Cytogenet Genome Res 108:139–160 (2005)
DOI: 10.1159/000080811

Cytogenetic and Genome Research

Fluorescence in situ hybridization to chromosomes as a tool to understand human and primate genome evolution

J. Wienberg

Institute of Human Genetics, GSF National Research Center for Environment and Health, and Institute for Anthropology and Human Genetics, Department Biology II, Ludwig Maximilian University, Munich (Germany)

Abstract. For the last 15 years molecular cytogenetic techniques have been extensively used to study primate evolution. Molecular probes were helpful to distinguish mammalian chromosomes and chromosome segments on the basis of their DNA content rather than solely on morphological features such as banding patterns. Various landmark rearrangements have been identified for most of the nodes in primate phylogeny while chromosome banding still provides helpful reference maps. Fluorescence in situ hybridization (FISH) techniques were used with probes of different complexity including chromosome painting probes, probes derived from chromosome subregions and in the size of a single gene. Since more recently, in silico techniques have been applied to trace down evolutionarily derived chromosome rearrangements by searching the human and mouse genome sequence databases. More detailed breakpoint analyses of chromosome rearrangements that occurred during higher primate evolution also gave some insights into the molecular changes in chromosome rearrangements that occurred in evolution. Hardly any "fusion genes" as known from chromosome rearrangements in cancer cells or dramatic "position effects" of genes transferred to new sites in primate genomes have been reported yet. Most breakpoint regions have been identified within gene poor areas rich in repetitive elements and/or low copy repeats (segmental duplications). The progress in various molecular and molecular-cytogenetic approaches including the recently launched chimpanzee genome project suggests that these new tools will have a significant impact on the further understanding of human genome evolution.

The evolution of the basic organization of the human genome into chromosomes has fascinated several generations of geneticists over the last decades. Primate chromosome analysis started with the description of uniformly stained metaphase chromosomes. Banding techniques allowed a more detailed insight into what shaped the human genome over the 50 or more million years of primate evolution. It soon became clear that human and great apes may only differ by few changes in chromosome morphology (Turleau et al., 1972; Dutrillaux, 1979; Seuánez, 1979; Yunis and Prakash, 1982; Clemente et al., 1990, for reviews). Further, various chromosomes of some Old World monkeys showed exactly the same banding patterns as humans and even outside the primate order chromosome banding patterns were observed in other mammals that were very similar to human chromosomes (Dutrillaux, 1979; Nash and O'Brien, 1982). However, although many comparative maps based on chromosome banding patterns were confirmed by comparative gene mapping, there was still some reservation in the scientific community about the level of confidence in primate cytogenetics as a tool for evolutionary studies. Thus, when molecular probes became increasingly available there was great demand for techniques that would overcome the limitations describing mammalian chromosomes on morphology alone. Molecular probes for the first time distinguished mammalian chromosomes on the basis of their DNA content rather than solely on morphological features such as banding patterns.

With the recent advances in different genome projects, numerous new molecular tools and DNA probes are now avail-

Received 7 February 2004; revision accepted 12 May 2004.

Request reprints from: Johannes Wienberg, Ingolstädter Landstr. 1
D–85764 Neuherberg (Germany); telephone: +49 8035 984928
fax: +49 8035 984930; e-mail: j.wienberg@lrz.uni-muenchen.de

able for chromosome studies that would define homologies in different mammalian taxa with high confidence. Now, the most informative technique to study chromosome evolution is fluorescence in situ hybridization (FISH) of these probes to chromosomes fixed on slides. Thereby, chromosome banding is still an important tool providing a reference map.

First molecular cytogenetic comparisons between human and different primates used highly repetitive sequences such as satellite DNAs and alphoid sequences. However, it soon became clear that satellite DNAs are highly dynamic in evolution and a certain human alphoid sequence would not always hybridize to the homologous primate chromosome (Warburton and Willard, 1996; Alexandrov et al., 2001, for reviews).

Comparative gene mapping with somatic hybrid cell lines was used to define homologies between different primate species. This technique, however, is highly laborious and except for some model species, originally only patchy maps could be established between mammalian species. Within primates this technique has extensively been used in a gibbon, some Old World monkeys and in New World monkeys, especially in the owl monkey (*Aotus*), but resulting maps were far from complete to allow a more detailed phylogenetic analysis of these species (see O'Brien, 1984, for review). Recently various species high resolution maps have been obtained from "radiation reduced hybrid mapping" (RH mapping, Hawken et al., 1999; Watanabe et al., 1999; Band et al., 2000; Mellersh et al., 2000). The disadvantage of this approach, however, is that for each new species a specific new panel of hybrids has to be established.

During the early stages of the human genome project various DNA probes were developed that would cover the euchromatin rather than the heterochromatic part of chromosomes. One approach was sorting to facilitate purification of chromosomes based on DNA staining with fluorochromes (Gray et al., 1987). Chromosome suspensions of single types with a purity of up to 90% were obtained and cloned DNA libraries were used for chromosome painting (Lichter et al., 1988; Pinkel et al., 1988). These techniques also allowed demonstration of changes in karyotypes that occurred during evolution (Wienberg et al., 1990). Another approach to produce chromosome paints used micro-dissection of chromosome segments with glass needles to obtain chromosome painting probes for comparative studies (Lengauer et al., 1991). Chromosome painting provides one of the few techniques by which basic syntenic homology data can be obtained without the development of specific conserved markers or specialized mapping systems for each new species.

More recently, ordered clone sets from entire genomes from different species are public domain and cytogeneticists can physically map any given sequence on the human genome and on those of various other species. By focusing on the evolution of autosomes the aim of this review is to illustrate how these new techniques helped in our understanding of the phylogeny and evolution of the gross architecture of the human genome. Although this survey is far from complete and does not include primate sex chromosome evolution, the data currently available allow some general hypotheses to be developed about the origin and evolution of the primate genome.

Molecular cytogenetic techniques to study primate evolution

The first chromosome painting experiments on non-human primates revealed the molecular proof for several hypotheses on primate genome evolution (Wienberg et al., 1990). (i) Hybridizations of various human chromosome-specific painting probes to their great ape homologs produced only one pair of signals each, except for the human chromosome 2 probe that delineated two pairs of homologs in all great apes. Further, a reciprocal translocation between the homologs of human chromosomes 5 and 17 in the gorilla karyotype was demonstrated. These rearrangements were already suggested from previous classical chromosome studies (Dutrillaux, 1979; Yunis and Prakash, 1982). The translocation in the gorilla, however, was not supported by previous gene mapping techniques (O'Brien et al., 1985), which at that time were suggested as being more reliable than interpretations from banded chromosomes. (ii) Many years ago S. Ohno postulated that the placental mammalian X chromosome was evolutionarily conserved (Ohno, 1973). He argued that this conservation was due to the dosage compensation mechanism for X-linked genes in placental mammals that rely on the functional inactivation of X chromosomes in somatic cells. In the first painting study with various different primate species the human X chromosome-specific probe proved "Ohno's law". The painting was found exclusively on the homologous X chromosome in all primates analyzed. (iii) Gibbons are classified with great apes and humans in the same primate superfamily Hominoidea. With chromosomal banding, however, they do not show any karyological relationship with great apes, humans or any other primate species. Thus it was suggested that gibbons had experienced rapid and massive chromosome evolution and as a consequence had highly derived karyotypes (Dutrillaux et al., 1975; Marks, 1982; Van Tuinen and Ledbetter, 1983; Stanyon et al., 1987). The first painting experiments with some human chromosome-specific probes on gibbon chromosomes confirmed this assumption and mapped various massive rearrangements (Wienberg et al., 1990).

A more complete study using all 24 different painting probes from human autosomes as well as the two sex chromosomes revealed no further rearrangements in the chimpanzee, gorilla, or the orangutan karyotype (Jauch et al., 1992). The complete set of human probes was also hybridized to three gibbon species from the *Hylobates lar* species group *(Hylobates lar, H. klossi, H. moloch)*. All three species have almost the same karyotype with 2n = 44 chromosomes and revealed the same chromosome painting patterns. The human chromosome-specific probes delineated various segments on the autosomes and for the first time allowed the complete reconstruction of the extreme reshuffling of a gibbon genome (Jauch et al., 1992). The Japanese macaque, *Macaca fuscata*, was the first monkey to be studied with all human chromosome painting probes (Wienberg et al., 1992). Strikingly, all human chromosome syntenies except for the homologs for human chromosome 2 were found intact in the macaque karyotype. However, three macaque chromosomes were found that were painted by two human chromosome specific probes each, indicating chromo-

Table 1. Primate species that have been analyzed by chromosome painting at the time of this writing (full karyotypes)

Prosimii	Lemuriformes	*Eulemur macaco macaco*	Müller et al., 1997b
		E. fulvus mayottensis	Müller et al., 1997b
		Lemur catta	Cardone et al., 2002
	Lorisidae	*Otolemur crassicaudatus*	Stanyon et al., 2002
		Galago moholi	Stanyon et al., 2002
New World monkeys	Callitrichidae	*Callithrix jacchus*	Sherlock et al., 1996; Neusser et al., 2001
		C. argentata	Neusser et al., 2001
		Cebuella pygmaea	Neusser et al., 2001
		Callimico goeldii	Neusser et al., 2001
		Saguinus oedipus	Müller et al., 2001
	Cebidae	*Cebus capucinus*	Richard et al., 1996
		C. apella	Garcia et al., 2000
		C. nigrivittatus	Garcia et al., 2002
		Saimiri sciureus	Stanyon et al., 2000
		Callicebus moloch	Stanyon et al., 2000
		C. donacophilus	Barros et al., 2003
		C. lugens	Stanyon et al., 2003a
	Atelidae	*Lagothrix lagothricha*	Stanyon et al., 2001
		Ateles geoffroyi	Morescalchi et al., 1997
		A. belzebuth hybridus	Garcia et al., 2002
		Alouatta seniculus arctoidea	Consigliere et al., 1996
		A. seniculus sara	Consigliere et al., 1996
		A. seniculus macconnelli	de Oliveira et al., 2002
		A. caraya	de Oliveira et al., 2002
		A. belzebul	Consigliere et al., 1998
		A. fusca fusca	de Oliveira et al., 2002
		A. fusca clamitans	de Oliveira et al., 2002
Old World monkeys	Cercopithecidae	*Macaca fuscata*	Wienberg et al., 1992
		Papio anubis	Best et al., 1998
		Cercopithecus aethiops	Finelli et al., 1999
	Colobidae	*Colobus guereza*	Bigoni et al., 1997b
	(Leaf eaters)	*Trachypithecus* (*Presbytis*) *cristatus*	Bigoni et al., 1997a
		T. (*P.*) *francoisi*	Nie et al., 1998
		T. (*P.*) *phayrei*	Nie et al., 1998
		Pygathrix nemaeus	Bigoni et al., 2004
		Nasalis larvatus	Bigoni et al., 2003
Apes (Hominoidea)	Gibbons	*Hylobates lar*	Jauch et al., 1992; Müller et al., 2002; Mrasek et al., 2003
	(Hylobatidae)	*H. moloch*	Jauch et al., 1992
		H. klossi	Jauch et al., 1992
		H. hoolock	Yu et al., 1997; Nie et al., 2001
		H. syndactylus	Koehler et al., 1995a
		Nomascus (*H.*) *concolor*	Koehler et al., 1995b; Schröck et al., 1996; Rens et al., 2001
	Great apes	*Pan troglodytes*	Jauch et al., 1992
	(Hominidae)	*Pan paniscus*	Jauch et al., 1992
		Gorilla gorilla	Jauch et al., 1992
		Pongo pygmaeus	Jauch et al., 1992

some-fusion or fission events (see below). Since these early chromosome painting studies, the karyotypes of nearly 50 primates have been completely analyzed with human chromosome-specific painting probes up to now (Table 1), that elucidated various new aspects of primate evolution.

In human molecular cytogenetics various improvements of the original chromosome painting protocol were developed in recent years. Most importantly, multi-color approaches were applied that used probes labeled with different fluorophores in single hybridization experiments (Ried et al., 1992). It is now possible to discriminate the entire human chromosome set with 24 different colored chromosomes in a single experiment and to define chromosome rearrangements for the entire human genome with ease (Schröck et al., 1996; Speicher et al., 1996). Although this approach also allowed delineating the entire karyotype of a gibbon species *(H. concolor)* in a single hybridization by spectral karyotyping (SKY) (Schröck et al., 1996), in general practice smaller probe pools are now being used in cross species hybridization (Fig. 1). This is due to the complexity of the probe sets and the difficulties in the interpretation of complex rearrangements especially in "multi-directional" chromosome painting experiments (see below). Further, because increasing DNA sequence divergence makes painting with human probes on chromosomes of more distantly related species less consistent, less complex chromosome painting sets are preferred (Neusser et al., 2001; Murphy et al., 2003).

The resolution of chromosome painting patterns in cross species hybridization has been disputed on the background of detailed gene mapping data between some mammalian taxa. In the mouse gene mapping revealed more than 200 segments that

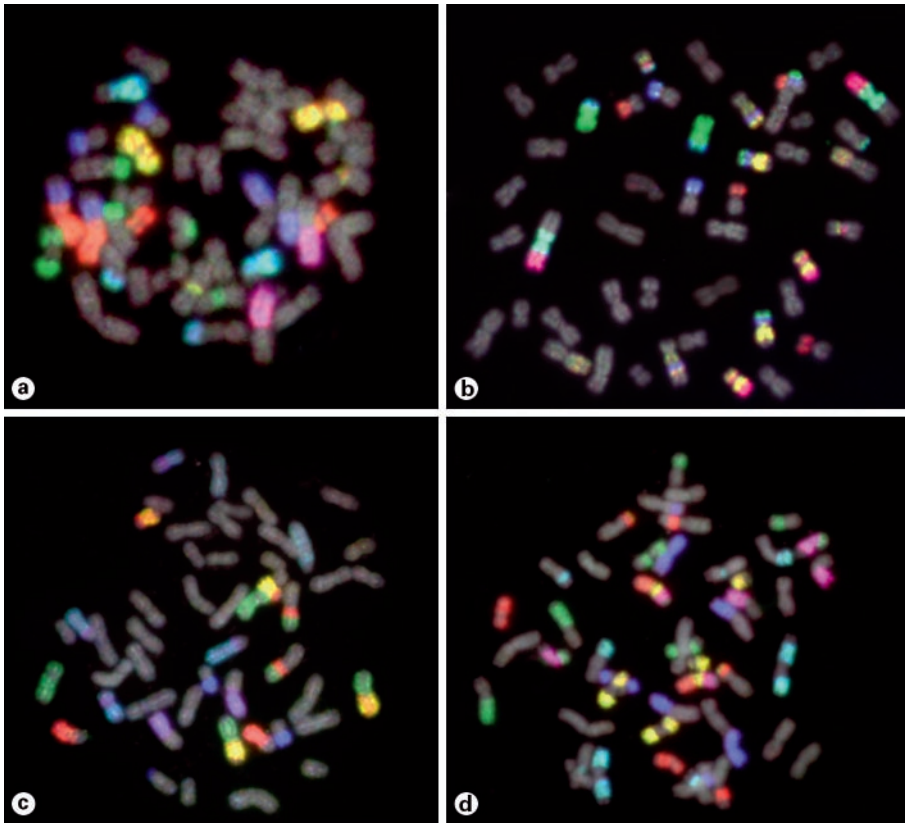


Fig. 1. Multi-directional chromosome painting using smaller pools of chromosome-specific probes between different primate species. Pools consist of 6 different probes labeled with three fluorochromes in Boolean combinations. (**a**) Human chromosome-specific paints hybridized to metaphase chromosomes of the Lar gibbon *(Hylobates lar)*. Various chromosome rearrangements are evident that disrupt synteny of gibbon chromosomes compared to humans. (**b–d**) Pools of gibbon-specific probes derived from *Nomascus (Hylobates) concolor* hybridized to metaphase chromosomes of other gibbon species, (**b**) *H. lar*, (**c**) *H. syndactylus* and (**d**) to a macaque *(Macaca fascicularis)* serving as outgroup. The hybridization demonstrates that gibbon karyotypes differ dramatically not only from apes (human), but also between gibbons. A detailed inspection of rearrangements allowed defining subregional homologies and the direction of changes in evolution (kindly provided by S. Müller and M. Neusser).

disrupted the synteny compared to human chromosomes. In the comparison of human and cat, however, only about 30 homologous segments were observed by reciprocal chromosome painting (Wienberg et al., 1997). With only some few additional segments this was recently confirmed by high resolution radiation hybrid mapping of cat chromosomes (Murphy et al., 2000). Thus, the high number of disrupted syntenies may be special to some rodent karyotypes rather than a general feature of mammalian chromosome evolution (Stanyon et al., 1999, 2003b; Yang et al., 2000). Analysis of highly rearranged gibbon karyotypes (see above) allowed us to estimate the resolution of chromosome painting. In closely related species such as between primates, translocations of the size of only one chromosome band (5–10 Mbp) could be visualized by this technique (Schröck et al., 1996). Again, because of increasing DNA sequence divergence, the resolution may decline with increasing phylogenetic distance of the species compared.

