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Analysis of chromosome conservation in *Lemur catta* studied by chromosome paints and BAC/PAC probes

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Abstract A panel of human chromosome painting probes and bacterial and P1 artificial chromosome (BAC/PAC) clones were used in fluorescence in situ hybridization (FISH) experiments to investigate the chromosome conservation of the ring-tailed lemur (*Lemur catta*, LCA) with respect to human. Whole chromosome paints specific for human chromosomes 7, 9, 11, 13, 14, 17, 18, 20, 21, and X were found to identify a single chromosome or an uninterrupted chromosomal region in LCA. A large set of partial chromosome paints and BAC/PAC probes were then used to refine the characterization of the rearrangements differentiating the two karyotypes. The results were also used to reconstruct the ancestral Lemuridae karyotype. *Lemur catta*, indeed, can be used as an outgroup, allowing symplesiomorphic (ancestral) rearrangements to be distinguished from apomorphic (derived) rearrangements in lemurs. Some LCA chromosomes are difficult to distinguish morphologically. The 'anchorage' of most LCA chromosomes to specific probes will contribute to the standardization of the karyotype of this species.

Introduction

Whole chromosome paints (WCP) and multidirectional chromosome painting have been advantageously used to delineate chromosome conservation in primates and in mammals. Yeast artificial chromosome (YAC) and bacterial and P1 artificial chromosome (BAC/PAC) probes, utilized in fluorescence in situ hybridization (FISH) experiments, can considerably improve the resolution of the cytogenetic analysis. This resolving power derives primarily from the fact that ordered BAC/PAC probe

contigs are the backbone on which the almost completed human genome sequence is based. However, BAC/PAC probes have been used only occasionally in non-human primates (Ventura et al. 2001).

Karyotype evolution in lemurs was first investigated using banding techniques (Rumpler and Dutrillaux 1976) and, more recently, using FISH (Apiou et al. 1996; Muller et al. 1997; Vezuli et al. 1997; Vezuli and Rumpler 2000). Ring-tailed lemur (*Lemur catta*, LCA; $2n=56$) is the only species of the genus *Lemur* and it represents a useful outgroup in defining the chromosome evolution of Lemuridae. Chromosome conservation studies in this species using a limited set of WCPs have been reported by Apiou et al. (1996) and Vezuli et al. (1997).

In order to define chromosome conservation precisely in LCA and to delineate the Lemuridae ancestral karyotype, we undertook a systematic analysis of LCA chromosomes using a set of molecular cytogenetic tools including WCPs, partial chromosome paints (PCPs) and BAC/PAC clones used as single probes or in pools.

Materials and methods

Metaphases were obtained from fibroblast cell lines of LCA, Brown lemur (*Eulemur fulvus*, EFU) and Black lemur (*Eulemur macaco*, EMA) by standard procedures.

Whole chromosome paints, derived from flow-sorted chromosomes, were a gift of the Sanger Centre (Dr. N.P. Carter). Partial chromosomal paints, derived from somatic cell hybrids or microdissection, were generated in our laboratory (Antonacci et al. 1995; see also our Web site <http://www.biologia.uniba.it/rmc/>). The specificities of the PCPs (codes according to our Web site; specificity in parenthesis) are as follows: 368 (2p25–p22/2p16–p11.2), 113 (2p), 162 (2q), 161 (2q12–qter), 409 (4p), 412 (4p), 414 (4q), 430 (4q24–qter), 611 (6p), 621 (6p), 614 (6q12–qter), PCP1p (microdissected 1p), PCP1q (microdissected 1q), PCP2p (microdissected 2p) and PCP2q (microdissected 2q). Most of the BAC/PAC probes used in this study (Table 1) belong to the RPC11 de Jong library (<http://www.chori.org/bacpac/>), with the exception of PACs 130G2, 13D10, 915N17, which belong to the RPC1 library and the four chromosome 22 BACs, which were from the Caltech library. These probes were obtained from the YAC Screening Centre (Milan) (<http://www.spr.it/iger/home.html>) or from the Sanger Centre (<http://www.sanger.ac.uk>). All the probes were first tested

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Table 1 The bacterial and P1 artificial chromosome (BAC/PAC) probes used in the study. All probes belong to the RP11 library (<http://www.chori.org/bacpac/>, release April 2002), with the exception of probes marked by an asterisk, which are from the RP1 library, and those with a double asterisk, from the California

Institute of Technology library. (FISH, fluorescence in situ hybridization; HSA, *Homo sapiens*; LCA, *Lemur catta*; UCSC, position on human genome sequence from University of California Santa Cruz database)

