Chromosome Painting Reveals That Galagos Have Highly Derived Karyotypes

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ABSTRACT The differences in chromosome number between *Otolemur crassicaudatus* (2n = 62) and *Galago moholi* (2n = 38) are dramatic. However, the total number of signals given by hybridizing human chromosome paints to galago metaphases is similar: 42 in *O. crassicaudatus* and 38 *G. moholi*. Many human chromosome homologs are found fragmented in each species, and numerous translocations have resulted in chromosomal syntenies or hybridization associations which differ from those found in humans. Only 7 human autosomes showed conserved synteny in *O. crassicaudatus*, and 9 in *G. moholi*. Both galago species have numerous associations or syntenies not found in humans: *O. crassicaudatus* has 11, and *G. moholi* has 21. The phylogenetic line leading to the last common ancestor of the two galago species accumulated 6 synapomorphic fissions and 5 synapomorphic fusions. Since the divergence of the two galago species, 10 Robertsonian translocations have further transformed the *G. moholi* karyotype, and 2 fissions have been incorporated into the *O. crassicaudatus* karyotype. Comparison with other primates, tree shrews, and other mammals shows that both galagos have karyotypes which are a mixture of derived and conserved chromosomes, and neither has a karyotype close to that of the proposed ancestor of all primates. Am J Phys Anthropol 117:319–326, 2002.
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With the introduction of molecular techniques, cytogenetic studies promise to provide more reliable data for a range of evolutionary problems (Wienberg and Stanyon, 1997). Chromosome painting data are now considered a first step in the reconstruction of genome evolution, and can often provide a broad overview of phylogenetic and taxonomic relationships (O'Brien et al., 1999a,b). Chromosome evolution within the galagos has drawn the attention of many investigators, due to the dramatic differences in their karyotypes. Cytogenetic studies in galagos have also contributed to raising and resolving phylogenetic and taxonomic questions. The karyological differences discovered within greater galagos (Primates, Prosimii, Lorisidae) were among the first pieces of evidence that eventually led to the recognition of two species now known as *Otolemur crassicaudatus* and *O. garnettii* (Masters et al., 1987). Likewise the karyological data suggest that multiple species are hidden within the taxon *Galagoides demidoff*, but this has yet to be confirmed by other methods (Stanyon et al., 1992).

TAXONOMY OF THE GALAGOS

Galagos are taxonomically much more complex than previously thought: the exact designation of genera, subgenera, and species is still a matter of disagreement. The classical taxonomy of galagos was that of Hill (1953); since then, numerous proposals have followed (Napier and Napier, 1967; Nash et al., 1989; Olson, 1986; Wolfheim, 1983). One recent classification recognized four genera (Groves, 1989). This classification, however, is not universally accepted. Some authors consider *Galago*, *Otolemur*, *Galagoides*, and *Euoticus* as subgenera of a single genus, *Galago.* There is no consensus over the exact number of species and subspecies.

KARYOLOGICAL STUDIES OF THE GENUS *OTOLEMUR*

Prior to the 1980s, taxonomists assumed that the sibling species *Otolemur crassicaudatus* and *O. garnettii* belonged to a single species, then referred as *Galago crassicaudatus* (Hill, 1953; Schwarz, 1931). Cytogenetics offered the first evidence of the exis-

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tence of two species: after the pioneering work of Chu and Bender (1961), studies of karyotypic variations within the group showed the presence of two different karyotypes. The first karyotype (*O. crassicaudatus*) had a diploid number of 2n = 62 and contained six pairs of meta- and submetacentrics, a large submetacentric X, and a small acrocentric Y; the fundamental number (FN) was FN = 75,76 m/f. The second karyotype (*O. garnettii*) had the same diploid number and the same sex chromosomes, but showed 13 pairs of biarmed autosomes and 17 pairs of acrocentrics. Therefore, the FN = 89/90 m/f was significantly different (de Boer, 1973; Egozcue, 1970; Hayata et al., 1971; Pasztor and Van Horn, 1977). The mechanisms proposed (de Boer, 1973) in order to explain the differences were translocations and pericentric inversions. The hypothesis of pericentric inversions was subsequently confirmed by karyological studies of *O. c. argentatus* (Pasztor and Van Horn, 1977) and by the utilization of banding techniques (Masters et al., 1987; Poorman, 1982). The existence of two species was later confirmed by a wealth of different studies on morphology, reproductive, social, and various other biological parameters (Dixson and Van Horn, 1977; Eaglen and Simons, 1980; Masters and Lubinsky, 1988; Masters and Dunn, 1988; Pasztor and Van Horn, 1976).

