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Reconstruction of genomic rearrangements in great apes and gibbons by chromosome painting

(primates/phylogeny/comparative cytogenetics/chromosome aberrations/chromosome-specific staining)

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ABSTRACT **The homology between hylobatid chromosomes and other primates has long remained elusive. We used chromosomal *in situ* suppression hybridization of all human chromosome-specific DNA libraries to "paint" the chromosomes of primates and establish homologies between the human, great ape (chimpanzee, gorilla, and orangutan), and gibbon karyotypes (*Hylobates lar* species group, $2n = 44$). The hybridization patterns unequivocally demonstrate the high degree of chromosomal homology and synteny of great ape and human chromosomes. Relative to human, no translocations were detected in great apes, except for the well-known fusion-origin of human chromosome 2 and a 5;17 translocation in the gorilla. In contrast, numerous translocations were detected that have led to the massive reorganization of the gibbon karyotype: the 22 autosomal human chromosomes have been divided into 51 elements to compose the 21 gibbon autosomes. Molecular cytogenetics promises to finally allow hylobatids to be integrated into the overall picture of chromosomal evolution in the primates.**

The origin of human and great ape chromosomes is well understood from comparative chromosome-banding analysis and has been widely confirmed by gene mapping (1–4). Yet the lesser apes, which are classified with great apes and human in the same primate superfamily Hominoidea, apparently show no karyological relationship with any other primate species (5–7). Only very few gibbon chromosomes appear to have a similar banding pattern compared with other catarrhine chromosomes. Even within hylobatids very few chromosome homologies can be found between species differing in diploid number ($2n = 38$, 44, 50, and 52), and there is little gene-mapping data supporting chromosome homologies between human and gibbon species (8). Gene-mapping data for the *Hylobates lar* species group ($2n = 44$) has, to our knowledge, not yet been reported. In contrast to the highly heterogeneous karyotypes, the hylobatids are fairly homogeneous in most other biological characteristics that also reveal their close relationship to great apes and humans (9–13). Molecular studies place gibbon divergence from pongids and humans at 16–23 million years, whereas orangutans diverged 12–16 million years ago, and human and African apes diverged ≈5–10 million years ago (13, 14).

Recently, chromosomal *in situ* suppression (CISS) hybridization (15–17) has been applied to establish homologies between human and primate chromosomes (18–21). In contrast to previous comparative gene-mapping experiments that have been restricted to single-copy sequences, this approach provides an overall comparison of DNA sequence homologies for complete chromosomes and extended chromosome subregions. In the present study phage or plasmid libraries derived from all 24 flow-sorted human chromosomes were hybridized to chromosome preparations of chimpanzee, gorilla, orangutan, and three of the 44-chromosome gibbon species (*H. lar*, *Hylobates moloch*, and *Hylobates klossii*).

MATERIALS AND METHODS

Cell Lines and Sample Preparation. Metaphase chromosome spreads for *in situ* hybridization experiments were prepared and stored as reported (18). Great ape chromosome preparations were obtained from lymphocyte or fibroblast cultures established from material provided by the Heidelberg Zoo (courtesy of H. Wiesner, Munich, and A. Poley, Heidelberg). Gibbon chromosomes were prepared from lymphoblastoid cell lines HylE-7, HylE-5, and HykE-1 established from a male *H. lar*, a female *H. moloch*, and a female *H. klossii*, respectively. Cell lines were immortalized with Epstein–Barr virus derived from the B95-8 cell line. (For further information about the origin of the individuals write to T.I.). The G-banded karyotypes of all three cell lines are the same and appeared to be normal diploid ($2n = 44$), including a polymorphism for chromosome 8. These hylobatids are known to be polymorphic within and between species for this chromosome (22). For gibbon chromosome 8 the following karyotypes were found: 8b/8c, 8a/8c, and 8b/8b. Chromosome banding before CISS hybridization was done as described (23).