BAC/PAC	Pool	HSA mapping	UCSC (kb)	LCA mapping	BAC/PAC	Pool	HSA mapping	UCSC (kb)	LCA mapping
62M23	1A	1p36	3,500	3q	348N12	4F	4q25	112,040	4p
447M5		1p36	3,925		21H22		4q27	122,106	
161A11	1B	1p34	28,807		381N20		4q28.1	125,502	
88O2		1p34.3	30,704		510D4		4q28.1	127,964	
91A21	1C	1p21.31	107,563		92K5		4q28.2	133,603	
29M22	1D	1p12	121,256		315C10	4G	4q31.1	147,839	4q
79E5		1q12	FISH		481K16		4q31.1	146,234	
165A8	1E	1q23.3	153,682		557J10		4q31.1	152,765	
137M19		1q23.1	156,317		503I23	4H	4q33	164,950	4q
124A11	1F	1q32.3	205,442	No signal	234O6		4q32.1	167,416	
131M16		1q32.3	205,904		218F10		4q32.2	170,118	
260A10		1q32.3	209,312		493C20		4q32.2	170,279	
915N17*	1G	1q42.13	225,376	No signal	512C14		4q32.2	174,431	
210E16		1q42.2	229,227		597P9	4I	4q35	191,335	
217J12	1H	1q43	237,867	No signal	661C8	5A	5p15.33	2,817	6
212E22		1q43	239,495		773M18		5p15.33	2,397	
438F14		1q44	245,490		94J21		5p15.33	11,326	
352J11	2A	2p25.3	789	5	19F12	5B	5p13.3	43,329	
379N10	2B	2p25.1	9,015		203O23		5q11.2	56,981	
198N12	2C	2p22.2	36,460		143O12		5q11.2	57,808	
480A6		2p21	46,346		47L19	5C	5q21.2	126,648	
299C5		2p21	48,087		265M23		5q23.2	132,018	
431B6	2D	2q11.2	98,218		115I4	5D	5q31.3	144,598	4q
34L23	2E	2q21.3	130,126	7	14K13		5q31.3	157,201	
107E5		2q22.3	144,186		14K9	5E	5q35.1	175,248	
389E16	2F	2q24.1	159,778		15F10		5q35.1	179,044	
818E9	2G	2q24.3	170,993		51D11		5q35.3	187,791	
559J5	2H	2q34	213,686		274H24	6A	6p25.1	10,829	4p
69J7		2q35	224,096		4A24		6p24.1	18,541	
558E6	2I	2q35	225,222		13D10*		6p22.3	19,200	
52C8		2q35	227,719		130G2*	6B'	6p22.1	24,690	
305J20		2q35	228,122		209A2	6B''	6p21.32	33,615	
124F22	2J	2q37.3	244,652		175A4	6B'''	6p21.31	39,833	2q
107B21		2q37.3	244,733		28O17*	6C	6p11.2	54,200	
206J15		2q37.3	245,799		10D8	7A	7q22.1	94,987	9
279L16	3A	3p25.1	15,703	1q	80P24		7q22.1	99,415	
255O19		3p25.1	16,270		72J24	7B	7q22.3	105,085	
11L6	3B	3p24.2	27,074	20	354H2	7C	7q31.2	116,123	
209O16		3p22.2	38,324		3L10		7q31.32	121,204	
189H19		3p21.32	40,984		286H14	7D	7q32.3	128,880	
119L2		3p21.32	41,391		4G15		7q33	153,903	
120C2		3p14.3	56,387		338B22	8A	8p23.3	165	25
70P20		3p14.2	60,262		18D5		8p23.3	313	
129B22		3p14.1	64,999		203E8		8p21.2	30,567	
271M21	3C	3p12	98,662	1qter	275E10		8p12	32,489	
145F16	3D	3q25.1	157,068	1q	10D7		8p12	33,878	
91L9		3q25.2	161,206		89M20		8p11.2	34,689	
436H7	3E	3q28	FISH	1q	262I23		8p11.22	40,310	
143P4		3q29	199,319		10D13	10A	10p15	166	1
778E2		3q29	202,006		195B3		10p15	3,193	
308K1	4A	4p16.3	519	15	454I3	10B	10p14	7,541	
310H1		4p16.3	519		379F12		10p14	7,927	
386I15		4p16.3	1,356		397O4		10p14	10,999	
262P20		4p16.3	1,881		120C13		10p14	11,099	
778B12	4B	4p15.31	20,471	5	30A6	10C	10p11.1	33,224	
497E20		4p15.2	25,635		241I20		10p11.1	36,248	
472B18	4C	4p14	38,055	5	60H16		10p11.1	38,465	
177C12		4p14	41,688		134A8		10p11.1	39,653	
89N9	4D	4p13	44,108	6	351D16	10D	10q11.21	44,431	12
188G15	4E	4q22	FISH		402D21	10E	10q23.32	95,594	
					119K6		10q23.33	98,199	
					7D5		10q24.1	99,286	
					88I10	10F	10q26.12	127,196	
					296H2		10q26.13	133,508	
					283C16		10q26.2	135,283	

Table 1 (continued)

BAC/PAC	Pool	HSA mapping	UCSC (kb)	LCA mapping
19E5	15A	15q25.3	79,280	11
91B15		15q25.3	88,568	
57P19		15q26.1	94,248	
292B10	16A	16p13.3	17,805	2p
450G5	16B	16p12.2	26,935	
360L15	16C	16p11.2	FISH	
497D8	16D	16q12	FISH	16
564F10	16E	16q22.3	79,852	
457K7	16F	16q24.1	88,226	
565J3	19A	19p13.3	6,947	1p
451E20		19p13.12	17,248	
91L5		19p13.1	20,926	
38C1	19B	19q13.2	49,057	19
25A12		19q13.3	63,943	
541M19		19q13.43	72,538	
115F6**	22A	22q11.1	1,700	10
154M4**		22q11.21	2,400	
433F6**		22q11.21	4,300	
268H5**	22B	22q13.3	29,000	21

on human metaphases as reported on our Web site. Their position on the human genome sequence was directly derived from the University of California Santa Cruz (UCSC) database (<http://genome.ucsc.edu/>) through their sequence or through their sequence-tagged site content. For a few of them only, FISH data were available.

