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Evolutionary history of chromosome 11 featuring four distinct centromere repositioning events in Catarrhini

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Abstract

Panels of BAC clones used in FISH experiments allow a detailed definition of chromosomal marker arrangement and orientation during evolution. This approach has disclosed the centromere repositioning phenomenon, consisting in the activation of a novel, fully functional centromere in an ectopic location, concomitant with the inactivation of the old centromere. In this study, appropriate panels of BAC clones were used to track the chromosome 11 evolutionary history in primates and nonprimate boreoeutherian mammals. Chromosome 11 synteny was found to be highly conserved in both primate and boreoeutherian mammalian ancestors. Amazingly, we detected four centromere repositioning events in primates (in Old World monkeys, in gibbons, in orangutans, and in the *Homo–Pan–Gorilla* (H-P-G) clade ancestor), and one in Equidae. Both H-P-G and Lar gibbon novel centromeres were flanked by large duplicons with high sequence similarity. Outgroup species analysis revealed that this duplicon was absent in phylogenetically more distant primates. The chromosome 11 ancestral centromere was probably located near the HSA11q telomere. The domain of this inactivated centromere, in humans, is almost devoid of segmental duplications. An inversion occurred in chromosome 11 in the common ancestor of H-P-G. A large duplicon, again absent in outgroup species, was found located adjacent to the inversion breakpoints. In Hominoidea, almost all the five largest duplicons of this chromosome appeared involved in significant evolutionary architectural changes.

Keywords: Chromosome 11; Primate evolution; Centromere repositioning; Segmental duplications

Karyotype evolution was first studied using classical cytogenetics and, more recently, using molecular cytogenetic tools. Chromosome painting and reciprocal chromosome painting, in particular, have delineated the organization of the ancestral karyotypes of both primates [1–3] and eutherians [2,4–7]. Genome sequencing projects are producing sequence drafts of an increasing number of vertebrate species, and new methods have been developed to track chromosomal rearrangements at this level [8–11]. However, the number of species whose genome has been fully sequenced is limited and not always phylogenetically appropriate. Fortunately, BAC libraries are available for a larger number of phylogenetically informative species. FISH with panels of BAC clones permits a detailed study of marker order. In primates these studies disclosed an unprecedented phenomenon, centromere repositioning (CR), the movement of the centromere along the chromosome without marker order variation [12–17]. This biological phenomenon also appears widespread in nonprimate mammals [17–19].

In this study, we report on the evolutionary history of chromosome 11 in primates and in selected other mammals. Our analysis showed that marker order organization in primates and the boreoeutherian ancestor was substantially conserved and differed from the human homolog for a single, large inversion. In contrast to this conservative scenario, the position of the centromere, ancestrally located at HSA11qter, moved four times in Catarrhini: a CR event occurred independently in the Old World monkeys (OWM) ancestor, in gibbons, in orangutan, and in the ancestor of the *Homo–Pan–Gorilla* (H-P-G) clade.

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This high number of CR events represented an opportunity to address some of the many unanswered questions raised by this phenomenon, in particular, its relationship to segmental duplications and to acentric fragments resulting from chromosomal breakages.

Results

To establish the evolutionary history of chromosome 11, a panel of 13 human BAC clones (Table 1) distributed along human chromosome 11 was used in cohybridization FISH. Marker order and orientation were determined in all great apes and in selected species of gibbons, OWM, New World monkeys (NWM), and Strepsirhini (see Materials and methods). The success rate of BAC hybridization in the ring-tailed lemur (LCA) was low probably due to the great phylogenetic distance. To overcome this problem we used a pool of two or three overlapping clones encompassing the same locus (see Table 1, sixth column). Occasionally, "overgo" probes were used to identify specific lemur BAC probes by screening high-density filters of the LBNL-2 lemur library (see below). A graphical summary of FISH results is displayed in Fig. 1.

The analysis was extended to selected nonprimate boreoeutherian mammals. The sequence encompassed by each BAC, composing the basic panel, was searched for conservation against the mouse and rat genomes. Overgo probes were designed on the most conserved region of each BAC or very close to it and were then used to screen high-density BAC libraries of pig (CHORI-242), horse (CHORI-241), and cat (RPCI-86). This approach was aimed at assembling a panel of BAC clones mapping to chromosomal loci orthologous to the loci defined by human BACs, thus significantly facilitating mapping comparison. BACs identified by these screenings and the corresponding overgo probes are reported in Table 2. Radiation hybrid maps of cattle [18,20,21], horse [22,23], cat [24], and pig [25,26] were also taken into account. In particular, they were utilized to compensate for the lack of marker order information, resulting from occasional library screening failure (see Table 2). This failure was the case, for example, of marker M in pig. Cattle probe identification took advantage of the collection of BAC clones positioned on the human sequence by BAC-end sequencing, as reported by Larkin et al. [18] and Everts-van der Wind et al. [27] (see Table 2).

Fig. 1 summarizes the results based on this first round of FISH experiments. These data constituted the starting point, in primates, for reiterative FISH experiments, using additional appropriate human BAC clones, aimed at precisely defining each "chromosomal event" (essentially rearrangement breakpoints and evolutionarily new centromere (ENC) seeding points). The most informative clones are reported in italic in Table 1. The complete list of all BAC clones used in the FISH experiments is reported in Supplemental Table 1.