DNA Probes and *in Situ* Hybridization. CISS hybridization of DNA library probes to human and primate chromosomes was done as described (15, 16). Briefly, DNA prepared from chromosome-specific human bacteriophage libraries (American Type Culture Collection nos. LA01NS01, LL02NS01, LA03NS02, LA04NS02, LA05NS01, LL06NS01, LA07NS01, LL08NS02, LL09NS01, LL10NS01, LL11NS01, LA12NS01, LL13NS02, LL14NS01, LL15NS01, LL16NS03, LL17NS02, LL18NS01, LL19NS01, LL20NS01, LA21NS01, LL22NS01, LA0XNS01, and LL0YNS01) or plasmid libraries (pBS1 to pBS4, pBS6 to pBS22, pBSX, and pBSY) were used as probes. The plasmid libraries were provided by J. Gray (University of California, San Francisco) and are described in detail by Collins *et al.* (24). In some experiments human genomic DNA obtained from the blood of a male individual (46,XY) was hybridized to gibbon chromosome preparations.

Library DNA was labeled with either biotin or digoxigenin by standard nick-translation assays. CISS hybridization was done as described in detail elsewhere (16). Briefly, 10 μl of the hybridization mixture [50% (vol/vol) formamide/1× stan-

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Abbreviations: AMCA, 7-amino-4-methylcoumarin-3-acetic acid; CISS, chromosomal *in situ* suppression; FITC, fluorescein isothiocyanate; *PTR*, *Pan troglodytes* (chimpanzee); *GGO*, *Gorilla gorilla*; *PPY*, *Pongo pygmaeus* (orangutan).

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dard saline citrate/10% (wt/vol) dextran sulfate] containing 1–2 μg of labeled phage library DNA or 300–500 ng of labeled plasmid library DNA, 4–8 μg of human genomic competitor DNA, and 10 μg of salmon sperm DNA was heated 5 min at 75°C. The denatured DNA was allowed to preanneal for 20 min at 37°C before the hybridization mixture was dropped on heat-denatured primate chromosome preparations and covered with 18 mm × 18 mm coverslips. After hybridization and washing of the slides, biotinylated DNA probes were detected with fluorescein isothiocyanate (FITC) coupled with avidin (Vector Laboratories). In double-hybridization experiments digoxigenin-labeled probes were detected with FITC-conjugated mouse anti-digoxigenin antibodies (Boehringer Mannheim), whereas biotin-labeled probes were detected with avidin coupled with AMCA (7-amino-4-methylcoumarin-3-acetic acid; Vector Laboratories). AMCA signals were amplified once as previously described for FITC signals (25).

RESULTS

We analyzed the hybridization pattern of all 24 human chromosome-specific libraries in chimpanzee (*Pan troglodytes; PTR*), gorilla (*Gorilla gorilla; GGO*), orangutan (*Pongo pygmaeus; PPY*) and in gibbons (*H. lar, H. klossii*, and *H. moloch*). The resulting hybridization pattern is summarized in Table 1.

Painting of Great Ape Chromosomes. In all great apes the human chromosome 2 library painted two chromosome pairs. The only other translocation found was a reciprocal translocation between chromosomes homologous to human chromosomes 5 and 17 (Fig. 1 *a* and *b*; for further details see ref. 21). Other interchromosomal rearrangements that have been proposed (for review see ref. 26) could be ruled out. In particular, no hybridization signals were observed that could be expected from the proposed translocation in the orangutan between chromosomes homologous to human chromosomes 8 and 20 (3) (Fig. 1 *c* and *d*). Still very small interstitial or terminal translocations may only be identified with appropriate, subregional DNA probes.