Details for DNA extraction from BACs and PACs have been previously reported (Ventura et al. 2001). Fluorescence in situ hybridization experiments were performed as described by Lichter et al. (1990) with slight modifications: hybridization time was prolonged to 3 days. Digital images were obtained using a Leica DMRXA epifluorescence microscope equipped with a cooled CCD camera (Princeton Instruments, N.J.). Cy3, Fluor-X, and 4',6-diamidino-2-phenylindole (DAPI) fluorescence signals, detected with specific filters, were recorded separately as gray-scale images. Pseudocoloring and merging of images were performed using Adobe Photoshop.

Results

The LCA karyotype is composed of 56 pairs of homologs. The chromosomes in Fig. 1 have been arranged using the karyotype reported by Rumpel and Dutrillaux (1976) as a reference. The arrangement of the smaller chromosome pairs (14–27), however, was difficult, since the picture quality of the reference karyotype was poor. The 14–27 pairs, therefore, were simply ordered by size. Heterochromatic blocks are usually evident in DAPI-stained metaphases that have undergone FISH. Major non-centromeric blocks, detected in this way, were evident on the short arm of the LCA3 chromosome and interstitially located in LCA4, LCA8, and LCA13 (Fig. 1). In accordance with their heterochromatic nature none of these regions was ever detected by any human chromosome probe.

Conservation of synteny in *Homo sapiens* (HSA) and LCA was first investigated using a complete panel of human autosomal WCPs. Data on chromosome X have been previously reported (Ventura et al. 2001). Ten WCPs identified a single chromosome or an uninterrupted

chromosomal region in LCA. Other WCPs showed homology to more than one chromosome. No satisfactory signals were obtained by WCPs specific for human chromosomes 2 and 4. In turn, some LCA chromosomes or chromosomal regions (LCA4 short arm, LCA5, LCA20) as well as some minichromosomes (from LCA22 to LCA27) did not show hybridization signals with any human WCP probe.

To overcome the problems encountered with WCPs and to obtain a more detailed picture of chromosome conservation, we utilized PCPs and BAC/PAC probes, singly or in pools (Table 1), with special attention to those LCA chromosomes not corresponding to a single human chromosome. When necessary, cohybridization experiments were carried out in order to establish association and/or probe order. Additional FISH experiments with BAC/PAC probes were occasionally performed on EMA and EFU to elucidate suspected inconsistencies arising from the comparison of our data on LCA with literature data on EMA and EFU (Muller et al. 1997). The results, described below, are summarized in Fig. 2.

Chromosome HSA1

The WCP specific for HSA1 painted the whole LCA3 chromosome. The PCP1p, PCP1q and BAC probes allowed us to define the LCA3 long arm as corresponding to HSA1pter–q23. The marker order appears to be conserved, LCA3qter corresponding to HSA1pter. The remaining HSA1q probes failed to give detectable signals.

Chromosome HSA2

The WCP specific for HSA2, as well as HSA2q PCP, repeatedly failed to generate any LCA FISH signal. The HSA2p microdissection library painted LCA5 almost entirely. Pools of BACs of HSA2p confirmed that LCA5 contains HSA2p sequences in addition to the HSA2q11–q12 region. Pools of BAC from HSA2q21–qter yielded signals on LCA7.

Chromosome HSA3

The strong WCP3 signal covered the LCA1 long arm almost completely. Weak signals were observed on LCA20. Probes mapping on LCA1 were not colinear as in human. Pool 3B defined the LCA20 as corresponding to the HSA3p14–p24 (Fig. 2).

Chromosome HSA4

No painting with WCP4 or PCPs was observed on LCA chromosomes. The BAC probes showed a complex pattern of signals. The HSA4 sequences are scattered on four different LCA chromosomes: LCA4p (HSA4q25–

Fig. 1 The *Lemur catta* (LCA) karyotype (56, XX) after 4',6-diamidino-2-phenylindole (DAPI) and QM staining. For each homologous pair one example of DAPI (*left*) and one of QM (*right*) is displayed. *Insert* DAPI-stained LCA chromosomes that show non-centromeric heterochromatin after fluorescence in situ hybridization (FISH)

