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Cytotaxonomy of Colobinae Primates with Reference to Reciprocal Chromosome Painting of *Colobus guereza* and Humans

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ABSTRACT: *Colobine phylogeny and evolution is little understood and poorly studied. We established* C. guereza *(2n=44) chromosome paint probes by flow sorting and reciprocally hybridized them to human chromosomes. The bivariate flow karyotype of* Colobus guereza *was resolved into 22 peaks. Paints of* C. guereza *were hybridized to human metaphases and 32 clear signals were detected. The reciprocal painting data allowed us to assign subchromosomal homologies between* C. guereza *and human chromosomes. A comparison of these data with previous chromosome painting and banding supported the monophyly of Colobinae and their division in an African and an Asian clade.* Pygathrix nemaeus *is karyologically the most conservative colobine species studied and it probably diverged early after the separation of Asian and African colobines. In contrast, chromosome painting shows that* Nasalis larvatus*, often considered the most primitive and isolated colobine, is karyologically derived and phylogenetically nested within Asian colobines. Both the painting and banding data support the taxonomic grouping of* Trachypithecus, *but would exclude the purple faced leaf monkey and align this species with the Hanuman langur in* Semnopithecus.

KEYWORDS: *molecular cytogenetics, chromosome rearrangements, genome evolution, phylogeny.*

INTRODUCTION

Colobine monkeys, also known as leaf-eaters, are a group of morphologically highly specialized catarrhines. Their latin name Colobinae, is derived from the Greek word for mutilated (kolobos), because these monkeys are characterized by a very short and in some case absent thumb. In contrast, the other fingers, especially the third and the fourth are longer then in cercopithecines (Strasser and Delson, 1987). Their dimensions vary from 4 kg in the African olive colobus (*Procolobus verus*), to 20 kg of the adult

males of the proboscis monkey in Borneo (*Nasalis larvatus*). Their diet consists primarily of mature leaves, but may also include other parts of plants, fruits, seeds and flowers, with variations among different species (Kay and Davies, 1994). Sometimes animal proteins in the form of insects, larvae and spiders are also included in the diet.

Their adaptation to the leaf eating niche explains their specialized morphology as well their digestive physiology. The colobine digestive system is unique among primates. Salivary glands are overdeveloped and produce a high quantity of saliva. The stomach is large and multi-chambered and symbiotic bacteria are present in the forestomach to digest cellulose (Oates *et al.*, 1994), therefore these monkeys are often indicated as 'foregut fermenters'. The stomach is divided in four chambers (*presaccus*, *saccus gastricus*, *tubus gastricus* and *pars pilorica*). In the first two chambers symbiotic bacteria are present and it appears that the saliva also acts as a buffer keeping the acidity in these first two chambers at an acceptable level for the survival of the bacteria. These bacteria are then digested by various enzymes in the next two stomach sections. The lysosomes involved are a striking case of adaptive convergence with ruminants (Stewart *et al.*, 1987). Recently, digestive RNases were studied in a colobine (*Pygathrix nemaeus*) and a clear case of duplicate gene evolution was found (Zhang, 2003).

The number of teeth and the dental formula are the same as in the other Catarrhinae (2-1-2-3), but important differences are present. The incisors are smaller then in cercopithecines and a high frequency of underbite was found. The masticatory system is overall powerful with teeth characterized by high cusps and cutting crests, also molars have high and sharp cusps linked by transversal crests (*bilophodontia*) (Richard, 1985). Colobines also differ from Cercopithecinae because they do not have cheek pouches.

