

Centromere Repositioning

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ABSTRACT

Primate pericentromeric regions have been recently shown to exhibit an extraordinary evolutionary plasticity. In the present paper we report an additional peculiar feature of these regions which we discovered while analysing, by FISH, the evolutionary conservation of primate phylogenetic chromosome IX. If the position of the centromere is not taken into account a relatively small number of rearrangements must be invoked to account for interspecific differences. If the centromere, conversely, is included in the analysis a paradox emerges: the position of the centromere seems to have undergone, in some species, an evolutionary history independent from the surrounding markers. Additional rearrangements are then necessary to reconcile the order of the markers with centromere position. Alternatively, the evolutionary emergence of neocentromeres can be postulated.

INTRODUCTION

The molecular structure and evolution of the eukaryotic centromere was remained very elusive. Despite its importance in cell division, the nature of the centromere remains poorly understood. Typically, the centromeres of primate chromosomes are composed of long arrays of alphoid sequences, organized in tandemly repeated monomers of approximately 171bp (Maio 1991; Willard and Wayne 1987; Choo et al. 1997). The evolution of alphoid DNA has been very rapid. Comparative Fluorescence In Situ Hybridization (FISH) studies in great apes using human alphoid probes have revealed substantial divergence in both the nature of the sequence as well as its location among chromosomes belonging to the same phylogenetic group (Archidiacono et al. 1995; Warburton et al. 1996). Pericentromeric regions exhibit even more complex evolution. We have investigated the organization and recent evolution of the pericentromeric region of chromosome 10, chosen as a model since it was the only chromosome for which a detailed physical map was available (Jackson et al. 1999). The results have indicated that this region has undergone an unprecedented level of rearrangements including duplications, transpositions, inversions and deletions. Although the data are limited, this plasticity seems to be a general feature of many different pericentromeric regions (Murphy and Karpen 1998; Eichler et al. 1999). Here we report a study on the evolutionary organization of the phylogenetic chromosome IX in primates suggesting an additional peculiar property of these regions: in some species the centromere position exhibits an evolutionary history which appear to be independent from the flanking chromosomal markers.

RESULTS

Nine primate species were studied:

- HSA (*Homo sapiens*);
- 3 great apes: common chimpanzee (*Pan troglodytes*, PTR), gorilla (*Gorilla gorilla*, GGO), and orangutan (*Pongo pygmaeus*, PPY);
- 1 Cercopithecidae (Old World Monkey, OWM): silvered leaf-monkey (*Presbytis cristata*, PCR);
- 4 Platyrrhinae (New World Monkeys, NWM): dusky titi (*Callicebus molloch*, CMO, Callicebinae), spider monkey (*Ateles geoffroyi*, AGE, Atelinae); common marmoset (*Callithrix jacchus*, CJA, Callitrichinae), and squirrel monkey (*Saimiri sciureus*, SSC, Saimirinae).

The *Presbytis cristata* (PCR) was chosen as the sole representative of the Cercopithecidae since previous unpublished data from our laboratory, based on Partial Chromosome Paints (PCP) and appropriate YAC probes, have shown that chromosome IX of PCR (Colobinae), CAE (*Cercopithecus aethiops*, Cercopithecinae), and MMU (*Macaca mulatta*, Cercopithecinae) appear perfectly alike (data not shown).

Fig. 1a shows a sample of DAPI banded chromosome IX from each species. In AGE, SSC, and CJA the chromosome IX lies uninterrupted within a larger chromosome (Sherlock et al. 1996; Morescalchi et al. 1997). In both AGE and SSC the additional cytogenetic material is positioned at one side, with the centromere defining the boundary. In CJA this chromosome is encompassed on both sides by additional cytogenetic material of different chromosomal origin, with the centromere lying within chromosome IX.

Evolution of chromosome IX in great apes have been investigated by Yunis and Prakash (1982) using banding techniques. Data on evolutionary conservation of chromosome IX in Old and New World monkeys have been obtained using whole chromosome paints, which, however, are not able to detect intrachromosomal rearrangements (Sherlock et al. 1996; Morescalchi et al. 1997).