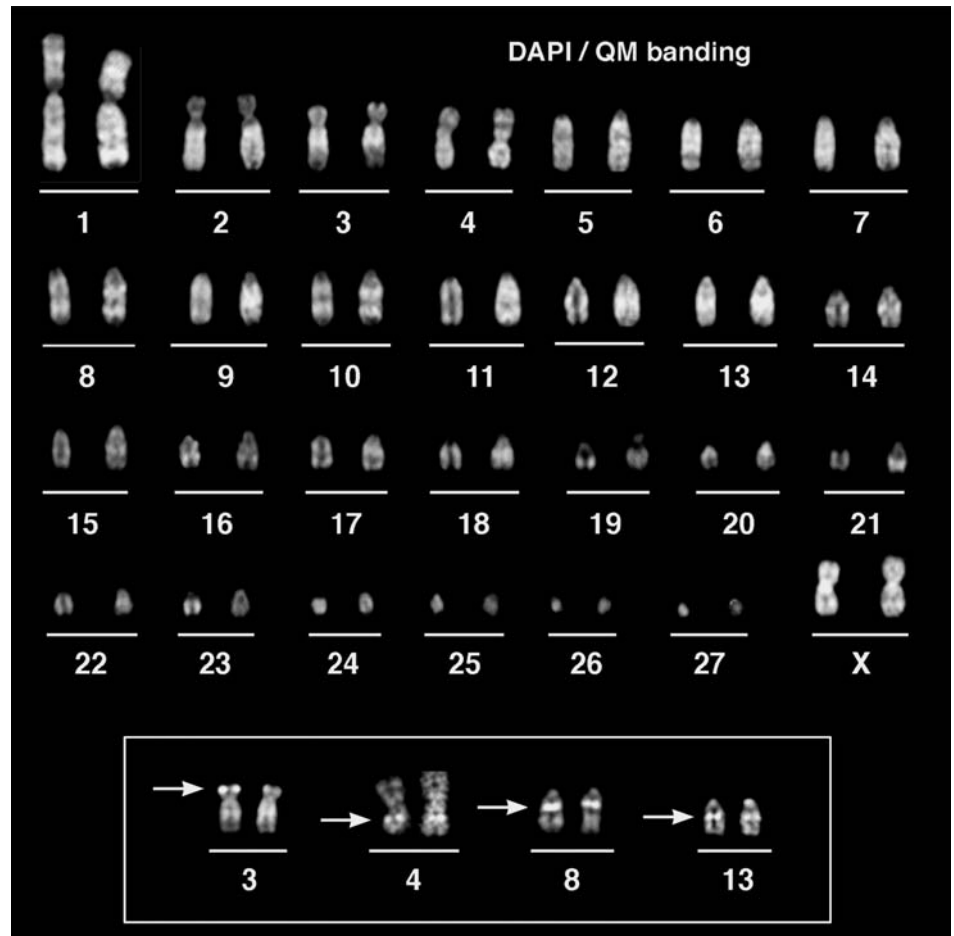
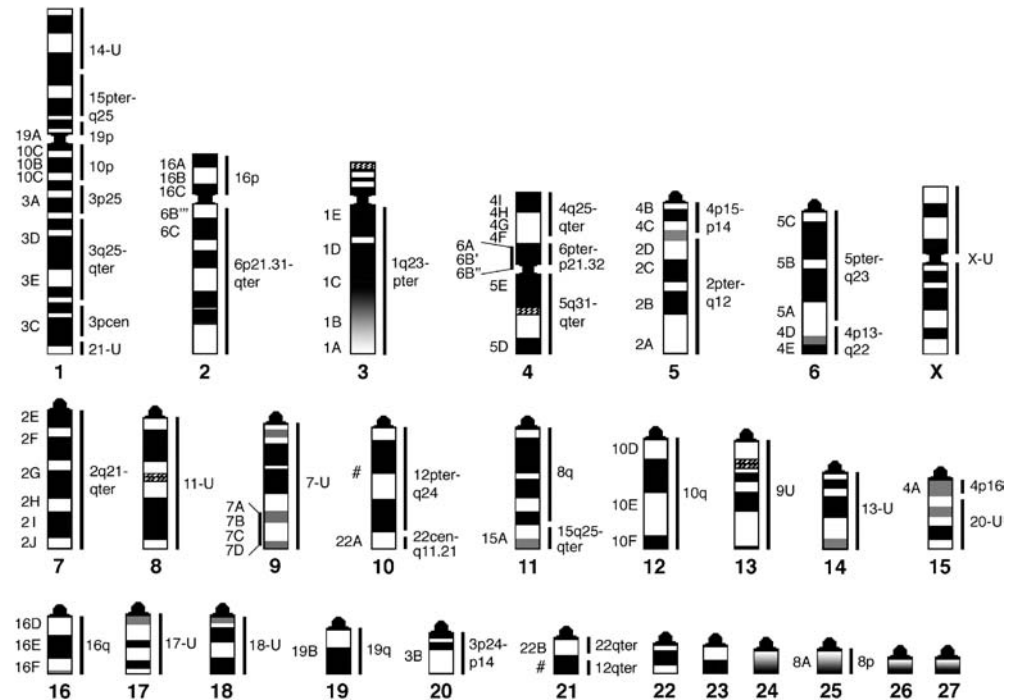


Fig. 2 Schematic representation of FISH results on *Lemur catta*. On the *left* of each ideogram are the results obtained after hybridization with single and/or pooled bacterial and P1 artificial chromosome (BAC/PAC) probes. Homologies with human chromosomes derived from whole chromosome paints (WCPs), partial chromosome paints (PCPs) and BAC/PAC probes are reported on the *right*. The BAC/PAC FISH data allow, in many cases, the definition of the orientation of markers along the chromosome or chromosomal fragment. The letter *U* to the right of a chromosome number indicates that the human WCP identified a unique LCA chromosome or a unique uninterrupted fragment