Despite a recent accumulation of data on various features of these monkeys, Colobinae phylogeny and evolution are still not well understood, partly because they do not survive well in captivity, and there is no consensus on the taxonomy. Groves (1989) proposed a basic division between *Nasalis* (including *Simias*) and the other species, while Strasser and Delson (1987) preferred a split between African and Asian colobines, a scenario confirmed by molecular studies and accepted in recent taxonomy (Collura *et al.*, 1996; Collura and Stewart, 1995; Disotell, 1996; Messier and Stewart, 1994; Page *et al.*, 1999; Sarich, 1970). The African group was divided by Oates, Davies and Delson (1994) in two genera: *Colobus* (black and white colobus monkey) and *Procolobus* (red and olive colobus monkeys). *Colobus* included five species: *satanas*, *angolensis*, *polykomos*, *guereza* and *vellerosus*. *Procolobus* comprehended the subgenus *procolobus* (olive colobus, one species) and *piliocolobus* (red colobus, also monospecific). Groves (2001) described three genera of African colobines (*Colobus*, *Piliocolobus* and *Procolobus*) and a total of 15 species.

Asian colobines taxonomy has gone through even more extensive changes. Groves (1989) described five genera *Nasalis*, *Pygathrix*, *Presbytis*, *Trachypithecus* and *Semnopithecus*. Oates, Davies and Delson (1994) separated the genus *Simias* from *Nasalis*. In 2001 Groves agreed on the generic status of *Simias*. Groves also took in account morphological studies (Jablonski and Yan-Zhang, 1993) supporting the recognition of *Rhinopithecus* as full genus and not just as subgenus of *Pygathrix*, for a total of seven

Asian colobine genera: *Nasalis*, *Pygathrix*, *Presbytis*, *Trachypithecus*, *Semnopithecus*, *Simias* and *Rhinopithecus*. The number of species listed in the taxonomy of Asian colobines was also dramatically increased from 24 in Oates *et al.* (1994) to 43 according to Groves (2001).

Cytogenetics of Colobines

It is widely recognized that chromosomal events are linked to molecular divergence and to the speciation process (Navarro and Barton, 2003; Rieseberg and Livingstone, 2003). Therefore data on colobine karyotypes should provide hints for phylogenetic reconstruction and help to tease out evolutionary relationships. From studies using classical staining, the diploid number of both African (genus *Colobus*) and Asian (genus *Presbytis*) colobines was found to be 2n=44 (Chiarelli, 1963; Ushijima *et al.*, 1964). The karyotype of the genus *Colobus* was composed by all metacentric and submetacentric chromosomes, including one pair bearing the nucleolar organizer region (NOR). The karyotypes of the three species studied (*Colobus polykomos*, *C. badius* and *C. kirkii)* appeared similar if not identical (Chiarelli, 1963). Through classical staining, the karyotype of the genus *Presbytis* appeared to be almost the same as the karyotype of the African colobines with the only difference of a small acrocentric pair of chromosomes.

Banding techniques introduced in the 1970s improved the possibility of identifying differences between karyotypes, but only a few species of Colobinae have been studied with banding. A report on G- and Q-banding on *Trachypithecus cristatus* (*Presbytis cristata* in the original pububication) was reported with the description of two variant forms of chromosome 1 in the same female studied (Ponsa *et al.*, 1983). Three studies also present the R-banding of various species of of *Colobus* and *Trachypithecus cristatus* making clear that many differences were present among these species despite the same diploid number and apparent similar chromosome morphology (Dutrillaux *et al.*, 1981; Dutrillaux *et al.*, 1984; Muleris *et al.*, 1986). In particular a translocation involving an autosome and the Y chromosome was described in *T. cristatus*, the only case reported in catarrhine primates (Dutrillaux *et al.*, 1984).

Chromosomes are a useful tool for evolutionary studies and phylogeny when homologous structures are compared. Unfortunately, banding techniques, while providing good indicators of the morphology of chromosomes, are sometimes insufficient when used for comparisons between species. Banding provides a hypothesis of chromosomal homology between species, which should then be confirmed at the DNA level. Chromosome painting is a now well-established method for determining chromosomal homology. By firmly establishing homology, modern molecular cytogenetics offers powerful tools to investigate and clarify phylogenetic relationships by tracing the genome evolution of species. Recently we used flourescent *in situ* hybridization (FISH) of human chromosome paints on metaphases of various colobines to establish chromosomal homology at the DNA level between human chromosomes and chromosomes of four colobine species. These studies included *Colobus guereza* (Bigoni *et al.*, 1997b),

an African colobine (2n=44), and three species of Asian colobines: *Trachypithecus cristatus* (2n=44) (Bigoni *et al.*, 1997a), *Nasalis larvatus* (2n=48) (Bigoni *et al.*, 2003) and *Pygathrix nemaeus* (2n=44) (Bigoni *et al.*, 2004). Other authors have also reported on the hybridization of human chromosome paints to Asian colobines *T. francoisi* and *T. phayrei* (Nie *et al.*, 1998).