KARYOLOGICAL STUDIES OF THE GENUS *GALAGO*

Karyological studies in the genus *Galago* have also been marked by the discovery of chromosome variability. A very early pioneering work by Matthey (1955) reported that *Galago senegalensis* had a diploid number of 2n = 38, with fundamental number FN = 64,12 autosomal submetacentric pairs and 6 autosomal acrocentric pairs. Chu and Bender (1961) described 30 submetacentric and 6 acrocentric autosomes, which brought the fundamental number to FN = 70. According to de Boer (1973), the differences could have been due to technical difficulties in identifying the short arms of the smaller chromosomes.

Chromosomal variability was also found in *G. senegalensis zanzibaricus* (now *Galagoides zanzibaricus*), with diploid number 2n = 36, and in *G. s. braccatus*, with diploid numbers 2n = 36, 37, and 38 (de Boer, 1973; Ying and Butler, 1971). The authors explained the difference in the number of chromosomes by a Robertsonian fusion involving a subtelocentric and an acrocentric chromosome, which formed a large metacentric. The difference in diploid number would then have been due to the absence of the translocation (2n = 38), or to its presence in heterozygous (2n = 37) or homozygous (2n = 36) form.

It now seems likely that the differences reported in the karyotypes were due to taxonomic confusion; different species (*Galago gallarum*, *G. moholi*, *G. matschiei*, and *Galagoides zanzibaricus*) were clustered together as subspecies of *Galago senegalensis*, even though one or more of these species may never have been karyotyped. But their assessment as distinct species was recent (Groves, 1989; Nash et al., 1989; Zimmermann et al., 1988).

TRADITIONAL INTERPRETATIONS OF THE KARYOLOGICAL DIFFERENCES BETWEEN THE GENERA *OTOLEMUR* AND *GALAGO*

Some authors (de Boer, 1972, 1973; Dutrillaux et al., 1982; Dutrillaux and Rumpler, 1995; Rumpler et al., 1983) explained the great difference in the number of chromosomes between *Otolemur crassicaudatus* (2n = 62) and *Galago moholi* (2n = 38) by simple Robertsonian chromosomal rearrangements. Robertsonian rearrangements are either fissions or translocations that involve break points in the centromeres. Polyploidy was ruled out by studies showing that the DNA content of nuclei of the two species was very similar (Manfredi-Romanini et al., 1972).

Given the similarity in fundamental number, two hypotheses can explain the contrast of many metacentrics in *G. moholi* and many acrocentrics in *O. crassicaudatus*. The fusion hypothesis was first proposed by de Boer (1973) on the basis of classic staining of *O. crassicaudatus* and *G. moholi* metaphases, and subsequently by Rumpler et al. (1983, 1989) on the basis of R-banding. The fission hypothesis, although logically equivalent to the first, was always considered much less probable because some authors considered fission products less stable (de Boer, 1973). The preference for fusion over fission stems led to the conclusion that *Otolemur crassicaudatus* with its high diploid number was karyologically and morphologically primitive both in respect to *Galago moholi* and in respect to the other Lorisidae. *Otolemur* has been described as plesiomorphic (Groves, 1989) and its karyotype was considered to be very similar to the ancestral one of the Lorisidae (Rumpler et al., 1983, 1989). According to this reconstruction, the *Galago* karyotype must have originated through multiple centric fusions.

Chromosome painting can provide data on chromosomal homology between the two genera needed to test these hypotheses. However, before our report only one human chromosome paint had been hybridized to galago metaphases (Healy, 1995). Here we report on the complete chromosomal homology between humans, *Galago moholi* and *Otolemur crassicaudatus*. In a similar fashion, chromosome painting helped to clarify the cytogenetic mechanisms responsible for the differences between the karyotypes of the African green monkey (2n = 62) and humans (2n = 46). Previously it was believed that the great differences in diploid numbers between these two species were due to Robertsonian transformations, but in situ hybridization showed that many rearrangements were non-Robertsonian fissions (Finelli et al., 1999). Indeed, chromosome painting in the galagos has helped to clarify the mechanisms of genome evolution in these prosimians.