Twelve human probes distributed along chromosome 9 were utilized in the study (Table 1 and Figure 1b). Each probe was used in FISH experiments on each species. Partial Chromosome Paints (PCP) specific for 9p (PCP #502) and 9q (PCP #29) (Antonacci et al. 1995) have been also used to grossly define the constitution of chromosome IX in the different species (Figure 1c). In several instances cohybridization experiments were performed in order to assess with certainty the relative order of probes. An example is shown in Figure 1d where cohybridization experiments using probes M and N

against metaphases from PCR and CMO were performed to unambiguously determine order. The results obtained have been summarized in Figure 2, bottom part. The position of each probe has been reported on the left of the chromosome IX ideograms, using the corresponding letter (see Table 1).

The order of the twelve markers was found to be identical in PCR (OWM), CMO and AGE (both NWM) and therefore was assumed to descend unchanged from an hypothesized Primate Common Ancestor (PCA, Figure 2). A paracentric inversion spanning markers A->H defines a Pongidae Ancestor (PA) whose chromosomal constitution was retained in GGO and PPY. A further pericentric inversion (see Figure 2) give rise to HPA (HSA/PTR common ancestor) whose constitution is unchanged in HSA. PTR derives from HPA through a pericentric inversion. One breakpoint of this inversion is detected by marker B (YAC 945F5) (Figure 1e). The splitting of this probe in PTR has been previously reported by Nickerson and Nelson (1998). The reconstruction of the evolutive pathways linking present day great apes to PA are in perfect agreement with data from Yunis and Prakash (1982). The markers' arrangement found in SSC and CJA can be derived from the PCA by hypothesizing a specific inversion in each lineage. The breakpoints of the inversion leading to SSC occurred between probes C/D and M/N respectively. One breakpoint of the inversion leading to CJA falls between probes D/E; the second lies inside marker B (YAC 945F5)(Figure 1e), which is the marker also involved in the inversion leading to PTR (see above).

The hypothesized phylogenetic pathways illustrated in Figure 2 do not take into account, intentionally, the position of the centromere. If the centromere is included in the analysis, indeed, a paradox emerges: in several instances its evolutive history seems to behave independently from the surrounding markers. The position of the centromere sorts the species under study into five groups: HSA-PTR-GGO-PPY, PCR, CMO-SSC, AGE, and CJA, as indicated in Figure 2 by a black line underlining each group. The differences in centromere position among the groups can not be easily reconciled with each other: an additional series of rearrangements must be postulated to fully account for the differences we have documented, as discussed below.

DISCUSSION

We have studied the evolutionary conservation of chromosome IX in nine primate species, using 12 molecular markers whose mapping in humans is well documented. Figure 2 summarizes the most parsimonious set of chromosomal inversions we propose to explain the constitution of chromosome IX in each species. Primate centromeric and pericentromeric regions have been shown to exhibit extraordinary evolutionary plasticity. Our findings add further complexity to the already complex evolutionary history of these chromosomal regions. The position of the centromere in some species, indeed, appears to have followed an independent evolutive path in respect to the flanking markers. Two different hypotheses can be proposed to reconcile these discrepancies:

- (i) Additional inversions have occurred in the evolutionary history of chromosome IX of these species. The ultimate results of these rearrangements would be the repositioning of the centromere leaving unchanged the markers' order.
- (ii) Alternatively, the evolutionary emergence of neocentromeres can be hypothesized.

A detailed series of hypothetical inversions needed to relocate the centromere to its present-day location through chromosomal rearrangements is schematized in Figure 3. In several instances the inversion breakpoints involve pericentromeric and telomeric regions. In two instances (PCR and CJA) the mechanism acts in a flip-flop mode (double inversion), the breakpoints in the pericentromeric region being once distal and the second time proximal to the centromere (or vice versa), so that the only detectable result would be the repositioning of the centromere.