Chromosome painting reveals changes in chromosome numbers and translocations but not changes within chromosomes. The latter can be achieved using sub-regional probes such as obtained by micro-dissection of chromosome arms or bands. One of the first human band-specific probes derived from micro-dissection came from band 8q21 spanning the Langer-Giedion syndrome region (Lüdecke et al., 1989). Hybridization of this probe to primate chromosomes demonstrated that it was sufficiently complex to paint the homologous bands in great ape, gibbon and Old World monkey chromosomes (Lengauer et al., 1991). Another probe derived by dissection of the long arm of human chromosome 2 was used for a more detailed analysis of the pericentromeric region of the homologous chromosomes in great apes and Old World monkeys. Inversions were observed in great apes and the hybridization pattern demonstrated the fusion of the human chromosome 2 homologs as proposed by banding studies (Wienberg et al., 1994). More complex sets of micro-dissection probes have recently been used to produce a banding pattern on human and primate chromosomes (Mrasek et al., 2001, 2003). The experiments gave proof that these probes are able to delineate rather small and complex intra-chromosomal rearrangements that occurred during primate evolution that may not be easily visible with chromosome banding.

Another source of probes for chromosome sub-regions are human/rodent somatic cell hybrids (Müller et al., 1996). Especially when using Chinese hamster cells as "host", human chromosomes tend to get fragmented in these cell lines. The DNA of human chromosomes can then be extracted from the hamster background by *ALU*-PCR. More than 300 different probes for fragments of human chromosomes have been described (Antonacci et al., 1995) and various probes have been used to identify chromosome homologies and rearrangements in different primate species.

Region-specific probes have also been established from non-human primates that show highly rearranged karyotypes compared to humans. Flow sorting of chromosomes has been used to establish paints from highly rearranged karyotypes in painting experiments on primates (Ferguson-Smith, 1997, for re-

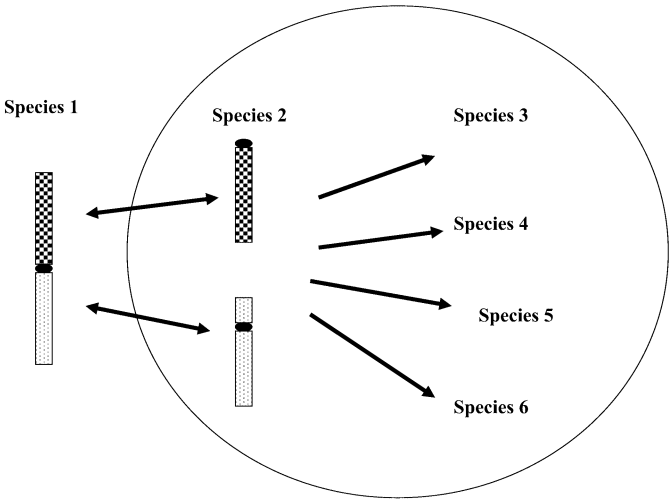


Fig. 2. Multi-directional chromosome painting: Detailed homology information is obtained by reciprocal chromosome painting between two species belonging to two different taxa (species 1 and 2; for example human and a New World monkey). Since hybridization efficiency of complex probes within the given taxa is better than between more distantly related species the analysis will be drastically simplified and more secure. Further, in case species 2 has a highly derived karyotype, many subregional homologies can be identified between species belonging to taxon 1 and 2.

view). These include probes from various gibbon species, from Old World monkeys such as the African green monkey *(Cercopithecus aethiops)*, or from New World monkeys, prosimians and non-primate mammals, such as the tree shrew (Müller et al., 1997a, b, 1998, 1999, 2001; Finelli et al., 1999). Especially probes derived from the two species *Saguinus oedipus* (Müller et al., 2001) and *Lagothrix lagothricha* (Stanyon et al., 2001) were very much helpful for the analysis of various complex karyotypes observed within New World monkeys. These probes were used together with human paints in "reciprocal" or even "multi-directional" approaches to better define homologies and breakpoints between New World monkeys species (Fig. 2; Neusser et al., 2001; Stanyon et al., 2001).

As the human genome project proceeded, various cloned DNA probes became available that were also used in comparative cytogenetics. Early studies included individual clones derived from the analysis of certain regions of the human genome such as clones containing oncogenes and other sequences important in human genetic pathology (Matera and Marks, 1992; Ried et al., 1993; Arnold et al., 1995). Later, well-defined cloned DNAs of high complexity became public domain such as yeast artificial chromosomes (YACs) that were used to delineate homologies in various primate species (Arnold et al., 1996; Haaf and Bray-Ward, 1996).

Recently, the entire human DNA sequence was aligned into a tile path of DNA cloned into some several thousand bacterial artificial chromosomes (BACs) and P1 derived artificial chromosomes (PACs), which now provide cytogenetic landmarks for the draft sequence of the human genome (Cheung et al., 2001). These resources are now of outstanding importance to define the homology of any human chromosome segment with their primate homolog by FISH. Similar resources were recently established for the chimpanzee genome (Fujiyama et al., 2002) and are currently developed for other primates and various non-primate mammals as well. To date, various comparative FISH studies use all these resources including probes of different complexity such as YACs/BACs/PACs, probes derived by micro-dissection or from rearranged somatic cell hybrids and from flow sorted chromosomes of non-human primates and other mammals. These approaches are now providing a more complete picture about how the organization of the human genome was shaped and promise insight into the dynamics and mechanisms of human genome evolution in general.

Phylogenetic techniques to study chromosome evolution

Some changes in the genome during evolution are events that are unlikely to happen twice and independently in different phylogenies. These "rare events" include for example insertions of LINEs or SINEs into distinct regions of the genome. Gene duplications or intron insertions and deletions can also be considered as unique events, which would then be shared by descendant species (Rokas and Holland, 2000, for review). Chromosome rearrangements represent a member of these "rare events" in which homoplasy is unlikely and their analysis can be fruitfully used for the reconstruction of phylogenetic relationships.

When using these rare changes the first step in the analysis of the phylogeny of a given taxon is the inspection for ancestral and derived traits and their distribution within and between the different species. The ancestral state can be assumed in cases when a certain trait is not only found within a given taxon but also in more distantly related species that may serve as an "outgroup". For example, the human chromosome 2 has two homologous pairs in great apes. Two chromosome pairs are also found in the karyotypes of apes and Old World monkeys and even in various non-primate mammals. Thus, two chromosomes should be considered as the ancestral state, while the human karyotype should have experienced an evolutionarily derived fusion of these two chromosomes. Similarly, in case two species share a derived chromosome rearrangement they should be linked phylogenetically by a common ancestor. In a systematic analysis including appropriate "outgroup" species we can now identify the ancestral or "primitive" as well as the derived karyotypes for all main divisions in primate evolution. This will then be of use to draw general conclusions about the direction of changes and primate chromosome evolution in general.

A quantification of chromosome rearrangements for establishing phylogenies was used in which chromosome rearrangements were treated as discrete characters in a parsimony analysis (PAUP, Phylogenetic Analysis Using Parsimony, Swofford, 1998). Using painting probes from highly rearranged karyotypes (for example from the Concolor gibbon, see Fig. 1) a chromosome painting unit termed "chromosomal segment homolog" (CSH) was introduced to quantify defined sub-regional chromosome homologies (Nash et al., 1998; de Oliveira et al.,

2002; Müller et al., 2003). A CSH for any group of species is that set of chromosome segments that all hybridize to the homologous region of a reference species. The size and numbers of CSHs are dependent upon the resolution of the subregional probes used. Adjacent homologous CSHs were recorded and associations of CSHs were used as discrete phylogenetic characters to be used in a PAUP analysis.

The ancestral karyotype of all primates

The presentation of the ancestral karyotype for all primates based on molecular probes became possible after chromosome painting techniques were successfully applied to non-primate mammals that can serve as outgroup (Scherthan et al., 1994). Since most experiments used human paints the interpretation of the findings is rather straight forward: in case a human chromosome-specific painting probe stains only one homologous pair in some other primates as well as in some non-primate mammals, this chromosome form should be considered ancestral for all primates (Haig, 1999; Müller et al., 1999). This was true for homologs to human autosomes 1, 4, 5, 6, 8, 9, 10, 11, 13, 17, 18, and 20. The situation for the human chromosome 1 homolog was not clear in earlier studies, because it was found fragmented in all primates with the exclusion of apes and most higher Old World monkeys that would suggest a more recent fusion of chromosome segments (Wienberg et al., 2000). A complete human chromosome 1 homolog, however, was found in whales and dolphins, and more recently in Afrotheria (aardvark) and in Xenarthra (two-toed sloth; see Froenicke et al., 2003; Yang et al., 2003; Svartman et al., 2004). Thus, it now seems likely that this chromosome was already part of the ancestral primate karyotype but was independently disrupted in various mammalian phylogenies including prosimians and New World monkeys. Thus, the disruption of ancestral syntenies may show convergence on the chromosome banding level, however different breakpoints can be observed with molecular tools (Murphy et al., 2003).

Another group of chromosomes that should be considered as ancestral for all primates are chromosome forms found in some primates as well as in non-primate mammals, but show a derived condition in humans. For example, homologs to human chromosomes 14 and 15 form a single chromosome in Old World monkeys and, although further rearranged, in New World monkeys and prosimians. Human painting probes for 14 and 15 together also delineate a single chromosome in various non-primate mammals and thus should be considered ancestral for all primates (Müller et al., 1999). Human chromosomes 14 and 15 arose by a simple fission of this ancestral homolog. The same situation is found for the association of entire homologs to human chromosomes 3 and 21, which is part of the karyotype of many non-primate mammals as well as those of prosimians (Richard and Dutrillaux, 1998; Müller et al., 2000). An association of a fragment of human chromosome 3 and 21 homologs can also be observed in New World monkeys suggesting that they have been derived from the ancestral 3/21 chromosome (Neusser et al., 2001).

For most of these chromosomes the synteny of their genes was not changed for more than 50 million years of human genome evolution. Changes in synteny, however, occurred by fusion of chromosomes and by some translocations. Fusion of six chromosomes, homologous to 2p and 2q, 16p and 16q, and 19p and 19q, respectively, occurred in the origin of human chromosomes 2, 16 and 19. The ancestral two chromosomes with homology to human chromosomes 12 and 22 experienced a reciprocal translocation. Human chromosomes 7 arose from two chromosomes that consisted of fragments (7a and 7b/16) that were later reshuffled by intra-chromosomal rearrangements. Thus, the human karyotype differs from that of the ancestor of all primates by six new syntenies only (chromosomes 2, 7, 12, 16, 19, and 22) and six new chromosomes in which ancestral syntenies were disrupted (chromosomes 3, 21 and 14, 15 derived from fissions and again 12 and 22 derived from a reciprocal translocation; see Müller et al., 1999). Compared to the ancestral primate karyotype the human complement has gained two chromosomes by two fissions and lost three by fusions. Together with the 12 human homologous autosomes that were not changed, and the sex chromosomes, the ancestral karyotype should have had a diploid number of 2n = 48 (Haig, 1999; Müller et al., 1999).

Landmark rearrangements in the evolution of the human karyotype

Since chromosome rearrangements can be considered as unique events with low level of convergence, they can present various landmarks in the evolution of the human karyotype. The succession of these landmark rearrangements can then be dated during primate evolution since derived rearrangements are generally shared by the members of a given taxon. For example, the ancestral 12/22 associations are still present in lemurs (Müller et al., 1997b), but the reciprocal translocation that formed the human chromosome 12 and 22 homologs is shared by all higher primates. These chromosomes, however, may have experienced further derived changes in some individual species. Figure 3 gives a summary of the landmark events that formed the human karyotype. Early in primate evolution the reorganization of homologous segments to human chromosomes 7, 16 and 19 started. In prosimians we still find an association of a chromosome 7 fragment (7b) with the p arm of the chromosome 16 homolog that was disrupted after divergence of prosimians and simians. At the same node the two arms homologous to human 19p and 19q fused and a reciprocal translocation formed the chromosome 12 and 22 homologs (Müller et al., 1997b; Cardone et al., 2002). The split of New World monkeys and higher Old World primates was followed by a fusion of each of the two fragments of the human chromosome 7 and 16 homologs, respectively. Further, this node is characterized by the fission that formed the homologs of human chromosomes 3 and 21 (Richard and Dutrillaux, 1998; Müller et al., 2000). Another fission gave origin of homologs of human chromosomes 14 and 15 after divergence of higher Old World monkeys and apes. The final landmark in the evolution of the human karyotype was the fusion of two chromosomes that formed

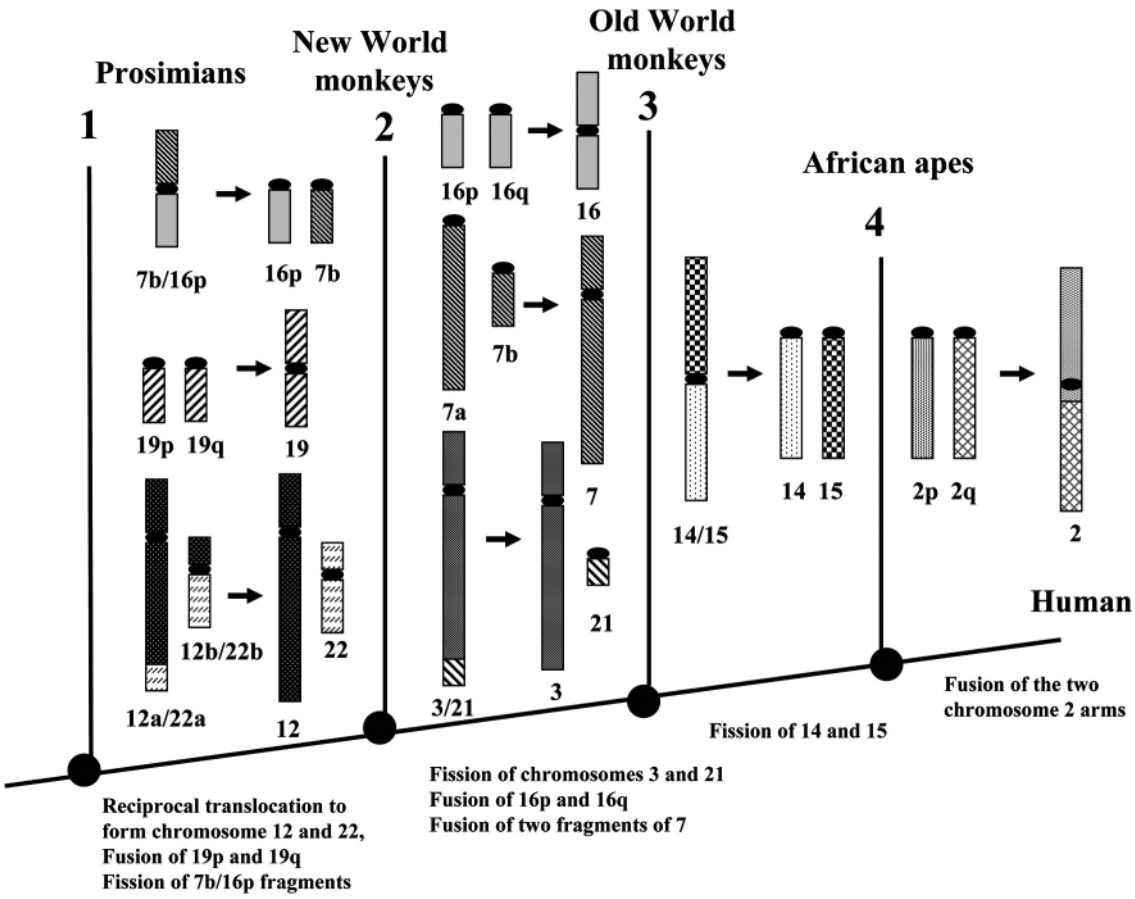


Fig. 3. Landmark chromosome rearrangements in primate and human evolution at each main divergence node. **1** After the divergence of prosimians and simians three main changes can be observed that would link all higher primates phylogenetically. These include a reciprocal translocation that formed the human chromosome 12 and 22 homologs, a fusion that produced the human chromosome 19 homolog and a fission that produced the "precursor" of chromosomes 7 and 16. **2** After the split of New World monkeys and higher Old World primates human chromosomes 3 and 21 emerged from a fission and chromosomes 7 and 16 from a fusion each. **3** The divergence of higher Old World monkeys from hominoids is marked by the origin of the homologous chromosomes 14 and 15. **4** The only derived rearrangement that distinguishes humans from apes is the fusion that led to human chromosome 2.

human chromosome 2 which distinguishes the human karyotype from great apes (Wienberg et al., 2000, for review). Since at the time of writing this article only a limited number of primates have been analyzed by chromosome painting, additional landmark rearrangements for the human karyotype may be identified in further studies. This should be especially true for prosimians for which only very few species have been analyzed up to now. For some chromosome forms observed in the boreoeutherian ancestor (associations of chromosome 4/8, 12/22/10, 19q/16q homologs) we do not know yet whether they were rearranged during primate chromosome evolution or before the origin of extant primates. They may be found in some not yet analyzed prosimian species and could be added to the ancestral karyotype of all primates. Many more landmark rearrangements can be observed within the individual phylogenies of the different primate taxa (see below), which makes this approach highly attractive for evolutionary studies.

