

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 $X_1X_2Y_1Y_2/X_1X_1X_2X_2$ Sex-Chromosome System

F. BIGONI,¹ U. KOEHLER,² R. STANYON,^{1*} T. ISHIDA,³ AND J. WIENBERG⁴

¹Department of Anthropological Sciences, University of Genoa, 16126 Genoa, Italy; ²Institute for Anthropology and Human Genetics, University of Munich, Munich, Germany; ³Department of Anthropology, University of Tokyo, Tokyo, Japan; ⁴Department of Pathology, Cambridge University, Cambridge, United Kingdom

KEY WORDS *Presbytis cristata*; catarrhine; primate; chromosome painting; polymorphism; evolution; speciation

ABSTRACT We employed in situ hybridization of chromosome-specific DNA probes ("chromosome painting") of all human chromosomes to establish homologies between the human and the silvered leaf monkey karyotypes (*Presbytis cristata* $2n=44$). The 24 human paints gave 30 signals on the haploid female chromosome set and 34 signals on the haploid male chromosome set. This difference is due to a reciprocal translocation between the Y and an autosome homologous to human chromosome 5. This Y/autosome reciprocal translocation which is unique among catarrhine primates has produced a $X_1X_2Y_1Y_2/X_1X_1X_2X_2$ sex-chromosome system. Although most human syntenic groups have been maintained in the silvered leaf monkey chromosomes homologous to human chromosomes 14 and 15, 21 and 22 have experienced Robertsonian fusions. Further, the multiple FISH signals provided by libraries to human chromosomes 1/9, 6/16 indicate that these chromosomes have been split by reciprocal translocations. G-banding analysis shows three different forms of chromosome 1 (X_2) which differ by a complex series of inversions in the 10 individuals karyotyped. Comparisons with the hybridization patterns in hylobatids (gibbons and siamang) demonstrate that resemblances in chromosomal morphology and banding previously taken to indicate a special phylogenetic relationship between gibbons and colobines are due to convergence. *A. J. Phys. Anthropol.* 102:315-327, 1997. © 1997 Wiley-Liss, Inc.

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; Ushima et al., 1964). In the genus *Colobus* the karyotype was found to be composed of all metacentric and submetacentric chromosomes which included one nucleolar organizer region (NOR)-bearing "marked pair." With classical staining, the karyotype of the

genus *Presbytis* appeared to be identical to that of the African colobines with the exception of one pair of small chromosomes which were acrocentric.

There are only a few publications on

*Correspondence to: R. Stanyon, Department of Anthropological Sciences, University of Genoa, Via Balbi 4, 16126 Genoa, Italy.
Received December 8, 1995; accepted August 4, 1996.

chromosome banding in colobines. The reports on African colobines, genus *Colobus*, are limited to R-banding (Dutrillaux et al., 1982; Muleris et al., 1986). There are more reports on chromosome banding in Asian colobines, i.e., genus *Presbytis* (Sharma et al., 1972; Krishna-Murthy et al., 1979; Ponsà et al., 1983; Dutrillaux et al., 1984). Ponsà et al. (1983) reported on the G and Q banding of *Presbytis obscurus* and *Presbytis cristata*. They described the banding pattern of only one female of *P. cristata* and noted the presence of two variant forms of chromosome 1. The publications of Dutrillaux et al. (1982) and Muleris et al. (1986) using R-banding compared three different species of genus *Colobus* with *P. cristata*. In contrast to the conclusion reached from classical staining they noted numerous chromosomal differences between all these species. Further, they described a Y/autosomal translocation in *P. cristata*, the only case known in catarrhine primates (Dutrillaux et al., 1984).

Although the majority of colobines species have yet to be studied by any cytogenetic method, the data are sufficient to conclude that more complete karyological information would be useful for clarifying a range of evolutionary problems concerning colobines and other primates. These include the origin and radiation of colobines, the phylogenetic relationships between Asiatic and African colobines, and the relationships between colobines and other primates (Sarich, 1970; Peng et al., 1993; Jablonski and Peng, 1993; Stanyon et al., 1992b, 1995a). Some cytogeneticists have even proposed a strict relationship between colobines and hylobatids as (Chiarelli, 1963, 1972). Finally, the role of chromosomes in colobine speciation needs examination.

The classification and taxonomy of colobines is unsettled and has been subjected to continued revision (Napier and Napier, 1967, 1985; Vogel and Winkler, 1990; Groves, 1970, 1993; Oates et al., 1994). For instance, there is no consensus even on the number of genera or species (see Table 1). The silvered leaf monkey is classified as either *P. cristata* (Napier and Napier, 1967, 1985; Thorington and Groves, 1970; Vogel and Winkler, 1990) or *Trachypithecus*

cristatus (Hooijer, 1962, Groves, 1989; Oates et al., 1994). Here we use the name *P. cristata*.

We hoped that more complete and reliable data on colobine chromosomes using both chromosome banding and molecular methods could be used to examine these diverse taxonomic schemes. However, chromosomes can be a useful tool in evolutionary studies only if some fundamental criteria are respected. First, it is absolutely essential to be sure that homologous structures are compared. Comparative cytogenetics have always been limited by difficulties in establishing between species chromosomal homology (Stanyon et al., 1995a). With the introduction of molecular methods, such as in situ hybridization of human chromosome-specific DNA libraries or probes ("chromosome painting": Lichter et al., 1988; Pinkel et al., 1988; Collins et al., 1991), it is now possible to unequivocally establish chromosomal homology between any two primate species (Wienberg et al., 1990, 1992; Stanyon et al., 1992a).

Recently, in situ hybridization was successfully used to establish the homologies between karyotypes of humans and great apes (chimpanzees, gorilla, orangutan), lesser apes (*Hylobates lar*, *Hylobates syndactylus*, and *Hylobates concolor*) and macaques (Wienberg et al., 1990, 1992; Jauch et al., 1992; Stanyon et al., 1992a, 1995a; Koehler et al., 1995a, 1995b). The data on colobine karyotypes will be also useful as an "out-group" to distinguish ancestral, primitive conditions (plesiomorphism), from derived characters (apomorphisms) and thereby establish the polarity of chromosomal differences among hominoids.