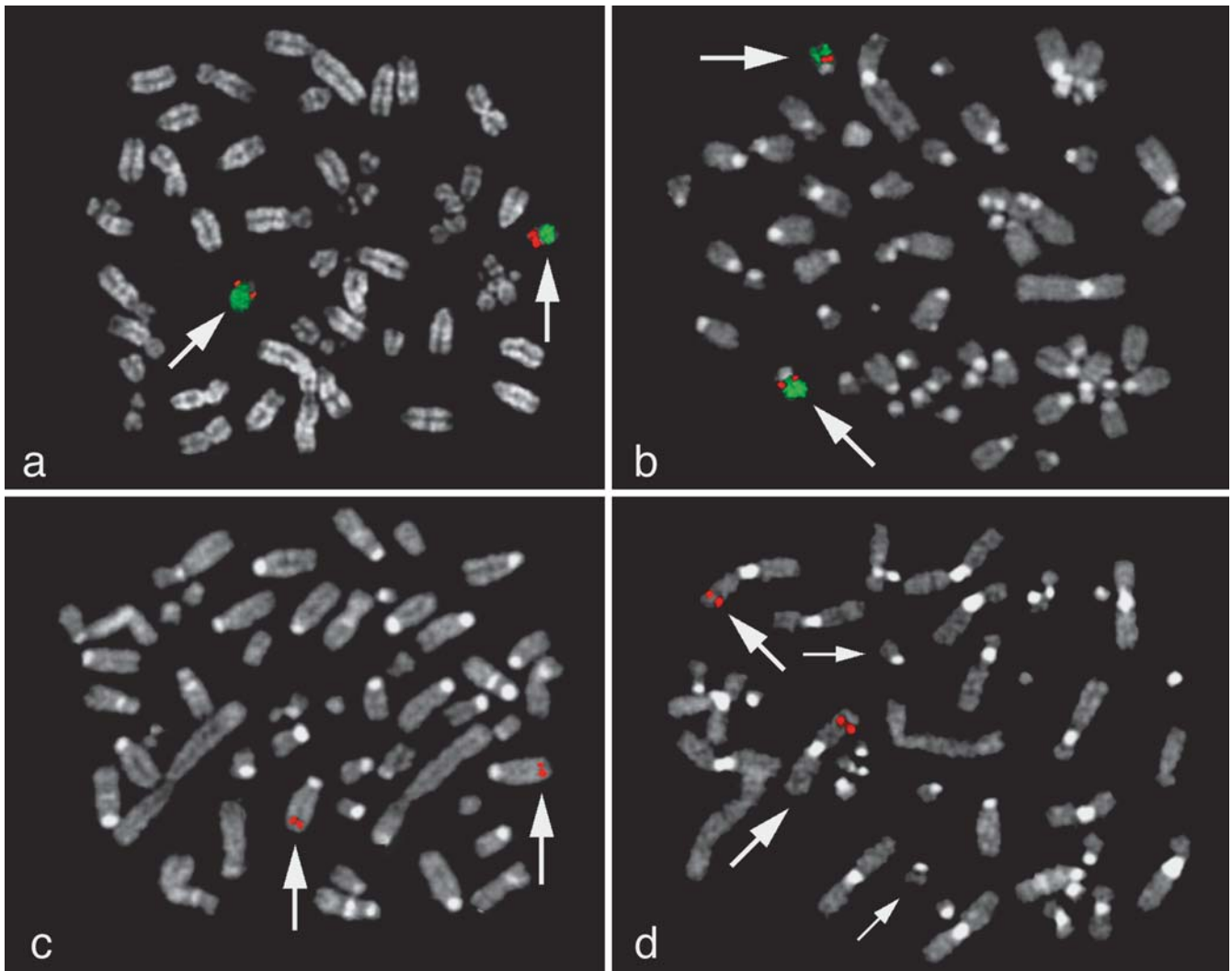


Fig. 3 Examples of FISH experiments using the Cy3-labeled Pool 4A probe (red), and the Fluor-X-labeled human WCP specific for chromosome 20 (green) cohybridized on LCA (a) and on *Eulemur fulvus* (EFU) (b) chromosomes. Association of signals of HSA4p16 (close to the centromere) with HSA20 WCP on LCA15 (a), and on

EFU17 (b) was seen. **c, d** Hybridization experiments using probes from the region HSA7q22-q33 on LCA (c) and *Eulemur macaco* (EMA) (d). Signals are present on LCA9 (c) and EMA3 (d), but absent on EMA19 (d small arrows)

qter), LCA5 (HSA4p15-p14), LCA6 (HSA4p13-q22), and LCA15 (HSA4p16) (Figs. 2, 3a). A pool of BACs from HSA4p16 was hybridized also on EMA and EFU metaphases to enable better tracking of the conservation of HSA4 in Lemuridae. The pool gave signals on EFU17 and EMA6p, which are mainly composed of HSA20-homologous regions (Fig. 3b). These results were inconsistent with those reported by Muller et al. (1997) on the latter chromosomes.