Libraries from acrocentric human chromosomes showed occasional cross hybridization to the short arms of other acrocentric human and great ape chromosomes, possibly due to sequence homologies in nuclear organizer regions and/or pericentric heterochromatin (24). In addition, several other chromosomal subregions in hominoid chromosomes remained unlabeled. These regions include telomeric heterochromatin in the chimpanzee and gorilla, Y chromosome heterochromatin in gorilla and orangutan, and an interstitial heterochromatic band on chimpanzee chromosome 14. Intriguingly, reduced hybridization was noted on the heterochromatic short arms of chimpanzee chromosomes 12 and 13, suggesting loss of some genetic material during the formation of human chromosome 2.

Painting of Gibbon Chromosomes. All three 44-chromosome gibbon species analyzed showed the same G-banded karyotype and the same hybridization pattern with the human chromosome libraries, except for gibbon chromosome 8. In contrast to the stability of great ape karyotypes the human libraries detected many translocations in the gibbon karyotypes (for examples, see Fig. 1 *f–j*). For all libraries, the classical G-banding was done before *in situ* hybridization; photographs were taken and compared thereafter to the CISS hybridization patterns. Fig. 2 shows the identification of four gibbon chromosome segments homologous to the human chromosome 1 library. Only gibbon chromosomes labeled with human chromosome libraries 11, 14, 20, X, and Y were not involved in interchromosomal rearrangements (for example, see Fig. 1*e*). Chromosomes homologous to human chromosomes 7, 13, 15, 18, 21, and 22 were translocated to another gibbon chromosome (for examples, see Fig. 1 *f* and *g*). Other hylobatid chromosomes showed multiple translocations composed of up to five different human chromosome segments (mode = 2) (for examples, see Fig. 1 *h–j*). Accordingly, the 22-autosomal human chromosomes could be divided into 51 segments to compose the 21 gibbon autosomes (Table 1).

CISS hybridization with the human chromosome 5 library showed a heterozygous pericentric inversion of gibbon chromosome 8 (type 8c) in cell line HylE-7 (Fig. 1*j*) as previously described in *H. lar* individuals. Instead, type 8a (found in cell line HykE-1 from the *H. klossii* individual) hybridization indicated a translocation between gibbon chromosomes 8 and 13 (data not shown).

Table 1. Chromosome homologies between human and hominoids (chimpanzee, gorilla, orangutan, and gibbons) revealed by chromosome painting with human chromosome-specific DNA libraries

Human chromosome-specific DNA library	Homologies detected in hominoid species			
	Chimpanzee	Gorilla	Orangutan	Gibbon*
Chromosome				
1	*PTR* 1	*GGO* 1	*PPY* 1	*HLA* 5q; 7q; 9p; 19
2	*PTR* 12; 13	*GGO* 12; 11	*PPY* 12; 11	*HLA* 1p; 10p; 12p; 16p; 16q
3	*PTR* 2	*GGO* 2	*PPY* 2	*HLA* 4p; 7q; 10q; 12q
4	*PTR* 3	*GGO* 3	*PPY* 3	*HLA* 2q; 3q; 18q
5	*PTR* 4	*GGO* 4q; 19p; 19q	*PPY* 4	*HLA* 6p; 6q; 8p or 8q†; 18p
6	*PTR* 5	*GGO* 5	*PPY* 5	*HLA* 3p; 3q 20
7	*PTR* 6	*GGO* 6	*PPY* 10	*HLA* 1p; 1q
8	*PTR* 7	*GGO* 7	*PPY* 6	*HLA* 7q; 9p; 9q
9	*PTR* 11	*GGO* 13	*PPY* 13	*HLA* 8q; 13q
10	*PTR* 8	*GGO* 8	*PPY* 7	*HLA* 2p; 2q; 3q
11	*PTR* 9	*GGO* 9	*PPY* 8	*HLA* 11
12	*PTR* 10	*GGO* 10	*PPY* 9	*HLA* 7p; 7q; 10p; 10q; 14p; 14q
13	*PTR* 14	*GGO* 14	*PPY* 14	*HLA* 4q
14	*PTR* 15	*GGO* 18	*PPY* 15	*HLA* 17
15	*PTR* 16	*GGO* 15	*PPY* 16	*HLA* 15p; 15q
16	*PTR* 18	*GGO* 17	*PPY* 18	*HLA* 6p; 6q; 8p
17	*PTR* 19	*GGO* 4p; 4q; 19q	*PPY* 19	*HLA* 8q; 13p; 16p; 16q
18	*PTR* 17	*GGO* 16	*PPY* 17	*HLA* 5p
19	*PTR* 20	*GGO* 20	*PPY* 20	*HLA* 10p; 14p; 14q; 16q
20	*PTR* 21	*GGO* 21	*PPY* 21	*HLA* 21
21	*PTR* 22	*GGO* 22	*PPY* 22	*HLA* 15q
22	*PTR* 23	*GGO* 23	*PPY* 23	*HLA* 8p; 8q
X	*PTR* X	*GGO* X	*PPY* X	*HLA* X
Y	*PTR* Y	*GGO* Y	*PPY* Y	*HLA* Y