Here we report on "chromosome painting" of human chromosome-specific DNA probes to establish homologies between the human and the silvered leaf monkey karyotypes. This technique allows a comparison of complete chromosomal homology directly at the DNA level. We were able to establish the homology between all human and silvered leaf monkey chromosomes. This pattern was then compared with that of previously studied primates with particular reference to gibbons. Further, we report on G-banded karyotype of 10 *P. cristata* (four males, six females)

TABLE 1. Two currently used taxonomic classifications of colobine monkeys

Colobine classification according to		
Napier and Napier (1985)	Oates et al. (1994)	Common name
African colobines		
<i>Colobus guereza</i>	<i>Colobus guereza</i>	black and white colobus
<i>Colobus polykomos</i>	<i>Colobus polykomos</i>	king or ursine colobus
	<i>Colobus vellerosus</i>	white-thighed colobus
<i>Colobus angolensis</i>	<i>Colobus angolensis</i>	Angolan colobus
<i>Colobus satanas</i>	<i>Colobus satanas</i>	black colobus
<i>Colobus badius</i>	<i>Procolobus badius</i>	red colobus
<i>Colobus verus</i>	<i>Procolobus verus</i>	olive colobus
Asian colobines		
<i>Presbytis melalophos</i>	<i>Presbytis melalophos</i>	banded leaf monkey
<i>Presbytis comata</i>	<i>Presbytis comata</i>	Sunda Island leaf monkey
<i>Presbytis frontata</i>	<i>Presbytis frontata</i>	white-fronted leaf monkey
<i>Presbytis hosei</i>	<i>Presbytis hosei</i>	Hose's leaf monkey
<i>Presbytis potenziani</i>	<i>Presbytis potenziani</i>	Mentawai leaf monkey
<i>Presbytis rubicunda</i>	<i>Presbytis rubicunda</i>	maroon leaf monkey
<i>Presbytis thomasi</i>	<i>Presbytis thomasi</i>	Thomas's leaf monkey
<i>Presbytis cristata</i>	<i>Trachypithecus cristatus</i>	silvered leaf monkey
	<i>Trachypithecus auratus</i>	lutung
<i>Presbytis francoisi</i>	<i>Trachypithecus francoisi</i>	Francois's leaf monkey
<i>Presbytis geei</i>	<i>Trachypithecus geei</i>	golden leaf monkey
<i>Presbytis obscurus</i>	<i>Trachypithecus obscurus</i>	dusky leaf monkey
<i>Presbytis phayrei</i>	<i>Trachypithecus phayrei</i>	Phayre's leaf monkey
<i>Presbytis pileatus</i>	<i>Trachypithecus pileatus</i>	capped leaf monkey
<i>Presbytis johnii</i>	<i>Trachypithecus johnii</i>	John's leaf monkey
<i>Presbytis vetulus</i>	<i>Trachypithecus vetulus</i>	purple faced leaf monkey
<i>Presbytis entellus</i>	<i>Semnopithecus entellus</i>	Hanuman langur
<i>Simias concolor</i>	<i>Simias concolor</i>	simakobu
<i>Nasalis larvatus</i>	<i>Nasalis larvatus</i>	proboscis monkey
<i>Pygathrix nemaeus</i>	<i>Pygathrix nemaeus</i>	Douc langur
<i>Rhinopithecus roxellana</i>	<i>Pygathrix roxellana</i>	golden snub-nosed monkey
	<i>Pygathrix bieti</i>	Yunnan snub-nosed monkey
	<i>Pygathrix brelichi</i>	Guizhou snub-nosed monkey
<i>Rhinopithecus avunculus</i>	<i>Pygathrix avunculus</i>	Tonkin snub-nosed monkey

and present a standardized ideogram based on chromosome measurements.

MATERIALS AND METHODS

Presbytis cristata chromosomes were prepared from lymphoblast cell lines and whole blood cultures; altogether 10 individuals were studied. The whole blood samples from six recently caught wild monkeys attributed to two subspecies came from Djakarta zoo and were kindly provided by A. Camperio-Ciani: *P. cristata cristata* (samples 9, 36, females; 37, 38, males) and *P. cristata pyrrhus* (samples 8, 23, females). Cell culture of these whole blood samples was according to Small et al. (1985). Chromosome preparations from these samples were used only in banding pattern comparisons.

Chromosome preparations used in hybridization experiments came from lymphoblast cultures of one male (PCR1) and one female (PCR2) from Indonesia, and one male (106) and one female (108) from Thailand. The

subspecies of these animals is unfortunately not known.

Hybridization of human specific chromosome probes has now become routine in comparative primate cytogenetics. We followed in situ hybridization as previously reported (Stanyon et al., 1992a, 1995a; Koehler et al., 1995a, 1995b). All hybridized (i.e., "painted") *P. cristata* chromosomes were identified with trypsin G-banding before hybridization. In addition to the G-banding protocol, the identification of chromosomes was also facilitated by DAPI-banding concurrently with in situ hybridization. This double banding system allowed us to easily assign the in situ hybridization signal to the correct *P. cristata* chromosomes or chromosome segments. Microphotography and image processing was the same as previously reported (Koehler et al., 1995a, 1995b).

The ideogram was prepared measuring 10 metaphase spreads. The numbering of chromosomes was according to the relative

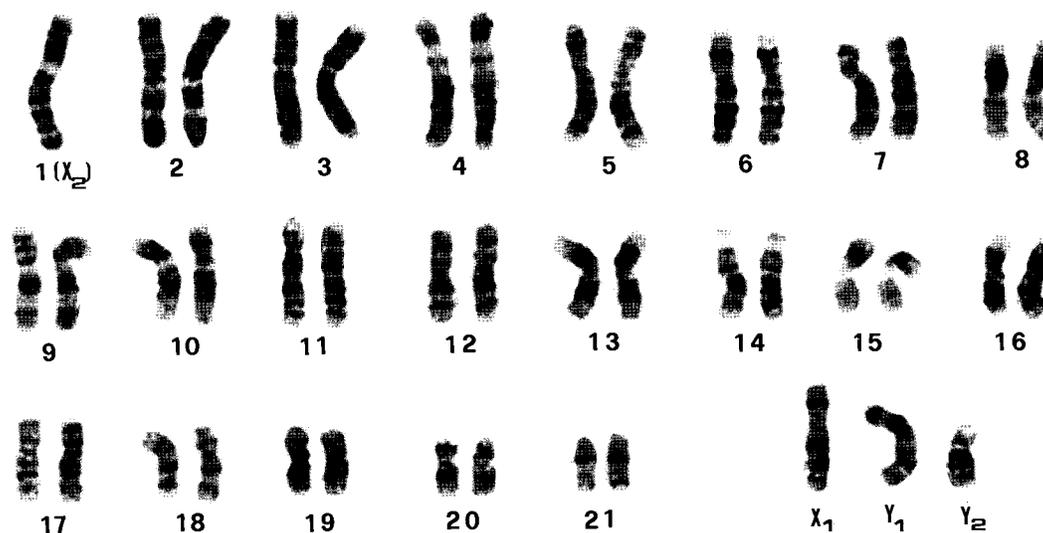


Fig. 1. A G-banded male karyotype of *P. cristata* $2n=44$. Four chromosomes have no homologs. One of them is the typical mammalian X that we called X_1 . Only one chromosome 1 is present because of the Y/autosome translocation. The chromosome 1 was hybridized by the human chromosome 5 probe and was also called X_2 . The human library Y hybridized two chromosomes that were hybridized also by the human chromosome 5 probe. These chromosomes were labelled Y_1 and Y_2 .

length of chromosomes. The chromosome banding represented in the ideogram is an idealized pattern derived from a number of medium resolution karyotypes.