In light of the data recently reported by du Sart et al. (1997) and Barry et al. (1999), the hypothesis of neocentromere emergence can not a priori be readily eliminated. The fact that all primate centromeres are defined by the presence of considerable amounts of alpha-satellite does not negate this hypothesis. It has been suggested that the accumulation of alpha satellite DNA at centromeres may simply be a consequence of its function and not a prerequisite to its origin (review by Eichler 1999). One obvious consequence of the birth of a neocentromere is the inactivation of the previously active centromere. Such centromere inactivation is a common event among human dicentric chromosomes resulting from chromosomal rearrangement (Sullivan and Willard 1998). What about the relics of these events? The incredible plasticity of these regions and our poor knowledge of primate genomes have made the identification of these remnants difficult. The only available example in this respect is the human ancestral centromere present at 2q21. This region was the domain of a normal centromere which was inactivated following the telomere-telomere fusion of the two ancestral chromosomes (phylogenetic IIp and IIq) which gave rise to the present-day human chromosome 2 (Ijdo et al. 1992). The fusion occurred at most 3-5 million years ago, which is the estimated date of the human-chimpanzee divergence (Andrews 1992; Li 1997). Despite its recent origin, relics of alphoid sequences are hardly detectable at this site (Avarello et al. 1992; Baldini et al. 1993), nor is there any evidence of C-banded material commonly associated with centromeric regions. These considerations suggest that the degradation of the ancestral centromere toward simple DNA has been extremely rapid. Relic sequences after such centromere inactivation events, therefore, can be very difficult to identify. The actual involvement of the two mechanisms (birth of a neocentromere and flip-flop processes) to centromere repositioning can not be easily distinguished at present. The flip-flop model might explain why pericentromeric and telomeric sequences share sometimes common sequences (Jackson et al. 1999; Puechberty et al. 1999).

An additional interesting observation we have documented concerns the two breakpoints identified in PTR and CJA, both lying inside the YAC 945F5 (Figure 1e). Both breakpoints appear to be asymmetrically located within the YAC, as revealed by the substantial difference in the intensity ratio between the two FISH signals, and are similarly oriented in respect to the flanking markers. We have documented, in a recent study, that the YAC 695H10 detects a breakpoint in the

phylogenetic chromosome IV of PTR and MMU (*Macaca mulatta*) (Marzella et al. 1999). It could be suggested that the breakpoint sites detected by YACs 945F5 and 695H10 has been utilized more than one time during evolution as a consequence of sequence intrinsic features. This conclusion, however, requires validation at the molecular level. Recurrence of chromosomal rearrangements due to intrinsic sequence features is now well documented in humans (Christian et al. 1999, and references therein).

Concluding remarks. It is becoming increasingly apparent that there are peculiar regions of the primate genome which exhibit an extraordinary degree of evolutionary plasticity. Such regions are in stark contrast to the bulk of euchromatic DNA which appears evolutionary stable. High evolutionary plasticity has been documented on centromeric and pericentromeric domains (Archidiacono et al. 1995; Jackson et al. 1999), and on the chromosome Y-specific chromosomal segment (Archidiacono et al. 1998). It is noteworthy that these regions share a very low or absence of meiotic recombination (Puechberty et al. 1999). At present we are investigating the evolutionary history of additional primate chromosomes in order to establish if the paradox documented for the centromere of chromosome IX is shared by other centromeres. Murphy and Karpen (1998) have proposed that the centromere function could be the result of an epigenetic mark. This hypothesis is very appealing in explaining the emergence of neocentromeres. In this respect studies at the molecular level on the phenomena we have documented, now in progress in our laboratory, could be crucial in substantiating this hypothesis.

METHODS

Probes. YACs are from the CEPH megalibrary; PAC 835J22 is from the PAC library described by Ioannou and de Jong (1996). YAC and PAC clones have been kindly provided by the YAC Screening Centre, Milan (<http://www.spr.it/iger/home.html>). The PAC 835J22 was identified by primers specific for the ABL locus at 9q34 (see our Web site <http://bioserver.uniba.it/fish/Cytogenetics/webbari/YAC-TUMORS/project/abl-bcr.html>). All probes used are listed in Table 1.