MATERIALS AND METHODS

Samples consisted of ear punches of one male and one female per species, kindly provided by the Duke Primate Center (Durham, NC). The samples were listed as *Galago senegalensis moholi* (now *Galago moholi*) GSE 2-3084 (Walnut, male) and GSE 3-3130 (Snowball, female), and *Galago crassicaudatus monteiri* (now *Otolemur crassicaudatus monteiri*) GCR 6-2789 (Chong, male) and GCR 7-2805 (Sadiki, female).

Standard procedures for fibroblast culture were followed, and chromosomes were prepared and stored in a fixative at −20°C. G-banding prior to in situ hybridization and destaining were performed as previously described (Stanyon et al., 2000). Chromosome identification and numbering in *Otolemur crassicaudatus* followed (Masters et al., 1987). Chromosomal painting with human chromosome-specific DNA probe paints was as described in Stanyon et al. (2000). Paints were labeled with biotin or digoxigenin by degenerate oligonucleotide primel-PCR (DOP-PCR). After hybridization and washing of slides, biotinylated or digoxigeninated DNA probes were detected with avidin (Vector Laboratories) or anti-digoxigenin (Boeringer Mannheim) antibodies, coupled with fluorescein isothiocyanate (FITC), tetramethyl-rhodamine-5-isothiocyanate (TRITC) or rodamine.

G-banded metaphases were photographed on Agfaortho 25 or Kodak Technical Pan film. Photographs of hybridized metaphases were taken with Agfachrome (ASA 1000) color slide film or Kodak T-max (ASA 400) black and white film. Digital images were taken using SmartCapture and a cooled CCD camera coupled to the microscope (Stanyon et al., 2000).

RESULTS

Hybridizations were obtained from 23 human chromosome paints on all autosomes and the X-chromosome for both galago species. The Y-chromosome did not give any hybridization signal. The hybridization signals obtained on these two prosimians had higher background levels and were less bright than those obtained on simian primates. Figure 1 shows typical examples of in situ hybridization signals in *O. crassicaudatus* and *G. moholi* chromosomes with human chromosome-specific painting probes.

Karyotype and hybridization pattern of *Otolemur crassicaudatus*

Our results confirm the diploid number, fundamental number, and the banding pattern of *Otolemur crassicaudatus* (OCR) (Masters et al., 1987). The diploid number is 2n = 62, with a normal XX/XY sex chromosome system. The autosomes are composed of 24 acrocentric and 6 submetacentric chromosomes. The submetacentric X chromosome is the largest chromosome in the karyotype, and is more acrocentric than the usual mammalian X chromosome. The Y is a small acrocentric. The fundamental number therefore is FN = 75 in males and FN = 76 in females.

The karyotype shown in Figure 2 summarizes the hybridization results of human chromosome-specific paints on *O. crassicaudatus* chromosomes. The total number of hybridization signals obtained was 42. Every galago chromosome except the Y was hybridized by at least one chromosome paint. DNA paints from 7 human autosomes showed conserved synteny: 5 human autosomes (paints 10, 13, 17, 18, and 20) each completely hybridized only one homolog, while 2 human chromosome paints (9 and 21) hybridized an *O. crassicaudatus* chromosome along with other human paints. The remaining 15 autosomal probes gave multiple signals on a number of different chromosomes; 12 human chromosome paints (2, 4–8, 11, 14, 15, 16, 19, and 22) gave signals on two chromosomes per haploid set; human chromosome paints 1 and 3 labeled three chromosomes per haploid set; and human chromosome paint 12 gave four signals per haploid set. Human X-chromosome paint completely hybridized the *Otolemur* X.

Twenty-one *O. crassicaudatus* autosomes (4–7, 9, 12–15, 17–23, and 25–30) were completely hybridized by one human autosomal paint. Nine chromosomes had two or more signals (1, 2, 3, 7, 8, 10, 11, 16, and 24), producing 11 chromosomal syntenies or hybridization associations which differ from those found in humans: 1/19, 2/12, 3/21, 6/14, 7/12, 7/16, 9/15, 10/19, 12/22 (twice), 12/16, and 14/15.

Karyotype and hybridization pattern of *Galago moholi*

The karyotype of *Galago moholi* (GMO) is shown in Figure 3. The diploid number is 2n = 38, with an XX/XY sex-chromosome system. Among the autosomes, 15 pairs are metacentric or submetacentric, and only 3 are acrocentric. The X chromosome is identical to that of *Otolemur crassicaudatus*. The Y is a small submetacentric. The fundamental number is therefore FN = 70 for both females and males.

The karyotype shown in Figure 3 summarizes the hybridization results of human chromosome-specific paints on *G. moholi* chromosomes. With the exception of the human Y-chromosome probe, all human paints provided hybridization signals (Fig. 1).