Assignment of great ape chromosomes was made by simultaneous 4',6-diamidino-2-phenylindole (DAPI) banding (data not shown); assignment of gibbon chromosomes was achieved by G-banding before CISS hybridization (compare Fig. 2 and text). Great ape and gibbon chromosomes or chromosome arms with complete or partial homology to specific human chromosomes are indicated. Many gibbon chromosome arms are painted with more than one library. For details of the extension of the homologies, see Fig. 3. *HLA, H. lar.*

**Hylobates* sp. ($2n = 44$).

†Gibbon chromosome 8 is polymorphic (see text).

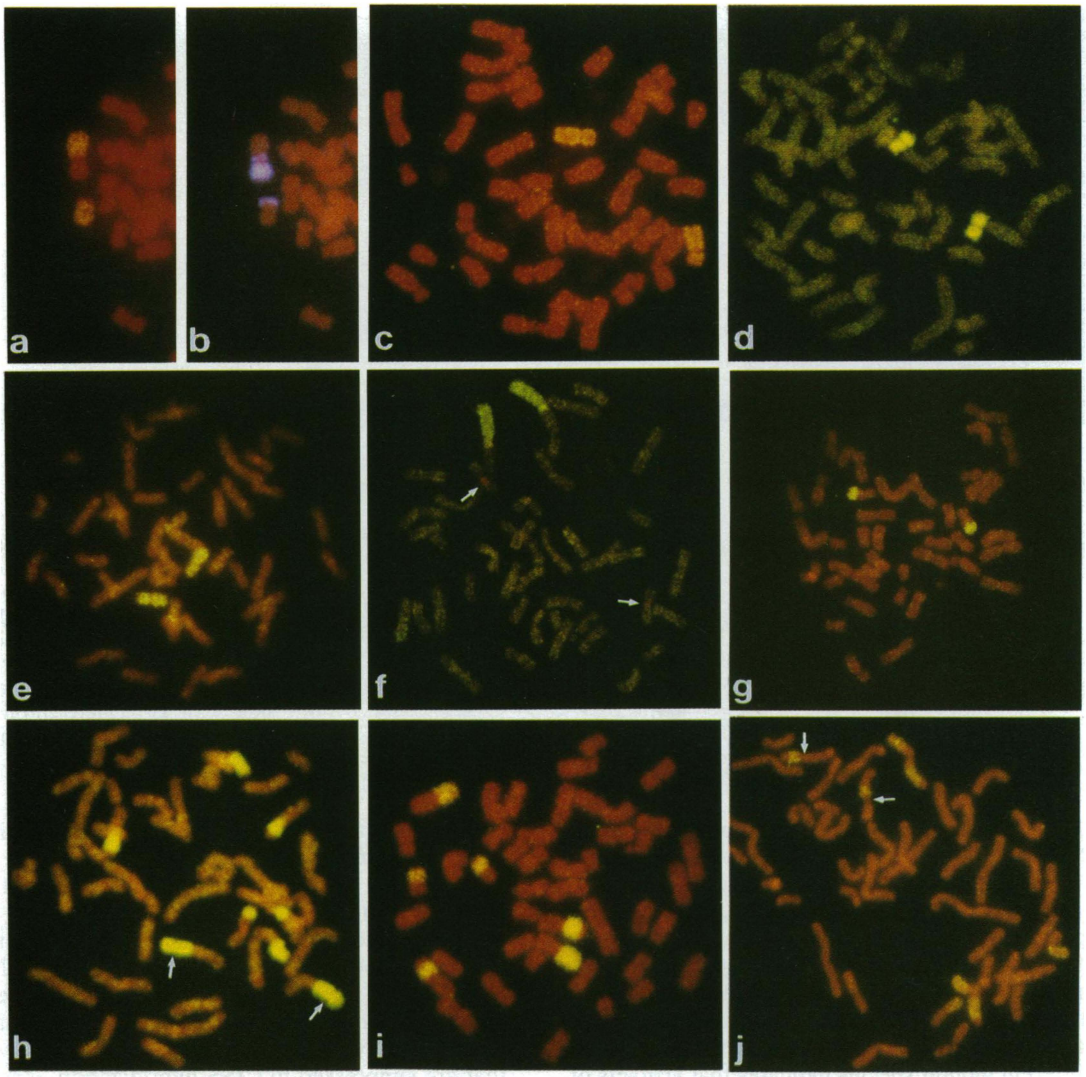

FIG. 1. Metaphase chromosomes of *GGO* (*a* and *b*), *PPY* (*c* and *d*) and *H. lar* (*e–j*) painted with biotin- or digoxigenin-labeled human chromosome-specific phage or plasmid DNA libraries. Hybridization sites were detected with FITC-conjugated antibodies against digoxigenin (*a*), avidin conjugated with 7-amino-4-methylcoumarin-3-acetic acid (*b*), or avidin-conjugated with FITC (*c–j*). Chromosomes were counterstained with propidium iodide. For chromosome identification G-banding was done before CISS-hybridization (data not shown). (*a* and *b*) Partial metaphase spread from *GGO* after two-color CISS-hybridization with libraries for human chromosomes 5 (*a*) and 17 (*b*), respectively. Note the reciprocal translocation detected by these libraries on gorilla chromosomes 4 and 19. (*c* and *d*) Orangutan chromosomes 6 and 21 painted with human chromosome 8 and 20 libraries, respectively. (*e*) Human chromosome 11 DNA library delineates gibbon chromosome 11. Painting with human chromosome 7 (*f*) and