Table 1
Relevant BAC clones used in the study

Code	BAC	Acc. No.	Map	UCSC May 2004	LCA
A	RP11-401C19	AC083984	11p15.5	896,316–1,008,135	RP11-613G2, RP11-496F2
A1	*RP11-1079D17*	BES	11p15.4	3,048,710–3,220,981	
A2	*RP11-640J8*	BES	11p15.4	3,857,738–4,031,847	
A3	*RP11-625D10*	BES	11p15.4	5,667,339–5,864,725	
A4	*RP11-661M13*	BES	11p15.4	5,856,181–6,043,020	
B	RP11-645I8	AC021935	11p15.4	6,071,593–6,234,222	RP11-645I8
C	RP11-56J22	BES	11p15.1	20,180,423–20,332,556	RP11-698J9, RP11-56J22
D	RP11-103P20	BES	11p13	36,021,056–36,180,792	RP11-1005H11, RP11-103P20
E	RP11-150D18	BES	11p12	41,858,281–42,020,207	RP11-937A2, RP11-150D18
F	RP11-29O22	BES	11p11.2	46,582,988–46,722,148	RP11-402C19, RP11-209O22
G	RP11-318O24	BES	11p11.12	50,545,853–50,719,949	RP11-368A23, RP11-318O24
Cen				50,740,430–54,450,781	
H	RP11-217G11	BES	11q12.1	56,609,801–56,610,186	RP11-644A8, RP11-217G11
H1	*RP11-955G14*	BES	11q13.4	71,190,153–71,377,632	
H2	*RP11-757C15*	AP000719	11q13.4	71,236,122–71,432,551	
H3	*RP11-586C4*	BES	11q13.5	75,974,968–76,152,559	
H4	*RP11-7H7*	BES	11q14.1	78,034,239–78,206,818	
I	RP11-119M23	BES	11q14.2	85,346,396–85,346,523	RP11-598K11, RP11-878E11
I2	*RP11-725D13*	BES	11q14.3	89,364,978–89,564,832	
I3	*RP11-692G6*	BES	11q14.3	89,719,943–89,890,899	
J1	*RP11-732A21*	AP001527	11q22.1	101,397,613–101,564,917	
J2	*RP11-864G5*	AP000942	11q22.1	101,600,598–101,786,581	
J	RP11-276O11	BES	11q22.3	105,262,408–105,262,775	RP11-817I5, RP11-276O11
K	RP11-100J10	BES	11q23.1	112,570,374–112,735,819	RP11-667I23, RP11-100J10
L	RP11-90A13	BES	11q25	130,889,653–131,037,422	RP11-368I14, RP11-90A13
L1	RP11-8J13	AC013591	11q25	133,659,867–133,807,866	
M1	RP11-627G23	BES	11q25	133,721,900–133,909,776	
M	RP11-265F9	BES	11q25	134,272,267–134,441,179	
End				134,452,384	

Probes in regular font were used to characterize all primate species. Probes in italic were used to define specific rearrangements or to restrict the evolutionarily new centromere location. BES, BAC end sequence. For details see text.