Prosimians

Banded chromosomes of numerous prosimian species have been analyzed by Y. Rumpler and colleagues. Banding analyses suggested that some lemur species may have a karyotype that is close to that of the assumed ancestral primate (Rumpler and Dutrillaux, 1976; Dutrillaux, 1979). This was consistent with the idea that prosimians in general are assumed to be more primitive and therefore more similar to the ancestor of all primates than monkeys, apes and humans. However, chromosome painting of some prosimians now demonstrates that within their 50 million years or more of evolution after the divergence leading to humans they may have accumulated even more rearrangements than various non-primate mammals (Müller et al., 1997b; Stanyon et al., 2002).

The two main divisions of Prosimii are the Lemuriformes and Lorisidae. At the time of writing this article, chromosome painting results of complete karyotypes of only three and two

species, respectively, have been published for these taxa (Müller et al., 1997b; Cardone et al., 2002; Stanyon et al., 2002; Table 1). One of the lemurs analyzed by chromosome painting *(E. fulvus mayottensis)* has a banded karyotype that is very close to *M. murinus,* the lemur species believed to have maintained, almost intact, the ancestral primate karyotype (Dutrillaux, 1979; Rumpler et al., 1989). Chromosome painting gave a detailed reconstruction of chromosomal changes in these species and demonstrated various derived rearrangements from the supposed ancestral primate karyotype.

Lemuriformes

Reciprocal painting of human probes to lemur chromosome preparations and vice versa was used to delineate the homology between the human karyotype and those of two lemur species (*Eulemur macaco macaco* and *E. fulvus mayottensis*; see Müller et al., 1997b). The reciprocal painting approach made sure that also various intra-chromosomal homologies could be detected. Considering that both human and lemurs should have their own evolutionary history of about 40–50 million years each, modest numbers of chromosome rearrangements were observed that happened during this period of closely 100 million years. The results further support earlier assumptions made on the basis of chromosome banding that showed that the karyotypes of the two lemur species have evolved exclusively by Robertsonian translocations. All probes derived from *E. f. mayottensis* specific for homologs involved in rearrangements in *E. m. macaco,* exclusively painted entire chromosome arms. When using human as the outgroup for lemurs, the results further indicate that *E. f. mayottensis* has a more ancestral karyotype than *E. m. macaco*: *E. f. mayottensis* has 6 and *E. m. macaco* has 15 derived associations of segments homologous to human chromosomes. The lemur painting probes were one of the very first established from a non-human primate by flow sorting. Since hybridization signals are generally much stronger between closely related species, this probe set was superior to human paints and may be an important tool for a more detailed analysis of other prosimian species (Müller et al., 1997b).

Another approach used human chromosome painting specific for entire chromosomes and chromosome fragments together with locus-specific probes derived from the human BAC/PAC libraries to define rearrangements in the karyotype of the ring-tailed lemur *(Lemur catta)* (Cardone et al., 2002). At the time of writing this article *L. catta* is the only member of the genus Lemur from which complete karyotypes have been analyzed by painting. Part of the karyotype was already analyzed with some chromosome paints before (Apiou et al., 1996). To obtain significant signals on lemur chromosomes BAC/PACs specific for certain gene-rich chromosome bands were pooled for hybridization. Thus, homologies between this lemur and humans could also be demonstrated for numerous chromosome sub-regions. The results show that for some chromosomes this species conserved a more ancestral form while other chromosomes were more derived than in Eulemurs (Cardone et al., 2002).

Lorisidae

Up to now, only two African species of the Lorisidae have been fully analyzed with chromosome painting, *Galago moholi* (2n = 38) and *Otolemur crassicaudatus* (2n = 62) (Stanyon et al., 2002). Chromosome banding data suggested that the differences between the karyotypes of these closely related species are simple Robertsonian translocations (de Boer, 1973; Dutrillaux et al., 1982; Rumpler et al., 1983). Chromosome painting confirmed this hypothesis, and comparing the results with the ancestral primate karyotype provided the information about the direction of changes in galagos. The numbers of hybridization signals with human paints were about the same in both species in spite of the great differences in chromosome numbers. A total of six synapomorphic associations were observed that would link both species. The apomorphic rearrangements in both species, however, are in fact simple Robertsonian translocations, ten fusions in *G. moholi* and two fissions in *O. crassicaudatus*. Thus, both species have karyotypes that present a mixture of derived and conserved features. When compared with lemurs there may be another set of six rearrangements indicative of a common phylogenetic root linking lemurs and lorises. These data suggest that both galago species as well as galagos and lemurs may have shared a long period of common ancestry after the divergence of prosimians from anthropoids. The data also demonstrate that the prosimians analyzed up to now are considerably derived from the ancestral primate karyotype (Stanyon et al., 2002).

Tarsier

The systematics of tarsier was highly disputed. In one hypothesis, tarsiers are closely related to lemurs and lorises, and thus primates should be divided into Prosimii (lorises, lemurs, and tarsiers) and Anthropoidea (Platyrrhini and Catarrhini, i.e., monkeys, apes, and humans) (Shoshani et al., 1996, for review). Recent molecular data, however, place Tarsius as a sister group to anthropoids (Koop et al., 1989a, b; Zietkiewicz et al., 1999; Schmitz et al., 2001). Classical staining of chromosomes and banding studies suggest that they may have extremely derived karyotypes (Klinger, 1963; Dutrillaux and Rumpler, 1988). Currently, no data are yet available with chromosome painting that should elucidate the reshuffling of these karyotypes and may contribute to a better understanding of the evolution of this taxon and its relationship to other primates.

New World monkeys

Within primates New World monkeys represent the taxon with the highest number of species and the highest variability in chromosome numbers as well as polymorphisms. Recent suggestion would propose about 110 species and 95 subspecies that would make up nearly half of the species of the primate order (Rylands et al., 2000). There is evidence that there are still undiscovered or not yet recognized species. Traditionally, New World monkeys are divided into three main families, the Callitrichidae, Cebidae, and Atelidae (Ford, 1986). Alternatively,

they are divided into two families, the Cebidae and Atelidae that may have four subfamilies such as the Callitrichinae, Cebinae, Atelinae and Pitheciinae (Rosenberger, 1981). The systematics of these taxa are highly disputed and was recently reviewed by Hugot (1998), Schneider (2000), and Schneider et al. (2001).

Chromosome numbers range from 2n = 16 in *Callicebus lugens* to 62 in *Lagothrix lagotricha*. In some species the karyotype is highly variable such as in the owl monkey *(Aotus trivirgatus)*. At least 12 different karyotypes have been observed by chromosome banding (Ma and Lin, 1992) within its distribution area ranging from Panama to Argentina. Other species, although showing little morphological differences may have dramatically different karyotypes such as some howler monkeys (*Alouatta*). In *A. sara* and *A. seniculus arctoidea* which were traditionally classified as *A. seniculus* subspecies the conservative estimate obtained by chromosome painting included two Robertsonian translocations, five tandem translocations and five intra-chromosomal rearrangements (Consigliere et al., 1996).

The ancestral karyotype of New World monkeys

Chromosome painting demonstrates that karyotypes of New World monkeys have been considerably reshuffled compared to the ancestral karyotype of all primates. Up to now close members of all of the supposed subfamilies have been fully analyzed by chromosome painting (Table 1). There are numerous chromosome rearrangements in various taxa that may serve as landmarks for a better understanding of the phylogeny within New World monkeys.

An ancestral karyotype on the basis of chromosome painting has been proposed by Stanyon et al. (2000) that recently was revised based on the analysis of further species (Neusser et al., 2001). When including our yet unpublished data on a broader number of species, we can conclude that New World monkeys should be indeed of monophyletic origin. When comparing their karyotypes with those of prosimians and higher Old World primates some commonly derived chromosome changes can be observed that link all anthropoids including New World monkeys. These include the origin of chromosomes homologous to human 12, 19, 22 and the fragments homologous to 7a and 16q (see Fig. 3). Figure 4 presents a suggestion about the ancestral karyotype of all New World primates with 2n = 54 chromosomes based on the species currently analyzed by chromosome painting (Neusser et al., 2001). According to this karyotype, 14 chromosomes are commonly derived for all New World primates that include chromosome fragments or associations of fragments homologous to human 1a, 1b, 1c, 2b, 3a, 3b, 3c/21, 5/7a, 8a, 8b/18, 10a, 10b/16b, 14/15a and 15b. Within different clades of New World monkeys, however, some of them experienced further massive rearrangements.

Callitrichidae

The Callitrichidae traditionally include marmosets and tamarins. There was still controversy about the placement of the Goeldi's marmoset *(Callimico goeldii)* which was only discovered in 1904. *C. goeldii* exhibits several anatomical features, which it shares either with callitrichids or cebids. The

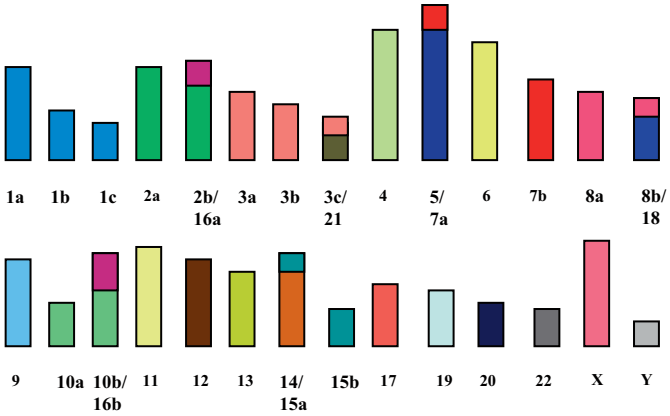


Fig. 4. The ancestral karyotype for all New World monkeys (2n = 54) up to now analyzed by chromosome painting. The ordering of chromosomes follows the numbering of human homologs. There are 14 chromosomes commonly derived for New World primates including chromosome fragments or associations of fragments homologous to human 1a, 1b, 1c, 2b/16a, 3a, 3b, 3c/21, 5/7a, 8a, 8b/18, 10a, 10b/16b, 14/15a and 15b. All other chromosomes were already present in the ancestral karyotype for all living primates (according to Neusser et al., 2001).

chromosome painting data show that *C. goeldii* has no derived chromosome rearrangements in common with any Cebidae monkey (Neusser et al., 2001). Common derived chromosome rearrangements, however, would link this species with all the Callitrichidae. Callitrichidae have diploid numbers ranging from 2n = 44 to 48 with not too many differences in their banding patterns (Canavez et al., 1996; Nagamachi et al., 1997). Currently, molecular cytogenetic data of this taxon have been published on *Callithrix jacchus, C. argentata, Cebuella pygmaea, Saguinus oedipus,* and *Callimico goeldii* (Table 1).

Using *Cebus* as outgroup and comparing with the proposed ancestral Platyrrhini karyotype various shared ancestral traits can be found in all Callitrichidae (Neusser et al., 2001). The relevant common chromosome forms that would distinguish Callitrichidae including *Callimico* from other New World primates consists of associations of fragments homologous to human 13/17/20, 13/9/22, 1a/10b, and 2a/15b.

Cebidae

The three capuchin monkeys analyzed with chromosome painting up to now include *Cebus capucinus, C. apella* and *C. nigrivittatus* (Table 1). They may have conserved almost completely the ancestral karyotype for all New World primates. *C. capucinus* and *C. apella* obviously share an identical karyotype, while *C. nigrivittatus* has one derived fused chromosome (Richard et al., 1996; Garcia et al., 2000, 2002).

Squirrel monkeys *(Saimiri)* are traditionally grouped within Cebidae. *S. sciureus* was completely analyzed by chromosome painting (Stanyon et al., 2000). Compared with other New World monkeys it shows a moderately derived karyotype with mostly autapomorphic rearrangements. One rearrangement, however, the association of human 2a/15b homologous segments, is also observed in all Callitrichidae and is most probably a common derived trait of both *Saimiri* and Callitrichidae.

This landmark would give evidence for a closer relationship of *S. sciureus* to Callitrichidae than to any other group of Platyrrhini, a conclusion also supported by some morphological comparisons (Kay, 1990; Rosenberger, 1981).

Callicebus species (titi monkeys) show a large variation in chromosome numbers ranging from 2n = 16 to 2n = 50 (Rodrigues et al., 2001; Stanyon et al., 2003a). Some authors include the titi monkeys into a family Pithecidae (see Schneider et al., 2001, for review). Three *Callicebus* species have been analyzed by chromosome painting up to now, including *C. moloch* and *C. donacophilus* (both 2n = 50) and *C. lugens*, the species with the lowest chromosome number known for primates (2n = 16; Barros et al., 2003; Stanyon et al., 2000, 2003a). As can be expected from the low chromosome number, *C. lugens* has a highly derived karyotype which shows mainly chromosome fusions compared to the other *Callicebus* species. However, the painting pattern also identified various fissions and inversions that changed this karyotype (Stanyon et al., 2003a). All three species were linked by three common derived associations, while *C. moloch* and *C. donacophilus* share at least seven derived chromosome rearrangements that would distinguish them from the ancestral karyotype. This finding suggests a closer relationship of *C. moloch* and *C. donacophilus.* There are no common derived rearrangements that would link them to any other genus within the Cebidae analyzed by chromosome painting up to now.

Atelidae

Among others, Atelidae include the large and prehensile-tailed genera *Alouatta* (howler monkeys), *Ateles* (spider monkeys), *Lagothrix* (woolly monkeys), which have been analyzed by chromosome painting (Table 1). From about half of these taxa chromosome painting data are still missing. These species attracted cytogeneticists because of their karyotype variability with chromosome numbers ranging from 2n = 32 to 62. The genus *Alouatta* gained special interest because of the extreme chromosomal variability and complex sex chromosome system found within this genus. Six species have recently been analyzed by chromosome painting including *A. fusca, A. caraya, A. sara, A. seniculus arctoidea, A. seniculus macconnelli* and *A. belzebul* (Consigliere et al., 1996, 1998; de Oliveira et al., 2002). To better define breakpoints the analysis included not only paints from human chromosomes but in part also those from other New World monkeys, *Saguinus oedipus* and *Lagothrix lagothricha* (Müller et al., 2001; Stanyon et al., 2001).

The six species analyzed differed from the inferred Platyrrhini ancestor by a total of 62 chromosomal rearrangements (Consigliere et al., 1996, 1998; de Oliveira et al., 2002). Of these, a high level of species-specific (autapomorphic) rearrangements were observed, reflecting the extensive karyological variation within this genus. There were, however, also various rearrangements that provided information on their evolutionary relationship. The high number of rearrangements allowed a PAUP analysis to be performed with binary characters extracted from cross species chromosome painting data (see above). High bootstrap values and three synapomorphies support the monophyly of the genus *Alouatta*, which is subsequently split into two main lineages (de Oliveira et al., 2002).

Ateles has one of the lowest chromosome numbers among Platyrrhini monkeys, which ranges from 32 in *A. paniscus paniscus* to 34 in all the other *Ateles* species so far karyotyped (Medeiros et al., 1997). Chromosome painting was performed for two species, the black-handed spider monkey *A. geoffroyi* and the long-haired spider monkey *A. belzebuth*, both with diploid numbers of 2n = 34 and very similar G-banded karyotypes (Morescalchi et al., 1997; Garcia et al., 2002 #196). Chromosome painting demonstrates that both species have highly derived karyotypes. The hybridization patterns with human paints are closely identical in both species that differ only by one fusion followed by an insertion. When compared to the ancestral New World monkey karyotype about 20 fusions/fissions, nine centromeric shifts, and five inversions were reported (Morescalchi et al., 1997; Garcia et al., 2002 #196).