chromosome 21 DNA libraries (*g*) indicates that the entire homologous chromosomes were translocated to gibbon chromosomes 1 and 15, respectively. Note that the nuclear organizer region (*f*, arrows) on gibbon chromosome 12 was not labeled by any of the human DNA libraries hybridized under suppression conditions. (*h–j*) Painting with human DNA libraries from chromosome 3 (*h*), chromosome 4 (*i*), and chromosome 5 (*j*) demonstrates the occurrence of multiple reciprocal translocations. In *h* the arrows point to a small unlabeled subregion in gibbon chromosome 4. In *j* arrows indicate a heterozygous pericentric inversion in gibbon chromosome 8 after painting with the human chromosome 5 library. Note that the painted region is located on the short arm of one homolog and on the long arm of the other.

For identification of the apparent breakpoints a comparison of G-banding and CISS hybridization patterns was done in 5–10 metaphase spreads for each human library and investigated 44-chromosome gibbon species. An idiogram summarizing all detected breakpoints is provided in Fig. 3. Still the localization of these breakpoints should be considered approximate for several reasons. (*i*) The banding resolution of the lymphoblastoid samples was limited to ≈300–400 bands. (*ii*) The borders of painted versus nonpainted chromosome regions were occasionally somewhat fuzzy, probably due to the denaturation of chromosomes. (*iii*) Some swelling of the chromosomes was noticed after the *in situ* hybridization procedure that also slightly impaired a precise overlay of the pictures from a given chromosome after G-banding and subsequent painting.