Cell lines. Human metaphase spreads were obtained from PHA-stimulated peripheral blood lymphocytes of a normal human donor. Cell lines from nine primates species have been previously described (Archidiacono et al. 1998).

FISH. Probes were labeled with biotin by nick-translation and hybridized in situ essentially as described by Lichter et al. (1990) with minor modifications. Detection was performed using avidin-conjugated Cy3 (Amersham). Chromosome identification was obtained by simultaneous DAPI staining. Co-hybridization experiments were accomplished by labeling the second probe with FluorX-dCTP (Amersham). Digital images were obtained using a Leica DMRXA epifluorescence microscope equipped with a cooled CCD camera (Princeton Instruments, NJ). Cy3, FluorX, and DAPI fluorescence signals, detected using specific filters, were recorded separately as gray scale images. Pseudocoloring and merging of images were performed using the Adobe PhotoshopTM commercial software.

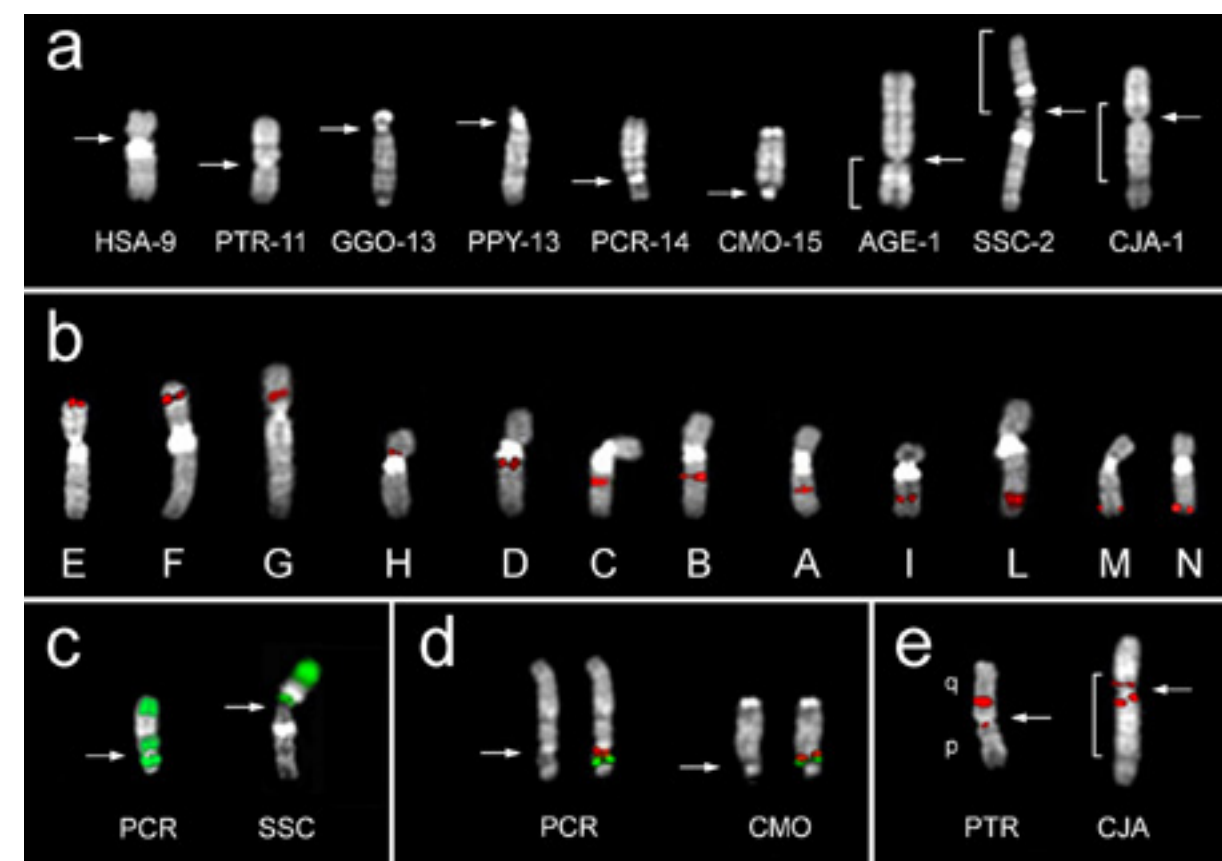


Figure 1
Examples of DAPI-banded phylogenetic chromosome IX from each species under study (a). Chromosome IX in AGE,