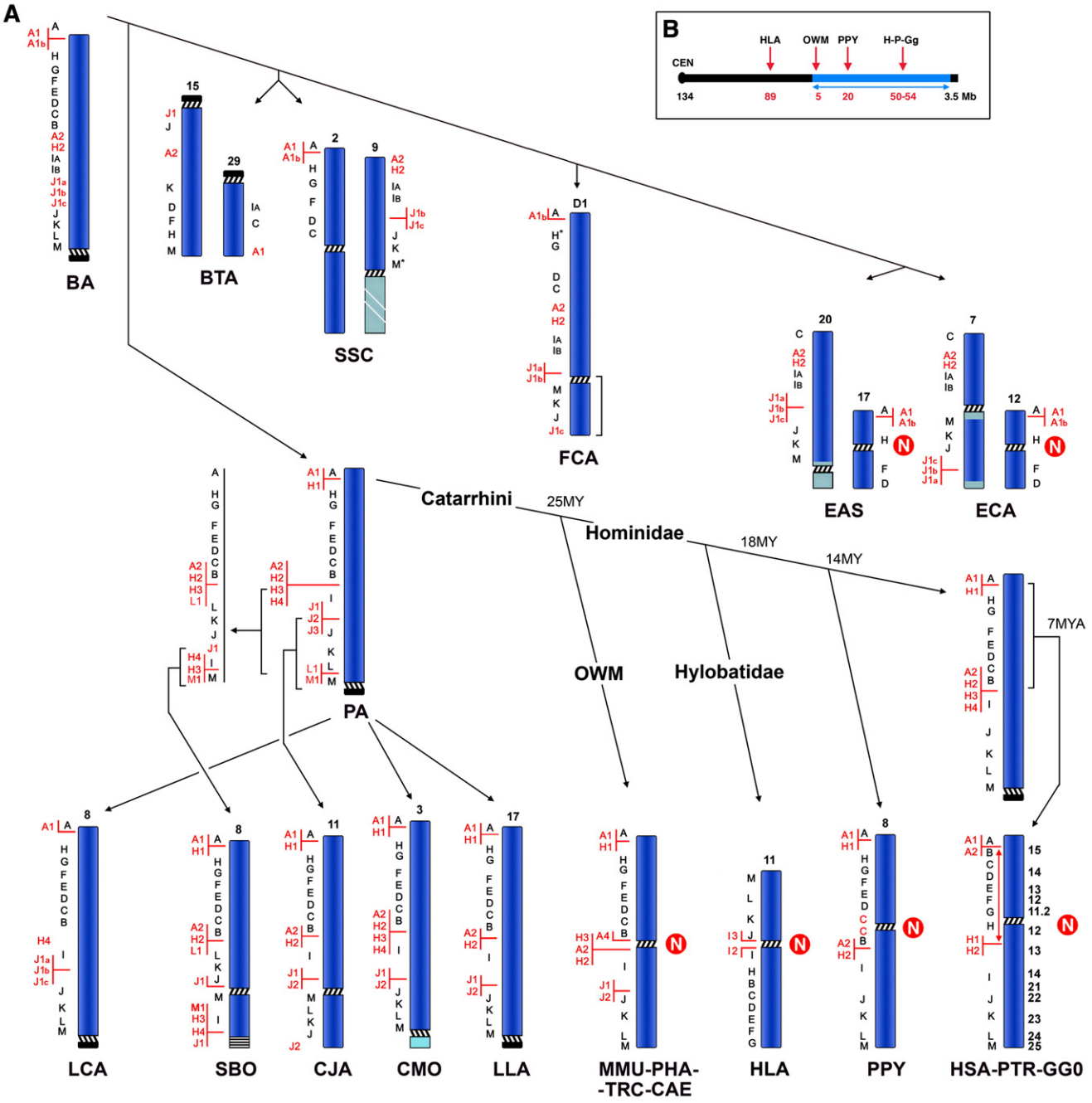

Fig. 1. (A) A summary of the marker order arrangement found in the studied species, from which the arrangements of the primate ancestor (PA) and boreoeutherian ancestor (BA) were derived (see text). N in a red circle stands for new centromere. The number that identifies the chromosome in each species is reported on top of each chromosome. The black letters on the left of each primate chromosome refer to the panel of BAC probes reported in Table 1 (human BACs); letters on cat (FCA), donkey (EAS), horse (ECA), pig (SSC), and cattle (BTA) chromosomes refer to BACs reported in Table 2 (see text). The position of marker M in pig was derived from radiation hybrid data from [25]. Letters in red are the most informative probes used to delimit chromosomal breakpoints or evolutionarily new centromeres. Nonprimate mammal phylogeny is reported according to [40]. Primate divergence time (in million years) is reported according to [41]. Most of human chromosome 11 sequences are located on Lar gibbon chromosome 11. Small segments, present on HLA chromosomes 4 and 5, are not reported. (B) The distribution of evolutionarily new centromeres on the hypothetical chromosome 11 ancestral form. The blue segment corresponds to the inverted region in human, chimpanzee, and gorilla. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Centromere repositioning

Comparison of marker order arrangements reported in Fig. 1A revealed that the centromere of chromosome 11 underwent repositioning events in the ancestor of the H-P-G clade, in orangutan, in gibbons, and in OWM. A CR event also occurred in Equidae. FISH experiments with appropriate BAC clones were undertaken, in primates, to map these evolutionarily new centromeres precisely. The most informative results are graphically reported in Fig. 1A (see Table 1 for details on the clones). Fig. 1B shows the relative positions of the evolutionarily new centromeres with respect to the ancestral form of

Table 2
Clones identified by library screening using overgo probes

Code	LCA LBNL-2	BTA [a] CHORI-240	SSC CHORI-242	ECA CHORI-241	FCA RPCI-86	Overgo sequence	Overgo location (UCSC, MAY 2004 RELEASE)
A			58H15	159N7	46J4	TGCTCAACTGCTTCCAACGTTGAAAGAAATCCTGAG	CHR 11: 999,180–999,215
A1		100H8	88I16			ATTTCAGACATCTCAACAACACAGGCCCTCCAAGTG	CHR 11: 1,988,306–1,988,341
A1b			40G20	34E24	81M19	TCCTGGAAACTCTCGCCAACTGCTGGCCTTTGGAAA	CHR 11: 2,652,576–2,652,611
A2		60I17	95C18	228E18	194I13	GATGGTTCATCGTCATCCAGCCCATCAAAGAGATGT	CHR 11: 3,709,224–3,709,259
C		33L23	92C9	300G11	57J2	CCCAAAACAGCTGGCAAGGGTGAATTAAACCCATTA	CHR 11: 20,142,375–20,142,410
D		62E8	136M6	199O13	200H12	CAGGAATGTGTGTCAAGACCCACCCTGCTAAATTTA	CHR 11: 35,985,378–35,985,413
F		92M9	3H5	199N11		AGCTTCATTTGCGACTGCTCTTTATTCTCTCCAGTC	CHR 11: 46,836,445–46,836,480
G			3K9		59N22	GTCAACATCCAGCTGTACCTCAGTCAAACAGATGGG	CHR 11: 49,346,869–49,346,904
H		16K15	196N18	202H13		ATAGACTGCTCCTGGCCCCGCTGAGGCATCTTTTCT	CHR 11: 56,649,209–56,649,244
H2			41N4	399K1	42C15	ACAGCTGAAGTCTGGAAACGCATGAACATCAAGCCC	CHR 11: 71,484,146–71,484,181
I A		93L12	29I12	234H19	62D7	GCTTTCCTACAATGAACTGTCCTTCTTAAGGCCCCT	CHR 11: 85,346,500–85,346,535
I B			42J19	76E11	54O1	CAGAGCAATTACTCATGTCCTCAGTACACTGCCTCC	CHR 11: 90,110,426–90,110,461
J1a	88O16			329H17	146J19	GAATAAACAAACAGCTGCGGTTAGTGCGTAGGCATT	CHR 11: 100,047,596–100,047,631
J1b	283L12		19L24	67K13	54O1	TTACCTCCAAGGACCATGCCAGCCTGACAGCACTTT	CHR 11: 100,467,697–100,467,732
J1c	242P9	106N12	1E9	367P18	73K3	AAGGGGAAGGGAGTGGAAACAAACCAGGTTGTTAGG	CHR 11: 101,491,743–101,491,778
J		75H3	30N18	476I6	25D12	GCACATGATCTTGTATGGGTTCAGGCTCATTCTTGG	CHR 11: 105,320,284–105,320,319
K		92L1	62E18	306E14	294P14	AGTCATCTCACTGTTTGGCATGCCTGTGAATGACAG	CHR 11: 116,106,496–116,106,531
M	23J12	112P5		219C2	193C10	AGCTCACTTAGCTGCCTTGACATCGATTTTCACCTG	CHR 11: 133,888,691–133,888,726