Notably, cross-hybridization events as found with libraries from human acrocentric chromosomes in great apes (see above) were not apparent when these libraries were hybridized to gibbon chromosomes. Furthermore, the single nuclear organizer region of the "marker chromosome" (gibbon chromosome 12) (Fig. 1*f*), as well as several pericentromeric and interstitial bands in the gibbons, remained unlabeled when hybridized under suppression conditions with any of the human chromosome-specific DNA libraries (Fig. 3) or total human DNA. In contrast, in the absence of competitor DNA at least some of these regions, especially the nuclear organizer region, became strongly painted with human genomic DNA.

DISCUSSION

In the great apes our results mostly confirm chromosome homologies previously suggested by chromosome banding and gene mapping (1–4). Conflicting hypotheses concerning particular interchromosomal rearrangements were resolved.

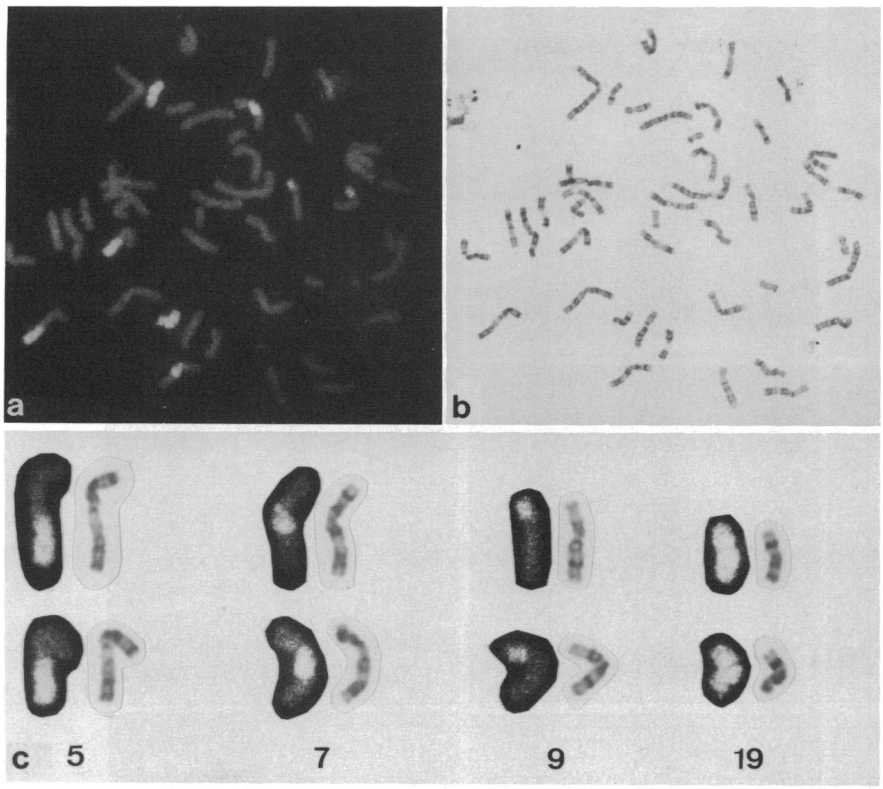

FIG. 2. Identification of painted chromosome regions on G-banded chromosomes from *H. lar*. (*a*) Metaphase spread painted with a plasmid DNA library from flow-sorted human chromosomes 1. (*b*) Same metaphase spread after G-banding. (*c*) Painted chromosomes shown side by side with G-banded chromosomes at higher magnification. The gibbon chromosome number is indicated below each pair (compare with Fig. 3).