RESULTS

G banded *P. cristata* karyotypes

We found that all 10 *P. cristata* had a diploid number of $2n=44$. The smallest autosomal pair was acrocentric; all the other chromosomes were submetacentric or metacentric. In the females two homologs for all chromosomes were identified. In all the males, four chromosomes had no homologs. One of these chromosomes clearly corresponds to the typical primate X chromosome. Another chromosome was homologous to the longest chromosome present in the female karyotype (chromosome 1 or X_2). The other two chromosomes, a metacentric and an acrocentric, both of medium length, did not show any immediate similarity to any chromosome found in the karyotype of females. There was no typical small primate Y-chromosome. A karyotype of a male is shown in Figure 1.

Variant forms of chromosome 1 (X_2)

Three different forms of chromosome 1 were found in the 10 individuals karyotyped here (Fig. 2). These three chromosome forms, designated 1a, 1b, and 1c, appear to differ by a complex series of inversions, but the exact break points and the number of inversions necessary to derive all three forms are not clear. The forms found in the 10 monkeys are shown in Table 2.

Localization of human chromosome specific probes in the karyotype of *P. cristata*

All 24 human chromosome-specific DNA probes gave bright paintings (i.e., hybridized) on the silvered leaf monkey chromosomes. Figure 3 shows examples of the hybridization signals obtained while Figure 4 presents a summary of the in situ hybridization results on a G-banded idiogram of the *P. cristata*. The 24 probes gave 30 signals on the haploid female chromosome set and 34 signals on the haploid male chromosome set (Fig. 5).

The difference in the number of signals



Fig. 2. Three different forms of chromosome 1 were found in 10 individuals. Only forms 1a and 1b were present in the Indonesian animals. Two females were heterozygous for 1a1b. Only form 1c was found in the two Thailand monkeys.

between male and females was due to the fact that the human Y chromosome probe hybridized to segments of two chromosomes that were also hybridized by the human chromosome 5 probe. These chromosomes have been labelled Y_1 and Y_2 . The other unpaired chromosome in males was homologous only to human chromosome 5 and has been alternately labelled as X_2 or chromosome 1.

Twelve human probes (3, 4, 7, 8, 9, 10, 11, 12, 13, 17, 18, 20) completely hybridized a single *P. cristata* autosome. In six cases a single *P. cristata* chromosome was hybridized by two human probes providing new chromosomal synteny: human 14/15, 21/22 provided signals on one *P. cristata* chromosome while probes 1/19 and 6/16 each hybridized with two different *P. cristata* chromosomes. Chromosome probes 6 and 16 are found on two *P. cristata*. Further, silvered leaf monkey 8 is divided in five signals of probes from human 1 and human 19.

Unhybridized chromosome segments

The centromeres of most chromosomes were not hybridized. The terminal segments of chromosomes 4p, 10p, 11p, 14p, 17p, 18p, the short arms of 21p and Y_1 did not hybridize with any human paint.

DISCUSSION

G-bands

Our results confirm and extend the only previous report on G-banding of a single female *P. cristata* (Ponsà et al., 1983). All indi-

TABLE 2. Variant forms of chromosome 1 (X_2) in each of ten monkeys (*P. cristata*)

<i>P. cristata</i> ¹	Origin	Sex	Chromosome 1 (X_2) karyotype ²
PCR1	Indonesia	M	a
PCR2	Indonesia	F	ab
8	Indonesia	F	aa
9	Indonesia	F	bb
23	Indonesia	F	bb
36	Indonesia	F	ab
37	Indonesia	M	b
38	Indonesia	M	b
106	Thailand	M	c
108	Thailand	M	cc

¹ Individual animals (samples) were numbered. See text.

² Three forms, a, b, and c, of chromosome 1 (X_2) were found that differ by a complex series of yet unclear inversions. As a result of a reciprocal translocation between this chromosome and the Y-chromosome, males have only one intact chromosome 1 or X_2 (see text).

viduals karyotyped had $2n=44$. Additionally, we found a third variant of the chromosome 1 (X_2). We confirmed the R-banding results which indicated the presence of a Y-autosomal translocation (Dutrillaux et al., 1984). However, our numbering system does not follow published reports. The publications of Dutrillaux et al. (1984) and Muleris et al. (1986) are based on R-banding and are difficult to compare with G-banding. The publication of Ponsà et al. (1983) showed a Q-banded karyotype that was not sufficient to identify all our G-banded chromosomes.

Hybridization of human chromosome specific DNA probes

The hybridization of probes to the chromosomes of *P. cristata* allowed us to establish the chromosomal homology between Asian colobine monkey and humans (Table 3). This technique has been previously used to establish the chromosomal homologies between humans and other hominoids (*ss*), hylobatids (gibbons and siamang) and a cercopithecine monkey (Japanese macaque). Our results can be used to make more secure karyological comparisons between a colobine and all the other catarrhine subfamilies.

The hybridization data indicate that most human chromosomal synteny are also present in *P. cristata*. Many of the same chromosomal synteny are common to all catarrhine species studied so far (great apes, macaques, and the silvered leaf monkey) and such synteny may be considered conserved

or ancestral for Old World primates. The hylobatids, however, are an exception and have highly rearranged genomes (Jauch et al., 1992; Koehler et al., 1995a, 1995b).

Out of 22 human autosomes, 18 are found intact in *P. cristata*. However, of these 18, the hybridization pattern shows the existence of two different syntenic groups in *P. cristata* due to association between human 14 and 15 to form silvered leaf monkey 5, and 21 and 22 to form silvered leaf monkey 15, the "marked" NOR chromosome.