These studies supported the monophyly of Colobinae and their division in an African and an Asian clade. *P. nemaeus* is karyologically the most conservative of the Colobinae studied and possibly splitting soon after the divergence of Asian and African colobines (Bigoni *et al.*, 2004). On the other hand, chromosome painting shows that *N. larvatus*, often considered the most primitive and isolated colobine, is karyologically derived and phylogenetically nested within Asian colobines (Bigoni *et al.*, 2003). *T. cristatus* appears to be karyologically the most derived among the Asian colobines. This colobine has a reciprocal translocation of human 6 and 16 and is one of the very few primates and the only catarrhine showing a reciprocal translocation involving the Y chromosome and an autosome (Bigoni *et al.*, 1997a).

Detailed data of chromosomal homology are necessary to delineate karyological events and, to provide a worthwhile contribution to the evolutionary history and phylogeny of species. However, unidirectional painting (i.e. human probes hybridized to monkey metaphases) does not provide any information on subchromosomal homology, which can be particularly important when translocations have transformed the karyotype. Reciprocal chromosome painting in which chromosomal paints from two species are hybridized to metaphases of the other species can assign subchromosomal homology and helps locate breakpoints. Such information is helpful in determining if disrupted chromosomal synteny or syntenic associations found in two or more species derive from the same cytogenetic event. Therefore in this study we used reciprocal chromosome painting, a technique that allows a more precise localization of breakpoints and the detailed identification of segments involved in chromosome rearrangements.

There are only three reports available on reciprocal chromosome painting between humans and Old World monkeys: *Chlorocebus aethiops* (Finelli *et al.*, 1999), *Erythrocebus patas* and *Cercopithecus neglectus* (Stanyon *et al.*, 2005). Here we present the first report of reciprocal chromosome painting between a colobine monkey and humans. We established a set of whole chromosome painting probes of *Colobus guereza* by fluorescence activated chromosome sorting and DOP-PCR (degenerate oligonucleotide primed PCR). We then hybridized the *C. guereza* painting probes to human metaphases to define subchromosomal homology of the rearranged monkey chromosomes.

Materials and Methods

Chromosome preparations of a female *Colobus guereza* (CGU) were obtained by standard procedures from fibroblasts established by a skin biopsy kindly provided by Dr S. O'Brien (Laboratory of Genomic Diversity, National Cancer Institute-Frederick). Chromosomes of *C. guereza* were numbered according to Bigoni *et al.* (1997a).

C. guereza chromosome specific-probes were obtained by DOP-PCR from flow sorted chromosomes by PCR amplification and labeling conditions as previously described (Telenius *et al.*, 1992; Wienberg and Stanyon, 1998). Chromosome sorting was performed using a dual laser cell sorter (FACSDiVa) that allows a bivariate analysis of chromosomes by size and base-pair composition. From each peak in the flow karyotype about five hundred chromosomes were sorted directly in PCR tubes containing 30 l of distilled water. The 6MW primer (5'-ccgactcgagnnnnnnatgtgg-3') described by Telenius *et al.* (1992) was used in the primary reaction and to label the chromosomal DNA with biotin dUTP or digoxigenin-dUTP in a secondary PCR for indirect detection. These paints were first hybridized to *C. guereza* metaphases to identify the chromosome content of each peak of the flow karyotype and then to human chromosomes. Common FISH procedures were followed performing *in situ* hybridization and probe detection. About 300 ng of each PCR product per probe, together with 10 g of human Cot-1 (Invitrogen) were precipitated and then dissolved in 14 l hybridization buffer. After hybridization and washing of the slides, biotinylated DNA probes were detected with avidin coupled with fluorescein isothiocyanate (FITC, Vector). Digoxigenin-labeled probes were detected with antidigoxigenin antibodies conjugated with Rodamine (Roche).