[a] BTA clones were derived from Larkin et al. [18] (see text).

chromosome 11 for primates. In humans, a large sequence block flanking the chromosome 11 centromere (DUP-cen; Table 3) is duplicated at 11q14.3 (DUP-4; 1.2 Mb; see Table 3). BAC clone RP11-725D13 (I2; Table 1), encompassing the telomeric boundary of the 11q14.3 duplicon, was found to face the 11q side of the Lar centromere exactly (Fig. 1A), while clone I3 (Table 1), 155 kb apart from I2, yielded a FISH signal on the opposite side of the HLA11 centromere (Fig. 1A) (data not shown). The same clones were used in macaque, orangutan, gorilla, and chimpanzee. On chimpanzee and gorilla they gave signals as in humans (data not shown). In orangutan and macaque the clone RP11-725D13 (I2) yielded signals only at regions orthologous to 11q14.3, indicating that this was the ancestral location of these sequences. No signals were detected on regions corresponding to the human centromere (examples are reported in Fig. 2B). Sequence analysis in macaque (UCSC, January 2006 release) perfectly matched the FISH results. Sequence comparisons have indicated that the duplication arose about 14 million years ago [28]. Our FISH analysis is in agreement with this duplication timing.

In donkey (*Equus asinus*, EAS) and horse (*Equus caballus*, ECA), the synteny of human chromosome 11 is disrupted and

Table 3
FISH probes used to characterize the H-P-G inversion and gibbon evolutionarily new centromere

Code	BAC	Acc. No.	Map	UCSC May 2004	H-P-G group	PPY [a]	MMU [a]	HLA [a]
A1	RP11-1079D17	BES	11p15.4	3,048,710–3,220,981	11p15.4	XIp	11p	5q [b]
	DUP-1			**3,364,782–3,579,756**				
A2	RP11-640J8	BES	11p15.4	3,857,738–4,031,847	11p15.4	XIq	11q	11q
	RP11-299D16	BES	11p11.12	48,756,360–48,962,389	11p11.2+11q14.3	XIq	11q	11q
	DUP-CEN			**48,845,000–49,876,000**				
	RP11-646P1	BES	11p11.12	48,991,499–49,172,530	11p11.2+11q14.3	XIq	11q	11q
	RP11-977L13	BES	11q13.2	67,007,487–67,191,071	11q13.2	XIp	11p	
	DUP-2			**67,230,613–67,520,168**				
	RP11-783C9	BES	11q13.2	67,921,051–68,120,386	11q13.2	XIp	11p	
	RP11-916J3	BES	11q13.4	70,224,518–70,415,390	11q13.4	XIp	11p	
	DUP-3			**70,914,582–71,302,090**				
	RP11-769L22	BES	11q13.4	71,003,604–71,180,927	m.s. (Fig. 2A)	XVI+VIII	20p	7qtel+8ptel
H2	RP11-757C15	AP000719	11q13.4	71,236,122–71,432,551	11q13.4	XIq	11q	11q
	RP11-357B14	BES	11q14.3	87,885,697–88,051,561	11q14.3	XIq	11q	11q
	RP11-962B21	BES	11q14.3	88,163,001–88,352,527	11p11.2+11q14.3 [c]	XIq		11q
	DUP-4		**11q14.3**	**88,206,678–89,470,331**				
I2	RP11-725D13	BES	11q14.3	89,364,978–89,564,832	11p11.2+11q14.3	XIq	11q	11q
I3	RP11-692G6	BES	11q14.3	89,719,943–89,890,899	11q14.3	XIq	11q	11p

[a] Results on orangutan (PPY), macaque (MMU), and gibbon (HLA) have to be evaluated considering the H-P-G-specific inversion and the centromere repositioning events.
[b] A small region of 11p15.5 is on HLA5q.
[c] A single signal on XIq 14.3 on PTR.