Human syntenic groups disturbed in *P. cristata*

The hybridization pattern demonstrates that five human syntenic groups (1, 2, 6, 9, 19) are disturbed, because some human chromosome probes hybridized to more than one *P. cristata* chromosome. However, it is well known that human chromosome 2 was derived by an apomorphic tandem fusion after the divergence of humans from African apes. The fragmentation of the other four human chromosomes can be derived in the *P. cristata* karyotype by reciprocal translocations: silvered leaf monkey chromosome 6 and 8 may have been produced by reciprocal translocation between human chromosomes 19 and 1, while silvered leaf monkey chromosome 9 and 16 have probably been produced by reciprocal translocation between human chromosomes 16 and 6. Further, the alternating pattern between chromosome segments homologous to human chromosomes 1 and 19 on silvered leaf monkey 8 indicates that a pericentric inversion followed the translocation.

Sex chromosomes

The hybridization results combined with G-banding allowed a precise description of the Y-autosomal translocation. It is a reciprocal translocation involving a chromosome homologous to the entire human chromosome 5. Strictly speaking the sex chromosomes system in *P. cristata* should be described as $X_1X_2Y_1Y_2$, where X_1 equals the original X, X_2 equals the intact homolog to human chromosome 5 (silvered leaf monkey 1), and Y_1 and Y_2 represent the reciprocal translocation products of silvered leaf monkey 1a and Y. This reciprocal translocation

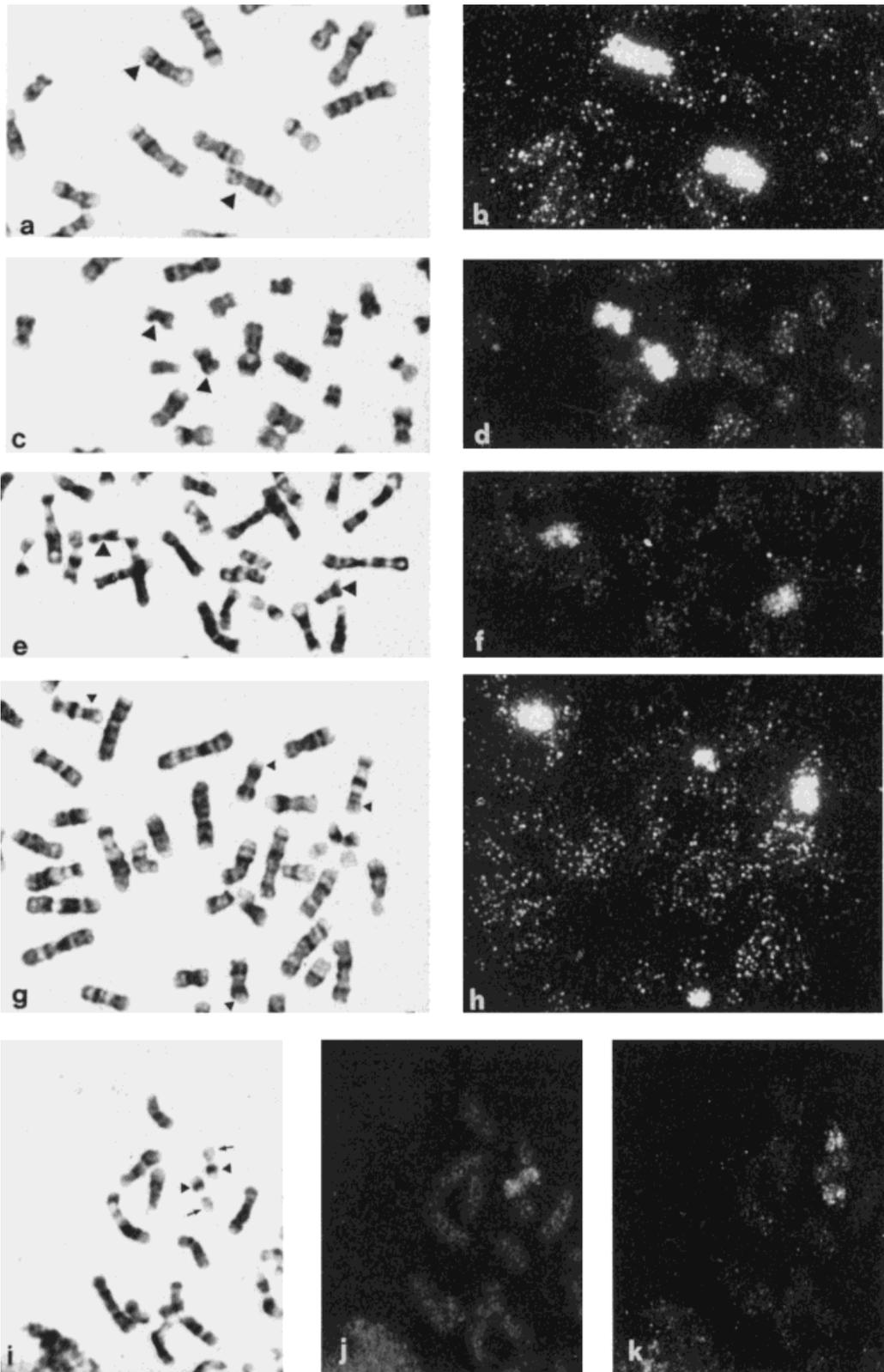
was further confirmed by probes specific to *P. cristata* Y_1 and Y_2 chromosome to metaphases of *Cercopithecus aethiops*, the African green monkey. The long and short arms respectively of the green monkey Y chromosome were hybridized by the two probes (unpublished data).

Translocations between the Y chromosome and an autosome are rare in primates. In catarrhines, *P. cristata* is the only species known to have a Y/autosome translocation. However, a number of platyrrhines have Y/autosome translocations (cf. Stanyon et al., 1995b). Red howler monkeys (*Alouatta seniculus*) also have an $X_1X_2Y_1Y_2/X_1X_1X_2X_2$ sex-chromosome system. Meiotic studies of red howler spermatocytes by Lima and Seuànez (1991) showed that in this case pairing occurs sequentially end to end to form a chain configuration in the order: $X_1-Y_1-X_2-Y_2$.

Unfortunately, we were unable to make meiotic studies (testicular biopsies are difficult to obtain) and consequently we do not know if a chain configurations are found in *P. cristata*. However, chain configurations have been found owl monkeys and Goeldi's marmoset which have a Y/autosome translocation and a $X_1X_2Y/X_1X_1X_2X_2$ sex-chromosome system (Ma et al., 1976).

