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Homologies between Human and Marmoset (*Callithrix jacchus*) Chromosomes Revealed by Comparative Chromosome Painting

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Regions of DNA homology between human and marmoset (*Callithrix jacchus*) chromosomes have been demonstrated using fluorescence *in situ* hybridization. All 24 chromosome paints and two centromere repeat sequences from *Homo sapiens* (HSA) have been annealed to previously G-banded metaphase spreads of *Callithrix jacchus*. All human paint probes, except Y, successfully hybridized to marmoset chromosomes. Fifteen of them hybridized to one region only, seven to two regions, and paint 1 to three regions. Homologies proposed from previous banding comparisons have been confirmed for HSA 2, 4–6, 10–12, 18, 19, 21, and X and partially confirmed for HSA 1 and 3, but were not in agreement for HSA 14 and 17. Human centromere repeat sequences for X and 18 did not hybridize to marmoset chromosomes. Because, at present, there is the confusing situation of several different numbering systems for marmoset chromosomes, we propose a new simpler nomenclature based on descending order of chromosome size.

INTRODUCTION

Comparison of banded karyotypes of humans and other primates has been used for a number of years as a basis for studying primate phylogenies (Turleau and de Grouchy, 1973; Turleau *et al.*, 1972; Dutrillaux and Couturier, 1981). The advent of fluorescence *in situ* hybridization (FISH) and the availability of human chromosome-specific probes have enabled this comparison to be much more precise (Wienberg *et al.*, 1990). The chromosomes of humans can now be compared with those of more distantly related primates such as the marmoset.

The marmoset (*Callithrix jacchus*) and *Homo sapiens* are thought to have diverged from a common ancestor, a primitive anthropoid, approximately 30 million years ago (Strickberger, 1990). The karyotype of *Callithrix jacchus* was first studied in 1962 by Benirschke *et al.*, who showed this species to have a diploid number of 46: 22 pairs of autosomes, a submetacentric X, and an acrocentric Y. The chromosomes were first banded in 1974 by Perrotez, who carried out R-banding and proposed a basic nomenclature. In 1981, Dutrillaux and Couturier, using various banding techniques, verified the universal presence of 22 pairs of autosomes and two sex chromosomes per cell. They proposed an alternative nomenclature based on euchromatin segments, which are not variable. They also proposed a possible evolutionary process deriving the present human and marmoset karyotypes, by rearrangements of ancestral chromosomes. In 1982, Soares *et al.* published a different nomenclature, which was also followed by Seuanez *et al.* in 1988. A further G-band nomenclature was presented by Nagamachi *et al.* (1988), which was followed in a more recent study in 1990 by de Souza Barros *et al.*

Linkage studies have progressed in parallel with the cytogenetics. By 1991, a closely related marmoset had 16 human linkage groups attributed to its genome, comprising 27 genes, homologous to the human equivalent (O'Brien and Marshall Graves, 1991). Although these human genes are known to be present in the marmoset genome, none have as yet been assigned to any one particular marmoset chromosome.

Following Wienberg *et al.* (1990), Jauch *et al.* (1992) used FISH with human chromosome-specific DNA libraries (paints) as probes, establishing homologies between the karyotypes of humans, great apes, and gibbons. FISH with all 24 human chromosome paints was also carried out on the Macaque (*Macaca fuscala*) karyotype (Wienberg *et al.*, 1992) and that of the chimpanzee (*Pan troglodytes*) (Luke and Verma, 1993). Scherthan *et al.* (1994) hybridized selected human chromosome paints to the karyotypes of rodents, even-toed ungulates, and whales. They described the technique as "ZOO FISH."

By using ZOO FISH on G-banded marmoset metaphase chromosome spreads, we have been able to visu-

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alize the DNA sequences homologous to specific human chromosomes and locate their karyotypic positions. These results can be used to verify or refute comparisons made from banding analysis, which by their nature are speculative and may not reflect syntenic homology at the DNA level.