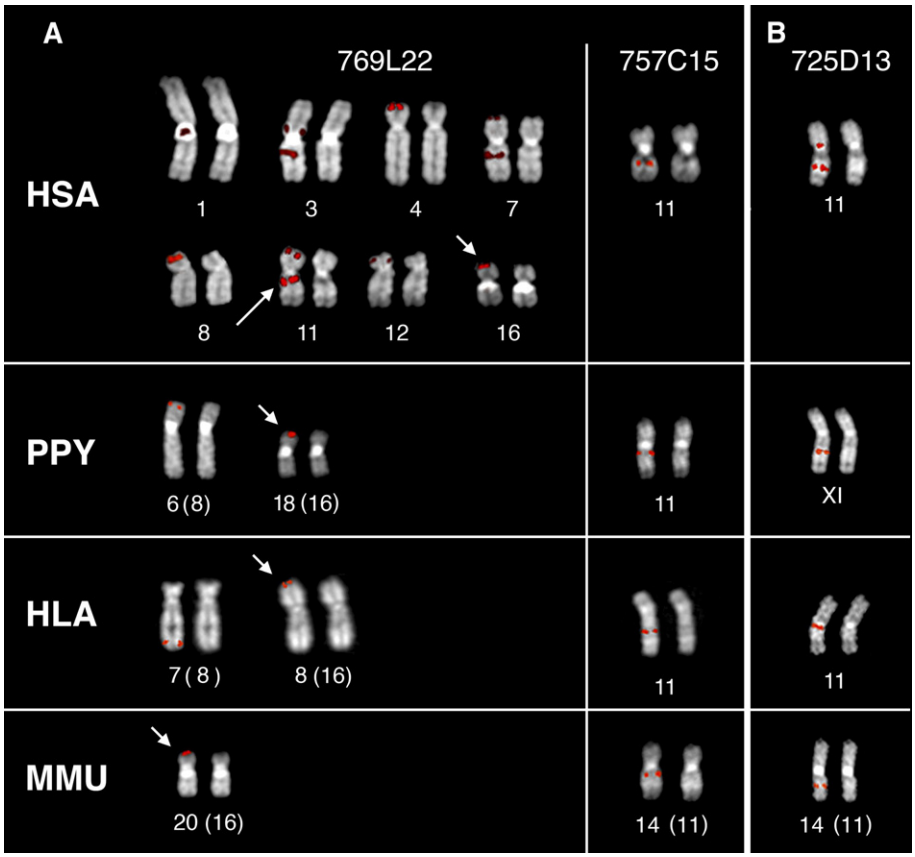

Fig. 2. (A) Partial metaphases show FISH experiments using BAC clones RP11-769L22 and RP11-757C15. RP11-769L22 maps inside the DUP-3. The big signal on 11q13 (long arrow) is due to both DUP-2 and DUP-3, which are ~3.3 Mb apart. For details see text. RP11-757C15 maps in a unique region close and telomeric to RP11-769L22. (B) Partial metaphases showing FISH results of the BAC clone RP11-725D13 mapping, in humans, at 11q14.3 and giving a second signal just above the centromere (11p11.2). This second signal is not present in orangutan, Lar gibbon, and macaque. The macaque chromosome nomenclature is according to Rogers [42]; MMU14 corresponds to human 11.

splits into two chromosomes (Fig. 1A). Very likely, the acentric fragment, generated by a break between markers C and D in the ancestral *Equus* genus, was rescued by the emergence of a novel centromere that was seeded in a region adjacent to marker H, which also flanks the human centromere (Fig. 1A).

11q telomeric region

The analysis of the evolutionary history of chromosome 11 indicated that the ancestral centromere was located in a region corresponding to 11qter in humans. Remains of duplicons in domains where ancestral centromeres were inactivated have been reported for three cases: 2q21, following the well-known tandem fusion that generated human chromosome 2; 6p22.1; and 15q24–q26 [14,16]. Therefore, we searched for duplicons in the 11q25 region, using a specific software (GenAlyzer [29]) and by querying the UCSC browser. The region, in humans, was found to be almost devoid of segmental duplications. The analysis was extended to the corresponding regions of chimpanzee and macaque (UCSC genome browser). The regions were also devoid of segmental duplications. The in silico analysis was coupled with FISH experiments using BAC clones from these two species. Telomeric macaque BAC clones were identified using the MMU integrated comparative map at Baylor College (http://brl.bcm.tmc.edu/pgi/rhesus/). The most telomeric macaque clones CH250-61C9 and CH250-143C7, while yielding a unique signal in macaque and in great apes, in humans produced, in addition to the expected signal at 11q25, a cross-hybridization signal on the pericentromeric heterochromatic blocks of chromosomes 1, 9, and 16 (data not shown). Similar results were obtained with the telomeric chimpanzee clones RP43-33M1, RP43-33B10, and RP43-64E24 (data not shown). Analysis of the Repeating Elements track of the human (UCSC genome browser May 2004 release) and chimpanzee (UCSC March 2006 release) did not reveal, in these BACs, any satellite sequence that could explain the results.

Inversion in the H-P-G clade

The H-P-G clade showed a large inversion with respect to the orangutan and OWMs, which maintain the ancestral form of chromosome 11 (see Discussion). Several human clones were used to localize the breakpoints of the inversion. The most informative clones are reported in Table 3. The definition of the breakpoints was complicated by the presence, on chromosome 11, of three large duplicon clusters with high sequence similarity, containing olfactory receptor genes, at 11p15.4 (DUP-1, ~215 kb in size), at 11q13.2 (DUP-2, ~290 kb), and at 11q13.4 (DUP-3,

~388 kb) (see Table 3; chromosome 11 is well known as enriched in segmental duplications, mostly composed of olfactory receptor gene clusters [30]). These duplicons are distinct from the DUP-cen/DUP-4 reported above. DUP-1, -2, and -3 were compared using GenAlyzer software [29]. DUP-1 and -2 are in direct orientation, while DUP-3 is in inverted orientation with respect to the other two DUPs. In silico and FISH analysis revealed that highly similar duplicon clusters are present on other chromosomes, including the one at 16p13.3 (chromosome 16: 5,070,810–5,285,333; 215 kb). FISH experiments using BACs flanking DUP-1 and DUP-3 (Table 3) indicated that the proximal (11p15.4) and distal (11q13.4) breakpoints mapped inside these duplicons, respectively (see Table 3). The analysis of the draft sequence assembly of the macaque genome, as reported at UCSC (January 2006 release), showed that all three DUPs were absent from macaque chromosome 11, while a related duplicon cluster was found on the tip of chromosome 20 (human 16), in a position corresponding to the human 16p13.3 (see above). To corroborate these in silico findings, the BAC RP11-769L22, completely contained within DUP-3 and covering a major part of it (see Table 3), was used in FISH experiments. It yielded the expected signals in human, a single signal on MMU20 (orthologous to human 16), and two signals in the orangutan on chromosomes PPY6 (HSA8) and PPY18 (HSA16) [31] (Fig. 2A). FISH results on chimpanzee and gorilla were identical with those of human (data not shown). The same BAC clone was hybridized on gibbon, where it yielded two signals, on chromosomes HLA7qter (HSA8) and HLA8pter (HSA16) (Fig. 2A). RP11-757C15, mapping in a unique region close to RP11-769L22, gave a single signal on all species (Fig. 2A).