Female ($X_1X_1X_2X_2$) silvered leaf monkeys produce only one gamete type (X_1X_2). In males only one type of segregation in the

Fig. 3. Examples of the sequential G-banding and hybridization signals obtained with probes of DNA libraries specific to human chromosomes on metaphase of the silvered leaf monkey. Conserved chromosomal synteny between humans and the silvered leaf monkey is shown by double hybridization signals for human chromosome 8 library (a,b), human chromosome 13 library (c,d), and human chromosome 18 library (e,f). Disrupted chromosomal synteny is shown by the four hybridization signals present for the human chromosome 16 library (g,h) which results from a reciprocal translocation with chromosome segments homologous to human chromosome 6. In the above examples biotinylated probes were detected with avidin conjugated to FITC. A double hybridization experiment on the same metaphase is shown in G-bands (i), human chromosome 21 detected by avidin FITC (j), and human chromosome 22 library labelled with digoxigenin and detected with TRITC-labeled antibodies (k). Note that the "marked" NOR bearing chromosomes are hybridized. All images in this figure were recorded directly on black and white photographic film without computer image processing.



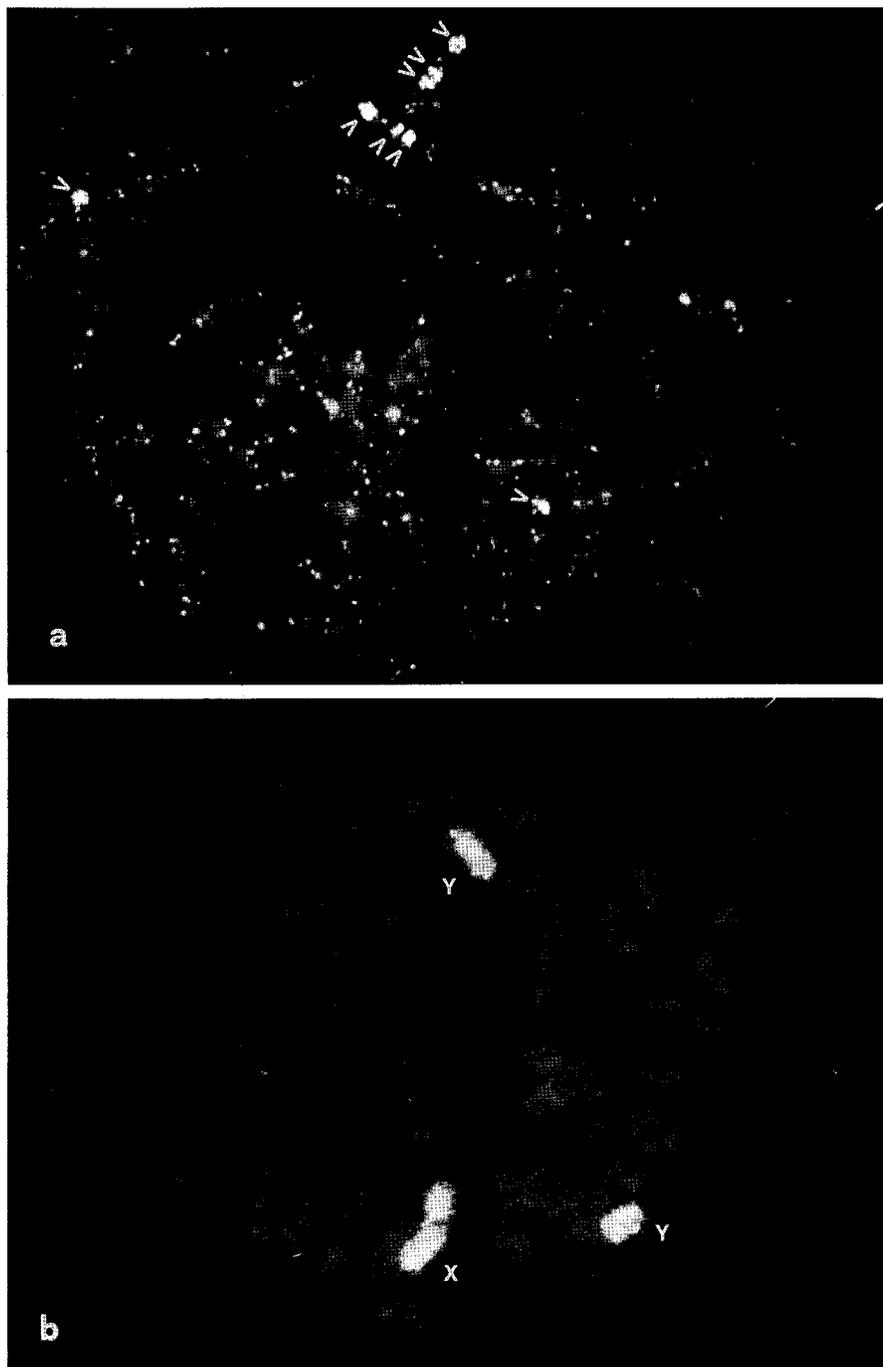


Fig. 4. Examples of the images obtained with a cooled CCD camera and image processing as previously described (Koehler et al., 1995): Images were a result of merging DAPI banding and hybridization signals obtained with a probes specific to human chromosome 19 (a) and human chromosome 5 (b). Note the multiple signals for chromosome segments homologous to human chromosome 19 (a) showing that this chromosome is

highly rearranged in silver leaf monkeys. The unusual three signal pattern of hybridization of human chromosome 5 DNA libraries in male silvered leaf monkeys is due to a reciprocal translocation with the Y-chromosome and has produced a $X_1X_2Y_1Y_2/X_1X_1X_2X_2$ sex-chromosome system. The largest hybridization signal labels X_2 and the other two signals label Y_1 and Y_2 .