SSC, and CJA are part of a larger chromosome. In all cases, however, chromosome IX is uninterrupted. The square parentheses indicate the portion of chromosome IX. Some chromosomes are presented in an inverted orientation, in respect to the position of the centromere, to match the orientation reported in Fig. 2. The actual chromosome number in each species is also reported, on the right of the species acronym. (b) FISH signal of the 12 probes on human chromosome 9. The examples have been arranged from left to right in increasing mapping distance from 9pter. (c) Example of FISH signals (green) of PCP #29, specific for human 9q, on PCR (left) and SSC (right). (d) Example of a cohybridization experiment performed to establish the relative order of probes M (red) and N (green) in PCR and CMO. The DAPI-banded chromosome IX without signals is on the left, to better show the morphology and centromere position. (e) The figure shows the splitting of probe B in PTR and CJA (see text). In all Figures the arrows point to the centromere.

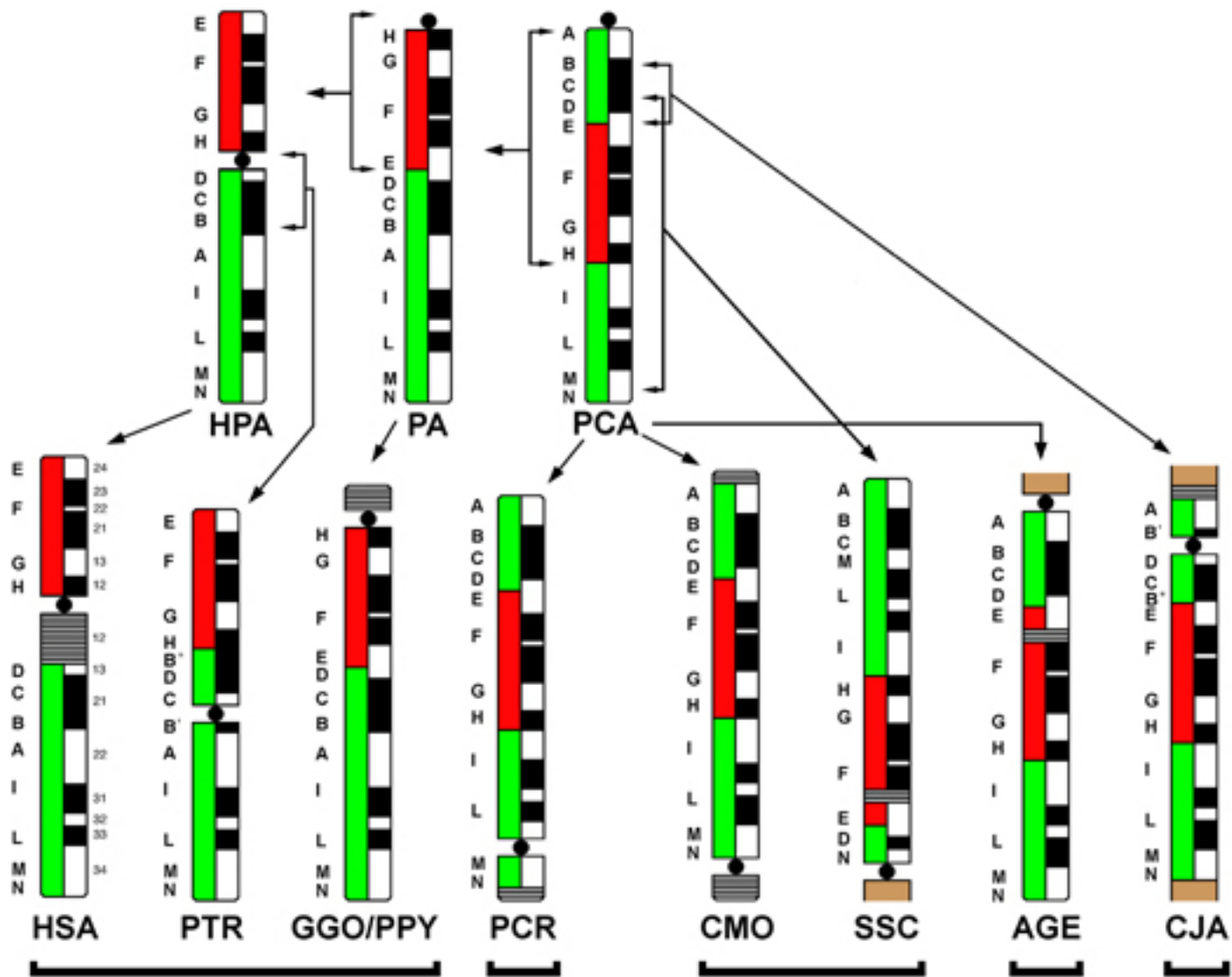


Figure 2
The diagram schematically summarizes the results obtained by hybridizing the 12 markers on each species under study. GGO and PPY turned out to be identical and have been grouped. Regions homologous to the human 9p (red) and 9q (green) are shown on the left of each ideogram which shows, on the right, the G-banding pattern. PCA stands for the hypothesized Primate Common Ancestor, PA for Pongidae Ancestor, HPA