Chromosome HSA5

Signals generated by WCP HSA5 were found on the long arm of LCA4 chromosome and on LCA6. Fluorescence in situ hybridization experiments using BAC pools from HSA5p and HSA5q showed that sequences homologous

to HSA5pter-q23 were on LCA6, and HSA5q31-qter sequences mapped on the long arm of LCA4.

Chromosome HSA6

The HSA6 WCP yielded a signal on the LCA2 long arm and a weak signal on LCA4, close to the centromere-short arm. The HSA6q PCP painted the LCA2 long arm. No signals were obtained using a PCP specific for HSA6p. Pools of BAC/PAC spanning the region HSA6pter-p21 (6A and 6B) and a single BAC, RP11-28O17, mapping on HSA6p11 were used. Pool 6A (HSA6pter-p24) mapped on LCA4p near the centromere in accordance with the weak signals obtained with WCP6; pool 6B showed two signals around the centromere of LCA4 and a small but sharp signal close to the centromere on LCA2q. In this

regard BACs from the 6B pool revealed a breakpoint between bA209A2 and bA175A4 (6p21.32–p21.31). Probe RP11-28O17 gave a signal at LCA2q.

Chromosome HSA7

The HSA7 WCP recognized LCA9 as a single counterpart. The BAC probes from HSA7q22.1–q33 were then used on EMA and LCA metaphases to confirm the HSA7 WCP signals also on EMA19 and EFU27 (Muller et al. 1997). The HSA7q22.1–q33 BACs yielded signals only on LCA9 (Fig. 3c). Similarly, in EMA they gave signals on EMA3, but failed to detect homologous sequences on EMA19 (Fig. 3d).

Chromosome HSA8

LCA11 was the only chromosome detected by HSA8 WCP. In contrast, BACs from HSA8p hybridizing to LCA25, not detected by the WCP, were reconfirmed as hybridizing on EMA and EFU as Muller et al. (1997) reported. They indeed reported HSA8 signals on EMA16 and EMA2q and on EFU21 and EFU6.

Chromosome HSA10

HSA10 signals were found on LCA1 and LCA12. Using specific BACs, the two blocks (LCA1q close to the centromere, and the entire LCA12) were found to correspond exactly to HSA10p and HSA10q, respectively. The detailed evolutionary history of chromosome 10 has been investigated using a large panel of probes and has been described separately (Carbone et al. 2002).

Chromosome HSA15

The WCP specific for HSA15 gave a main signal on LCA1p and a second very weak signal on the telomeric region of LCA11. Muller et al. (1997) identified an HSA15q25–qter-homologous region on EMA2 and EFU6. The corresponding LCA11 telomeric region was detected by the HSA15q25–qter BAC pool. Therefore, EMA2, EFU6, and LCA11 have a synapomorphic arrangement.

Chromosome HSA16

WCP16 revealed two distinct regions of homology on LCA metaphases: LCA2p and LCA16. Hybridization with pools and single BAC probes indicated that the two regions correspond exactly to HSA16p and HSA16q, respectively.

Chromosome HSA19

A small region close to the centromere of LCA1p and the entire chromosome LCA19 were detected by WCP19. Appropriate pools of BACs defined the two homologous region as corresponding to HSA19p and HSA19q, respectively.