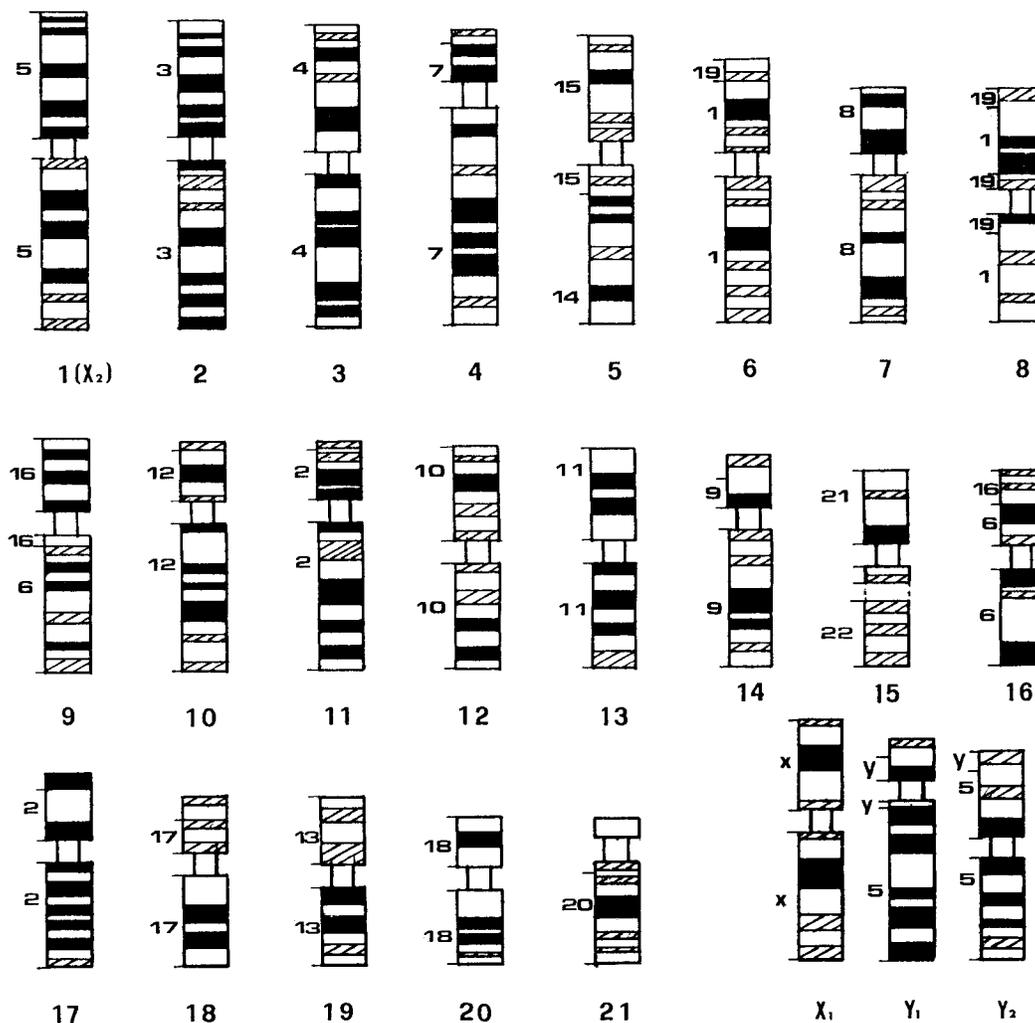


Fig. 5. A summary of the in situ hybridization results on a G-banded ideogram of the *P. cristata*.

first meiotic division can produce balanced gametes (X_1X_2 or Y_1Y_2). Other types of segregation would produce unbalanced gametes or disrupt the sex determining system (Lima and Seuànez, 1991). The consequences of sex chromosome rearrangements have been extensively discussed (King, 1993). Generally, the repercussions of X/autosome translocations are considered to be quite profound, but Y/autosome translocations do not necessarily have a detrimental reproductive impact (Charlesworth et al., 1987; Ford, 1994).

Much further data will be necessary to determine if male silvered leaf monkeys

have fertility problems due to the production of unbalanced gametes. However, because all males examined so far are $X_1X_2Y_1Y_2$, it appears that this sex determining system is stable in the species.

Variants of chromosome 1 (X_2)

Usually karyological reports are based on very limited samples, often as few as one or two individuals per species are studied. Clearly chromosomal polymorphisms can be easily overlooked in such small samples. Our study of 10 individuals showed three forms of chromosome 1 (X_2) apparently due to se-

TABLE 3. Homology¹ between human and *P. cristata* chromosomes or chromosome segments

Human chromosome	<i>P. cristata</i> chromosome(s)
1	6, 8
2	11, 17
3	2
4	3
5	1(X ₂), Y1, Y2
6	9, 16
7	4
8	7
9	14
10	12
11	13
12	10
13	19
14	5
15	5
16	9
17	16
18	20
19	6, 8
20	21
21	15
22	15
X	X ₁
Y	Y ₁ , Y ₂

¹Homology was established at the DNA level by hybridization with human chromosome specific probes subsequent to trypsin G-banding.

ries of, as yet unclear, inversions. Two of these forms probably are similar to those found by Ponsà et al. (1983) in a single female. In the males only one intact chromosome 1 is present due to a reciprocal translocation with the Y. Eight of our monkeys came from Indonesia and in these monkeys two forms were present, a and b. Of the five Indonesian females, two were found to be heterozygous, two were homozygous for form b, and one was homozygous for form a. Of the three Indonesian males, two had form b and one had form a. We studied only one male and one female from Thailand and found only form c. It is tantalizing to hypothesize that form c is present only in continental silvered leaf monkeys. However, this possibility must be confirmed by adequate samples.

The Indonesian monkeys studied were captured in the wild and so we are sure that this variability is not a consequence of hybridization in captivity. The frequency of inversion variants for chromosome 1 (X₂) in *P. cristata* appears quite high. In humans, chromosome 9 is the best-known inversion polymorphism with frequencies of up to 4% in some populations. Inversion polymorphisms with much higher frequencies are

found in non-human primates. A complex inversion polymorphism of chromosome 9 (homologous to human 12) is present in high frequencies in both Bornean and Sumatran orangutans (de Boer and Seuànez, 1982). In gibbons, three forms of chromosome 8 (homologous to segments of humans chromosomes 5, 9, 17, 16 and 22) are also found at high frequencies (Stanyon et al., 1987).

Every time that reasonable samples of species are karyotyped the concept of "one karyotype one species" as stated by Dutrillaux (1979) and Lejeune (1983) is clearly shown to be a gross oversimplification (Stanyon, 1992). It is now clear that chromosomal polymorphisms can exist for considerable time and even survive speciation events (Stanyon et al., 1987). Clearly pericentric inversions have played an important part in differentiating primate karyotypes. Inversions have been fundamental in the karyotypic evolution of Hominoidea and in the origin of the human genome.

The exact role of chromosome variability in speciation is still an open question. However, recent models of chromosome speciation give inversion polymorphisms a primary role in population divergence and isolation (Rumpler et al., 1995). Only more complete data on karyotype evolution will determine if chromosome polymorphisms have played an important role in leaf eater speciation.