Results

The bivariate flow karyotype of *C. guereza* was resolved into 22 peaks (Figure 1). Flow sorting and DOP-PCR provided chromosome paints from each peak. These paints

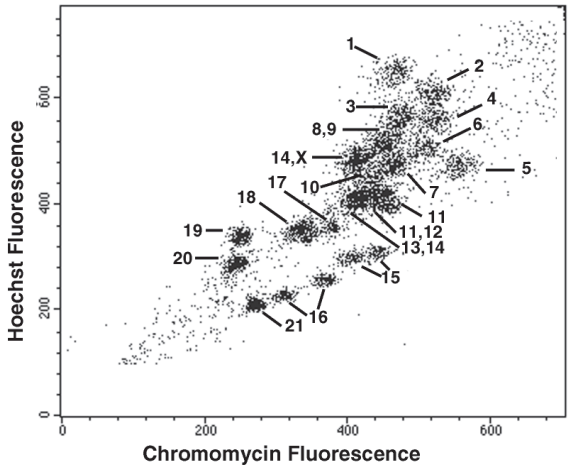

Figure 1 – This figure shows the flow karyotype of *Colobus guereza*. Chromosome were stained with a combination of Hoechst and Chromomycin-A which allowed a bivariate plot. The chromosomes were distributed in 22 peaks.

were then hybridized to *C. guereza* metaphases to identify the chromosome content of each peak of the flow karyotype. All but four peaks contained a single chromosome. Chromosomes 8 and 9 were contained in a single peak. Chromosomes 11 and 14 were present in two different peaks. Chromosome 11 once alone and the other in combination with chromosomes 12. Chromosome 14 was found once with 13 and the X-chromosome. Chromosomes 15 and 16 were each present in two different peaks. All peaks provided good chromosome paints. Colobine paints were then used to hybridize human metaphases and 32 clear signals were detected on the human karyotype (Figures 2 and 3). We had no Y-chromosome probe since a female cell line was used for sorting.

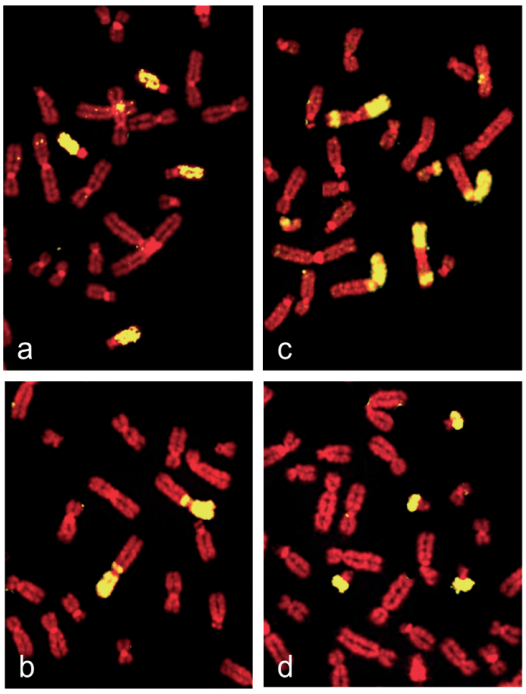

Figure 2 – Examples of hybridization of colobine paints to human metaphases a) CGU 6 hybridized to HSA 14 and 15; b) CGU 17 hybridized to HSA 2p and small part of 2q; c) CGU 18 hybridized to HSA 3 and 19; d) CGU16 hybridized to HSA 21 and 22.