The hybridization pattern found in the common woolly monkey *Lagothrix lagothricha* is less complex than that of other Atelidae (Stanyon et al., 2001). Changes are restricted to about nine rearrangements derived from the ancestral New World primate karyotype that were already identified in *Alouatta* and *Ateles* and would link all Atelidae up to now analyzed. These landmarks include a fission of the 1a fragment into two chromosomes ($1a_1$, $1a_2$), fission of the human chromosome 4 homolog into three chromosomes (4a, 4c) including a translocation of the 14/15a with 4b ($14/15b_1$, $4b/15a_2$), fission of the 5/7a chromosome and inversion within the 7a segment (7a/5a/7a, 5b), and finally, an inversion in 10b/16b (10b/16b/10b/16b) (Stanyon et al., 2001).

In summary, neotropical primates experienced various chromosome reorganizations compared to the ancestral karyotype of all primates, many of them in a common ancestor of all New World monkey species yet analyzed. In some clades chromosome rearrangements were so frequent, that various forms were identified that would serve as distinct landmarks and valuable markers for the understanding of the highly disputed taxonomy of these primates. Chromosome painting already added valuable information to this puzzle. Since data on some clades are still missing, however, a final picture cannot be provided yet.

Old World monkeys

Apes and higher Old World monkeys together form the clade Catarrhini. Their ancestral karyotype includes only a very few gross changes derived from the ancestal karyotype of all primates. Common to all higher Old World monkeys is that the chromosomes homologous to human 14 and 15 still form a unique entity that is split up in apes. All other inter-chromosomal rearrangements found in higher Old World monkeys should be considered as evolutionarily derived within their individual branches.

Higher Old World monkeys are traditionally divided into two families: Cercopithecidae (the cheek pouch monkeys including baboons, macaques, guenons and others) and Colobidae (African and Asian leaf-eating monkeys). From both of these taxonomic divisions chromosome painting data from complete karyotypes are now available for several species that

would complement previous G-banding results and would allow a preliminary discussion about the dynamics of chromosome evolution in these taxa.

Cercopithecidae

Chromosome banding on karyotypes of the Papionini, which include more than 30 different species and among others the genera *Papio, Macaca, Cercocebus*, showed that they all share a very similar karyotype with 2n = 42 chromosomes (Dutrillaux, 1979; Clemente et al., 1990, for reviews). Chromosome painting in the Japanese macaque *(Macaca fuscata)* demonstrated that except for the homolog of human chromosome 2 the synteny of all autosomes was found conserved compared to the human karyotype (Wienberg et al., 1992). However, three macaque chromosomes were painted with two human chromosome-specific paints each (paints for chromosomes 7/21, 14/15 and 20/22) indicating fusion/fission events of entire chromosomes. The 14/15 association was already described as ancestral of all primates (see above) while the two other rearrangements have to be considered as derived traits for Papionini. A baboon recently analyzed by SKY gave the same results as for the macaque (Best et al., 1998) confirming the similar karyotypes that are evident by chromosome banding.

Guenons are the only group within the Cercopithecidae with highly variable karyotypes. This taxon can be divided into about 20 species (Fleagle, 1988) and their chromosome numbers range from 2n = 48 to 72 (Dutrillaux, 1979; Ponsa et al., 1980). Chromosome painting data have been published on the African green monkey (*Cercopithecus aethiops*, 2n = 60; see Finelli et al., 1999), which is an important animal model in biomedical research. Many chromosomes of *C. aethiops* show a close resemblance to single arms of human or Papionini banded chromosomes, indicating Robertsonian fissions. Chromosome painting of *C. aethiops* probes back on human chromosomes, however, demonstrate that the karyological differences are not always simple centromeric fissions but more complex rearrangements have to be assumed (Finelli et al., 1999).

There is no evidence for any reciprocal translocation in the phylogenetic lines leading to humans and *C. aethiops*. However, the multiple hybridization patterns of some *C. aethiops* paints on human chromosomes 3 and 7 suggest that inversions have occurred during the evolution of both species. The ancestral association of human chromosome 14 and 15 homologs was disrupted into three chromosomes with breakpoints different from the fission that occurred in early hominoid evolution. An association between human chromosome 20 and 21 homologs as found in chromosome 2 of *C. aethiops* should be considered as a derived trait since both chromosomes are separate in the ancestral Catarrhine karyotype. The two derived chromosome associations, 7/21 and 20/22 that appear to link all Papionini are not observed in the African green monkey. These associations may have occurred in Papionini after the divergence of both phylogenies (Finelli et al., 1999). To better define the exact origin of this genus it would be of great benefit to have more chromosome painting data from a number of *Cercopithecus* species.

Colobidae

As for Papionini, African and Asian leaf-eating monkeys (Colobidae) have rather conserved karyotypes. With the exception of *Nasalis larvatus* (2n = 48), all have an identical diploid number, 2n = 44. Up to now only one African colobine species, the Abyssinian black-and-white colobus monkey *(Colobus guereza)* has been analyzed by chromosome painting (Bigoni et al., 1997b). All rearrangements observed were derived traits from the ancestral catarrhine karyotype and no common derived rearrangements were observed with Cercopithecidae. Reciprocal translocations were found between homologs of 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 indicate that a reciprocal translocation was followed by a pericentric inversion.

Very little is known about the phylogeny of Asian colobines. Chromosome painting data are now available for five species including the silvered leaf monkey *(Trachypithecus (Presbytis) cristatus*; see Bigoni et al., 1997a), two Chinese langurs *(T. (P.) francoisi* and *T. (P.) phayrei*; Nie et al., 1998), and two species from the odd-nosed group, the douc langur (*Pygathrix nemaeus*; Bigoni et al., 2004) and the proboscus monkey (*Nasalis larvatus*; Bigoni et al., 2003). Chromosome painting data indicate that the Asian colobines analyzed up to now have more derived karyotypes compared to African colobines. Except for the fusion of the homologs of human chromosomes 21 and 22 African and Asian colobines show no common derived characters in their karyotypes. A special derived character found in the karyotype of *T. cristatus* is a reciprocal translocation between the Y and an autosome homologous to human chromosome 5 that has produced a $X_1X_2Y_1Y_2/X_1X_1X_2X_2$ sex-chromosome system (Bigoni et al., 1997a). Translocations between the Y chromosome and an autosome are rare in primates. All Asian colobines analyzed share a reciprocal translocation of homologs of human chromosomes 1 and 19. The simple translocation pattern is found in *Pygathrix*. In *Nasalis* and the three *Trachypithecus* species this chromosome shows a more complex painting pattern that can be explained by a pericentric inversion which should have occurred in a common ancestor after the divergence from *Pygathrix*. Another rearrangement that may be informative for the phylogeny of Asian colobines is a reciprocal translocation between homologs of human chromosomes 6 and 16 that is common to all three species of *Trachypithecus* with the exclusion of *Pygathrix* and *Nasalis*. Figure 5 presents a summary of landmark chromosomes up to now identified in higher Old World monkeys.

Apes

Among apes (Hominoidea) we find two main taxa. Gibbons (Hylobatidae) are considered as the "lesser apes" while human and great apes form the clade Hominidae. As already discussed gibbons have highly derived karyotypes. Except for some few chromosomes, banding did not reveal the complex changes that reshuffled gibbon genomes (Dutrillaux et al., 1975; Stanyon et al., 1987; Van Tuinen and Ledbetter, 1983). Human and great apes, in contrast, have karyotypes that are very similar to var-

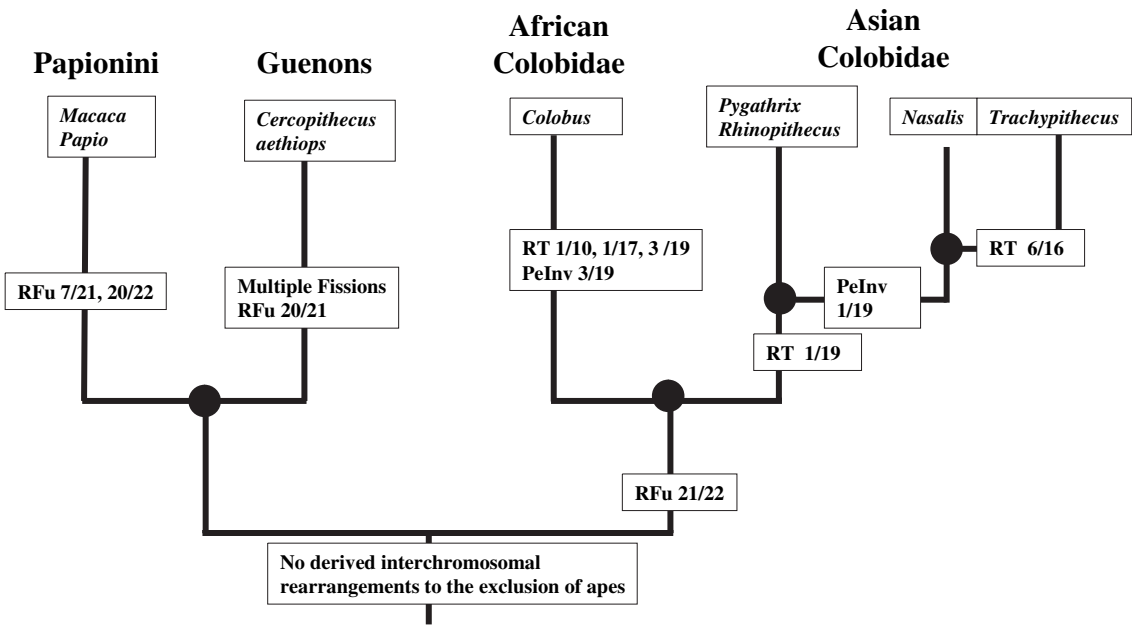


Fig. 5. Chromosome evolution in the higher Old World monkeys. There are no derived chromosome aberrations common to all species yet analyzed. Papionini all share Robertsonian translocations (RFu) of human chromosomes 7/21 and 20/22, respectively. Guenons show multiple derived fissions. Common to all Colobidae is an RFu of human chromosome 21 and 22 homologs. There are no further rearrangements common to African and Asian colobines. Within Asian colobines all species analyzed share a reciprocal translocation (RT) between human chromosome 1 and 19 homologs, which experienced an additional pericentric inversion (PeInv) in the ancestor of the odd nosed group of *Nasalis* and *Trachypithecus*.

ious higher Old World monkeys and even when compared to the ancestral karyotype of all primates only a few changes become evident by chromosome painting (Jauch et al., 1992; Wienberg et al., 1992; Müller et al., 1999).

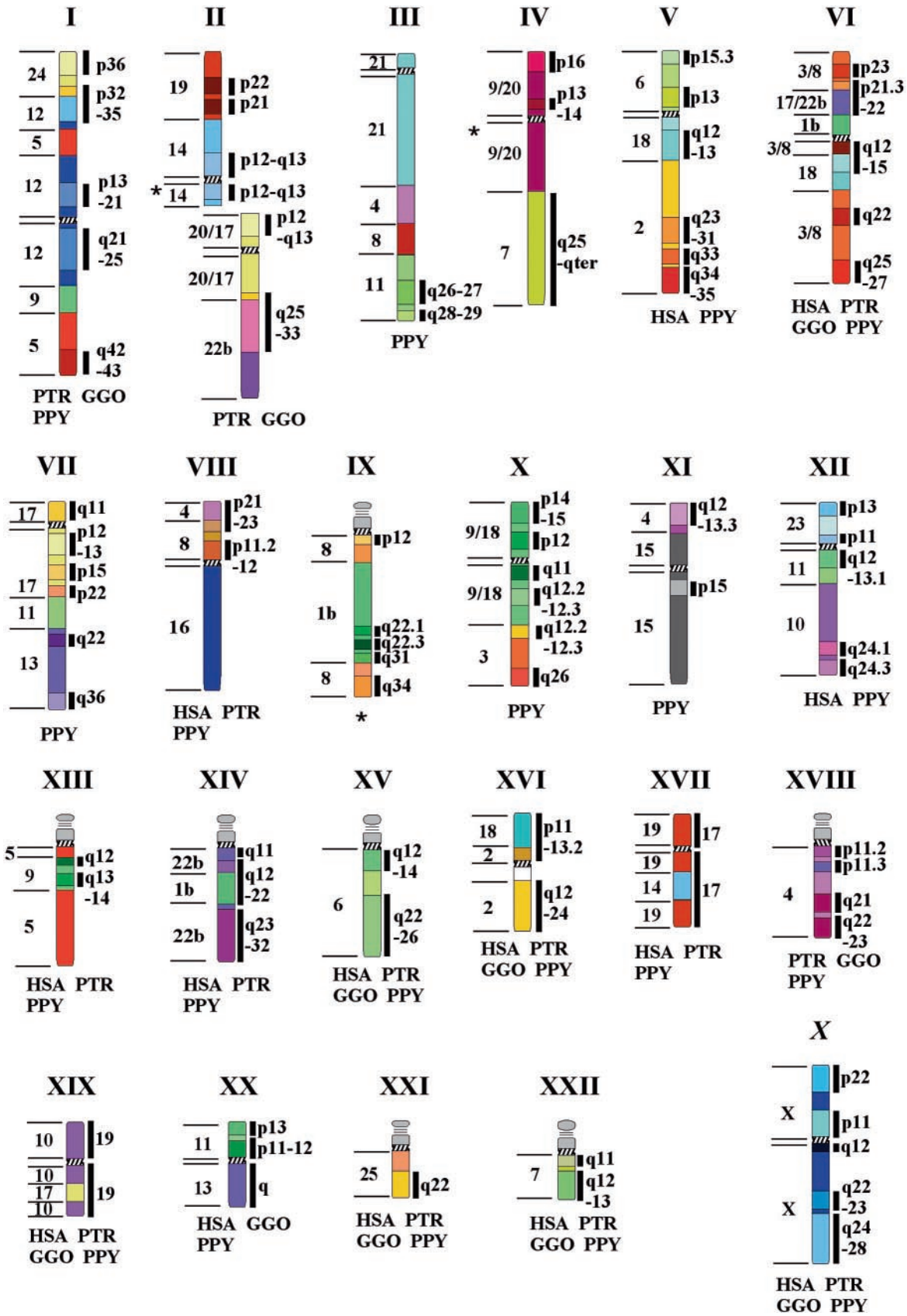
The ancestral karyotype for all hominoids

To define the ancestral karyotype for all hominoid apes chromosome painting patterns of great apes and humans can be compared with that of Papionini that serve as outgroup (Jauch et al., 1992; Wienberg et al., 1992; Müller and Wienberg, 2001 #73). The data suggest that conserved syntenies found in both higher Old World monkeys and in great apes should also be considered as ancestral for all hominoid apes while those of gibbons are derived. A common derived rearrangement of all hominoid apes is the fission of the homologs of human chromosomes 14 and 15 (see Fig. 3; Wienberg et al., 1992). The only derived changes in great apes and human karyotypes were the fusion of two chromosomes that formed human chromosome 2 and the reciprocal translocation of the homologs of human chromosomes 5 and 17 in the gorilla (Jauch et al., 1992).

Banding patterns already suggested that various inversions were the main changes in human and great ape karyotype evolution (Seuánez, 1979; Yunis and Prakash, 1982; Dutrillaux et al., 1986, for reviews). Rearrangements within chromosomes, however, naturally escape chromosome painting analysis and would need further delineation of chromosome sub-regions. A search for the ancestral karyotype of hominoid primates has been performed using chromosome painting probes obtained from the highly derived gibbon genomes and painting probes established from rearranged human/rodent somatic cell hybrids (Müller and Wienberg, 2001). Together, both probe sets yield approximately 160 molecular landmarks in multiple colors (Müller et al., 1997c, 1998) and were hybridized to human, great ape and the pigtailed macaque *(Macaca nemestrina)* serving as the outgroup for hominoid primates. The size of the probes ranged from one chromosome band to an entire chromosome arm.

Given an ancestral chromosome number of 2n = 48 for all hominoids (see above), 20 ancestral forms out of the 23 different autosomes were identified with this probe set (Müller and Wienberg, 2001). Three autosomes were not included in the final suggestion for the ancestral karyotype because of extensive rearrangements close to the centromeres (chromosomes 2p, 4, 9) where no probes were informative. From the remaining 20 autosomes for which ancestral chromosome forms can be defined, the orangutan conserved 19 while 13 are conserved in the chimpanzee, 13 by humans, and 10 by the gorilla (Fig. 6).

Fig. 6. Schematic presentation of the proposed ancestral human and great ape karyotype, based on a total of about 160 different molecular cytogenetic landmarks. Under each chromosome except for IIp, IV, and IX the species are indicated showing the proposed ancestral form. The Y chromosome is not shown since its complex rearrangements in human and apes are not discussed in this review. To the left of each chromosome homologous chromosome regions determined by probes of the Concolor gibbon are indicated, to the right segments and bands defined by "fragmented hybrids" (from Müller and Wienberg, 2001, with permission of Human Genetics).