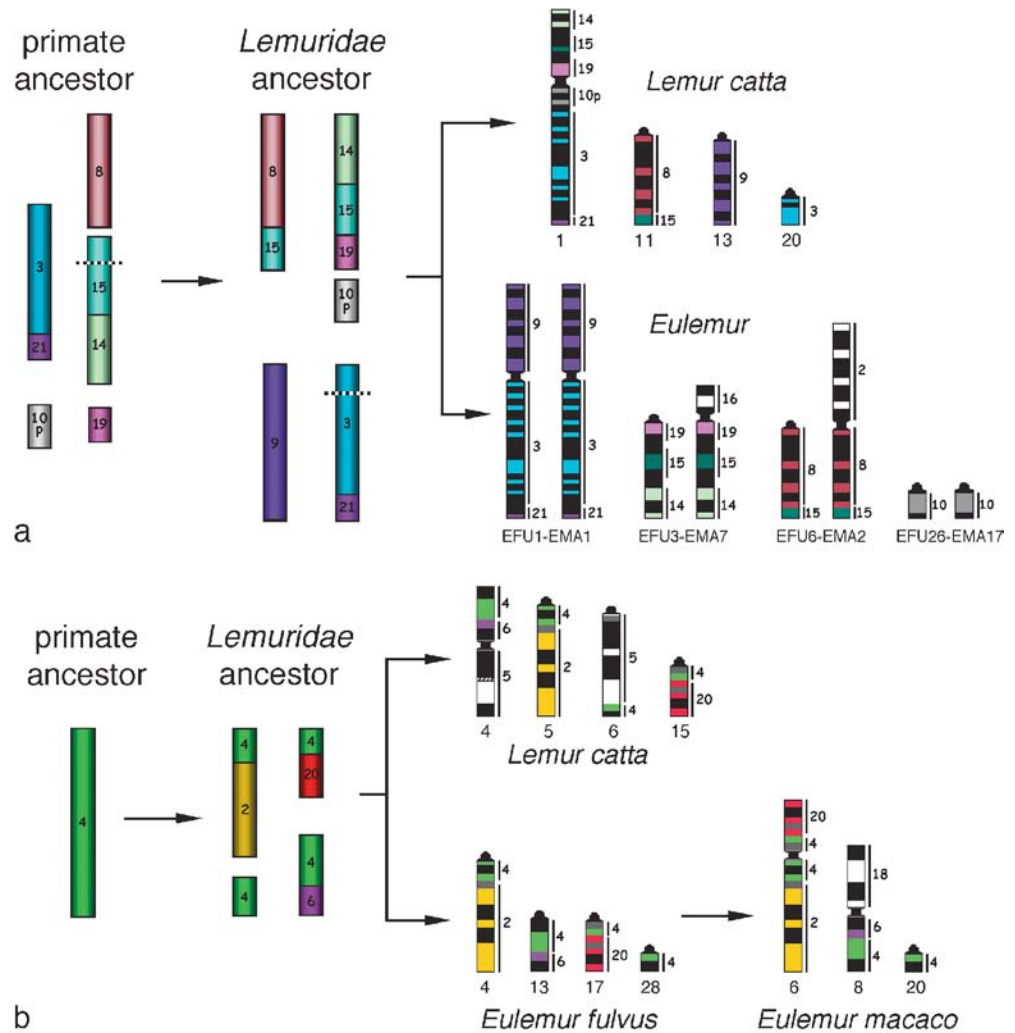
Chromosome HSA22

Signals of HSA22 were found on the distal region of LCA10 and on the proximal region of LCA21. Use of the BAC pool specific for HSA22q13–qter revealed that the distal region of LCA10 corresponds to HSA22cen–q11.21 and that the proximal part of LCA21 corresponds to HSA22q13–qter.

Discussion

The human genome sequencing effort has allowed investigations on the structural organization of our genome (Bailey et al. 2002). The architecture of genomes, however, is a complex mosaic puzzle where full understanding will be achieved only through the elucidation of their evolutionary history. In the present paper, we report a detailed analysis of karyotypic conservation in LCA with respect to human. The use of appropriate BAC/PAC probes turned out to be crucial in the precise characterization of chromosomal rearrangements, in solving inconsistencies with literature data and in discriminating between ambiguous WCP results. WCP probes have the advantage of depicting a broad view of chromosomal changes in a single experiment but their resolving power is low and in evolutionary distant species may encounter technical problems. This was the case for the WCP2 and WCP4 libraries. We do not know whether these problems could be ascribed to poor quality of the WCP or to a high extent of sequence divergence. Variability in FISH efficiency has been observed also among BAC/PAC probes. Better results were obtained by choosing BAC/PAC probes in gene-rich regions, which can be supposed to be under higher sequence conservation constraint. Gene richness, however, can vary greatly among chromosomes or along chromosomal regions. Single BAC probes from the very gene-rich chromosome 19, for example, never failed in giving satisfactory FISH signals. Knowledge of the evolutionary history of some chromosomal regions may also help in explaining the poor results with some probes. The best example is the region HSA2cen–q21.2. It is well known that the human chromosome 2 is the result of a telomere-telomere fusion by the short arms of the two acrocentric ancestral chromosomes (phylogenetic IIp and IIq). Segment HSA2cen–q21.2 roughly encompasses the region composed by the centromere-short arms of both phylogenetic IIp and IIq chromosomes. Centromeric and pericentromeric regions as well as the short arm of acrocentric

Fig. 4 a A diagrammatic summary of the complex evolution of the different *Homo sapiens* (HSA) synteny groups contributing to the LCA1 chromosome. The results on the conservation of HSA4 in *Lemuridae* are reported in **b**. Data from EFU and EMA are in part from Muller et al. (1997) and in part from the present paper. For details see text



chromosomes are known to be almost devoid of genes. The lack of hybridization signal of probes from these highly variable regions is therefore not unexpected.

Most of the results reported in Fig. 2 are self explanatory and will not be discussed in detail. We will focus on chromosomes with a more complex evolutionary history.

Several HSA synteny groups were involved in the genesis of LCA1, whose composition has been delineated by Vezuli et al. (1997). The long arm of this chromosome is composed of an HSA21/3(part)/10p association, while the short arm is composed of the association HSA19/15pter-q25/14. The HSA10p and HSA19p homologies, positioned close the LCA1 centromere and ambiguously detected by WCPs, were clearly identified by using PCPs and BAC/PAC probes. The HSA3/21 and the HSA14/15 associations appear to be ancestral to mammals (Muller et al. 1999; Murphy et al. 2001) but, contrary to our findings in LCA, each of the four chromosomes is involved as a whole. We have found HSA15q25-qter associated with HSA8 (LCA11) and HSA3p14-p24 as separate and single LCA11 and LCA20 chromosomes [the latter was reported as LCA17 by Vezuli et al. (1997)]. The marker order of

the portion of HSA3 present in LCA1 perfectly matches the ancestral primate marker arrangement hypothesized by Muller et al. (2000). Taking into account our data and all the available data in prosimians on these synteny groups (Muller et al. 1997; Vezuli et al. 1997), we have reconstructed their evolutionary relationship (Fig. 4a).