We show here that human chromosome homologies can be established for every part of the marmoset karyotype, enabling the rapid assignment of marmoset genes and linkage groups with human equivalents to their appropriate marmoset chromosome. Before this can be performed, a single satisfactory numbering system must be agreed upon to replace the multiple systems already in existence. We are proposing here that a system based on the original karyotype of Benirschke *et al.* (1962), where chromosomes are arranged in order of descending size, as has been done for the great apes (Paris Conference supplement, 1975; ISCN, 1985), would be the most satisfactory way of organizing the karyotype.

MATERIALS AND METHODS

Marmoset blood was obtained from two normal healthy males from the Institute of Zoology, Regents Park Zoo (London).

All chromosome-specific paints were isolated from flow-sorted human chromosome-specific libraries and supplied by "CAMBIO" (Cambridge). The probes provided already contained repetitive DNA and were ready to apply directly to slides. The alpha satellite repeat sequences used (HSA 18 and HSA X) were supplied by Oncor.

Marmoset blood was cultured using standard protocols, alongside normal human male control blood (Verma and Babu, 1989). To marmoset blood cultures only, an additional 50 μl of heparin (5000 units/ml) was added to 1 ml of blood to avoid clotting that was apparent in earlier cultures. The resulting lymphocyte metaphase cells were fixed in methanol:acetic acid (3:1) and dropped onto slides.

G-banding. After "aging" at 60°C overnight, the slides were incubated in 2× SSC for 2–4 h at 60°C, then placed in 2.8% Difco bactotrypsin for 15–30 s at 10°C. The slides were then washed in buffer (BDH G-banding buffer 6.8) before being placed in Giemsa stain (1:10 Giemsa:buffer) at room temperature for 5–10 min. After washing in tap water, the slides were mounted in buffer, and 6 to 20 G-banded metaphases were photographed. The positions of the cells on the slide were noted using a vernier scale, for future location after FISH.

To remove the Giemsa stain prior to *in situ* hybridization, the slides were dehydrated through 75, 90, and then 100% ethanol before being air-dried.

Fluorescence in situ hybridization. A standard FISH protocol (Pinkel *et al.*, 1986) was used to prepare the cells for addition of the paint or repeat probes. Ten nanograms of alpha satellite repeat probe and 7.5 μl of paint probe were used per slide. Slides were then placed on a hotplate in an 80°C oven for 3 min, denaturing both probe and chromosomes simultaneously. The slides were left to hybridize in a moist-chamber for about 36 h at 37°C, then exposed to three 5-min posthybridization washes in 65–70% formamide, 2× SSC at 37°C, before being exposed to 4× SSC 5% Marvel nonfat dried milk powder to block nonspecific hybridization. The signal was developed with successive layers of avidin–FITC, biotinylated anti-avidin, and avidin–FITC. The slides were finally mounted in propidium iodide counterstain containing antifade. Metaphases previously photographed were relocated on a Nikon Optiphot microscope equipped with a MRC 600 confocal attachment for image capture. Chromosomes displaying fluorescent signal were compared to the G-band prints to ascertain which marmoset chromosomes had been labeled by the human chromosome paints.

RESULTS

Marmoset metaphase spreads were consistently seen to contain 22 pairs of autosomes and two sex chromosomes, with no apparent mosaicism in the two individuals used in this study. A representative G-banded metaphase is shown in Fig. 1 using the proposed new chromosome numbering system. The SSC G-banding method, although a far more lengthy process, had the advantage over the Hanks G-banding method of preserving the marmoset lymphocyte metaphases on the slide. Since the marmoset cells were shown to be far more vulnerable than the human controls to removal from the slide during *in situ* hybridization, this method proved to be essential for achieving useful results.