The total number of hybridization signals obtained was 39. Every *G. moholi* chromosome was hybridized by at least one chromosome paint (excluding the Y). DNA paints from 9 human autosomes showed conserved synteny: human paint 17 completely hybridized only one *G. moholi* homolog, while 8 human chromosome paints (7, 9, 10, 13, 15, 18, 20, and 21) hybridized a *G. moholi* chromosome along with other human paints. The remaining 13 autosomal probes gave multiple signals on a number of different *G. moholi* chromosomes. Ten human chromosome paints (2–6, 8, 11, 14, 16, 19, and 22) gave signals on two chromosomes per haploid set; human chromosome paint 1 labeled three chromo-

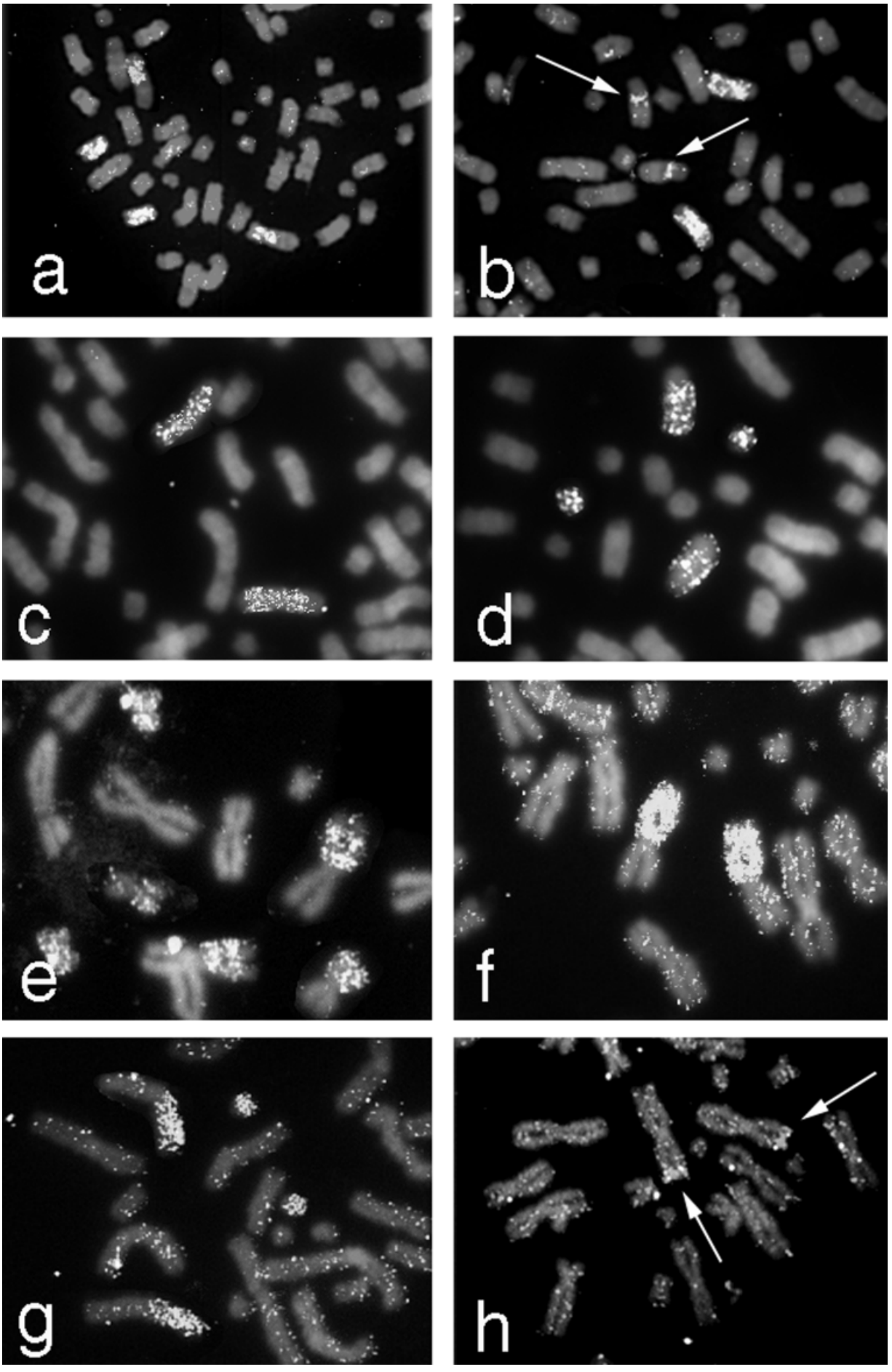

Fig. 1.