The LCA2 arrangement is exclusive with respect to EMA and EFU and is composed of HSA6p21.31-qter fused to HSA16p (Fig. 2). The remaining portion of HSA6 (6p21.32-pter) is associated with HSA4q25-qter to form the LCA4 short arm. HSA6 has been hypothesized as a separate chromosome in the primate ancestor (Murphy et al. 2001). Data on EMA and EFU (Muller et al. 1997) and the present data on LCA indicate that the HSA6p21.31-qter block and the HSA4/6 association (as in LCA) were present in prosimian ancestors as separate chromosomes. Subsequently, they fused in different combinations in EMA, EFU and LCA. We defined the HSA6 splitting in LCA as occurring in the region encompassed by BACs RP11-209A2 and RP11-175A4, which are approximately 6 Mb apart (UCSC; see Table 1). The use of probes utilized by the Human Genome Sequencing Project allows a very detailed characteriza-

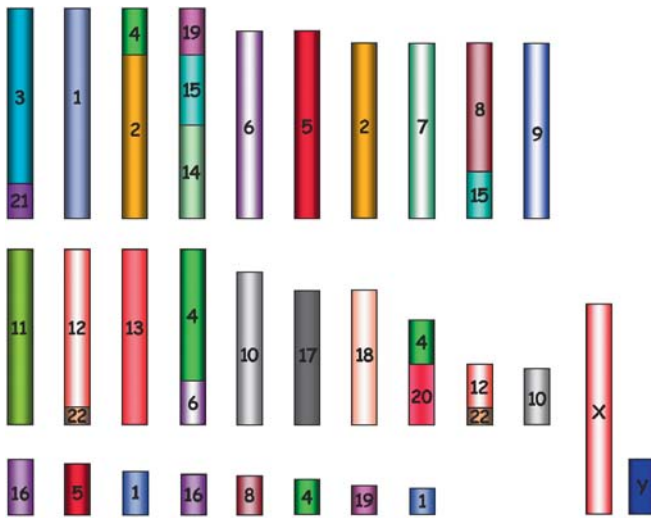


Fig. 5 The *Lemuridae* ancestral karyotype, reconstructed on the basis of the available data from the literature and data from the present paper (see Discussion)

tion of chromosomal rearrangement. In the present case the results we have obtained are a good starting point for a detailed molecular characterization of the breakpoint.

The HSA1q23–pter constitutes the entire LCA3. Its composition appears identical in EMA (EMA9) and EFU (EFU2) (Muller et al. 1997). The arrangement of HSA1qter–q23 in LCA remains unclear, since no unambiguous FISH signals were obtained with BAC/PAC probes belonging to this region. Most likely this region is scattered on microchromosomes, as in EMA and EFU (Muller et al. 1997).

The human chromosome 4 synteny group, as a whole, is considered ancestral to all primates (Murphy et al. 2001). In LCA, EMA, and EFU this chromosome shows a complex evolutionary history. Pieces of HSA4 were detected on LCA4, LCA5, LCA6, and LCA15 (Fig. 2). Difficulties in reconciling our data on LCA with literature data on EMA and EFU prompted us to use the HSA4-specific BACs also on the latter species (see above). The results solved the inconsistencies, as depicted in Fig. 4b. Three associations (HSA4/6; HSA4/2; HSA4/20) are shared by EMA, EFU and LCA. Very likely, therefore, they were present in the *Lemuridae* ancestor. Maximum parsimony, in addition, suggests that they were arranged as in EFU. Subsequently, chromosomal fusions specific for LCA and EMA created the LCA4, EMA6 and EMA8 chromosomal forms. The HSA4/5 association (LCA6) has not been reported in EFU and EMA (Muller et al. 1997). It can be considered therefore, an apomorphic trait.

The HSA8/15 association in LCA11 appears identical to the HSA8/15 association reported in EMA and EFU (Muller et al. 1997), and therefore has been assumed ancestral to *Lemuridae*. Chromosome HSA8 and the HSA14/15 association have been hypothesized as separate chromosomes in the primate ancestor (Murphy et al. 2001). The *Lemuridae* HSA8/15 (LCA11), as well as the

HSA14/15/19 (part of LCA1) associations are therefore derivative.

Minor inconsistencies were also found when comparing the organization of LCA9 with EFU12 and EMA3p. We found that the region HSA7q22–31 was also present on EFU12 and EMA3p. EFU27 and EMA19 were never detected by a complete set of HSA7 probes (see Results).

The overall evaluation of our data shows that the use of WCP probes, which provide an overview, can be advantageously combined with panels of well-characterized BAC/PAC probes in delineating a precise pathway of karyotypic evolution in primates. In the present study we have utilized this approach to characterize in detail the LCA karyotype. Taking into account the ancestral karyotype of primates (Murphy et al. 2001) and using LCA as an outgroup with respect to EFU and EMA, we were able to delineate the most likely chromosomal organization of the *Lemuridae* ancestor, as diagrammatically reported in Fig. 5. In addition, the anchorage of almost all LCA chromosomes to specific human probes will be of great help in the standardization of the karyotype of this species.

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