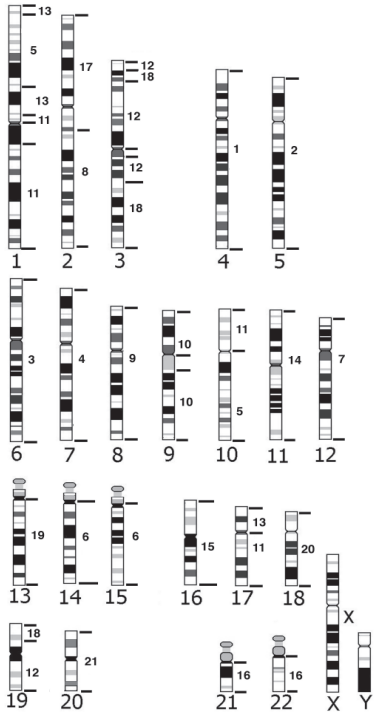

Figure 3 – Human idiogram, numbered below, with hybridization pattern of *Colobus guereza* chromosome paints to the left.

Twelve *C. guereza* chromosome paints hybridized completely a single human chromosome: CGU1 (HSA4), CGU2 (HSA5), CGU3 (HSA6), CGU4 (HSA7), CGU7 (HSA12), CGU9 (HSA8), CGU10 (HSA9), CGU14 (HSA11), CGU15 (HSA16), CGU19 (HSA13), CGU20 (HSA18), CGU21 (HSA20). Each of the following two *C. guereza* chromosome paints hybridized completely two human chromosomes: CGU6 (HSA14 and HSA15) and CGU16 (HSA21 and HSA22). Six human chromosomes

where painted by two or more *C. guereza* probes. HSA2, HSA10, HAS 17 and HSA19 where divided in two segments, HSA1 and HSA3 in four segments. On each human chromosome 1, 3 and 9 a subcentromeric heterocromatic band is present and was not painted. The following associations of *C. guereza* chromosomal segments were found on the human karyotype: 5/13 and 11/13 on HSA1, 8/17 on HSA2, 12/18 on HSA3, 5/11 on HSA10, 11/13 on HSA17 and 12/18 on HSA19.

Discussion

The reciprocal hybridization of *C. guereza* chromosome paints to human metaphases allowed us to assign the subchromosomal homology and breakpoints of fissioned chromosomal syntenies. The reciprocal painting also supported the conclusion previously reported on hybridizing human paints to *C. guereza* metaphases. In our previous report on hybridization of human probes on *C. guereza* metaphases (Bigoni *et al.*, 1997b) we showed that chromosomal synteny between humans and this species of colobine is generally well conserved, but with some exceptions. In *C. guereza* there are six human chromosomes (1, 2, 3, 10, 17 and 19) that are fragmented (the synteny is disrupted). These breaks and translocations have produced: CGU5 (HSA 1/10), CGU11 (HSA 17/1/10), CGU12 (HSA 3/19/3/19), CGU13 (HSA 1/17), and CGU18 (HSA 3/19). The alternating pattern of human segments 3 and 19 on CGU12 is best interpreted as a pericentric inversion that followed a translocation.

To draw phylogenetic information from the hybridization patterns found in colobine monkeys we must compare the chromosomes with the ancestral catarrhine karyotype (Stanyon *et al.*, 2004). There is good agreement that the ancestral catarrhine karyotype had a diploid number of 2n=46 with the following chromosomes: 1, 2a, 2b, 3-13, 14/15, 16-22, X and Y.

We can then compare the ancestral catarrhine karyotype with that of *C. guereza* and other colobines established through chromosome painting and secondarily by banding comparisons. In a previous study (Bigoni *et al.*, 2004) we established the chromosomal homology of *Pygathrix nemaeus* (douc) with human and other primates by *in situ* hybridization of human chromosome paints to douc metaphases. Our results indicated that *P. nemaeus* is karyologically the most conservative colobine species studied and that it probably diverged early after the separation of Asian and African colobines. These data reinforced the monophyly of the Colobinae and their division into an African and an Asian clade. When a human paint is found divided in two or more segments the genetic synteny is not maintained. The FISH data showed that three human syntenic groups are fragmented, as human paints of chromosomes 1, 2 and 19 are each present on two different douc chromosomes. Human chromosome paint 2 was divided on two douc chromosomes (12 and 13) as expected, since it is well known that an apomorphic tandem fusion gave origin to human chromosome 2. The other human syntenic groups fragmented are homologous to human chromosomes 1 and 19. The fragmentation and association of human chromosomes 1 and 19 can be explained with a reciprocal