In addition to the well-known fusion-origin of human chromosome 2, the evolutionary-derived reciprocal translocation in the gorilla between chromosomes homologous to human chromosomes 5 and 17 was confirmed (18, 21), whereas other previously proposed translocations were rejected.

Our results show that translocations have played a major role in the massive karyotypic reorganization in hylobatids. With exception of gibbon chromosome 8, the consistency of the translocation patterns in lymphoblastoid cell lines derived from three different gibbon species indicates that artifacts of cell transformation and *in vitro* cultivation can be ruled out. Although the CISS hybridization technique with human chromosome-specific libraries is ideally suited to identify translocations of homologous chromosome segments, its potential to detect intrachromosomal changes is limited. In spite of this limitation, our present data indicate the occurrence of numerous intrachromosomal rearrangements. To match the banding patterns of human autosome segments homologous with gibbon counterparts, intrachromosomal rearrangements have often to be assumed (data not shown). In several gibbon chromosomes—i.e., no. 6 (Figs. 1*j* and 3), 14 (Fig. 3), and 16 (Fig. 3), two chromosomal subregions painted by one human autosome library were interrupted by a region homologous to another human autosome. The assumption of a single translocation followed by a pericentric inversion appears to be the easiest explanation for these findings. It is likely that other intrachromosomal rearrangements have occurred in the gibbons but have not been identified so far. In the future a reliable estimate of the magnitude of such effects may be obtained by using multiple-color *in situ* hybridization with subregional probes.

Balanced inversion and translocation polymorphisms have been reported for different gibbon species (5, 6, 22, 27). Inversions were previously hypothesized to account for the three forms—a, b, and c—of gibbon chromosome 8 (22). Our data support this hypothesis for chromosome forms 8b and 8c. In contrast, the data suggest that a reciprocal translocation of gibbon chromosomes 8 and 13 led to form 8a. However, we cannot exclude the possibility that rearrangements restricted to one of the three lymphoblastoid gibbon cell lines investigated so far have occurred *in vitro*. Additional gibbon specimens must be analyzed with *in situ* hybridization to rule out this possibility.

Although some subregions in hominoid chromosomes remained unlabeled with chromosome-specific DNA libraries, hybridization experiments with human whole-genomic DNA labeled all chromosome regions. One explanation is that formerly unhybridized regions contain repetitive sequences that are suppressed in CISS-hybridization experiments.

The data support the hypothesis that changes in the gibbon karyotype are characterized by an extremely high evolutionary rate compared with other primates (7, 28). Papionini karyotypes (macaques, baboons, mandrills, and cercocebus monkeys) are conservative and nearly identical in all these species (29). These karyotypes have frequently been used as an "outgroup" for determining the direction of chromosomal rearrangements in hominoid species. Recently, we applied chromosome painting with all 24 human chromosome-specific DNA libraries to chromosome preparations of *Macaca fuscata* (20). Only three macaque chromosomes were each hybridized by two separate human libraries. Even though Papionini are evolutionarily more distant from humans than hylobatids, all other libraries painted an entire homologous counterpart in the macaque karyotype.

The mechanism of the rapid rate of chromosomal evolution in gibbons remains to be explained. It is not clear whether gibbons have a higher chromosomal mutation rate or whether chromosomal mutations have occurred at a normal rate but were more easily fixed, or both. Molecular data show that the evolutionary rate seen in the gibbon genome is within the range of other primates (13). Rates of chromosomal rearrangements and molecular evolution, however, may be independent.