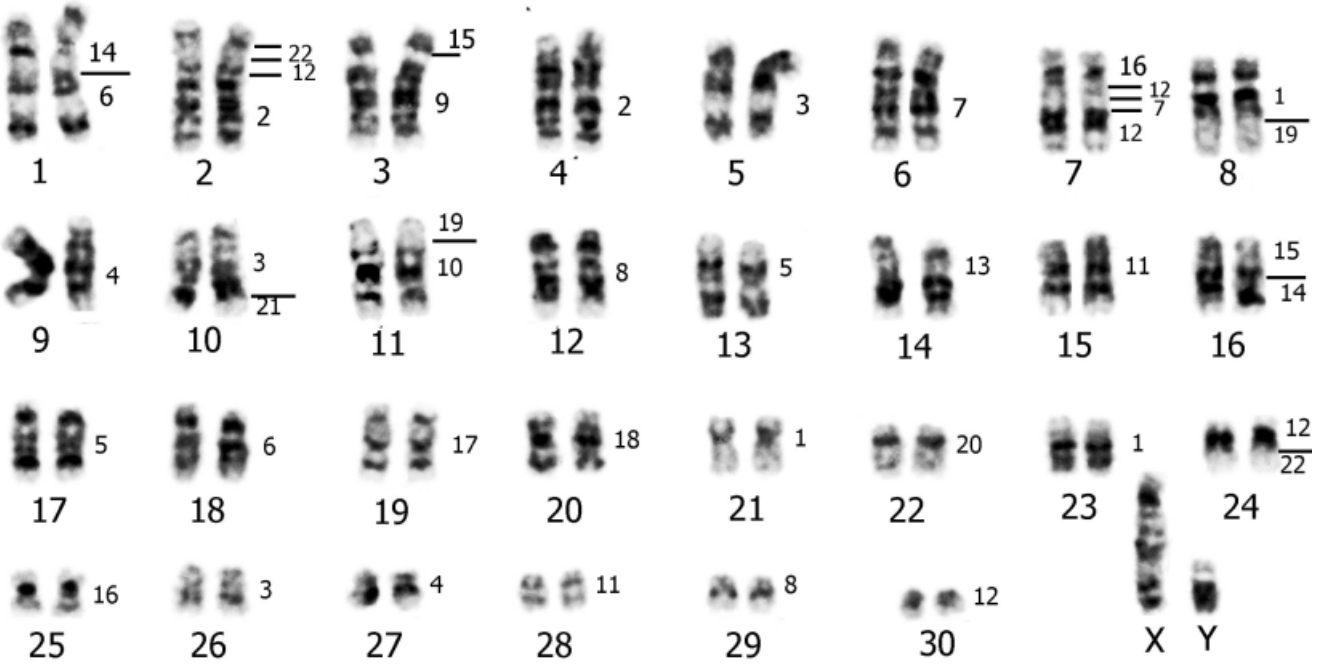

Fig. 2. G-banded karyotype of *O. crassicaudatus*, including a summary of chromosome painting results. Galago karyotype is numbered below, and human homologies are at right.

somes per haploid set; and chromosome 12 labeled four chromosomes. Human paint X completely hybridized the *G. moholi* X.

Seven *G. moholi* autosomes (11, and 13–18) were completely hybridized by one human autosomal paint; 5 (6, 8, 9, 10, and 12) had two signals; and 7 (1–5, 7, and 10) had three signals. The 11 *G. moholi* chromosomes which were hybridized by more than one human probe produced 17 chromosomal syntenies or hybridization associations which differed from those found in humans: 1/5, 1/12, 1/19, 2/12, 2/22, 3/7, 3/21, 4/6, 5/14, 6/14, 8/11, 9/15, 10/19, 12/16, 12/18, 12/22 (twice), 13/16, 14/15, 18/22, and 19/20.