translocation that produced the douc chromosomes. This association was found in all Asian colobines studied, but not in the African species *Colobus guereza*, where different translocations are present (Bigoni *et al.*, 1997a, 1997b). However, the karyotypes of *T. cristatus*, *T. francoisi*, *T. phayrei* and *N. larvatus* showed a more complicated pattern of four alternating segments of human chromosome paints 1 and 19 on the same colobine chromosome (Bigoni *et al.*, 2003, 2004; Nie *et al.*, 1998). The most parsimonious explanation is that a reciprocal translocation occurred in the lineage of the Asian colobines and distinguishes this group from the African colobines. Other rearrangements such as inversions may provide distinguishing traits between Asian and African colobines. *P. nemaeus* showed the primitive reciprocal translocation between 1 and 19 that was followed by a pericentric inversion linking the genus *Trachypithecus* with *Nasalis*.

The probe specific for the human chromosome 6 painted only one chromosome in the African colobine species *C. guereza* (Bigoni *et al.*, 1997b), and in *P. nemaeus* (Bigoni *et al.*, 2004). G-banding analyses and comparisons demonstrated that human chromosome 6 is also maintained in some other species of Asian colobines including *Semnopithecus entellus*, *Presbytis comata* and *Semnopithecus vetulus* (Bigoni, 1995). In *T. cristatus*, *T. francoisi* and *T. phayrei* the probe specific for human chromosome 6 painted two segments of two different chromosomes, but they are associated with a segment homologous to human chromosome 16, following a reciprocal translocation that involved human homologs 6 and 16.

It can be noted that the reciprocal translocation of 6 and 16 appears to be a distinguishing characteristic of the genus *Trachypithecus.* This rearrangement was found in all *Trachypithecus* species published so far. The only exception would be the purple-faced langur (*T. vetulus*): our unpublished FISH data show that this rearrangement is not present and a single syntenic homolog to human chromosome 6 was found. Therefore the cytogenetic data do not support the inclusion of the purple-faced langur in the genus *Trachypithecus* as suggested by Groves (1989).

Karyological data supporting a closer relationship between *S. entellus* (which also has a syntenic chromosome 6, unpublished data) and *vetulus* are not in contrast with geographical distribution of these two species (*entellus* in India and Sri Lanka, *vetulus* in Sri Lanka) and with observations on the color of infants, an important and variable character in colobines. In fact *vetulus* could be excluded from *Trachypithecus* on the basis that infants are not orange, but gray. On the same basis of a blackish color of newborn infants Groves (1989) argued that *entellus* is more primitive than *Trachypithecus*.

The exceptional diploid number of *Nasalis*, 2n=48 (Chiarelli, 1963, 1966; Soma *et al.*, 1974; Stanyon *et al.*, 1992) has played a pivotal role in phylogenies which view the proboscis monkey as the most primitive colobine, and a long isolated genus of the group (Giusto and Margulis, 1981; Groves, 1989; Peng *et al.*, 1993). Groves (1989) considered *Nasalis* primitive for a relevant number of morphological characters for the most part linked to the lack of masticatory specialization seen in other colobines and for the diploid number. He considered *Nasalis* as a sister species to all other African and Asian colobines and he divided the Colobidae into two subfamilies: Nasalinae and Colobinae. Harvati (2000) found support for Groves on the basis of colobine dental

eruption sequences. Peng *et al.* (1993) also claimed that *Nasalis* is the most primitive colobine genus on the basis of morphological measurements and again for the chromosome number. On the other hand, molecular studies support a monophyletic Asian clade including four lineages: *Nasalis*, *Rhinopithecus*/ *Pyghatrix*, *Semnopithecus entellus*/ *vetulus*, *Trachypithecus*/ *francoisi*/ *obscurus*/ *cristatus*. Zhang and Ryder (1998) supported the existence of a monophyletic Asian clade and suggested the possibility of a lineage including *Nasalis*, *Rhinopithecus* and *Pygathrix*.