for HSA-PTR Ancestor. The not detailed cytogenetic material from different chromosome(s) present in AGE, SSC, and CJA is in brown. Close horizontal lines indicate heterochromatin blocks. The hypothesized pericentric or paracentric inversions are indicated by square parentheses spanning the inverted cytogenetic segment. The split signals of marker B (YAC 945F5) are indicated as B' and B". In both cases signal of B" is much stronger than B' (see text and Fig. 1e).

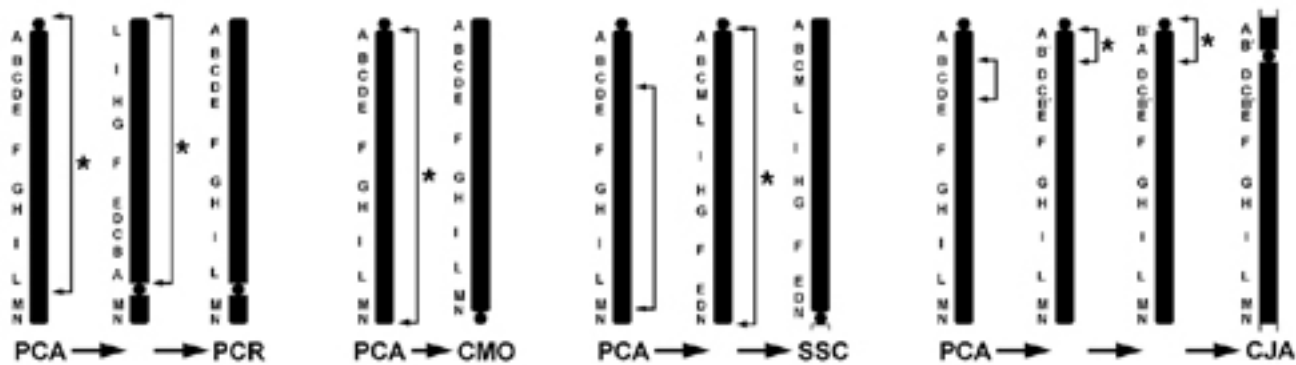


Figure 3
 The Figure schematically describes the most parsimonious series of hypothetical rearrangements that would be needed to reconcile the observed marker order and the position of the centromere s. They are based on the assumption that in PCA the centromere was positioned telomeric to marker A. This conclusion is drawn exclusively from the constraint imposed by the maximum parsimony. The inversions are indicated by square parenthesis. The inversions not present in Figure 2 have been specifically introduced to account for the paradoxal position of the centromere and are indicated by an asterisc. In AGE and SSC the centromere is positioned at the boundary between chromosome IX and the chromosome segment brought there by an interchromosomal rearrangement. It can not be excluded, therefore, that the centromere of these two species has originated from a different chromosome. The orientation of the chromosomes has been reported to match the orientation reported in Fig. 2.