All the human paint probes used, with the exception of the Y chromosome, successfully hybridized to marmoset chromosomes. Paints for human chromosomes 4–7, 11, 12, 19, 21, and X were found to hybridize to single whole marmoset chromosomes. An example of this is shown for the human X paint in Fig. 2a. Human chromosome paints 9, 14, 17, 18, 20, and 22 were each found to hybridize to a single contiguous region within a larger marmoset chromosome, as shown for paint 14 (Fig. 2b). Human chromosome paint 3 hybridized to two marmoset chromosomes in their entirety (Fig. 2c). Human chromosome paints 2, 8, 10, 13, 15, and 16 each annealed to segments of DNA in two marmoset chromosomes, as shown for paint 13 in Fig. 2d. Human chromosome 1 was homologous to three segments of marmoset DNA (Fig. 2e), while the paint probe for the human Y chromosome did not hybridize anywhere in the marmoset karyotype. The repetitive centromeric probes specific to human chromosomes X and 18 were found not to hybridize to marmoset DNA. The quality of the hybridization signals varied from one metaphase to another, especially in the resulting digital images captured in one plane of focus. These images appear sometimes to indicate the absence of fluorescent signal on the telomeres of marmoset chromosomes (Fig. 2b). When numerous metaphases are observed through all planes of focus, a more accurate assessment of the area of probe hybridization can be made. Some human paint probes annealed to marmoset chromosomes with a greater efficiency than others. In the cases of the weaker probes such as HSA 3 (Fig. 2c), numerous metaphases needed to be assessed before the position of the probe and the area of homology could be decided. Some human paints annealed to large areas of marmoset chromosomes, producing lengthy regions of fluorescent signal. Comparison of these results with those of other paints annealing to the same marmoset chromosome adjacent to these regions established the position where homology ceased. The complete hybridization pattern and the proposed new nomenclature is shown in Fig. 3. The numbering systems from previous studies are shown under each chromosome.

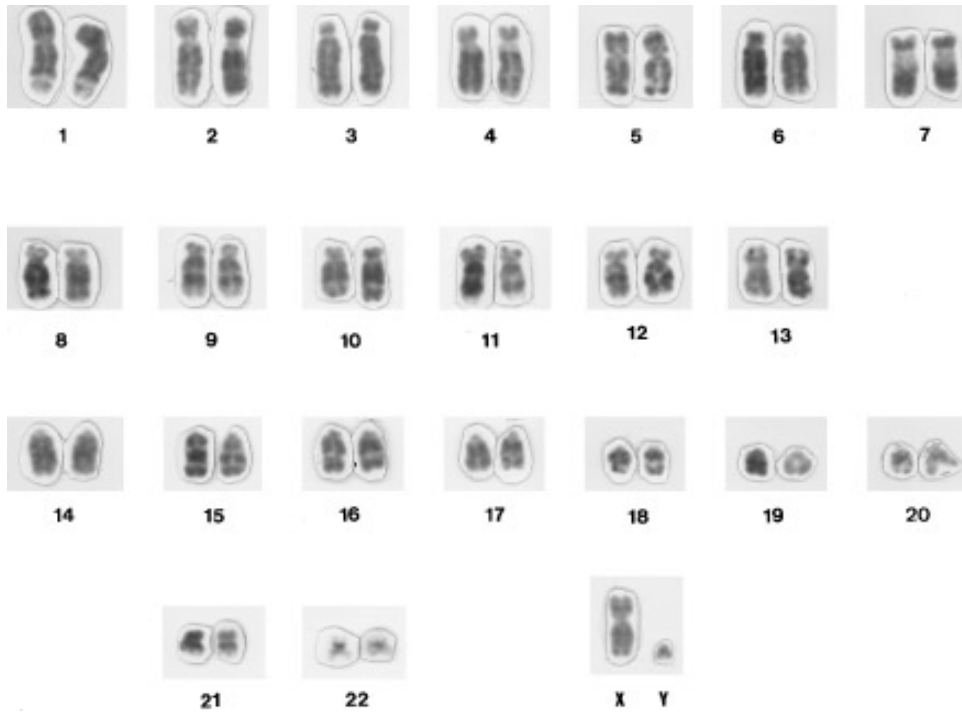


FIG. 1. Proposed new karyotype nomenclature for G-banded chromosomes of *Callithrix jacchus.*