DISCUSSION

Our results show that for many human chromosomes, the hybridization signals are fragmented both in *O. crassicaudatus* and in *G. moholi*. Many chromosomes in both species show signals from two or more human probes, producing linkage groups that are absent in humans. To evaluate the direction of evolutionary change, it is necessary to establish a comparison with an "outgroup" and to apply the criterion known as maximum parsimony. When the same character or character state is found in the outgroup, it can be considered plesiomorphic. Chromosome painting has provided over the last decade data on numerous primates and mammal species belonging to different orders (Haig, 1999; O'Brien et al., 1999a,b). Chromosome painting among tree shrews, lemurs, and humans was recently reported, and an ancestral karyotype for all primates was proposed (Müller et al., 1997, 1999). Tree shrews provide a reasonable outgroup to reconstruct the ancestral karyotype of all primates, and several hypotheses have been proposed (Müller et al., 1999; O'Brien and Stanyon, 1999).

Mechanisms and direction of change in galago karyotypes

The painting results in galagos can be compared to the proposed ancestral primate karyotype, and to the in situ hybridization results in other primates, especially lemurs. In situ hybridization very effectively reveals the fission and fusion of syntenies. The ancestral karyotype proposed on the basis of molecular cytogenetic analysis has a diploid number of 2n = 50. This ancestral karyotype has the following homologs to human chromosomes or chromosome segments: 1a, 1b, 2a, 2b, 3/21, 4–11, 12/22a, 12/22b, 13, 14/15 16a, 16b, 17, 18, 19a 19b, X, and Y (Müller et al., 1999). There are three common syntenic associations of human homologs present in galagos, lemurs, tree shrews, the ancestral primate karyotype, and many other mammals: 3/21, 12/22, and 14/15. These associations all represent ancestral syntenies (Haig, 1999; Müller et al., 1999).

A comparison of the two galago karyotypes, based on hybridization data and banding pattern, is shown in Figure 4. Six chromosome pairs are very similar if not identical between *O. crassicaudatus* and *G. mo-*

Fig. 1. Examples of hybridization signals produced by human chromosome probes in *O. crassicaudatus*: (**a**) human chromosome probe 6; (**b**) paint 7; (**c**) paint 10; and (**d**) paint 11. Also shown are hybridization signals produced in *G. moholi* by human chromosome probes (**e**) 1, (**f**) 7, (**g**) 11, and (**h**) 21.

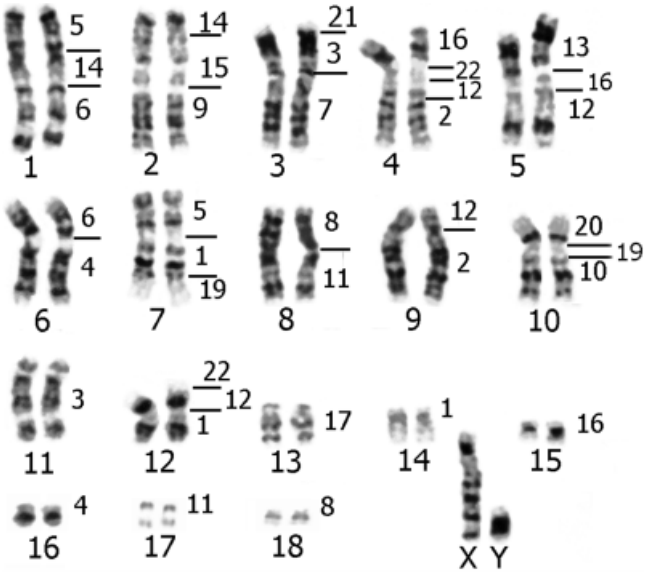

Fig. 3. G-banded karyotype of *G. moholi*, including a summary of chromosome painting results. Galago karyotype is numbered below, and human homologies are at right.