Table 1 Probes used in the study

	Probe	cM	cR
E	YAC 816E6	0	3
F	YAC 922A5		36
G	YAC 823G12	57	134-139
H	YAC 763A12	60	172
D	YAC 748D2	65	
C	YAC 906G6	84	
B	YAC 945F5	87	318
A	YAC 747B3	93-94	338
I	YAC 750C6	117	414
L	YAC 756E10	128	426
M	YAC 758F1	136-143	458
N	PAC 835J22	ABL locus	

Table 1
 The FISH probes are reported according to their order along human chromosome 9. The order has been confirmed by data derived from STSs lying inside each YAC (MIT database) and reported in the 3rd column (genetic data, in cM) and in the 4th column (radiation hybrids data, in cR). An upper case letter identifies each probe (1st column), and was arranged so that the ascending sequence from A to N corresponds to the hypothesized physical order in the ancestral chromosome IX (Fig. 2). The YACs 763A12 and 748D2 has been chosen because they are very close to the centromere on p and q side

respectively (see MIT database).

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REFERENCES

- Andrews, P. 1992. Evolution and environment in the Hominoidea. *Nature* **360**: 641-6.
- Antonacci, R., R. Marzella, P. Finelli, A. Lonoce, A. Forabosco, N. Archidiacono, and M. Rocchi. 1995. A panel of subchromosomal painting libraries representing over 300 regions of the human genome. *Cytogenet. Cell Genet.* **68**: 25-32.
- Archidiacono, N., R. Antonacci, R. Marzella, P. Finelli, A. Lonoce, and M. Rocchi. 1995. Comparative mapping of human alphoid sequences in great apes using fluorescence *in situ* hybridization. *Genomics* **25**: 477-484.
- Archidiacono, N., C.T. Storlazzi, C. Spalluto, A.S. Ricco, R. Marzella, and M. Rocchi. 1998. Evolution of chromosome Y in primates. *Chromosoma* **107**: 241-6.
- Avarello, R., A. Pedicini, A. Caiulo, O. Zuffardi, and M. Fraccaro. 1992. Evidence for an ancestral alphoid domain on the long arm of human chromosome-2. *Hum. Genet.* **89**: 247-249.
- Baldini, A., T. Ried, V. Shridhar, K. Ogura, L. D'Aiuto, M. Rocchi, and D.C. Ward. 1993. An alphoid DNA sequence conserved in all human and great ape chromosomes: evidence for ancient centromeric sequences at human chromosomal regions 2q21 and 9q13. *Hum. Genet.* **90**: 577-583.
- Barry, A.E., E.V. Howman, M.R. Cancilla, R. Saffery, and K.H.A. Choo. 1999. Sequence analysis of an 80 kb human neocentromere. *Hum. Mol. Genet.* **8**: 217-227.
- Choo, K.H., B. Vissel, A. Nagy, E. Earle, and P. Kalitsis. 1991. A survey of the genomic distribution of alpha satellite DNA on all the human chromosomes, and a derivation of a new consensus sequence. *Nucleic Acids Res.* **19**: 1179-1182.
- Christian, S.L., J.A. Fantes, S.K. Mewborn, B. Huang, and D.H. Ledbetter. 1999. Large genomic duplicons map to sites of instability in the Prader- Willi/Angelman syndrome chromosome region (15q11-q13). *Hum Mol Genet* **8**: 1025-1037.
- du Sart, D., M.R. Cancilla, E. Earle, J. Mao, R. Saffery, K.M. Tainton, P. Kalitsis, J. Martin, A.E. Barry, and K.H.A. Choo. 1997. A functional neo centromere formed through activation of a latent human centromere and consisting of non-alpha-satellite DNA. *Nature Genet.* **16**: 144-153.