Breakpoint definition in NWM

Marker arrangement in squirrel monkey (SBO, Cebidae) can be derived from the primate ancestral form assuming two successive inversions (see Fig. 1A). A split signal showed that one breakpoint of the first inversion (paracentric) fell within marker H3 (RP11-586C4), the second is encompassed by the overlapping clones L1 and M1. The second inversion was pericentric. One breakpoint was located within marker J1 (Table 1), which gave a split signal; the second breakpoint was probably located in the short arm of the ancestral chromosome, which is supposedly acrocentric. A single inversion was detected in the marmoset (CJA, Cebidae). A split signal showed that the euchromatic breakpoint fell within marker J2 (Table 1), the second breakpoint was probably located very close or inside the telomeric centromere (data not shown). Clones J1 and J2 are almost overlapping.

Discussion

In the present paper we have delineated the organization of chromosome 11 in primate and boreoeutherian mammal ancestors (BA in Fig. 1). The result was achieved by performing FISH cohybridization experiments of appropriate panels of BAC clones on metaphase chromosomes of 13 primate species and 5 nonprimate boreoeutherian mammals. Fig. 1 diagrammatically summarizes the FISH results. An identical marker order, if centromeres are not considered, was found to be shared by orangutan (great apes) and representatives of OWM (MMU, PHA, TRC, CAE), NWM (CMO and LLA), and Strepsirhini (LCA). This marker order was therefore assumed as ancestral to primates (PA in Fig. 1). The centromere in both primate and boreoeutherian ancestor (BA in Fig. 1) was hypothesized to be telomerically located, close to marker M. This conclusion was based on the fact that the centromere is close to this marker in all the studied NWM species and in lemur catta (LCA), pig (SSC), and cat (FCA) (see Fig. 1).

In cat (FCA), human chromosome 11 is syntenic to the D1 cat chromosome and shows an inversion of the segment encompassed by markers J-M. In donkey (EAS) and pig (SSC) chromosome 11 was split into two chromosomes that, in both cases, appear to derive from a chromosome 11 perfectly colinear with the primate ancestral form. These data strongly indicate that the boreoeutherian ancestor and primate ancestral chromosome 11 were substantially colinear. Painting library analysis has shown that chromosome 11 is syntenic in Afrotheria. In chicken, chromosome 11 is split in three chromosomes (1, 5, and 24; UCSC May 2006 release). If very small inversions are not considered, the marker order of these three fragments appears consistent with the marker order of the boreoeutherian ancestor. Altogether, these data suggest that the marker arrangement of chromosome 11 in mammalian ancestor was, very likely, identical to the form of the boreoeutherian ancestor.

Centromere repositioning

The most relevant piece of information of the present study is the high number of centromere repositioning events that occurred in Catarrhini lineages. None of the extant Catarrhini species conserved the ancestral centromere. Old World monkeys, gibbons, orangutans, and the H-P-G clade showed, indeed, a distinctly located centromere with respect to the hypothesized telomerically located ancestral centromere. In Fig. 1B these evolutionarily new centromeres are positioned on the human sequence map (in Mb) arranged as in the primate ancestor, the blue part of the chromosome representing the segment inverted in the H-P-G clade.

The emergence of novel centromeres in the H-P-G group and in gibbons is intriguing. In gibbon the ENC was seeded at 11q14.3. A segment (chromosome 11: 88,206,678–89,470,331; 1.2 Mb), facing the gibbon centromere, appears duplicated and reshuffled at 11p11.12, facing the H-P-G centromere. In humans, very small stretches of these sequences, below FISH resolution, are duplicated in pericentromeric regions of chromosomes 2, 9, 10, and 22 (UCSC, Duplications track). This duplication is H-P-G-specific, because it is absent in gibbon, orangutan, and macaque. It appears as if these duplicated sequences were independently involved in triggering neocentromere seeding in gibbons and, via a duplicative transposition, in the H-P-G ancestor. If this were the case, it would represent the first example of an ENC event triggered by a transpositive duplication. Unfortunately, we cannot date the

duplication event with respect to the centromere seeding. The hypothesis that the duplicon played a role in the centromere seeding is therefore purely speculative.

Several hypotheses to explain the emergence of a new centromere, in clinical cases or in evolution, have been formulated (for a review see Ferreri et al. [32]). Analysis of these cases suggests that this phenomenon is epigenetic and sequence-independent in nature [33]. In most cases, indeed, the neocentromere arises as an opportunistic event, while the primary event was, very likely, the rearrangement that generated the acentric fragment. On the other hand, there is an evident neocentromere clustering on specific chromosomal domains, in particular at 3q26, 13q21, 15q24–q26, and Yq, indicating that some regions have a peculiar potentiality in triggering neocentromeres. The hypothesis of "latent centromeres," first introduced by Choo [34], was corroborated by Ventura et al. [14], who found that the 15q24–q26 cluster region corresponds to the domain where an ancestral centromere was inactivated. More recent papers, in addition, have pointed out that novel centromeres at both 3q26 and 13q21 loci have occurred both in clinical cases and in evolution [15,17]. In this context is also worth noting that the "reuse" of the same sequences in genome "events" (rearrangements, centromeres, telomeres) appears quite common in evolution [10,35].