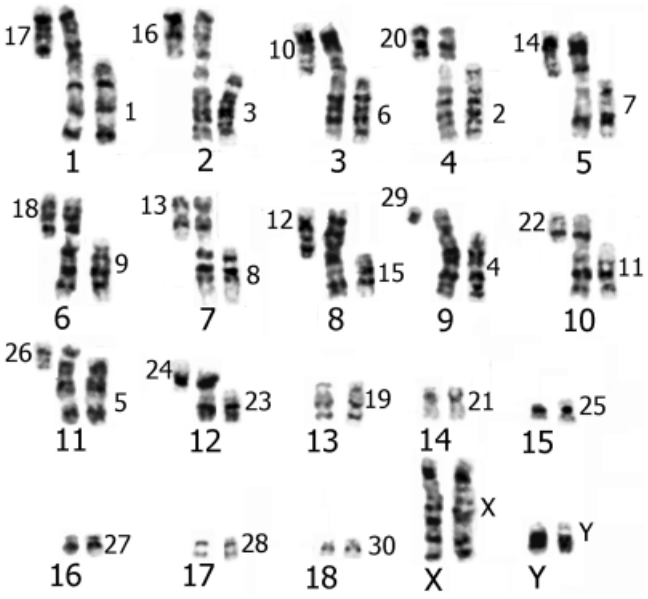

Fig. 4. Comparison of two G-banded galago karyotypes, based on in situ hybridization results. *G. moholi* chromosomes are numbered below, and *O. crasssicaudatus* homologs are numbered laterally. When two *O. crassicaudatus* chromosomes are homologous to a single *G. moholi* chromosome, the *G. moholi* chromosome is placed in the middle. When a single chromosome is homologous, *O. crassicaudatus* is placed to the right. Note that the very good match between bands suggests that only rarely have intrachromosomal rearrangements differentiated the chromosomes of these two species after their divergence.

holi: (GMO/OCR 13/19, 14/21, 15/25, 16/27, 17/28, and 18/29). Robertsonian fusions (10) can account for most of the difference between the two karyotypes. However, Robertsonian fusions are not the only mechanisms. The hybridization patterns of *G. moholi* chromosome 2 and *O. crassicaudatus* chromosome 3 and chromosome 16 can most easily be interpreted as the result of a Robertsonian fission of the *G. moholi* chromosome that produced the two *O. crassicaudatus* chromosomes. *O. crassicaudatus* chromosome 5 and chromosome 26 probably resulted from a non-Robertsonian fission of *G. moholi* chromosome 11. There are two signals for chromosome 7 in *O. crassicaudatus* and one signal in *G. moholi*. Usually additional signals are interpreted as evidence of chromosome fissioning, but the small signal homologous to a segment of human chromosome 7 in OCR 7 may have been missed in *G. moholi* 9q.

There are six synapomorphic associations linking *G. moholi* and *O. crassicadautus*: 1/19, 2/12, 6/14, 9/15, 10/19, and 12/16. Clearly the two galagos shared a relatively long period of common ancestry after the divergence of prosimians from anthropoids. Our hybridization results in the galagos show that the karyotypes of *Otolemur crassicaudatus* and *Galago moholi* differ principally by Robertsonian fusions. However, there are in addition probably two and possibly three fissions that have contributed to the differences between these two genomes. Therefore, the karyological evolution of these two species has not operated exclusively by Robertsonian fusion, as previously suggested by numerous authors on the basis of classical staining, and then banding (de Boer, 1972; Dutrillaux et al., 1982; Dutrillaux and Rumpler, 1995; Egozcue, 1970; Rumpler et al., 1983, 1989).

It is unknown whether the mechanisms and direction of change in galago karyotypes seen here for these two species are typical of galagos in general. We studied only two isolated members of a speciose group of primates. It would be informative to have molecular cytogenetic data on a number of other species, and to integrate these data with other molecular genetic studies such as those (e.g., DelPero et al., 2000) on mtDNA.

Mosaic karyotype evolution in galagos

Compared to the ancestral primate karyotype proposed by Müller et al. (1999) on the basis of chromosome painting data, *O. crassicaudatus* has 7, and *G. moholi* 17 derived associations. On this basis, *O. crassicaudatus* has a more conserved karyotype than *G. moholi*. However, if we consider the number of hybridization signals present, *O. crassicaudatus* has a more derived karyotype. A simple statistical parameter, the diversity index or Z statistic, which takes into account the fragmentation of human chromosome homologs in other species, clearly demonstrates this (Cavagna et al., 2000). This index can be calculated on the basis of two parameters: 1) number of conserved syntenies, K; and 2) total number of hybridization signals, T. We compute the index of distance as: $Z = (1 - K/T)$; complete conservation of syntenies would give $Z = 0$. Sex chromosomes are not considered in this analysis. The Z statistic is one measure of phenetic distance. In comparisons with

the proposed ancestral karyotype for *O. crassicaudatus*, Z = 1 − 7/42 or 0.83, while for *G. moholi*, Z = 1 − 9/38 or 0.76. In contrast to the conclusion from the number of derived chromosome syntenies, the lower value of Z in *G. moholi* suggests that it is less derived than *O. crassicaudatus*. We can conclude that both species are a mixture of derived and conserved karyological characters. Both galago species demonstrate mosaic chromosome evolution: *O. crassicaudatus* is more derived in terms of fragmentation of human homologs, while *G. moholi* is more derived in terms of fusions. Neither species has the intact ancestral primate karyotype. However, before we reconstruct the ancestral karyotype of all lorids or even galagos, more chromosome painting data, including a wide range of species, are needed.