- Eichler, E.E. 1999. Repetitive conundrums of centromere structure and function. *Hum. Mol. Genet.* **8**: 151-155.
- Ijdo, J.W., A. Baldini, D.C. Ward, S.T. Reeders, and R.A. Wells. 1991. Origin of human chromosome 2: an ancestral telomere-telomere fusion. *Proc. Natl. Acad. Sci. USA* **88**: 9051-9055.
- Ioannou, P.A., C.T. Amemiya, J. Garnes, P.M. Kroisel, H. Shizuya, C. Chen, M.A. Batzer, and P.J. de Jong. 1994. A new bacteriophage P1-derived vector for the propagation of large human DNA fragments. *Nature Genet.* **6**: 84-89.
- Jackson, M.S., M. Rocchi, G. Thompson, T. Hearn, M. Crosier, J. Guy, D. Kirk, L. Mulligan, A. Ricco, S. Piccininni, R. Marzella, L. Viggiano, and N. Archidiacono. 1999. 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.
- Li, W. 1997. Molecular Evolution . Sinauer Associates, Sunderland, MA.
- Lichter, P., C.-J. Tang Chang, K. Call, G. Hermanson, G.A. Evans, D. Housman, and D.C. Ward. 1990. High resolution mapping of human chromosomes 11 by *in situ* hybridization with cosmid clones. *Science* **247**: 64-69.
- Maio, J.J. 1971. DNA strand reassociation polyribonucleotide binding in African green monkey, *Cercopithecus aethiops*. *J. Mol. Biol.* **56**: 579-595.
- Marzella, R., L. Viggiano, V. Miolla, C.T. Storlazzi, A. Ricco, E. Gentile, R. Roberto, C. Surace, A. Fratello, M. Mancini, N. Archidiacono, and M. Rocchi. 1999. Molecular cytogenetic resources for chromosome 4 and comparative analysis of phylogenetic chromosome IV in great apes. *Submitted for publication*
- Morescalchi, M.A., W. Schempp, S. Consigliere, F. Bigoni, J. Wienberg, and R. Stanyon. 1997. Mapping chromosomal homology between humans and the black-handed spider. *Chrom. Res.* **5**: 527-536.
- Murphy, T.D. and G.H. Karpen. 1998. Centromeres take flight: alpha satellite and the quest for the human centromere. *Cell* **93**: 317-20.
- Nickerson, E. and D.L. Nelson. 1998. Molecular definition of pericentric inversion breakpoints occurring during the evolution of humans and chimpanzees. *Genomics* **50**: 368-372.
- Puechberty, J., A.M. Laurent, S. Gimenez, A. Billault, M.E. Brun-Laurent, A. Calenda, B. Marcais, C. Prades, P. Ioannou, Y. Yurov, and G. Roizes. 1999. Genetic and physical analyses of the centromeric and pericentromeric regions of human chromosome 5: recombination across 5cen. *Genomics* **56**: 274-87.
- Sherlock, J.K., D.K. Griffin, J.D.A. Delanthi, and J.M. Parrington. 1996. Homologies between human and marmoset (*Callitrix jacchus*) chromosomes revealed by comparative chromosome painting. *Genomics* **33**: 214-219.
- Sullivan, B.A. and H.F. Willard. 1998. Stable dicentric X chromosomes with two functional centromeres. *Nat. Genet.* **20**: 227-8.
- Warburton, P.E., T. Haaf, J. Gosden, D. Lawson, and H.F. Willard. 1996. Characterization of a chromosome specific chimpanzee alpha satellite subset: evolutionary relationship to subsets on human chromosomes. *Genomics* **33**: 220-228.
- Willard, H.F. and J.S. Waye. 1987. Hierarchical order in chromosome-specific human alpha satellite DNA. *Trends Genet.* **3**: 192-198.