssinicus* because this name is considered as a synonym of *C. guereza* [Groves, 1993]. A full comparison was not possible because of the different banding technique used, but it is evident that the morphology of some chromosomes are different. This diversity may be due to a poly-

morphism not yet identified (we studied only one individual) or to a different taxonomic status.

### Hybridization Pattern

The chromosome painting results show that chromosomal synteny between humans and *Colobus guereza* is fairly well conserved. It is well known that human chromosome 2 resulted from an apomorphic tandem fusion and, as expected, we found that the human chromosome 2 probed hybridized to two different pairs of CGU chromosomes. However, a number of additional interchromosomal rearrangements distinguished the human genome from that of CGU. There are two Robertsonian translocations (homologs 14/15 from CGU chromosome 6; 21/22 from CGU 16) and four reciprocal translocations (between segments homologous to human chromosomes 1/10, 1/17, 1/10/17 and 3/19) on various CGU chromosomes. The alternating hybridization signals between segments homologous to human chromosomes 3 and 19 on CGU chromosome 12 are clear evidence that a pericentric inversion followed the reciprocal translocation.

### Comparison of Chromosome Painting Pattern Between *C. guereza* and Other Primates

***P. cristata*.** Although the hybridization patterns between CGU and this Asian colobine are identical for the Robertsonian translocations, they differ for the reciprocal translocations. In both karyotypes Robertsonian translocations between human chromosomes 14/15 and 21/22 are present [Bigoni et al., 1997]. It should be noted that in both species the “marked” chromosome (NOR-bearing) is formed by segments homologous to human chromosomes 21 and 22 in both species of colobines. The 14/15 translocation may well be ancestral for Old World monkeys as it is also present in the Cercopithecinae (see below). In comparing *C. guereza* to *P. cristata*, it is remarkable to note that different reciprocal translocations are present in this Asian Colobine (1/19, 6/16, Y/5). Although the reciprocal translocations that distinguish these karyotypes did not change the diploid (2N) and Fundamental Number (FN) different complex, apomorphic rearrangements have lead to the actual karyotypes. The karyotypes, which seemed so similar on morphological grounds, are actually quite diverse.

Some authors [Szalay and Delson, 1979] suggest that the common ancestor of all colobines evolved in Africa and later spread to Asia. It would be intriguing to test this hypothesis with the chromosome data, but more cytogenetic research on a good number of Asian colobines, especially with molecular techniques, would be necessary.

***M. fuscata*.** *M. fuscata* presents three associations of whole human chromosomes due to translocations: 7/21, 20/22, 14/15 [Wienberg et al., 1992]. Only the translocation of 14/15 is common to both colobines and macaques; the other two translocations are not present in colobines. In colobines human chromosomes 7 and 20 are single independent chromosome and 21 is translocated to 22.

**Hylobatids.** The fact that African colobines and gibbons (*Hylobates* lar species group) have an identical diploid number (44) and FNs (88) and one pair of “marked chromosomes” led some cytogeneticists to consider them phylogenetically aligned. However, chromosome painting in *Hylobates* has shown that this genus of primates has an extremely high rate of chromosomal evolution characterized by many interchromosomal rearrangements (translocations) [Koehler et al., 1995a,b]. A comparison of the hybridization patterns with African colobines



show that there are no common associations of hybridization signals (translocations) and there are no chromosome rearrangements that could phylogenetically link hylobatids and colobines. Clearly, the similarities in the karyotypes previously cited by cytogeneticists resulted from convergence [Stanyon et al., 1995; Bigoni et al., 1997].

## CONCLUSIONS

1. In the karyotype of *Colobus guereza* ( $2n = 44$ ,  $FN = 88$ ) the 22 human autosome chromosomes are divided into 31 segments due to reciprocal translocations.

2. Six black and white colobine monkey chromosomes are formed by reciprocal translocations between segments homologous to human chromosomes 1/10, 1/17, 1/10/17, and 3/19, while two colobine chromosomes are the result of Robertsonian translocations between human chromosomes 14/15 and 21/22.

3. A comparison of the hybridization pattern between the black and white colobine monkey (African colobine) and the silvered leaf monkey (an Asian colobine) shows that the karyotypes, in spite of the similarities in diploid number and chromosome morphology, differ for 6 reciprocal translocations.

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