In a previous report (Bigoni *et al.*, 2003) we used molecular cytogenetic methods to map the chromosomal homology of the proboscis monkey *(N. larvatus*) in order to test these hypotheses. The use of *in situ* hybridization allowed us to establish homologies between the chromosomes of humans and the *N. larvatus* karyotype. Comparisons with molecular cytogenetic data in other primates show that the proboscis monkey genome is derived and not primitive. The diploid number of 2n=48 can be best explained by derived fissions of a segment of human chromosome 14 and the fission of human chromosome 6. Consequently the higher diploid number found in *Nasalis* is not, as mistakenly assumed, a primitive character. Our results supported the view that the *N. larvatus* karyotype is not primitive, but derived in respect to other colobines and most other Old World monkeys. In fact this karyotype is derived not only in chromosome number, but also for the syntenies present. In spite of these derived apomorphic characters, *Nasalis* is closely related to and nested within other species of Asian colobines.

We cannot exclude the possibility that the fission of homologs chromosome 6 links *N. larvatus* with some *Trachypithecus* species after the divergence of *Presbytis* and *Semnopithecus*. If this is the case, then *N. larvatus* would show an intermediate stage between all the colobine species with intact human syntenic group 6 and the group *T. cristata, T. phayrei* and *T. francoisi* bearing the reciprocal translocation involving human chromosomes 6 and 16. According to this hypothesis chromosome 6 would have been fissioned in a common ancestor of *Nasalis* and *Trachypithecus*. After the divergence of *N. larvatus* two fusion events involving chromosome 6 and 16 homologs would have occurred in the phylogenetic line leading to *Trachypithecus*. This hypothesis is a less parsimonious explanation then the alternative hypothesis, which we favor here: the fissions of chromosome 6 homologs in these taxa are independent events. However, to distinguish between these hypotheses we need to know if the breakpoints in homologs to chromosome 6 in *Nasalis* and *Trachypithecus* are the same or not and that the resulting segments are truly homologous. To test these different hypotheses, more detailed studies are necessary such as reciprocal in situ hybridization, use of subregional probes, cloning and eventually sequencing of the breakpoints.

Integration of Chromosome Painting and Banding

The number of segments or hybridization signals is a good indicator of the evolution of the karyotypes with regard to interchromosomal rearrangements. The human paints were split into 26 segments in *P. nemaeus*, 30 segments in *N. larvatus* and *T. cristatus*

and 32 segments in *Colobus guereza* (always considering the female karyotype). The same diploid chromosomal number 2n=44 shared by *P. nemaeus*, *C. guereza* and *T. cristatus* is the result of different interchromosomal rearrangements. Additionally, *T. cristatus*, *T. francoisi* and *T. phayrei* karyotypes are derived for a reciprocal translocation between homologs to human 6 and 16 (Bigoni *et al.*, 1997a; Nie *et al.*, 1998). The karyotype of *N. larvatus* is also derived for two fissions of the homologs to human chromosomes 14 and 6 (Bigoni *et al.*, 2003). Our results suggest that *P. nemaeus* is the most conservative of the Asian colobines and is phylogenetically basal to all other Asian colobine studied up to now.

We can integrate the molecular cytogenetic data with banding comparisons. Particular chromosomes can provide additional clues to Colobinae phylogeny which however should eventually be confirmed by molecular methods (Figure 4). There is no equivalent to HSA1 in any colobine species. In African colobines there are at least two translocations present yielding two derived syntenic associations 1/10 and 1/17: in Asian colobines 1/19 is present. The synteny of the chromosome homologous to HSA 3 is maintained in Asian colobines and the banding pattern is similar to that found in macaques. A derived pericentric inversion distinguishes this chromosome in the genus *Trachypithecus*. *C. guereza* is distinguished by a 3/19 reciprocal translocation.