Limited intrachromosomal evolution suggested by combining painting and chromosome banding results

Chromosome painting results, in general, provide data regarding interchromosomal rearrangements (translocations), and are only rarely informative about intrachromosomal rearrangements (i.e., inversions). A preliminary assessment of intrachromosomal rearrangements can be gained by combining chromosome painting data and G-banding data (Fig. 4). The high degree of correspondence in the banding patterns between the two galagos indicates that intrachromosomal rearrangements are probably limited (i.e., most karyological differences can be explained by translocations and fissions, with limited further rearrangements). This conclusion agrees with previous assessments based on chromosome banding alone (Dutrillaux and Rumpler, 1995; Rumpler et al., 1983). However, interpretations regarding the absence or presence of intrachromosomal rearrangements based on banding comparisons should be treated as hypotheses to be tested with subchromosomal DNA probes, which allow rearrangements such as inversions to be more securely recognized (Müller et al., 2000).

Common evolutionary stem between galagos and lemurs

There are no common derived associations of human homologs between galagos and lemurs, which would indicate a lengthy period of common ancestry after divergence from the anthropoid primates. There may be, however, a number of fissions of syntenies indicative of a common phylogenetic root linking lemurs and lorids. The fissioning of chromosomes 1, 4, 5, 6, 7, and 8 could be synapomorphic traits. However, further reciprocal chromosome painting with probes derived from these species will be necessary to support this conjecture.

Prosimians do not have primitive primate karyotypes

It has often been proposed that some prosimians have retained karyotypes that are close to the ancestral karyotype of all primates (Dutrillaux, 1979), reflecting a *scala naturae* attitude toward phylogenetic reconstruction that can be soundly rejected. To date, all the prosimian species studied with molecular cytogenetic techniques have highly derived karyotypes, both in diploid number and in apomophic syntenic associations. *Eulemur fulvus mayottensis* has 6 and *E. m. macaco* has 15 derived associations (both have Z = 0.76) (Müller et al., 1997). This conclusion becomes even more striking when we consider that some nonprimate mammals (carnivores) have karyotypes which are more similar to humans than are those of prosimians. Without doubt, the human karyotype is less derived and closer to the ancestral primate karyotype than are any prosimian karyotypes yet examined (Fig. 5).

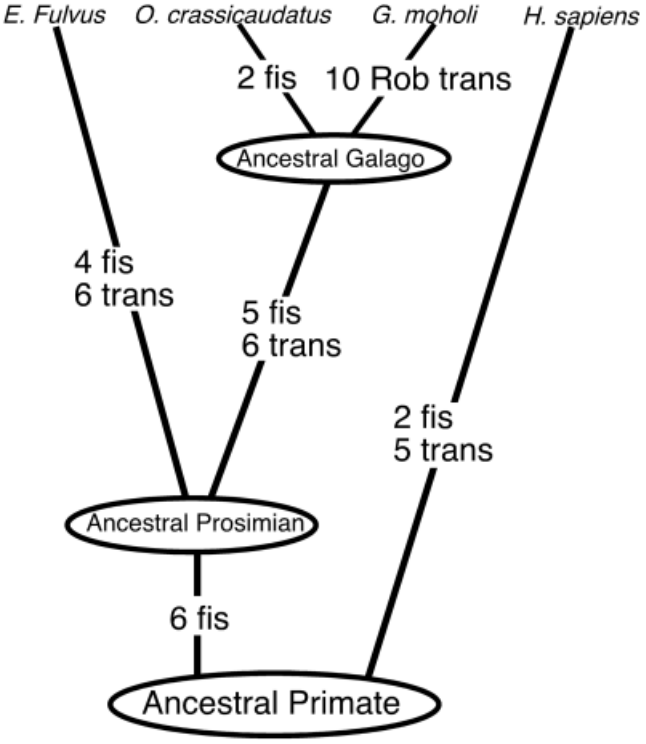

Fig. 5. Phylogeny of interchromosomal rearrangements in *Eulemur fulvus*, *Otolemur crassicaudatus*, *Galago moholi*, and humans, based on in situ hybridization results. This hypothesis is based on the reconstruction of an ancestral primate karyotype in Müller et al. (1999). It can be noted that the human karyotype is closer to the ancestral primate karyotype than any of the prosimians. Fis, fissions; trans, translocations; Rob trans, Robertsonian translocations.

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