I
24
12
5
12
12
9
5
p36
p32
-35
p13
-21
q21
-25
q42
-43
PTR GGO
PPY
II
19
14
*
14
20/17
20/17
22b
p22
p21
p12-q13
p12-q13
p12
-q13
q25
-33
PTR GGO
III
21
21
4
8
11
q26-27
q28-29
PPY
IV
9/20
*
9/20
7
p16
p13
-14
q25
-qter
V
6
18
2
p15.3
p13
q12
-13
q23
-31
q33
q34
-35
HSA PPY
VI
3/8
17/22b
1b
3/8
18
3/8
p23
p21.3
-22
q12
-15
q22
q25
-27
HSA PTR
GGO PPY
VII
17
17
11
13
q11
p12
-13
p15
p22
q22
q36
PPY
VIII
4
8
16
p21
-23
p11.2
-12
HSA PTR
PPY
IX
8
1b
8
p12
q22.1
q22.3
q31
q34
*
X
9/18
9/18
3
p14
-15
p12
q11
q12.2
-12.3
q12.2
-12.3
q26
PPY
XI
4
15
15
q12
-13.3
p15
PPY
XII
23
11
10
p13
p11
q12
-13.1
q24.1
q24.3
HSA PPY
XIII
5
9
5
q12
q13
-14
HSA PTR
PPY
XIV
22b
1b
22b
q11
q12
-22
q23
-32
HSA PTR
PPY
XV
6
q12
-14
q22
-26
HSA PTR
GGO PPY
XVI
18
2
2
p11
-13.2
q12
-24
HSA PTR
GGO PPY
XVII
19
19
14
19
17
17
HSA PTR
PPY
XVIII
4
p11.2
p11.3
q21
q22
-23
PTR GGO
PPY
XIX
10
10
17
10
19
19
HSA PTR
GGO PPY
XX
11
13
p13
p11-12
q
HSA GGO
PPY
XXI
25
q22
HSA PTR
GGO PPY
XXII
7
q11
q12
-13
HSA PTR
GGO PPY
X
X
X
p22
p11
q12
q22
-23
q24
-28
HSA PTR
GGO PPY

Thus, for more than 10 million years of human and great ape genome evolution most changes were derived rearrangements in African apes and human, while the orangutan retained a conservative karyotype (Müller and Wienberg, 2001). For few chromosomes, however, recent molecular breakpoint analysis gave more detailed information about the sequence of rearrangements (see below).

Gibbons

Early systematics divided gibbons into two distinct subgenera that would include the siamang (subgenus *Symphalangus*, one species, *Hylobates syndactylus*) and *Hylobates* (all other lesser apes). More recently it became evident from various biological traits that four distinct major divisions in lesser apes should be recognized which would include the subgenera *Bunopithecus, Hylobates, Symphalangus*, and *Nomascus* (see Roos and Geissmann, 2001, for recent review). This division is also reflected by the four different karyomorphs found in the four subgenera with representative species such as the Hoolock gibbon *(H. hoolock, Bunopithecus)* 2n = 38, the white-handed gibbon *(H. lar, Hylobates)* 2n = 44, the siamang *(H. syndactylus, Symphalangus)* 2n = 50, and the white-cheeked gibbon *(H. concolor, Nomascus)* 2n = 52.

Gibbon karyotypes do not only differ dramatically from that of other primates, but, as is suggested by their different diploid numbers, within this group highly variant chromosome sets can also be observed. Comparison of chromosome morphology between gibbon species revealed extensive differences in chromosome-banding patterns suggesting various translocations and other rearrangements between these species. Only a few chromosome homologies could be proposed on the basis of banding patterns. Thus, it became clear that gibbons experienced a dramatic change in chromosome morphology not found in other primates (Dutrillaux et al., 1975; Couturier et al., 1982; Marks, 1982; Stanyon and Chiarelli, 1982; Van Tuinen and Ledbetter, 1983). Examinations within gibbon species further revealed polymorphisms for inversions and translocations (Couturier et al., 1982; Stanyon et al., 1987; Couturier and Lernould, 1991). Since previously only a few individuals within each species had been analyzed, it was not clear whether these rearrangements are simple polymorphisms or defined karyological differences of sub-species (Couturier and Lernould, 1991). Alternatively, they could also represent karyotypes of not yet recognized species. Recently, however, more than 60 individuals of the subgenus *Hylobates* have been analyzed by chromosome banding and extensive inversion/translocation chromosome polymorphisms were described (Van Tuinen et al., 1999). There is no such extensive chromosome polymorphism known in other primates.

Plenty of chromosome painting data are now available that can contribute to elucidate the complex evolution of gibbon karyotypes. Most of the experiments used single chromosome painting probes to establish homologies with the human karyotype. When applied to entire gibbon karyotypes, all four karyomorphs were analyzed in detail with human chromosome-specific painting probes: three species of the subgenus *Hylobates (H. lar, H. agilis, H. klossii)* (Jauch et al., 1992), the white-cheeked gibbon (Koehler et al., 1995b; Schröck et al., 1996), the siamang (Koehler et al., 1995a) and more recently, the Hoolock gibbon (Yu et al., 1997; Nie et al., 2001). Some further experiments used multi-color chromosome painting with reciprocal painting, sub-regional probes, or SKY and/or M-FISH (Rens et al., 2001; Müller et al., 2002; Mrasek et al., 2003; see Fig. 1). Chromosome rearrangements, however, were still too complex to propose a firm phylogenetic interpretation of gibbon chromosome evolution using just human chromosome-specific painting probes. A phylogenetic interpretation became possible when gibbon karyotypes were compared to the orangutan instead of humans (Müller et al., 2003). Comparing gibbons with the orangutan instead of humans eliminated "noise" that stems from various additional derived chromosome rearrangements that occurred in human but not during orangutan genome evolution that may have conserved the ancestral chromosome from which gibbon karyotypes were derived.

A detailed comparison of chromosome painting data of all four gibbon karyomorphs with the karyotype of humans and the orangutan identified a suggested ancestral karyotype common to all extant gibbons. This karyotype is already highly reshuffled compared to other hominoids and would have had 2n = 66 chromosomes. The ancestral karyotype for all gibbons already differed from the putative ancestral hominoid by at least 24 rearrangements: 5 reciprocal translocations, 8 inversions, 10 fissions and 1 fusion (Müller et al., 2003). Both the visual inspection of derived and conserved rearrangements as well as a PAUP analysis resulted in the same most parsimonious phylogeny, with the branching sequence {Hoolock gibbon {white-handed gibbon {white-cheeked gibbon and siamang}}}. This phylogeny is consistent with findings based on other biological traits such as morphology and DNA sequence data with the close relationship of *Bunopithecus* and *Hylobates*, and *Symphalangus* and *Nomascus*, respectively. However, it contrasts with the position of the most basal group (Roos and Geissmann, 2001). Yet, since the Hoolock gibbon *(Bunopithecus)* does not share a single derived chromosome rearrangement with other gibbons whereas other nodes show several common derived traits, this is strong support for the basal position of *Bunopithecus* (Müller et al., 2003).

Gibbon phylogeny may be one of the examples where species diverged over very short evolutionary time in which chromosome rearrangements occurred at high frequencies. At the same short evolutionary time the divergence of DNA sequences and other biological traits may have been not as well informative to draw conclusions about their phylogeny. Thus, molecular cytogenetics could be an important tool to elucidate phylogenies also in other groups of species where speciation occurred over a short time not leaving too much information in DNA sequence divergence, but leaving considerable landmark chromosome changes for phylogenetic analysis.

Great apes and human

Recently, most molecular traits place the chimpanzee as the closest relative to human. Some more recent publications would even include the chimpanzee into *Homo* dividing this clade into the subgenera *H. (Homo)* for humans and *H. (Pan)* for chimpanzees and bonobos (Wildman et al., 2003). The phy-

logeny of human and great apes, however, has been a matter of controversial discussions for many years. The discussion focused on whether chimpanzees and gorilla had a common origin after the divergence from the phylogeny leading to humans or whether chimpanzees and humans would have shared a common ancestry after the divergence from the line leading to the gorilla. Cytogenetic approaches were of limited help to settle these disputes since they produced controversial results (Stanyon and Chiarelli, 1982; Marks, 1992).

Strong cytogenetic evidence for a link of gorilla and chimpanzees came from the distribution of heterochromatin in human and great apes where chimpanzees and gorilla share patterns on the Y chromosome and subtelomeric bands (Miller, 1977). Both, the human and the orangutan karyotype lack visible bands at telomeres after C-banding and after staining with certain fluorochromes such as DAPI. Thus, large subtelomeric heterochromatin bands were suggested as a common derived trait of both chimpanzee and gorilla. Subtelomeric heterochromatin bands, however, have also been observed in two of the four gibbon subgenera, as well as in some guenons, while others do not show this trait. Thus, subtelomeric heterochromatin bands should be considered as a highly variable character prone to convergence.

Some inversions that occurred during human and great ape evolution appeared to be informative as landmark changes that would help to define the correct sequence of species divergence (Stanyon and Chiarelli, 1982; Yunis and Prakash, 1982; Dutrillaux et al., 1986; Marks, 1992). However, interpretations derived from banding again were not conclusive. Mainly four inversions have been discussed as landmarks in human and great ape evolution which include those on human chromosomes 2, 7, 9, and 12, and their primate homologs. For example, high-resolution chromosome banding suggests a derived pericentric inversion in the homolog of human chromosome 2p in a common ancestor of human and chimpanzee. This inversion was then followed by the fusion event in the human karyotype. An alternative interpretation that would have linked the chimpanzee and gorilla after divergence from the lineage leading to humans was suggested from the banding pattern of the human chromosome 12 homologs. Both chimpanzee and gorilla share a pericentric inversion that, on the cytogenetic level, would have had included identical breakpoints. The chromosome 2p, 7 and 12 rearrangements and other human and great ape homologs have recently been subject to molecular-genetic and -cytogenetic analyses and some breakpoints have been characterized in detail.

Chromosome 2: No other chromosomes have been analyzed in such detail as the homologs that led to human chromosome 2. Banding analysis suggested a telomere fusion of two closely acrocentric chromosomes and the inactivation of one of the centromeres (Dutrillaux, 1979; Yunis and Prakash, 1982). In fact, molecular studies identified telomeric sequences in the band 2q13 by in situ hybridization of DNA probes containing inverted arrays of the vertebrate telomeric repeat in a head to head arrangement (Ijdo et al., 1991). This tandem repeat (5′-$(TTAGGG)_n$-$(CCCTAA)_m$-3′) can now be found on the human DNA sequence within the BAC clone RP11–395L14 of the human genome BAC tile path that contains 789 bp of degenerate telomere repeats organized in two head-to-head arrays (Fan et al., 2002a). Close to the assumed fusion point (2q21) a relict of an alphoid domain was found by in situ hybridization of a satellite DNA under low hybridization stringency (Baldini et al., 1991; Avarello et al., 1992). This alphoid domain indicates the presence of the ancestral centromere of the 2q homolog resulting in a single stable chromosome.

A painting probe derived from microdissection of the long arm of human chromosome 2 was used to analyze the sequence of the pericentric inversion in human, great apes and some Old World monkeys *(M. fuscata* and *C. aethiops)* serving as outgroup (Wienberg et al., 1994). The hybridization patterns obtained verified multiple rearrangements in the pericentric region of the human 2p homolog. Old World monkeys had a different pattern from that observed in the gorilla and the orangutan, but a pattern similar to that of chimpanzees. This result suggests convergence of chromosomal rearrangements in different phylogenetic lines. Thus, without detailed breakpoint analysis the chromosome 2 inversion would not contribute to the landmarks in human and great ape chromosome evolution.

The pericentric inversion was also verified using cosmid clones that mapped close to the assumed fusion point and originated from a segmental duplication of the V kappa gene cluster on human chromosome 2p12→p11 (Arnold et al., 1995). Yeast artificial chromosomes (YACs) that map approximately one every 5–10 cM evenly spaced over the human chromosome 2 were hybridized to human and great ape homologs to identify further rearrangements (Haaf and Bray-Ward, 1996). Except for the pericentromeric inversions no further rearrangements were observed. Further, a series of 38 cosmids derived from 2q12→q14 were hybridized to human and chimpanzee chromosomes (Kasai et al., 2000). The relative order of signals was the same on human chromosome 2 and the short arms of chimpanzee chromosomes 12 and 13 indicating that both chromosomes underwent no gross alteration in structure.

Recently, a genome-wide high-resolution scanning method called HAPPY mapping (Dear and Cook, 1989) was used to analyze the fine mapping of markers along human chromosome 2 and great ape homologs (de Pontbriand et al., 2002). The HAPPY mapping method is an acellular adaptation of the radiation hybrid (RH) method with even higher resolution. The authors confirmed the above discussed pericentric inversion. Another rearrangement observed was a paracentric inversion in the gorilla not yet identified with other methods. The simplicity of this method, however, promises that other human chromosomes and their primate homologs can now be scanned for aberrations with high resolution.

The suggested fusion region was recently subject of a more detailed molecular analysis. As for other subtelomeric regions in the human genome, the ancestral telomeric regions which are now interstitially located in 2q13→q14.1 had been extensively reshuffled by segmental duplications or low copy repeats (see Eichler, 2001; Mefford and Trask, 2002; Samonte and Eichler, 2002; Eichler and Sankoff, 2003, for recent reviews), with orthologous segments of 96–99% sequence identity mainly on chromosomes 9 and 22. About 360 kb of the region surrounding the fusion site was analyzed in more detail (Fan et al.,

2002a) and include segments that are duplicated to subtelomeric and pericentromeric locations at least once elsewhere in the genome. While the orthologous segments from the proximal region of the fusion point are mostly homologous to chromosome 9 pericentromeric and to 9p subtelomeric sites, the ancestral subtelomeric region at the distal side of the fusion site shows orthologous segments in the subtelomeric region on chromosome 22q. Sequence analysis of the duplicated segments revealed that the timing of some segmental duplications should have predated hominid divergence while others are of more recent origin still predating the fusion event, or were specific for humans and dated after the divergence from the chimpanzee. This was confirmed by searching for common derived insertions of repetitive elements and by the distribution of FISH signals in human and great apes. Interestingly, several genes have been duplicated during these events, nine of them are present in the human genome in more than one copy (Fan et al., 2002b). Since the duplications occurred at various times during hominoid evolution before the fusion event of the two ancestral chromosomes was fixed, they may have been the cause rather than the result of the chromosome rearrangement. Thus, today human chromosome 2 may have originated from non-homologous recombination between two chromosomes at a duplicated segment.

Chromosome 7: Human chromosome 7 homologs appear to contribute to landmarks at various nodes in primate evolution. These not only include gross morphological changes such as found after the divergence of prosimians and New World monkeys, and New World and Old World monkeys, respectively (see Fig. 3), but also changes within the chromosome as observed in human and great ape evolution. Banding analysis in human chromosome 7 and great ape homologs suggested that two inversions may have changed an ancestral chromosome found in the orangutan: A pericentric inversion with breakpoints at 7p22 and 7q11.23→q21would have created the chromosome form found in the karyotype of the gorilla. This chromosome was further changed by a paracentric inversion with breakpoints at 7q11.23→q21 and 7q22 that is shared by human and chimpanzees (Yunis and Prakash, 1982; Dutrillaux et al., 1986; Marks, 1992). Thus, these rearrangements would represent landmarks for human and African ape evolution that would link the phylogeny of human and chimpanzee as well as that of a last common ancestor of all African great apes and human to the exclusion of the orangutan. From the banding pattern it could not be determined whether the 7q11.23→q21 breakpoint was reused during great ape evolution.

A more detailed analysis of the breakpoints of these rearrangements was recently performed in a comparative analysis of the Williams-Beuren syndrome (WS) region. In humans this syndrome results from deletions of large segments (1.5–2 Mbp) in chromosome 7q11.23 close to one of the breakpoint regions involved in both the pericentric as well as the paracentric inversion (DeSilva et al., 1999). The human WS region revealed high frequencies of segmental duplications containing low copy repeats such as p47-*phox* sequences. Copies of this sequence are also found in the breakpoints of the other inversion regions involved in the evolution of this chromosome. The authors suggest a scenario in which a p47-*phox*-containing segment first duplicated locally in a common ancestor of hominoids and then some copies were distributed to three locations on chromosome 7 by the inversion events. Alternatively, as for the human chromosome 2 fusion the duplications may have been the cause rather than the result of these inversions (Müller et al., 2004).

Chromosome 9: According to banding interpretations the human chromosome 9 homolog would be another informative chromosome for human and great ape evolution (Yunis and Prakash, 1982; Dutrillaux et al., 1986; Marks, 1992). Both the orangutan and the gorilla would have shared the ancestral form from which the human homolog could be derived by a pericentric inversion. After divergence of human and chimpanzees this chromosome was further changed by another pericentric inversion in the chimpanzee.