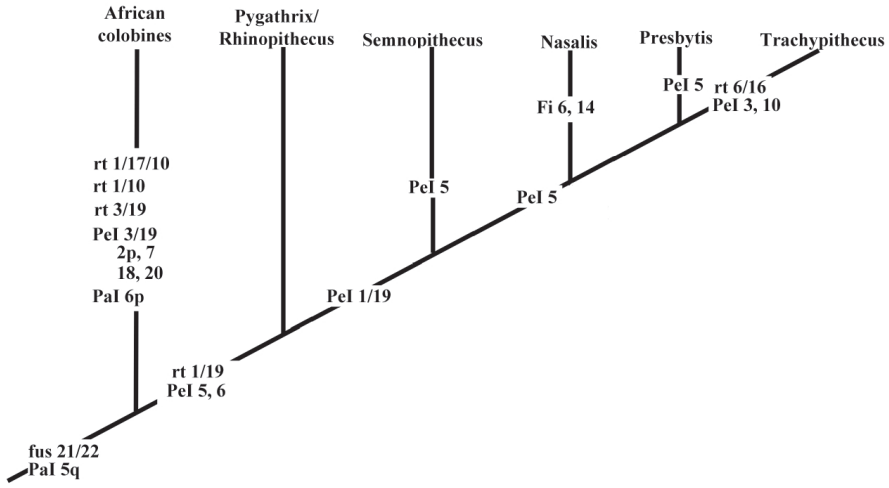

Figure 4 – A working hypothesis of the cytogenetic phylogeny of colobines monkeys based on a combination of chromosome painting and banding (rt=reciprocal translocation, PeI=pericentric inversion, PaI=paracentric inversion, fus=fusion, Fi=fission.

The banding pattern of chromosome 5 appears to provide phylogenetically relevant data. The banding pattern of HSA5 appears to be ancestral for catarrhines. A paracentric inversion on the terminal part of 5q appears to link both Asian and African

colobines. A subsequent pericentric inversion links all Asian colobines. Another pericentric inversion appears to link the genera *Nasalis* and *Trachypithecus*. Another pericentric inversion seems to link *S. entellus* and *S. vetelus*. Providing additional support that *vetulus* does not belong in *Trachypithecus*. Apparently apomorphic inversions derive the homologous chromosome in other Asian colobines.

The homolog to chromosome 10 is involved in a reciprocal translocation in the African colobines. A pericentric inversion derives the banding found in all *Trachypithecus* (but again not in *vetulus*). Different pericentric inversions of chromosomes 12, 18 and 20 appear to distinguish Asian and African colobines. In all Asian colobines, chromosome 19 is reciprocally translocated with chromosome 1, while a reciprocal translocation between 3 and 19 is found in African colobines. Finally, in both Asian and African colobines a derived fusion of 21 and 22 forms the marked (NOR bearing) chromosome.

Many of the hypotheses developed here should be tested with molecular methods. Further use of reciprocal painting in Asian colobines especially in *Trachypithecus* and *Nasalis* and additional African species could be highly informative concerning the phylogeny of leaf-eaters. Such data could help to clarify the exact position of *Nasalis* within the Asian colobines.

Use of subchromosomal probes of decreasing size such YACs, BACs and cosmids would effectively contribute to the study of colobines and help define breakpoints, which may have phylogenetic significance. It would be particularly interesting to have such data on chromosome 5 homologs as this chromosome appears to be rich in phylogenetic information. Finally there are no molecular cytogenetic data on *Rhinopithecus*. Such data could help clarify the phylogenetic position of the snub-nosed langurs and in particular their relationship to *Pygathrix*. Indeed the data presented here are only the beginning of the contribution modern cytogenetics can provide to probe colobine evolution.

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