As stated, most of the clinical neocentromeres arose in acentric fragments. This appears to be the case for the ENC seeded in horse and monkey ancestor. This event, therefore, can be considered additional indirect evidence that neocentromeres occurring in clinical cases and ENCs are essentially the same phenomenon at two different stages.

11q telomere

Segmental duplication analyses have documented remains of duplicon clusters, typical of pericentromeric regions, in domains where ancestral centromeres were inactivated (see 6p22.1 [16], 15q25 [14], and 2q21 [36]). We therefore searched for duplicons at the 11q telomeric region where the chromosome 11 ancestral centromere was located. Unexpectedly, in humans the region was found almost completely devoid of duplicons. The analysis was extended to the corresponding region in chimpanzee and macaque. The in silico investigation was coupled with FISH analysis. BACs encompassing the telomeric region of humans, chimpanzee, and macaque were hybridized to all three species. Most of the clones gave a single signal on the telomeric region of all species. The telomeric macaque clones CH250-61C9 and CH250-143C7, and chimpanzee clones RP43-33M1, RP43-33B10, and RP43-64E24, yielded noteworthy results. In addition to a specific signal at the 11q telomeres, they lit up, in humans, the pericentromeric heterochromatic blocks of chromosome 1, 9, and 16. Interestingly, similar findings have been reported for the human BAC RP11-498P15 (AC112906). This fully sequenced human clone maps to a neocentromeric region on chromosome 3 that corresponds to the functional centromere in macaque [15]. In humans, this clone hybridized the pericentromeric satellite block of chromosome 1. No known repeat that could justify the cross-hybridization signal was identified in the finished sequence of this clone, neither in the macaque nor in the chimpanzee above-reported clones, thus indicating that the unsequenced heterochromatic blocks of chromosomes 1, 9, and 16 contain, very likely, unknown sequences related, at least in part, to sequences present in these BACs, making apparent the limitations that still exist in the sequencing of centromeric–pericentromeric regions.

Homo–Pan–Gorilla group inversion

The lower part of Fig. 1A shows the most parsimonious chromosomal changes necessary to derive the chromosome 11 organization of the extant examined primate species from the ancestral primate form. A pericentric inversion occurred in the ancestor of the H-P-G clade after the orangutan divergence. The 11p and 11q inversion breakpoints, using human probes against orangutan and macaque, appeared as falling within duplicons DUP-1 and DUP-3, respectively. The high similarity between the two duplicons prevented the precise definition of the breaks. DUP-1, -2, and -3 are absent in orangutan, gibbon, and macaque, but human BACs containing them recognize a region orthologous to the human chromosome 16p13.3 that, therefore, can be considered the locus from where DUP-1, -2, and -3 originated. Human–macaque sequence comparison of the inversion breakpoints indicated that the duplicons inserted exactly at the two breaks. This information leads to the conclusion that the duplicon insertions occurred before or were concomitant with the rearrangement. The high homology between DUP-1 and DUP-3 and their opposite orientation prevented the identification of a potential break inside the duplications. Stankiewicz et al. [37] have shown that the rearrangement that gave rise to the species-specific 4/19 translocation in gorilla was accompanied by an ~250-kb duplication of the breakpoint region on phylogenetic chromosome XVII (17p11–p12 in humans). In our case the situation is complicated by the fact that this duplicon was not ancestrally present on chromosome 11. It could be hypothesized that the insertion was concomitant with the inversion that generated a duplication of the inserted segment.

Precise chromosomal breakpoints were determined for all the rearrangements found in the studied NWM species. Splitting signal results could not be interpreted as merely due to segmental duplications because the identification of the splitting BAC clone was obtained following reiterative FISH experiments that approached each breakpoint from both sides and gave, in all cases, consistent results.

Concluding remarks

We have shown that the chromosome 11 centromeres of all extant Catarrhini species are ENCs. These observations, together with data reported in the literature, indicate that centromere repositioning in evolution is a relatively frequent phenomenon. The observed ENCs, however, are only those that have been fixed in the population. Therefore, they can

be regarded as the tip of a large iceberg. The incidence of neocentromeres in the human population can provide estimates of its extent. In this respect, the ENC that appeared in Equidae, reported in the present paper, provides significant support to the view that human clinical neocentromeres and ENC are two faces of the same coin.

Materials and methods

Metaphase preparations were obtained from lymphoblastoid or fibroblast cell lines of the following species: Great apes—common chimpanzee (*Pan troglodytes,* PTR), gorilla (*Gorilla gorilla,* GGO), Borneo orangutan (*Pongo pygmaeus pygmaeus,* PPY); Hylobatidae—white-handed gibbon (*Hylobates lar,* HLA); OWM—rhesus monkey (*Macaca mulatta,* MMU, Cercopithecinae), sacred baboon (*Papio hamadryas,* PHA), African green monkey (*Cercopithecus aethiops,* CAE, Cercopithecinae), silvered leaf monkey (*Trachypithecus cristatus,* TCR, Colobinae); NWM—wooly monkey (*Lagothrix lagothricha,* LLA, Atelinae), common marmoset (*Callithrix jacchus,* CJA, Callitricinae), dusky titi (*Callicebus moloch,* CMO, Callicebinae), squirrel monkey (*Saimiri boliviensis boliviensis,* SBO, Callicebinae); Strepsirhini—ring-tailed lemur (*Lemur catta,* LCA, Lemurinae). Selected clones were also hybridized to metaphases of three Hylobatidae species. FISH experiments in nonprimate mammals were performed on metaphases obtained from fibroblasts of the following species: cattle (*Bos taurus,* BTA, Cetartiodactyla,), pig (*Sus scrofa,* SSC, Cetartiodactyla), horse (*Equus caballus,* ECA, Perissodactyla), donkey (*Equus asinus,* EAS, Perissodactyla), cat (*Felis catus domesticus,* FCA, Carnivora). Specific probes for nonprimate mammals were identified by screening high-density filters of specific libraries, as described under Results.