Comparison of chromosome painting pattern *P. cristata* and other primates

The hybridization pattern of *P. cristata* demonstrates the existence of the following associations: 14/15, 21/22 (due to simple Robertsonian changes), 1/19, 6/16 (due to reciprocal translocations). Out of these associations, only 14/15 was also found in macaques (Wienberg et al., 1992), but it is not present in gibbons, great apes, or humans. This character is common to both Cercopithecinae and Colobinae and was probably present in the karyotype of their common ancestor. Two hypotheses can be proposed concerning 14 and 15: 1) this syntenic group may be ancestral for all catarrhines and in this case a synapomorphic fission occurred in the common ancestor of gibbons, great apes, and humans; or 2) chromosomes 14

and 15 were independent chromosomes in the common catarrhine and a fusion occurred in the common ancestors of all Old World monkeys. Chromosome painting data from both cows and pigs and gene mapping data from the cat show that 14 and 15 are syntenic in these species and support the first hypothesis (Rettenberger et al., 1995; Solinas-Toldo et al., 1995).

The "marked" chromosome of *P. cristata* is formed by homologs to human 21 and 22. In macaques the "marked" chromosome is composed by segments homologous to human 20/22 while in gibbons (*H. lar*; 2n=44) the "marked" chromosome is composed by homologs to human chromosomes 2 and 3. The phylogenetic significance of marked chromosomes has been recently discussed by Stanyon et al. (1995a). Briefly, the "marked" chromosomes along with similar diploid numbers and FN (fundamental numbers, the number of chromosome arms) were taken by some cytogenetists as evidence of a special phylogenetic relationship between gibbons and colobines (Chiarelli, 1972). However, the chromosome painting data clearly show that the resemblance between marked chromosome in colobines and gibbons is the result of convergence. The "marked" chromosomes in these two groups are not homologous. There is a relationship between the "marked" chromosomes of colobines and cercopithecines; the presence of probe 22 in the "marked" chromosomes of both groups makes these chromosomes partly homologous. The phylogenetic significance of chromosomes 21 and 22 for hominoid phylogeny is not clear. It is possible that their independence in gibbons and great apes is a linking trait; however, 21 and 22 may well have been independent in ancestral catarrhine. As has been shown for the bovine karyotype a chromosome homologous to human chromosome 22 is a single element (Solinas-Toldo et al., 1995).

In *P. cristata* chromosome segments homologous to human chromosomes 6 and 16 are directly associated on two chromosomes due to a reciprocal translocation. In *H. concolor* two segments of paints 6 and 16 are also found on the same chromosome. However, this is not a trait which links gibbons and colobines because, first, these segments

are not directly associated in the concolor gibbons, but separated by two other segments homologous to human chromosomes 10 and 5, and, second, the association in gibbons is not the result of a reciprocal translocation, but probably of a secondary apomorphic rearrangement involving a primary product consisting of the two associations 5/16 and 6/10 which are also found in *H. lar* and *H. syndactylus* (Jauch et al., 1992; Koehler et al., 1995a, 1995b). It is safe to conclude that the presence of signals from paint 6 and 16 on one chromosome in gibbons and *P. cristata* is due to convergence. Further, the rapid and massive genomic reshuffling in gibbons also permits us to conclude that the similarity in diploid and fundamental numbers has equally resulted from convergence. There is no cytogenetic data to support a special phylogenetic relationship between gibbons and colobines.

ACKNOWLEDGMENTS

Partial funding was provided by MURST 60% and Vigoni programme grants. We thank P. Finelli for the data on the hybridization of chromosome libraries from *P. cristata* Y1 and Y2 chromosomes to *Cercopithecus aethiops* metaphases.

LITERATURE CITED

- Charlesworth B, Coyne JA, and Barton NH (1987) The relative rates of evolution of sex chromosomes and autosomes. *Am. Nat.* 130:113-146.
- Chiarelli B (1963) Comparative morphometric analysis of primate chromosomes. III. The chromosomes of the genera *Hylobates*, *Colobus*, and *Presbytis*. *Caryologia* 16:637-648.
- Chiarelli B (1972) The karyotypes of the gibbons. In DM Rumbaugh (ed.): *Gibbon and Siamang*. Basel: Karger 1:90-102.
- Collins C, Kuo WL, Segreaves R, Pinkel R, Fuscoe J, and Gray JW (1991) Construction and characterization of plasmid libraries enriched in sequences from single human chromosomes. *Genomics* 11:997-1006.
- de Boer LEM, and Seuanez HN (1982) The chromosomes of the orangutan and their relevance to the conservation of the species. In LEM de Boer (ed.): *The Orang Utan: Its Biology and Conservation*. The Hague: Junk.
- Dutrillaux B (1979) Chromosomal evolution in primates: Tentative phylogeny from *Microcebus murinus* (prosimian) to man. *Hum. Genet.* 48:251-314.
- Dutrillaux B, Couturier J, Muleris M, Lombard M, and Chauvier G (1982) Chromosomal phylogeny of forty-two species or subspecies of Cercopithecoids (Primates, Catarrhini). *Ann. Gnt.* 25:96-109.