The sequence of rearrangements proposed by banding was confirmed by a panel of YACs that were placed about every 10 Mbp along the human chromosome 9 and were hybridized to human and great ape chromosomes (Haaf and Bray-Ward, 1996; Montefalcone et al., 1999). No other rearrangements were found except for those predicted by chromosome banding. One of the breakpoints that would distinguish human and chimpanzee chromosomes was identified using a YAC contig and was narrowed down to a single breakpoint-spanning clone (Nickerson and Nelson, 1998).

Chromosome 12: The final chromosome that on the basis of banding has been considered to be informative for human and great ape phylogeny is human chromosome 12 (Yunis and Prakash, 1982; Dutrillaux et al., 1986; Marks, 1992). Since breakpoints of the inversion are close to the centromere, chromosome bar coding with molecular probes was not sufficient in resolution to determine whether the rearrangements in both great apes would share the same breakpoints (Müller and Wienberg, 2001).

YAC mapping with contigs narrowed down the breakpoints and indeed showed that the breakpoints on 12p12 were covered by a different YAC in both chimpanzee and gorilla indicating that breakpoints should differ by at least one Mbp (Nickerson and Nelson, 1998). Thus, the inversion in chimpanzees and gorilla definitively had an independent origin, which suggests two apomorphic rearrangements in both taxa not informative for hominid phylogeny. Recently, a preliminary first-generation genomic comparative sequence map between human and chimpanzee was published (Fujiyama et al., 2002). The map was constructed through paired alignment of more than 70,000 chimpanzee bacterial artificial chromosome end sequences with human genome sequences. With the first description of this library, the authors already described clones that would cover breakpoints on the inversion on the human chromosome 12 homolog in the chimpanzee. Thus with progress of the "chimpanzee genome project" we can expect that many more gross differences in chromosome structure will be elucidated within the near future.

Other chromosomes: Several other human chromosomes and their primate homologs have recently been analyzed by molecular probes, delineating evolutionarily derived chromosome breakpoints. Most interestingly, many of the rearrangement breakpoints contain segmental duplications with se-

quences also found within and/or between other chromosomes and obviously have been dispersed during hominoid or late Old World monkey evolution (Bailey et al., 2002; Mefford and Trask, 2002; Eichler and Sankoff, 2003, for recent reviews).

As already discussed, the human chromosome 3 homolog was involved in an association with chromosome 21 until its fission after the origin of higher primates (see Richard and Dutrillaux, 1998, for review). Except for human chromosome 1 the association of human chromosome 3 and 21 homologs may be the largest widely conserved syntenic block known for mammals (Murphy et al., 2003). The human chromosome 3 homolog has conserved its synteny but experienced massive intrachromosomal rearrangements during placental mammalian genome evolution. Recent comparative gene mapping studies in pig showed conserved synteny of human chromosome 3, but reorganization within the chromosome. More than ten large conserved segments were described that should have resulted from extensive intra-chromosomal rearrangements (Goureau et al., 2001).

Molecular cytogenetic analysis of intra-chromosomal rearrangements of the syntenic block 3/21 was performed with probes derived from chromosomal sub-regions with a resolution of nearly 10–15 Mbp (Müller et al., 2000). The ancestral segment order in both primates and carnivores is still found in some species of both the primate and carnivore orders. From the ancestral primate/carnivore condition at least one inversion is needed to derive the pig homolog. A fission of chromosome 21 and a pericentric inversion has to be predicted to derive the Bornean orangutan condition that may represent the ancestral form for all higher Old World primates. Two overlapping inversions in the chromosome 3 homolog would then lead to the chromosome form found in humans and African apes. From the ancestral form for Old World primates (now found in the Bornean orangutan) a pericentric inversion and centromere shift leads to the chromosome ancestral for all Old World monkeys (Müller et al., 2000). A more recent and more detailed analysis of the breakpoint regions would place an intermediate form (probably the macaque) as the ancestral chromosome for all higher Old World primates (Tsend-Ayush et al., 2004). At the time of writing this article breakpoint sequences of the different rearrangements have not yet been described in detail. However, various breakpoints in chromosome evolution can now be inferred "in silico" from the human and mouse genome sequence map. An association of sequences homologous to human chromosomes 3 and 21 can be found in the mouse genome sequence on chromosome 16. Thus, the fission point of these two chromosomes should be represented by this association and can be identified at 76.68 Mb in the mouse sequence map (http://www.ensembl.org/Mus_musculus/cytoview, Build 32).

Banding pattern analysis of the human chromosome 4 homolog identified several pericentric inversions (Dutrillaux et al., 1986; Yunis and Prakash, 1982) that were confirmed by molecular probes (Ryan et al., 1991). Recently, probes of different complexity identified species-specific small pericentric inversions with breakpoints close to the centromeres for all great apes as well as for the macaque. Thus, no ancestral chromosome form could be postulated yet. Interestingly, some of these probes were spanning one breakpoint in different inversions indicating recurrent breakage close to or maybe even at the same site (Marzella et al., 2000).

The only reciprocal translocation in human and great apes is the already mentioned apomorphic rearrangement of human chromosome 5 and 17 homologs in the gorilla (Jauch et al., 1992; Stanyon et al., 1992). Banding suggested the breakpoints at human chromosomes 5q13.3 and 17p12 → p11.2 (Yunis and Prakash, 1982). Recently, Stankiewicz et al. (2001) analyzed this translocation in more detail and identified various BAC clones that span the translocation breakpoints. One breakpoint occurred in the region homologous to human chromosome 5q13.3, between the HMG-CoA reductase gene (HMGCR) and RAS p21 protein activator 1 gene (RASA1). The second breakpoint was in a region homologous to human chromosome 17p12. This breakpoint was associated with a submicroscopic chromosome duplication involving a chromosome fragment homologous to the human chromosome region surrounding the proximal 24-kb region specific of low-copy repeats Charcot-Marie-Tooth disease type 1A REP segment (CMT1A-REP). The authors suggest that as for many human diseases, low copy repeats that have been distributed throughout the genome may have also mediated these chromosome rearrangements by non-homologous recombination.

The human chromosome 6 homolog has conserved its subregional organization throughout various mammalian taxa but experienced various shifts of the centromere (Eder et al., 2003). The emergence of new centromeres has been previously identified as an important change in primate karyotype evolution. For several chromosomes molecular probes as well as chromosome banding patterns did not reveal any gross changes in chromosome morphology except for changes in the position of centromeres (Montefalcone et al., 1999; Müller et al., 2000; Cardone et al., 2002; Ventura et al., 2003). In primates the position of the centromere of the human chromosome 6 homolog was found to be located in three distinct regions, without any evidence for chromosomal rearrangement that would account for its movement. In a common ancestor of great apes the centromere moved from 6p22.1 to the present day location. On human chromosome 6 a cluster of intrachromosomal segmental duplications is located at 6p22.1 and scattered in a region of about 9 Mb, which may represent remains of duplicons that flanked the ancestral centromere (Eder et al., 2003).

The ancestral chromosome for the human chromosome 10 homolog is found in the orangutan as well as in the macaque serving as outgroup (Müller and Wienberg, 2001). Homologs to 10p and 10q were already distinct chromosome segments in the primate ancestor (Cardone et al., 2002). Human and African apes share a derived inversion from which the homologous chromosome found in the gorilla experienced another inversion. Again, there are large sequence duplications flanking the centromeric satellites. FISH analysis indicates that these loci were duplicated before the divergence of orangutan from other apes and that a cytogenetically cryptic pericentric inversion may have been involved in the formation of the flanking duplications (Jackson et al., 1999).

The human chromosomes 14 and 15 emerged after the divergence of Old World monkeys and hominoids (see Fig. 3).

The fission point of the homolog that gave rise to human chromosomes 15 and 14 was recently analyzed by a radiation hybrid panel derived from a macaque (Murphy et al., 2001) and by FISH with a panel of human BAC clones (Ventura et al., 2003). The ancestral centromere as found in Old World primates was silenced and two new centromeres evolved on the "new" chromosome 14 and 15 homologs in hominoids. The site of the ancestral centromere is found in a region homologous to human chromosomal 15q24→q26, which is also known as a hot spot for neocentromere formation in human genetic pathology (Amor and Choo, 2002). This region is also well known for numerous segmental duplications (Bailey et al., 2001; Pujana et al., 2001). Interestingly, two neocentromeres identified in the karyotype of patients with genetic disorders were mapped not directly at the ancestral centromeric region but some few Mbp proximally and distally. They were, however, located within 500 kb of duplicons, copies of which flank the centromere in Old World monkeys. These results suggest that there may be a correlation between the origin of neocentromeres and ancestral centromere positions that may have been mediated through multiple segmental duplications. Human chromosome 15 also differs in a pericentric inversion from the homolog in the chimpanzee that should have occurred in chimpanzees after the divergence from humans (Yunis and Prakash, 1982; Dutrillaux et al., 1986). A breakpoint region in human 15q11→q13 of this rearrangement was analyzed in more detail and again was assigned to a region consisting of entirely duplicated segments in a 600-kb interval of the homologous human sequences (Locke et al., 2003).

The human chromosome 16 homologous p and q arms were on separate chromosomes in the ancestor of all primates (see Fig. 3). Various BACs have been used for FISH in different primate taxa indicating that gross chromosome morphology was conserved (Misceo et al., 2003). More detailed analyses for low copy repeats, however, demonstrate that this chromosome harbors various highly duplicated regions that should have emerged rather recently in human and African ape evolution (Eichler et al., 2001).

Human chromosome 17 differs from the chimpanzee homolog by a pericentric inversion with breakpoints assumed at 17p13 and 17q21.3. The breakpoints of this rearrangement have been analyzed in detail using a protocol that could be a model for further identification and analysis of breakpoint-spanning regions on other human and primate chromosomes (Kehrer-Sawatzki et al., 2002). BACs from the tile path of the human genome analysis were used to identify the breakpoint-spanning clone on the chimpanzee homolog by FISH. Two clones were identified that gave split signals at the predicted chromosome bands in the chimpanzee. From the chimpanzee BAC library, clones were then identified that span the homologous human "breakpoints". The breakpoints were further narrowed down with PCR products from these clones and by detailed DNA sequencing. The breakpoints, however, were finally identified in regions without any obvious segmental duplications but rich in repetitive elements that may have mediated the chromosome rearrangement.

FISH with various probes of different complexity confirm that human chromosome 18 differs from its homologs in great apes by a pericentric inversion (McConkey, 1997; Müller and Wienberg, 2001). Thus, the human chromosome is the derived rearrangement. The macaque shows further inversions or a centromere dislocation. Interspecies sequence comparisons indicate that the ancestral break occurred between the genes ROCK1 and USP14 (Dennehey et al., 2004). In humans, the inversion places ROCK1 near the centromeric heterochromatin and USP14 adjacent to highly repetitive subtelomeric repeats. Again, segmental duplications may have been involved in the formation of these rearrangements.

Conclusions

The present review should demonstrate that molecular cytogenetics combined with recently emerging tools from various genome projects have become a worthy tool to elucidate human genome evolution. For many nodes in primate phylogeny landmark chromosome rearrangements have been identified that would discriminate ancestral versus derived chromosome changes and can help to elucidate species phylogenies. Chromosome breakpoints are now being traced down to the DNA sequence level and analyzed by "in silico" techniques. Thus, chromosome analysis combined with molecular approaches should be considered a highly reproducible tool for cytotaxonomy in primates. Since chromosome rearrangements are rare events and not likely prone to convergence, their analyses will provide an attractive additional tool for a phylogenetic analysis where other techniques may fail. This should be especially helpful in species where speciation may have occurred over a very short evolutionary time. In those species DNA sequence divergence may not be informative enough for the discrimination of phylogenies.

Recent breakpoint analyses, however, also provide some insights into the molecular changes in evolutionarily derived chromosome rearrangements. Up to now, hardly any evidence for dramatic changes of genes at the sites of breakpoints such as new "fusion genes" has been found as known from chromosome rearrangements in cancer cells. Most breakpoint regions lie in gene-poor areas rich in repetitive elements or low copy repeats. Thus, previous assumptions that suggested drastic "position effects" in genes in rearranged chromosomes that may trigger large morphological differences and evolution in primate species were not verified.

Current efforts to establish BAC resources and to sequence the genome of other primates and non-primate mammals will be of fundamental impact on the understanding of human genome evolution (Eichler and DeJong, 2002). Recently BAC libraries have become available for representatives of all major primate taxa that could be used within and between these species to extract any region of homology to the human genome (see http://bacpac.chori.org/libraries.php). New techniques such as "array painting" (Gribble et al., 2004) and the application of "HAPPY mapping" to primate genome evolution (de Pontbriand et al., 2002) will allow us to pin down any rearrangement to a restricted segment of the genome or to a single defined cloned DNA sequence within a few experiments. For example, recent hybridization experiments using gibbon chro-

mosome-specific probes on human BAC arrays allowed identification of various breakpoint-spanning clones for further detailed analysis (Gribble et al., 2004). The use of these new technologies provided by the human genome project will further facilitate the understanding of primate genome organization and evolution.

Acknowledgement

I would very much like to thank S. Müller, M. Neusser and A. Kofler for their kind support and helpful comments.

References

Alexandrov I, Kazakov A, Tumeneva I, Shepelev V, Yurov Y: Alpha-satellite DNA of primates: old and new families. Chromosoma 110:253–266 (2001).

Amor DJ, Choo KH: Neocentromeres: role in human disease, evolution, and centromere study. Am J Hum Genet 71:695–714 (2002).

Antonacci R, Marzella R, Finelli P, Lonoce A, Forabosco A, Archidiacono N, Rocchi M: A panel of subchromosomal painting libraries representing over 300 regions of the human genome. Cytogenet Cell Genet 68:25–32 (1995).

Apiou F, Rumpler Y, Warter S, Vezuli A, Dutrillaux B: Demonstration of homoeologies between human and lemur chromosomes by chromosome painting. Cytogenet Cell Genet 72:50–52 (1996).

Arnold N, Wienberg J, Ermert K, Zachau HG: Comparative mapping of DNA probes derived from the V kappa immunoglobulin gene regions on human and great ape chromosomes by fluorescence in situ hybridization. Genomics 26:147–150 (1995).

Arnold N, Stanyon R, Jauch A, O'Brien P, Wienberg J: Identification of complex chromosome rearrangements in the gibbon by fluorescent in situ hybridization (FISH) of a human chromosome 2q specific microlibrary, yeast artificial chromosomes, and reciprocal chromosome painting. Cytogenet Cell Genet 74:80–85 (1996).

Avarello R, Pedicini A, Caiulo A, Zuffardi O, Fraccaro M: Evidence for an ancestral alphoid domain on the long arm of human chromosome 2. Hum Genet 89:247–249 (1992).

Bailey JA, Yavor AM, Massa HF, Trask BJ, Eichler EE: Segmental duplications: organization and impact within the current human genome project assembly. Genome Res 11:1005–1017 (2001).

Bailey JA, Gu Z, Clark RA, Reinert K, Samonte RV, Schwartz S, Adams MD, Myers EW, Li PW, Eichler EE: Recent segmental duplications in the human genome. Science 297:1003–1007 (2002).

Baldini A, Miller DA, Shridhar V, Rocchi M, Miller OJ, Ward DC: Comparative mapping of a gorilla-derived alpha satellite DNA clone on great ape and human chromosomes. Chromosoma 101:109–114 (1991).

Band MR, Larson JH, Rebeiz M, Green CA, Heyen DW, Donovan J, Windish R, Steining C, Mahyuddin P, Womack JE, Lewin HA: An ordered comparative map of the cattle and human genomes. Genome Res 10:1359–1368 (2000).

Barros RM, Nagamachi CY, Pieczarka JC, Rodrigues LR, Neusser M, de Oliveira EH, Wienberg J, Muniz JA, Rissino JD, Muller S: Chromosomal studies in *Callicebus donacophilus pallescens*, with classic and molecular cytogenetic approaches: multicolour FISH using human and *Saguinus oedipus* painting probes. Chromosome Res 11:327–334 (2003).

Best RG, Diamond D, Crawford E, Grass FS, Janish C, Lear TL, Soenksen D, Szalay AA, Moore CM: Baboon/human homologies examined by spectral karyotyping (SKY): a visual comparison. Cytogenet Cell Genet 82:83–87 (1998).

Bigoni F, Koehler U, Stanyon R, Ishida T, Wienberg J: Fluorescence in situ hybridization establishes homology between human and silvered leaf monkey chromosomes, reveals reciprocal translocations between chromosomes homologous to human Y/5, 1/9, and 6/16, and delineates an X1X2Y1Y2/X1X1X2X2 sex-chromosome system. Am J Phys Anthropol 102:315–327 (1997a).