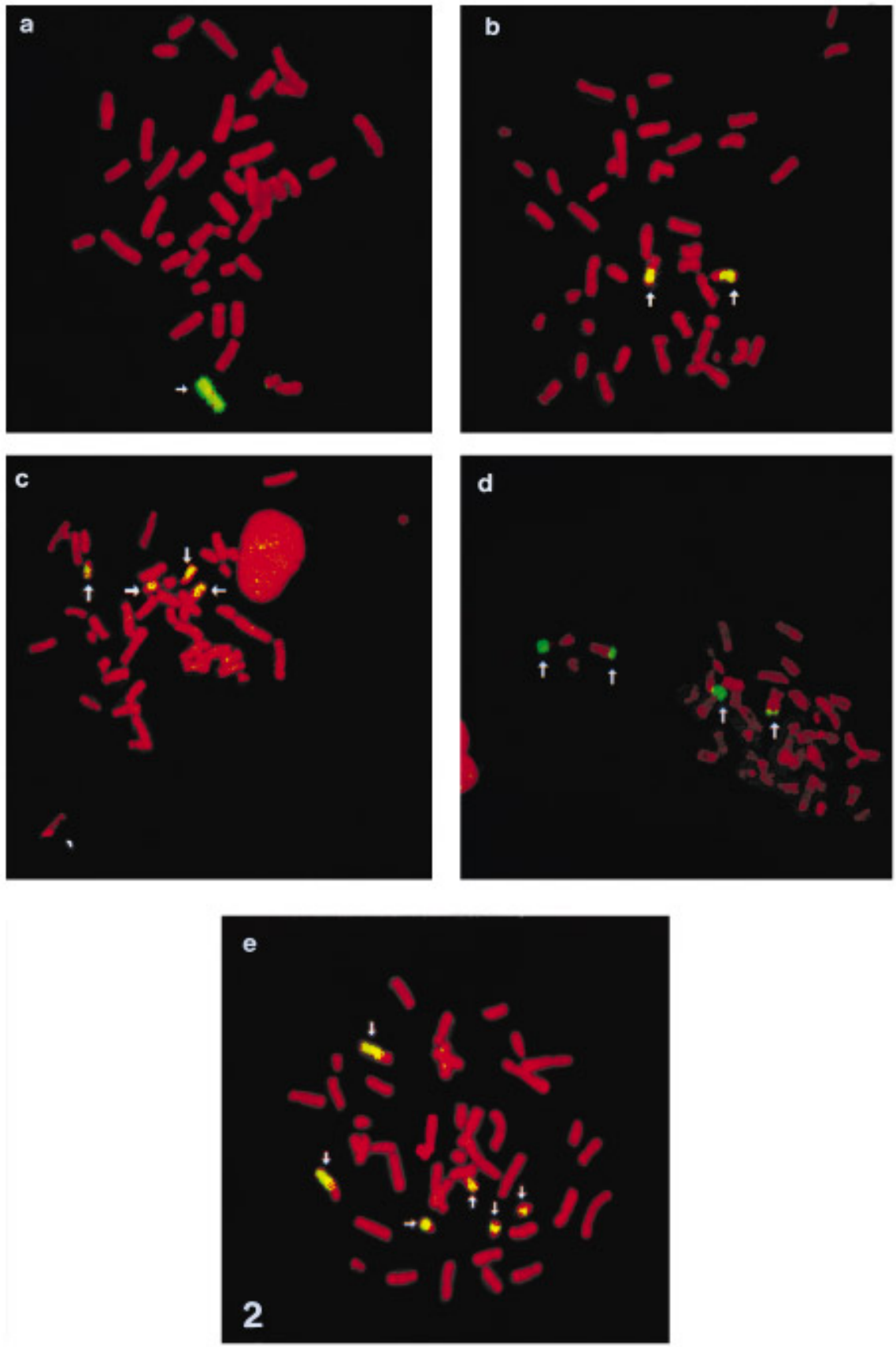
DISCUSSION

Despite 30 million years of evolution separating *Callithrix jacchus* and *Homo sapiens,* human DNA sequences are still similar enough to anneal to marmoset metaphases. The use of human chromosomal-specific paints applied to other species, coupled with G-banding analysis, is thus an effective means of establishing karyotype homology between species. It can be seen that fragmentation and rearrangement of chromosomes has occurred since the species diverged. Chromosomal homologies established using ZOO FISH between *C. jacchus* and *H. sapiens* can now be compared with those based on banding alone (Dutrillaux and Couturier, 1981). The suggested homologies for HSA 2, 4–6, 10–12, 18, 19, 21, and X were found to be entirely correct, those for HSA 1 and 3 only partially correct, and those for HSA 14 and 17 erroneous.

Despite the rearrangements from the ancestral karyotype, many chromosomal DNA regions have remained intact, either isolated or as part of a larger chromosome in both species: HSA 4–7, 9, 11, 12, 14, 17–22, and X. However, even when a human chromosome has homology with only one entire marmoset chromosome, the centromere positions and banding patterns are nearly always different from the human equivalent, suggesting that both pericentric and paracentric inversions have occurred during the evolutionary process. It should be noted that FISH painting, although able to detect chromosomal fissions, fusions, and translocations, cannot identify internal chromosomal rearrangements such as inversions or the loss or gain of genetic material. An