- Dutrillaux B, Webb G, Muleris M, Couturier J, and Butler R (1984) Chromosome study of *Presbytis cristatus*: Presence of a complex y-autosome rearrangement in the male. *Ann. Gnt.* 27:148–153.
- Ford SM (1994) Taxonomy and distribution of the owl monkey. In JF Baer, RE Weller, and I Kakoma (eds.): *Aotus: The Owl Monkey*. San Diego: Academic, pp. 1–57.
- Groves CP (1970) The forgotten leaf-eaters, and the phylogeny of the Colobinae. In JR Napier and PH Napier (eds.): *Old World Monkeys: Evolution, Systematics and Behavior*. London: Academic, pp. 555–587.
- Groves CP (1989) *A Theory of Human and Primate Evolution*. Oxford: Oxford University Press.
- Groves CP (1993) Order Primates. In DE Wilson and DM Reeder (eds.): *Mammal Species of the World*. London: Smithsonian Institution Press, pp. 243–277.
- Hoojer DA (1962) Quaternary langurs and macaques from the Malay Archipelago. *Zool. Verh. Leiden* 55:1–64.
- Jablonski NG, and Peng YZ (1993) The phylogenetic relationship and classification of the doucs and snub-nosed langurs of China and Vietnam. *Folia Primatol.* 60:36–55.
- Jauch A, Wienberg J, Stanyon R, Arnold N, Tofaneli S, Ishida T, and Cremer T (1992) Reconstruction of genomic rearrangements in great apes and gibbons by chromosome painting. *Proc. Natl. Acad. Sci. U.S.A.* 89:8611–8615.
- King M (1993) *Species Evolution: The Role of Chromosome Change*. Cambridge: Cambridge University Press.
- Koehler U, Arnold N, Wienberg J, Tofaneli S, and Stanyon R (1995a) Genomic reorganization and disrupted chromosomal synteny in the siamang (*Hylobates syndactylus*) revealed by fluorescence *in situ* hybridization. *Am. J. Phys. Anthropol.* 97:37–47.
- Koehler U, Bigoni F, Wienberg J, and Stanyon R (1995b) Genomic reorganization in the concolor gibbon revealed by chromosome painting. *Genomics* 30:287–292.
- Krishna-Murthy DS, Jayaraman S, and Ambani LM (1979) Giemsa banding pattern in the langur monkey *Presbytis entellus entellus* (Dufresne). *Curr. Sci.* 48:180–181.
- Lejeune J (1983) Les chromosomes et l'espece. In C Chagas (ed.): *Recent advances in the evolution of primates*. Vatican City, Pontificia Academia Scientiarum, pp. 145–152.
- Lichter P, Cremer T, Borden J, Manuelidis L, and Word DC (1988) Delineation of individual human chromosomes in metaphase and interphase cells by *in situ* suppression hybridization using recombinant DNA libraries. *Hum. Genet.* 80:224–234.
- Lima MMC, and Seuànez HN (1991) Chromosome studies in the red howler monkey, *Alouatta seniculus stramineus* (Platyrrhini, Primates): description of an X₁X₂Y₁Y₂X₁X₂X₂ sex-chromosome system and karyological comparison with other subspecies. *Cytogenet. Cell Genet.* 57:151–156.
- Ma NSF, Elliot MW, Morgan L, Miller A, and Jones TC (1976) Translocation of Y chromosome to an autosome in the Bolivian owl monkey, *Aotus*. *Am. J. Phys. Anthropol.* 45:191–202.
- Muleris M, Couturier J, and Dutrillaux B (1986) Phylogénie chromosomique des Cercopithecoidea. *Mammalia* 50:38–52.
- Napier JR, and Napier PH (1967) *A handbook of living primates*. London: Academic.
- Napier JR, and Napier PH (1985) *The Natural History of the Primates*. London: British Museum.
- Oates JF, Davies AG, and Delson E (1984) The diversity of living colobines. In JF Oates and AG Davies (eds.): *Colobine Monkeys: Their Ecology, Behaviour, and Evolution*. Cambridge: Cambridge University Press, pp. 45–73.
- Peng YZ, Pan RL, and Jablonski NG (1993) Classification and evolution of Asian Colobines. *Folia Primatol.* 60:106–117.
- Pinkel D, Straume T, and Gray J (1988) Cytogenetic analysis using quantitative high sensitivity fluorescence hybridisation. *Proc. Natl. Acad. Sci. U.S.A.* 83:2934–2938.
- Ponsà M, Boer de LEM, and Egozcue J (1983) Banding patterns of the chromosomes of *Presbytis cristatus pyrrhus* and *P. obscurus*. *Am. J. Primatol.* 4:165–169.
- Rettenberger G, Klett C, Zechner U, Kunz J, and Hameister H (1995) Visualization of the conservation of synteny between humans and pigs by heterologous chromosomal painting. *Genomics* 27:489–496.
- Rumpler Y, Gabriel-Robez O, Volobouev V, Yu W, Rasamimanana P, and de Perdigo A (1995) Male sterility and double heterozygosity for chromosomal inversion. *Cytogenet. Cell Genet.* 69:66–70.
- Sarich VM (1970) Primate systematics with special reference to Old World Monkeys: a protein perspective. In JR Napier and PH Napier (eds.): *Old World Monkeys: Evolution, Systematics and Behavior*. London: Academic, pp. 175–226.
- Sharma GP, Sobti RC, and Gupta CM (1972) An analysis of chromosomes in three primates from India. *J. Hum. Evol.* 2:283–287.
- Small MF, Stanyon R, Smith DG, and Sineo L (1985) High-resolution chromosomes of rhesus macaques (*Macaca mulatta*). *Am. J. Primatol.* 9:63–67.
- Solinas-Toldo S, Lengauer C, and Fries R (1995) Comparative Genomic map of human and cattle. *Genomics* 27:489–496.
- Stanyon R (1992) How polymorphisms and homoplasy can be informative about the evolution and phylogeny of humans and apes. *Proceedings XIII Congress of the International Primatology Society*. Kyoto: Kyoto University Press, pp. 423–439.
- Stanyon R, Sineo L, Chiarelli B, Camperio-Ciani A, Haimoff EH, Mootnick AR, and Sutarman Drh (1987) Banded karyotypes of the 44-chromosome gibbons. *Folia Primatol.* 48:56–64.
- Stanyon R, Wienberg J, Romagno D, Bigoni F, Jauch A, and Cremer T (1992a) Molecular and classical cytogenetic analyses demonstrate an apomorphic reciprocal chromosomal translocation in *Gorilla gorilla*. *Am. J. Phys. Anthropol.* 88:245–250.
- Stanyon R, Camperio Ciani A, Sineo L, and Morescalchi MA (1992b) The G-banded chromosomes of the Proboscis monkey (*Nasalis larvatus*) compared with the

- Macaque (*Macaca mulatta*). *Antropol. Contemp.* 15: 101–104.
- Stanyon R, Arnold N, Koehler U, Bigoni F, and Wienberg J (1995a) Chromosomal painting shows that “marked chromosomes” in lesser apes and Old World monkeys are not homologous and evolved by convergence. *Cytogenet. Cell Genet.* 68:74–78.
- Stanyon R, Tofanelli S, Morescalchi A, Agoormorthy G, Ryder O, and Wienberg J (1995b) Cytogenetic analysis shows extensive genomic rearrangements between *Alouatta* subspecies. *Am. J. Primatol.* 35:171–183.
- Thorington RW Jr, and Groves CP (1970) An annotated classification of the Cercopithecoidea. In JR Napier and PH Napier (eds.): *Old World Monkeys: Evolution, Systematics and Behavior*. London: Academic, pp. 629–647.
- Ushima RN, Shininger FS, and Grand T (1964) Chromosome complements of two species of primates: *Cynopithecus niger* and *Presbytis entellus*. *Science* 146: 78–79.
- Vogel C, and Winkler P (1990) Langurs and colobi. In Grzimek’s *Encyclopedia of Mammals*, vol. 2. London: McGraw-Hill, pp. 296–324.
- Wienberg J, Jauch A, Stanyon R, and Cremer T (1990) Molecular cytogenetics of primates by chromosomal *in situ* suppression hybridization. *Genomics* 8: 347–350.
- Wienberg J, Stanyon R, Jauch A, and Cremer T (1992) Homologies in human and *Macaca fuscata* chromosomes revealed by *in situ* suppression hybridization with human chromosome specific DNA libraries. *Chromosoma* 101:265–270.