Bigoni F, Stanyon R, Koehler U, Morescalchi AM, Wienberg J: Mapping homology between human and black and white colobine monkey chromosomes by fluorescent in situ hybridization. Am J Primatol 42:289–298 (1997b).

Bigoni F, Stanyon R, Wimmer R, Schempp W: Chromosome painting shows that the proboscis monkey *(Nasalis larvatus)* has a derived karyotype and is phylogenetically nested within Asian Colobines. Am J Primatol 60:85–93 (2003).

Bigoni F, Houck ML, Ryder OA, Wienberg J, Stanyon R: Chromosome painting shows that *Pygathrix nemaeus* has the most basal karyotype among Asian Colobinae. Int J Primatol 25:679–688 (2004).

Canavez F, Alves G, Fanning TG, Seuánez HN: Comparative karyology and evolution of the Amazonian *Callithrix* (Platyrrhini, Primates). Chromosoma 104:348–357 (1996).

Cardone MF, Ventura M, Tempesta S, Rocchi M, Archidiacono N: Analysis of chromosome conservation in *Lemur catta* studied by chromosome paints and BAC/PAC probes. Chromosoma 111: 348–356 (2002).

Cheung VG, Nowak N, Jang W, Kirsch IR, Zhao S, Chen XN, Furey TS, Kim UJ, et al: Integration of cytogenetic landmarks into the draft sequence of the human genome. Nature 409:953–958 (2001).

Clemente IC, Ponsa M, Garcia M, Egozcue J: Evolution of the Simiiformes and the phylogeny of human chromosomes. Hum Genet 84:493–506 (1990).

Consigliere S, Stanyon R, Koehler U, Agoramoorthy G, Wienberg J: Chromosome painting defines genomic rearrangements between red howler monkey subspecies. Chromosome Res 4:264–270 (1996).

Consigliere S, Stanyon R, Koehler U, Arnold N, Wienberg J: In situ hybridization (FISH) maps chromosomal homologies between *Alouatta belzebul* (Platyrrhini, Cebidae) and other primates and reveals extensive interchromosomal rearrangements between howler monkey genomes. Am J Primatol 46:119–133 (1998).

Couturier J, Lernould JM: Karyotypic study of four gibbon forms provisionally considered as subspecies of *Hylobates (Nomascus) concolor* (Primates, Hylobatidae). Folia Primatol 56:95–104 (1991).

Couturier J, Dutrillaux B, Turleau C, de Grouchy J: [Comparative karyotyping of our gibbon species or subspecies (author's transl)]. Ann Genet 25:5–10 (1982).

de Boer LEM: Cytotaxonomy of the Lorisoidea (Primates; Prosimii). I. Chromosome studies and karyological relationships in the Galagidae. Genetica 44:155–193 (1973).

de Oliveira EH, Neusser M, Figueiredo WB, Nagamachi C, Pieczarka JC, Sbalqueiro IJ, Wienberg J, Müller S: The phylogeny of howler monkeys (*Alouatta*, Platyrrhini): reconstruction by multicolor cross-species chromosome painting. Chromosome Res 10:669–683 (2002).

de Pontbriand A, Wang XP, Cavaloc Y, Mattei MG, Galibert F: Synteny comparison between apes and human using fine-mapping of the genome. Genomics 80:395–401 (2002).

Dear PH, Cook PR: Happy mapping: a proposal for linkage mapping the human genome. Nucleic Acids Res 17:6795–6807 (1989).

Dennehey BK, Gutches DG, McConkey EH, Krauter KS: Inversion, duplication, and changes in gene context are associated with human chromosome 18 evolution. Genomics 83:493–501 (2004).

DeSilva U, Massa H, Trask BJ, Green ED: Comparative mapping of the region of human chromosome 7 deleted in Williams syndrome. Genome Res 9: 428–436 (1999).

Dutrillaux B: Chromosomal evolution in primates: tentative phylogeny from *Microcebus murinus* (prosimian) to man. Hum Genet 48:251–314 (1979).

Dutrillaux B, Rumpler Y: Absence of chromosomal similarities between tarsiers *(Tarsius syrichta)* and other primates. Folia Primatol 50:130–133 (1988).

Dutrillaux B, Rethore MO, Aurias A, Goustard M: [Karyotype analysis of 2 species of gibbons *(Hylobates lar* and *H. concolor)* with different banding species]. Cytogenet Cell Genet 15:81–91 (1975).

Dutrillaux B, Couturier J, Warter S, Rumpler Y: Chromosomal evolution in "lemurs". VI. Chromosomal banding studies of *Galago senegalensis, Galago alleni, Galago demidovii* and *Euoticus elegantulus*. Folia Primatol 37:280–296 (1982).

Dutrillaux B, Couturier J, Sabatier L, Muleris M, Prieur M: Inversions in evolution of man and closely related species. Ann Genet 29:195–202 (1986).

Eder V, Ventura M, Ianigro M, Teti M, Rocchi M, Archidiacono N: Chromosome 6 phylogeny in primates and centromere repositioning. Mol Biol Evol 20:1506–1512 (2003).

Eichler EE: Recent duplication, domain accretion and the dynamic mutation of the human genome. Trends Genet 17:661–669 (2001).

Eichler EE, DeJong PJ: Biomedical applications and studies of molecular evolution: a proposal for a primate genomic library resource. Genome Res 12: 673–678 (2002).

Eichler EE, Sankoff D: Structural dynamics of eukaryotic chromosome evolution. Science 301:793–797 (2003).

Eichler EE, Johnson ME, Alkan C, Tuzun E, Sahinalp C, Misceo D, Archidiacono N, Rocchi M: Divergent origins and concerted expansion of two segmental duplications on chromosome 16. J Hered 92:462–468 (2001).

Fan Y, Linardopoulou E, Friedman C, Williams E, Trask BJ: Genomic structure and evolution of the ancestral chromosome fusion site in 2q13-2q14.1 and paralogous regions on other human chromosomes. Genome Res 12:1651–1662 (2002a).

Fan Y, Newman T, Linardopoulou E, Trask BJ: Gene content and function of the ancestral chromosome fusion site in human chromosome 2q13-2q14.1 and paralogous regions. Genome Res 12:1663–1672 (2002b).

Ferguson-Smith MA: Genetic analysis by chromosome sorting and painting: phylogenetic and diagnostic applications. Eur J Hum Genet 5:253–265 (1997).

Finelli P, Stanyon R, Plesker R, Ferguson-Smith MA, O'Brien PC, Wienberg J: Reciprocal chromosome painting shows that the great difference in diploid number between human and African green monkey is mostly due to non-Robertsonian fissions. Mamm Genome 10:713–718 (1999).

Fleagle JG: Primate Adaption and Evolution (Academic Press, San Diego 1988).

Ford SM: Systematics of the New World Monkeys (Alan R Liss, New York 1986).

Froenicke L, Wienberg J, Stone G, Adams L, Stanyon R: Towards the delineation of the ancestral eutherian genome organization: comparative genome maps of human and the African elephant (*Loxodonta africana*) generated by chromosome painting. Proc R Soc Lond B Biol Sci 270:1331–1340 (2003).

Fujiyama A, Watanabe H, Toyoda A, Taylor TD, Itoh T, Tsai SF, Park HS, Yaspo ML, Lehrach H, Chen Z, Fu G, Saitou N, Osoegawa K, de Jong PJ, Suto Y, Hattori M, Sakaki Y: Construction and analysis of a human-chimpanzee comparative clone map. Science 295:131–134 (2002).

Garcia F, Nogues C, Ponsa M, Ruiz-Herrera A, Egozcue J, Garcia Caldes M: Chromosomal homologies between humans and *Cebus apella* (Primates) revealed by ZOO-FISH. Mamm Genome 11:399–401 (2000).

Garcia F, Ruiz-Herrera A, Egozcue J, Ponsa M, Garcia M: Chromosomal homologies between *Cebus* and *Ateles* (primates) based on ZOO-FISH and G-banding comparisons. Am J Primatol 57:177–188 (2002).

Goureau A, Garrigues A, Tosser-Klopp G, Lahbib-Mansais Y, Chardon P, Yerle M: Conserved synteny and gene order difference between human chromosome 12 and pig chromosome 5. Cytogenet Cell Genet 94:49–54 (2001).

Gray JW, Dean PN, Fuscoe JC, Peters DC, Trask BJ, van den Engh GJ, Van Dilla MA: High-speed chromosome sorting. Science 238:323–329 (1987).

Gribble SM, Fiegler H, Burford DC, Prigmore E, Yang F, Carr P, Ng BL, Sun T, Kamberov ES, Makarov VL, Langmore JP, Carter NP: Applications of combined DNA microarray and chromosome sorting technologies. Chromosome Res 12:35–43 (2004).

Haaf T, Bray-Ward P: Region-specific YAC banding and painting probes for comparative genome mapping: implications for the evolution of human chromosome 2. Chromosoma 104:537–544 (1996).

Haig D: A brief history of human autosomes. Philos Trans R Soc Lond B Biol Sci 354:1447–1470 (1999).

Hawken RJ, Murtaugh J, Flickinger GH, Yerle M, Robic A, Milan D, Gellin J, Beattie CW, Schook LB, Alexander LJ: A first-generation porcine whole-genome radiation hybrid map. Mamm Genome 10:824–830 (1999).

Hugot JP: Phylogeny of neotropical monkeys: the interplay of morphological, molecular, and parasitological data. Mol Phylogenet Evol 9:408–413 (1998).

Ijdo JW, Baldini A, Ward DC, Reeders ST, Wells RA: Origin of human chromosome 2: an ancestral telomere-telomere fusion. Proc Natl Acad Sci USA 88:9051–9055 (1991).

Jackson MS, Rocchi M, Thompson G, Hearn T, Crosier M, Guy J, Kirk D, Mulligan L, Ricco A, Piccininni S, Marzella R, Viggiano L, Archidiacono N: Sequences flanking the centromere of human chromosome 10 are a complex patchwork of arm-specific sequences, stable duplications and unstable sequences with homologies to telomeric and other centromeric locations. Hum Mol Genet 8:205–215 (1999).

Jauch A, Wienberg J, Stanyon R, Arnold N, Tofanelli S, Ishida T, Cremer T: Reconstruction of genomic rearrangements in great apes and gibbons by chromosome painting. Proc Natl Acad Sci USA 89:8611–8615 (1992).

Kasai F, Takahashi E, Koyama K, Terao K, Suto Y, Tokunaga K, Nakamura Y, Hirai M: Comparative FISH mapping of the ancestral fusion point of human chromosome 2. Chromosome Res 8:727–735 (2000).

Kay RF: The phyletic relationships of extant and fossil Pitheciinae (Platyrrhini, Anthropoidea). J Hum Evol 19:175–208 (1990).

Kehrer-Sawatzki H, Schreiner B, Tanzer S, Platzer M, Müller S, Hameister H: Molecular characterization of the pericentric inversion that causes differences between chimpanzee chromosome 19 and human chromosome 17. Am J Hum Genet 71:375–388 (2002).

Klinger HP: The somatic chromosomes of some primates *(Tupaia glis, Nycticebus coucang, Tarsius bancanus, Cercocebus aterrimus, Symphalangus syndactylus)*. Cytogenetics 85:140–151 (1963).

Koehler U, Arnold N, Wienberg J, Tofanelli S, Stanyon R: Genomic reorganization and disrupted chromosomal synteny in the siamang *(Hylobates syndactylus)* revealed by fluorescence in situ hybridization. Am J Phys Anthropol 97:37–47 (1995a).

Koehler U, Bigoni F, Wienberg J, Stanyon R: Genomic reorganization in the concolor gibbon *(Hylobates concolor)* revealed by chromosome painting. Genomics 30:287–292 (1995b).

Koop BF, Siemieniak D, Slightom JL, Goodman M, Dunbar J, Wright PC, Simons EL: *Tarsius* delta- and beta-globin genes: conversions, evolution, and systematic implications. J Biol Chem 264:68–79 (1989a).

Koop BF, Tagle DA, Goodman M, Slightom JL: A molecular view of primate phylogeny and important systematic and evolutionary questions. Mol Biol Evol 6:580–612 (1989b).

Lengauer C, Wienberg J, Cremer T, Lüdecke H-J, Horsthemke B: Comparative chromosome band mapping in primates by in situ suppression hybridization of band specific DNA microlibraries. Hum Evol 6:67–71 (1991).

Lichter P, Cremer T, Borden J, Manuelidis L, Ward DC: Delineation of individual human chromosomes in metaphase and interphase cells by in situ suppression hybridization using recombinant DNA libraries. Hum Genet 80:224–234 (1988).

Locke DP, Archidiacono N, Misceo D, Cardone MF, Deschamps S, Roe B, Rocchi M, Eichler EE: Refinement of a chimpanzee pericentric inversion breakpoint to a segmental duplication cluster. Genome Biol 4:R50 (2003).

Lüdecke HJ, Senger G, Claussen U, Horsthemke B: Cloning defined regions of the human genome by microdissection of banded chromosomes and enzymatic amplification. Nature 338:348–350 (1989).

Ma NS, Lin KC: Chromosome mapping of the owl monkey CSF1R and IL5 genes. Genomics 13:1174–1177 (1992).

Marks J: Evolutionary tempo and phylogenetic inference based on primate karyotypes. Cytogenet Cell Genet 34:261–264 (1982).

Marks J: Evolution of human chromosomes, in Jones S, Martin R, Pilbeam D (eds): The Cambridge Encyclopedia of Human Evolution (Cambridge University Press, Cambridge 1992).

Marzella R, Viggiano L, Miolla V, Storlazzi CT, Ricco A, Gentile E, Roberto R, Surace C, Fratello A, Mancini M, Archidiacono N, Rocchi M: Molecular cytogenetic resources for chromosome 4 and comparative analysis of phylogenetic chromosome IV in great apes. Genomics 63:307–313 (2000).

Matera AG, Marks J: Complex rearrangements in the evolution of hominoid chromosome XVII. J Hum Evol 24:233–238 (1992).

McConkey EH: The origin of human chromosome 18 from a human/ape ancestor. Cytogenet Cell Genet 76:189–191 (1997).

Medeiros MA, Barros RM, Pieczarka JC, Nagamachi CY, Ponsa M, Garcia M, Garcia F, Egozcue J: Radiation and speciation of spider monkeys, genus *Ateles*, from the cytogenetic viewpoint. Am J Primatol 42:167–178 (1997).

Mefford HC, Trask BJ: The complex structure and dynamic evolution of human subtelomeres. Nat Rev Genet 3:91–102 (2002).

Mellersh CS, Hitte C, Richman M, Vignaux F, Priat C, Jouquand S, Werner P, Andre C, DeRose S, Patterson DF, Ostrander EA, Galibert F: An integrated linkage-radiation hybrid map of the canine genome. Mamm Genome 11:120–130 (2000).

Miller DA: Evolution of primate chromosomes. Science 198:1116–1124 (1977).

Misceo D, Ventura M, Eder V, Rocchi M, Archidiacono N: Human chromosome 16 conservation in primates. Chromosome Res 11:323–326 (2003).

Montefalcone G, Tempesta S, Rocchi M, Archidiacono N: Centromere repositioning. Genome Res 9:1184–1188 (1999).

Morescalchi MA, Schempp W, Consigliere S, Bigoni F, Wienberg J, Stanyon R: Mapping chromosomal homology between humans and the black-handed spider monkey by fluorescence in situ hybridization. Chromosome Res 5:527–536 (1997).

Mrasek K, Heller A, Rubtsov N, Trifonov V, Starke H, Rocchi M, Claussen U, Liehr T: Reconstruction of the female *Gorilla gorilla* karyotype using 25-color FISH and multicolor banding (MCB). Cytogenet Cell Genet 93:242–248 (2001).

Mrasek K, Heller A, Rubtsov N, Trifonov V, Starke H, Claussen U, Liehr T: Detailed *Hylobates lar* karyotype defined by 25-color FISH and multicolor banding. Int J Mol Med 12:139–146 (2003).

Müller S, Wienberg J: "Bar-coding" primate chromosomes: molecular cytogenetic screening for the ancestral hominoid karyotype. Hum Genet 109:85–94 (2001).

Müller S, Koehler U, Wienberg J, Marzella R, Finelli P, Antonacci R, Rocchi M, Archidiacono N: Comparative fluorescence in situ hybridization mapping of primate chromosomes with *Alu*-PCR generated probes from human/rodent somatic cell hybrids. Chromosome Res 4:38–42 (1996).

Müller S, O'Brien PC, Ferguson-Smith MA, Wienberg J: A novel source of highly specific chromosome painting probes for human karyotype analysis derived from primate homologues. Hum Genet 101:149–153 (1997a).