illustration of this is the HSA paint 4, mapping to marmoset chromosome 3, and the HSA paint 5, mapping to the entirety of marmoset chromosome 2. In the human karyotype, chromosome 4 is slightly larger than chromosome 5, whereas in marmosets, chromosome 2 is slightly larger than chromosome 3. This apparent anomaly can be explained by there being either a deletion of human chromosome 4 DNA in the marmoset karyotype or a duplication event within the human 5 chromosome homologue in marmosets. Marmoset chromosome 21, which is homologous to human chromosome 21, has a G-banding pattern that indicates that it has undergone a pericentric inversion with the breakpoints just within band q2.1. Comparing accurate G-banding patterns could provide more insight into these problems. Alternatively, one could employ the FISH technique using subregional multiple color probes to compare the order of gene loci in the two species.

By their very nature, chromosome paint probes cre-

FIG. 2. (a) Human chromosome X paint annealing to the entire marmoset chromosome X. (b) Human chromosome 14 paint annealing to a region within marmoset chromosome 10. (c) Human chromosome 3 paint annealing to the entirety of marmoset chromosomes 15 and 17. (d) Human chromosome 13 paint annealing to the entire short arm of marmoset chromosome 1 and the telomeric end of the long arm of marmoset chromosome 13. (e) Human chromosome 1 paint annealing to the whole of marmoset chromosomes 18 and 19 and the entire length of the long arm of marmoset chromosome 7.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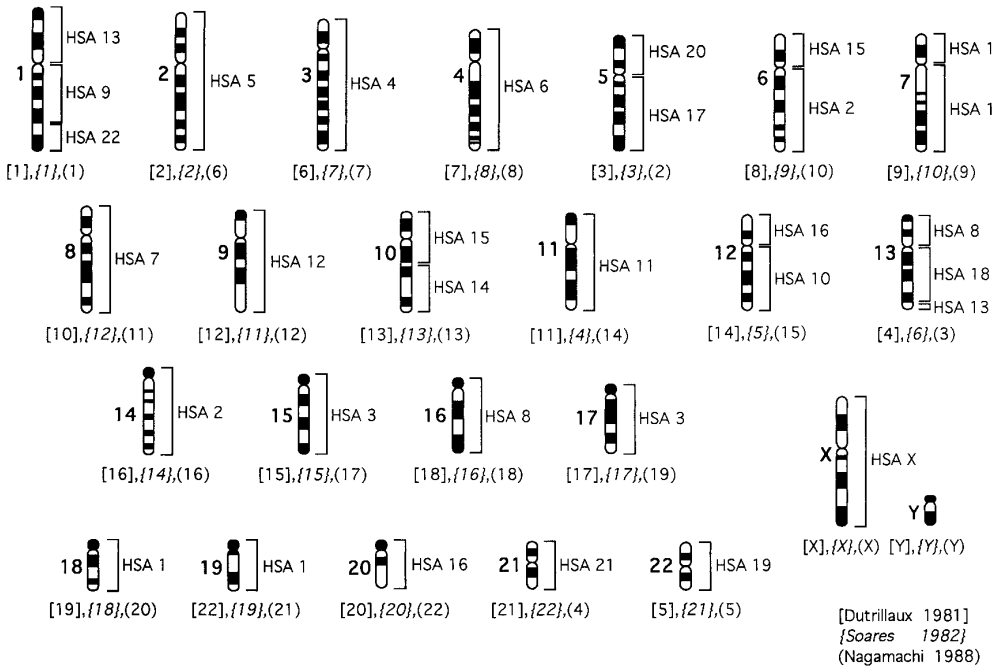