It is generally argued that the heterozygotes for chromosomal rearrangements (especially translocations) should have drastically reduced fertility due to the loss of genetically unbalanced offspring (30, 31). Population bottlenecks and inbreeding have been proposed to explain the rapid fixation of

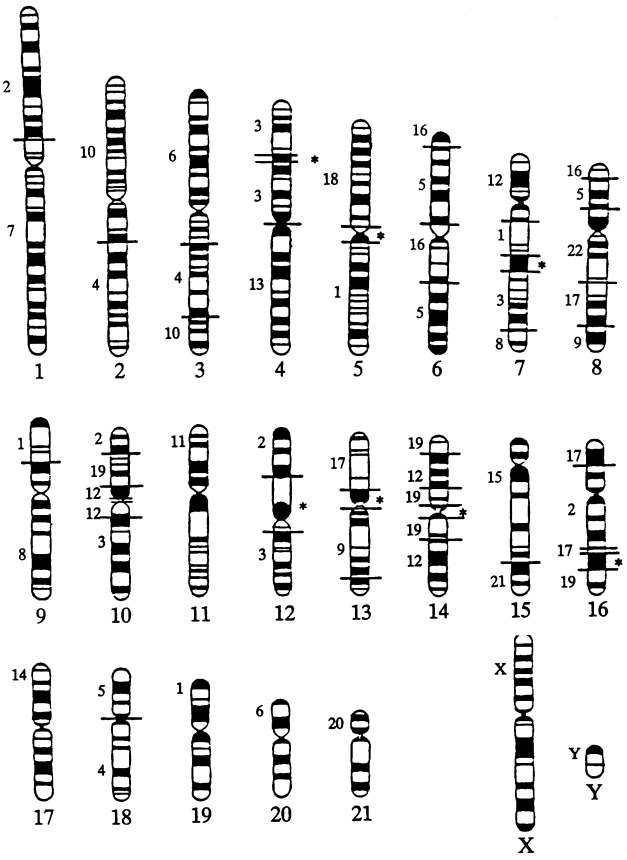

FIG. 3. Idiogramatic summary of the hybridization patterns of human chromosome-specific DNA libraries to G-banded chromosomes of *H. lar* [idiogram after Stanyon and Chiarelli (7)]. Gibbon chromosome numbers are given below each chromosome. The nuclear organizer region-bearing chromosome, which so far has been referred to as "marker chromosome" and not included into the numbering system, is designated here as chromosome 12. Numbers at left of each chromosome indicate the subregions painted with the respective human chromosome-specific library. Bars indicate the tentative translocation breakpoints identified by superimposition of G-banded chromosomes with the same chromosomes after CISS hybridization (≈300- to 400-band stage). Some chromosomal subregions indicated by asterisks remained unlabeled with any human library.

such rearrangements (32, 33). On the one hand, a rapid fixation may have been favored by gibbon social structure and ecology, including monogamous matings, nuclear family units, and an arboreal lifestyle. In contrast, the chromosomally conservative Papionini live in large terrestrial groups with multiple male and multiple female matings (28, 29). On the other hand, the reduction of fitness due to chromosomal rearrangements may be less drastic than normally proposed. Even though very few gibbons have been karyotyped, most species have chromosome polymorphisms. Apparently, all 44-chromosome gibbon species share such polymorphisms (22), indicating that they are not transient but have even survived speciation events. These findings argue against drastic bottlenecks during the divergence of the various 44-chromosome gibbon species. Other gibbon species have probable translocation polymorphisms (5, 6) or subspecies with different karyotypes (34), suggesting that the process of karyological transformation is still underway. CISS hybridization analysis of larger sample sizes, especially of free-ranging lesser apes, could provide further insight into the possible role of chromosome polymorphisms during speciation events.

The three other karyomorphs in hylobatids remain to be fully studied with the CISS hybridization technique. Preliminary data on *Hylobates syndactylus* ($2n = 50$) show translocations not present in the *H. lar* species group and *H. klossii*. For example, the human chromosome 7 library paints segments on three different chromosomes in *H. syndactylus* (18), in contrast to the 44-chromosome gibbon species, where only one chromosome is painted. These studies promise to help resolve the evolutionary relationships within hylobatids and with other primates and will finally allow hylobatids to be integrated into the overall picture of chromosomal evolution in the primates.

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