Müller S, O'Brien PC, Ferguson-Smith MA, Wienberg J: Reciprocal chromosome painting between human and prosimians *(Eulemur macaco macaco* and *E. fulvus mayottensis)*. Cytogenet Cell Genet 78:260–271 (1997b).

Müller S, Rocchi M, Ferguson-Smith MA, Wienberg J: Toward a multicolor chromosome bar code for the entire human karyotype by fluorescence in situ hybridization. Hum Genet 100:271–278 (1997c).

Müller S, O'Brien PC, Ferguson-Smith MA, Wienberg J: Cross-species colour segmenting: a novel tool in human karyotype analysis. Cytometry 33:445–452 (1998).

Müller S, Stanyon R, O'Brien PC, Ferguson-Smith MA, Plesker R, Wienberg J: Defining the ancestral karyotype for all primates by multidirectional chromosome painting between tree shrews, lemurs and humans. Chromosoma 108:393–400 (1999).
Müller S, Stanyon R, Finelli P, Archidiacono N, Wienberg J: Molecular cytogenetic dissection of human chromosomes 3 and 21 evolution. Proc Natl Acad Sci USA 97:206–211 (2000).
Müller S, Neusser M, O'Brien PC, Wienberg J: Molecular cytogenetic characterization of the EBV-producing cell line B95-8 (*Saguinus oedipus*, Platyrrhini) by chromosome sorting and painting. Chromosome Res 9:689–693 (2001).
Müller S, Neusser M, Wienberg J: Towards unlimited colors for fluorescence in-situ hybridization (FISH). Chromosome Res 10:223–232 (2002).
Müller S, Hollatz M, Wienberg J: Chromosomal phylogeny and evolution of gibbons (Hylobatidae). Hum Genet 113:493–501 (2003).
Müller S, Finelli P, Neusser M, Wienberg J: The evolutionary history of human chromosome 7. Genomics 84 (in press 2004).
Murphy WJ, Sun S, Chen Z, Yuhki N, Hirschmann D, Menotti-Raymond M, O'Brien SJ: A radiation hybrid map of the cat genome: implications for comparative mapping. Genome Res 10:691–702 (2000).
Murphy WJ, Page JE, Smith C Jr, Desrosiers RC, O'Brien SJ: A radiation hybrid mapping panel for the rhesus macaque. J Hered 92:516–519 (2001).
Murphy WJ, Froenicke L, O'Brien SJ, Stanyon R: The origin of human chromosome 1 and its homologs in placental mammals. Genome Res 13:1880–1888 (2003).
Nagamachi CY, Pieczarka JC, Schwarz M, Barros RM, Mattevi MS: Comparative chromosomal study of five taxa of genus *Callithrix*, group jacchus (Platyrrhini, Primates). Am J Primatol 41:53–60 (1997).
Nash WG, O'Brien SJ: Conserved regions of homologous G-banded chromosomes between orders in mammalian evolution: carnivores and primates. Proc Natl Acad Sci USA 79:6631–6635 (1982).
Nash WG, Wienberg J, Ferguson-Smith MA, Menninger JC, O'Brien SJ: Comparative genomics: tracking chromosome evolution in the family Ursidae using reciprocal chromosome painting. Cytogenet Cell Genet 83:182–192 (1998).
Neusser M, Stanyon R, Bigoni F, Wienberg J, Müller S: Molecular cytotaxonomy of New World monkeys (Platyrrhini) – comparative analysis of five species by multi-color chromosome painting gives evidence for a classification of *Callimico goeldii* within the family of Callitrichidae. Cytogenet Cell Genet 94:206–215 (2001).
Nickerson E, Nelson DL: Molecular definition of pericentric inversion breakpoints occurring during the evolution of humans and chimpanzees. Genomics 50:368–372 (1998).
Nie W, Liu R, Chen Y, Wang J, Yang F: Mapping chromosomal homologies between humans and two langurs *(Semnopithecus francoisi* and *S. phayrei)* by chromosome painting. Chromosome Res 6:447–453 (1998).
Nie W, Rens W, Wang J, Yang F: Conserved chromosome segments in *Hylobates hoolock* revealed by human and *H. leucogenys* paint probes. Cytogenet Cell Genet 92:248–253 (2001).
O'Brien SJ: Genetic Maps, vol 3 (Cold Spring Harbor Press, New York 1984).
O'Brien SJ, Seuanez H, Womack J: On the evolution of genome organization in mammals, in MacIntyre RJ (ed): Molecular Evolutionary Genetics, pp 519–589 (Plenum Press, New York 1985).
Ohno S: Ancient linkage groups and frozen accidents. Nature 244:259–262 (1973).
Pinkel D, Landegent J, Collins C, Fuscoe J, Segraves R, Lucas J, Gray J: Fluorescence in situ hybridization with human chromosome-specific libraries – detection of trisomy-21 and translocations of chromosome-4. Proc Natl Acad Sci USA 85:9138–9142 (1988).
Ponsa M, Estop AM, Miro R, Rubio A, Egozcue J: Banding patterns of the chromosomes of *Miopithecus talapoin* compared with *Macaca mulatta* and *Cercopithecus aethiops*. Cytogenet Cell Genet 28:41–46 (1980).
Pujana MA, Nadal M, Gratacos M, Peral B, Csiszar K, Gonzalez-Sarmiento R, Sumoy L, Estivill X: Additional complexity on human chromosome 15q: identification of a set of newly recognized duplicons (LCR15) on 15q11-q13, 15q24, and 15q26. Genome Res 11:98–111 (2001).
Rens W, Yang F, O'Brien PC, Solanky N, Ferguson-Smith MA: A classification efficiency test of spectral karyotyping and multiplex fluorescence in situ hybridization: identification of chromosome homologies between *Homo sapiens* and *Hylobates leucogenys*. Genes Chromosomes Cancer 31:65–74 (2001).
Richard F, Dutrillaux B: Origin of human chromosome 21 and its consequences: a 50-million-year-old story. Chromosome Res 6:263–268 (1998).
Richard F, Lombard M, Dutrillaux B: ZOO-FISH suggests a complete homology between human and capuchin monkey (Platyrrhini) euchromatin. Genomics 36:417–423 (1996).
Ried T, Baldini A, Rand TC, Ward DC: Simultaneous visualization of seven different DNA probes by in situ hybridization using combinatorial fluorescence and digital imaging microscopy. Proc Natl Acad Sci USA 89:1388–1392 (1992).
Ried T, Arnold N, Ward DC, Wienberg J: Comparative high-resolution mapping of human and primate chromosomes by fluorescence in situ hybridization. Genomics 18:381–386 (1993).
Rodrigues LR, Barros RM, Pissinati A, Pieczarka JC, Nagamachi CY: Cytogenetic study of *Callicebus hoffmannsii* (Cebidae, Primates) and comparison with *C. m. moloch*. Cytobios 105:137–145 (2001).
Rokas A, Holland PW: Rare genomic changes as a tool for phylogenetics. Trends Ecol Evol 15:454–459 (2000).
Roos C, Geissmann T: Molecular phylogeny of the major hylobatid divisions. Mol Phylogenet Evol 19:486–494 (2001).
Rosenberger AL: The Higher Taxa (Academia Brasiliera de Ciencias, Rio de Janeiro 1981).
Rumpler Y, Dutrillaux B: Chromosomal evolution in Malagasy lemurs. I. Chromosome banding studies in the genuses *Lemur* and *Microcebus*. Cytogenet Cell Genet 17:268–281 (1976).
Rumpler Y, Couturier J, Warter S, Dutrillaux B: The karyotype of *Galago crassicaudatus* is ancestral for lorisiforms. Folia Primatol 40:227–231 (1983).
Rumpler Y, Waters S, Ishak B, Dutrillaux B: Chromosomal evolution in prosimians. Hum Evol 4:157–170 (1989).
Ryan SC, Zielinski R, Dugaiczyk A: Structure of the gorilla alpha-fetoprotein gene and the divergence of primates. Genomics 9:60–72 (1991).
Rylands AB, Schneider H, Langguth A, Mittermeier RA, Groves CP, Rodrigues LR: An Assessment of the diversity of New World primates. Neotropical Primates 8:62–93 (2000).
Samonte RV, Eichler EE: Segmental duplications and the evolution of the primate genome. Nat Rev Genet 3:65–72 (2002).
Scherthan H, Cremer T, Arnason U, Weier HU, Lima-de-Faria A, Fronicke L: Comparative chromosome painting discloses homologous segments in distantly related mammals. Nat Genet 6:342–347 (1994).
Schmitz J, Ohme M, Zischler H: SINE insertions in cladistic analyses and the phylogenetic affiliations of *Tarsius bancanus* to other primates. Genetics 157:777–784 (2001).
Schneider H: The current status of the New World monkey phylogeny. An Acad Bras Cienc 72:165–172 (2000).
Schneider H, Canavez FC, Sampaio I, Moreira MA, Tagliaro CH, Seuánez HN: Can molecular data place each neotropical monkey in its own branch? Chromosoma 109:515–523 (2001).
Schröck E, du Manoir S, Veldman T, Schoell B, Wienberg J, Ferguson-Smith MA, Ning Y, Ledbetter DH, Bar-Am I, Soenksen D, Garini Y, Ried T: Multicolor spectral karyotyping of human chromosomes. Science 273:494–497 (1996).
Seuánez HN: The Phylogeny of Human Chromosomes (Springer, Berlin 1979).
Sherlock JK, Griffin DK, Delhanty JD, Parrington JM: Homologies between human and marmoset *(Callithrix jacchus)* chromosomes revealed by comparative chromosome painting. Genomics 33:214–219 (1996).
Shoshani J, Groves CP, Simons EL, Gunnell GF: Primate phylogeny: morphological vs. molecular results. Mol Phylogenet Evol 5:102–154 (1996).
Speicher MR, Gwyn Ballard S, Ward DC: Karyotyping human chromosomes by combinatorial multi-fluor FISH. Nat Genet 12:368–375 (1996).
Stankiewicz P, Park SS, Inoue K, Lupski JR: The evolutionary chromosome translocation 4;19 in *Gorilla gorilla* is associated with microduplication of the chromosome fragment syntenic to sequences surrounding the human proximal CMT1A-REP. Genome Res 11:1205–1210 (2001).
Stanyon R, Chiarelli B: Phylogeny of the Hominoidea: The chromosome evidence. J Hum Evol 11:493–504 (1982).
Stanyon R, Sineo L, Chiarelli B, Camperio-Ciani A, Haimoff AR, Mootnick EH, Sutarman D: Banded karyotypes of the 44-chromosome gibbons. Folia Primatol 48:56–64 (1987).
Stanyon R, Wienberg J, Romagno D, Bigoni F, Jauch A, Cremer T: Molecular and classical cytogenetic analyses demonstrate an apomorphic reciprocal chromosomal translocation in *Gorilla gorilla*. Am J Phys Anthropol 88:245–250 (1992).
Stanyon R, Yang F, Cavagna P, O'Brien PC, Bagga M, Ferguson-Smith MA, Wienberg J: Reciprocal chromosome painting shows that genomic rearrangement between rat and mouse proceeds ten times faster than between humans and cats. Cytogenet Cell Genet 84:150–155 (1999).
Stanyon R, Consigliere S, Müller S, Morescalchi A, Neusser M, Wienberg J: Fluorescence in situ hybridization (FISH) maps chromosomal homologies between the dusky titi and squirrel monkey. Am J Primatol 50:95–107 (2000).
Stanyon R, Consigliere S, Bigoni F, Ferguson-Smith M, O'Brien PC, Wienberg J: Reciprocal chromosome painting between a New World primate, the woolly monkey, and humans. Chromosome Res 9:97–106 (2001).
Stanyon R, Koehler U, Consigliere S: Chromosome painting reveals that galagos have highly derived karyotypes. Am J Phys Anthropol 117:319–326 (2002).
Stanyon R, Bonvicino CR, Svartman M, Seuánez HN: Chromosome painting in *Callicebus lugens*, the species with the lowest diploid number (2n = 16) known in primates. Chromosoma 112:201–206 (2003a).
Stanyon R, Stone G, Garcia M, Froenicke L: Reciprocal chromosome painting shows that squirrels, unlike murid rodents, have a highly conserved genome organization. Genomics 82:245–249 (2003b).

Svartman M, Stone G, Page JE, Stanyon R: A chromosome painting test of the basal eutherian karyotype. Chromosome Res 12:45–53 (2004).

Swofford D: PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4 (Sinauer Associates, Sunderland 1998).

Tsend-Ayush E, Grutzner F, Yue Y, Grossmann B, Hansel U, Sudbrak R, Haaf T: Plasticity of human chromosome 3 during primate evolution. Genomics 83:193–202 (2004).

Turleau C, De Grouchy J, Klein M: [Chromosomal phylogeny of man and the anthropomorphic primates. *(Pan troglodytes, Gorilla gorilla, Pongo pygmaeus)*. Attempt at reconstitution of the karyotype of the common ancestor]. Ann Genet 15:225–240 (1972).

Van Tuinen P, Ledbetter DH: Cytogenetic comparison and phylogeny of three species of Hylobatidae. Am J Phys Anthropol 61:453–466 (1983).

Van Tuinen P, Mootnick AR, Kingswood SC, Hale DW, Kumamoto AT: Complex, compound inversion/translocation polymorphism in an ape: presumptive intermediate stage in the karyotypic evolution of the agile gibbon *Hylobates agilis*. Am J Phys Anthropol 110:129–142 (1999).

Ventura M, Mudge JM, Palumbo V, Burn S, Blennow E, Pierluigi M, Giorda R, Zuffardi O, Archidiacono N, Jackson MS, Rocchi M: Neocentromeres in 15q24-26 map to duplicons which flanked an ancestral centromere in 15q25. Genome Res 13: 2059–2068 (2003).

Warburton PE, Willard HF: Evolution of centromeric alpha satellite DNA: molecular organization within and between human and primate chromosomes, in Jackson MS, Strachan T, Dover G (eds.): Human Genome Evolution, pp 121–146 (Bios, Oxford 1996).

Watanabe TK, Bihoreau MT, McCarthy LC, Kiguwa SL, Hishigaki H, Tsuji A, Browne J, et al: A radiation hybrid map of the rat genome containing 5,255 markers. Nat Genet 22:27–36 (1999).

Wienberg J, Jauch A, Stanyon R, Cremer T: Molecular cytotaxonomy of primates by chromosomal in situ suppression hybridization. Genomics 8:347–350 (1990).

Wienberg J, Stanyon R, Jauch A, Cremer T: Homologies in human and *Macaca fuscata* chromosomes revealed by in situ suppression hybridization with human chromosome specific DNA libraries. Chromosoma 101:265–270 (1992).

Wienberg J, Jauch A, Ludecke HJ, Senger G, Horsthemke B, Claussen U, Cremer T, Arnold N, Lengauer C: The origin of human chromosome 2 analyzed by comparative chromosome mapping with a DNA microlibrary. Chromosome Res 2:405–410 (1994).

Wienberg J, Stanyon R, Nash WG, O'Brien PC, Yang F, O'Brien SJ, Ferguson-Smith MA: Conservation of human vs. feline genome organization revealed by reciprocal chromosome painting. Cytogenet Cell Genet 77:211–217 (1997).

Wienberg J, Frönicke L, Stanyon R: Insights into mammalian genome organization and evolution by molecular cytogenetics, in Clark MS (ed): Comparative Genomics, pp 207–244 (Kluver Acad. Publ., Dordrecht 2000).

Wildman DE, Uddin M, Liu G, Grossman LI, Goodman M: Implications of natural selection in shaping 99.4 % nonsynonymous DNA identity between humans and chimpanzees: enlarging genus *Homo*. Proc Natl Acad Sci USA 100:7181–188 (2003).

Yang F, O'Brien PC, Ferguson-Smith MA: Comparative chromosome map of the laboratory mouse and Chinese hamster defined by reciprocal chromosome painting. Chromosome Res 8:219–227 (2000).

Yang F, Alkalaeva EZ, Perelman PL, Pardini AT, Harrison WR, O'Brien PC, Fu B, Graphodatsky AS, Ferguson-Smith MA, Robinson TJ: Reciprocal chromosome painting among human, aardvark, and elephant (superorder Afrotheria) reveals the likely eutherian ancestral karyotype. Proc Natl Acad Sci USA 100:1062–1066 (2003).

Yu D, Yang F, Liu R: [A comparative chromosome map between human and *Hylobates hoolock* built by chromosome painting]. Yi Chuan Xue Bao 24:417–423 (1997).

Yunis JJ, Prakash O: The origin of man: a chromosomal pictorial legacy. Science 215:1525–1530 (1982).

Zietkiewicz E, Richer C, Labuda D: Phylogenetic affinities of tarsier in the context of primate Alu repeats. Mol Phylogenet Evol 11:77–83 (1999).