FIG. 3. Idiogram of proposed new karyotype nomenclature of *Callithrix jacchus,* with other numbering systems in brackets, showing the regions of homology with human (HSA) chromosome paints.

ate large regions of fluorescence over long regions of DNA. As a result of this, it is possible that small regions within these fluorescent bands, which do not consist of homologous DNA, might be mistakenly identified as such. When a human chromosome has been fragmented in the marmoset karyotype, as with chromosome 1, it exists as more than one region of homologous DNA. As this technique causes universal fluorescent labeling of DNA homologous to the single chromosome, it is impossible to order or orientate the constituent fragments that make up the whole without additional regional probes.

Considering the evolutionary distance between marmosets and humans, the karyotypes appear surprisingly similar. Of the 24 human chromosome paints, 15 mapped to one chromosomal region within the marmoset karyotype. This compares with 23 in the chimpanzee, 21 in the gorilla, 23 in the orangutan, 21 in the macaque, and 8 in the gibbon (Jauch *et al.,* 1992), which supports the evidence that changes in the gibbon karyotype are characterized by an extremely high evolutionary rate compared to other primates (Stanyon and Chiarelli, 1993; Marks, 1982). It also shows that the extent of differences in the karyotype of species is not necessarily proportional to the time elapsed since the species diverged, as gibbons are more closely related to humans than marmosets (Sibley and Ahlquist, 1987).

Neither of the human alpha satellite repeat probes was seen to hybridize to the centromere of the homologous marmoset chromosomes. This indicates that whereas the euchromatin in *Callithrix jacchus* has remained very similar to that of *Homo sapiens,* the sequence of noncoding centromeric repeat sequences has diverged. This may be due to the constraints imposed upon coding DNA (euchromatin) during evolution, constraints absent for heterochromatin. This also explains why no hybridization was apparent in the marmoset when using the Y paint; the paint for the Y chromosome provided by CAMBIO consists entirely of a noncoding repeat sequence present in the heterochromatic region of the human Y chromosome.

The technique of ZOO FISH directly indicates regions of interspecies chromosomal homology at the DNA level. This process complements gene mapping and helps to coordinate it. Using human chromosome-specific DNA libraries and fluorescence *in situ* hybridization, extended homologous regions comprising several megabases of DNA can be unequivocally detected in primate chromosomes. Using present technology, regions homologous to human chromosomes in all other primate karyotypes can be ascertained. This will provide invaluable information in determining chromosomal evolutionary processes and in refining existing phylogenies. The process may even be extended into examining other mammalian, or even more distantly related, species (Scherthan *et al.,* 1994). It is hoped that the proposed new size order numbering system, together with the human homologies, will make it easier to recognize individual marmoset chromosomes and facilitate rapid production of a genetic map.

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