The identification of BAC clones for some species took advantage of specific genome browsers (rhesus macaque, http://brl.bcm.tmc.edu/pgi/rhesus/; chimpanzee, http://genome.ucsc.edu).

DNA extraction from BACs has already been reported [38]. FISH experiments were performed essentially as described by Lichter et al. [39] with minor modifications. Digital images were obtained using a Leica DMRXA2 epifluorescence microscope equipped with a cooled CCD camera (Princeton Instruments, NJ, USA). FITC, Cy3, DEAC, Cy5, and DAPI fluorescence signals, detected with specific filters, were recorded separately as gray-scale images. Pseudocoloring and merging of images were performed using Adobe PhotoShop software.

Evolutionary marker order reconstruction took advantage of the GRIMM software package [8] (http://www.cs.ucsd.edu/groups/bioinformatics/GRIMM/).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ygeno.2007.01.007.

References

[1] S. Muller, J. Wienberg, “Bar-coding” primate chromosomes: molecular cytogenetic screening for the ancestral hominoid karyotype, Hum. Genet. 109 (2001) 85–94.

[2] W.J. Murphy, R. Stanyon, S.J. O’Brien, Evolution of mammalian genome organization inferred from comparative gene mapping, Genome Biol. 2 (2001) REVIEWS0005.

[3] R. Stanyon, S. Consigliere, S. Muller, A. Morescalchi, M. Neusser, J. Wienberg, Fluorescence in situ hybridization (FISH) maps chromosomal homologies between the dusky titi and squirrel monkey, Am. J. Primatol. 50 (2000) 95–107.

[4] F. Richard, C. Messaoudi, A. Bonnet-Garnier, M. Lombard, B. Dutrillaux, Highly conserved chromosomes in an Asian squirrel (Menetes berdmorei, Rodentia: Sciuridae) as demonstrated by ZOO-FISH with human probes, Chromosome Res. 11 (2003) 597–603.

[5] F. Yang, E.Z. Alkalaeva, P.L. Perelman, A.T. Pardini, W.R. Harrison, P.C. O’Brien, B. Fu, A.S. Graphodatsky, M.A. Ferguson-Smith, T.J. Robinson, Reciprocal chromosome painting among human, aardvark, and elephant (superorder Afrotheria) reveals the likely eutherian ancestral karyotype, Proc. Natl. Acad. Sci. USA 100 (2003) 1062–1066.

[6] W.J. Murphy, P.A. Pevzner, J.S. O’Brien, Mammalian phylogenomics comes of age, Trends Genet. 20 (2004) 631–639.

[7] M. Svartman, G. Stone, J.E. Page, R. Stanyon, A chromosome painting test of the basal Eutherian karyotype, Chromosome Res. 12 (2004) 45–53.

[8] G. Bourque, P.A. Pevzner, Genome-scale evolution: reconstructing gene orders in the ancestral species, Genome Res. 12 (2002) 26–36.

[9] G. Bourque, P.A. Pevzner, G. Tesler, Reconstructing the genomic architecture of ancestral mammals: lessons from human, mouse, and rat genomes, Genome Res. 14 (2004) 507–516.

[10] W.J. Murphy, D.M. Larkin, A. Everts-van der Wind, G. Bourque, G. Tesler, L. Auvil, J.E. Beever, B.P. Chowdhary, F. Galibert, L. Gatzke, C. Hitte, S.N. Meyers, D. Milan, E.A. Ostrander, G. Pape, H.G. Parker, T. Raudsepp, M.B. Rogatcheva, L.B. Schook, L.C. Skow, M. Welge, J.E. Womack, J. O’Briens, P.A. Pevzner, H.A. Lewin, Dynamics of mammalian chromosome evolution inferred from multispecies comparative maps, Science 309 (2005) 613–617.

[11] A. Ma, L. Zhang, B.B. Suh, B.J. Raney, R.C. Burhans, W.J. Kent, M. Blanchette, D. Haussler, W. Miller, Reconstructing contiguous regions of an ancestral genome, Genome Res. 16 (2006).

[12] G. Montefalcone, S. Tempesta, M. Rocchi, N. Archidiacono, Centromere repositioning, Genome Res. 9 (1999) 1184–1188.

[13] M. Ventura, N. Archidiacono, M. Rocchi, Centromere emergence in evolution, Genome Res. 11 (2001) 595–599.

[14] M. Ventura, J.M. Mudge, V. Palumbo, S. Burn, E. Blennow, M. Pierluigi, R. Giorda, O. Zuffardi, N. Archidiacono, M.S. Jackson, M. Rocchi, Neocentromeres in 15q24–26 map to duplicons which flanked an ancestral centromere in 15q25, Genome Res. 13 (2003) 2059–2068.

[15] M. Ventura, S. Weigl, L. Carbone, M.F. Cardone, D. Misceo, M. Teti, P. D’Addabbo, A. Wandall, E. Björck, P. de Jong, X. She, E.E. Eichler, N. Archidiacono, M. Rocchi, Recurrent sites for new centromere seeding, Genome Res. 14 (2004) 1696–1703.

[16] V. Eder, M. Ventura, M. Ianigro, M. Teti, M. Rocchi, N. Archidiacono, Chromosome 6 phylogeny in primates and centromere repositioning, Mol. Biol. Evol. 20 (2003) 1506–1512.

[17] M.F. Cardone, A. Alonso, M. Pazienza, M. Ventura, G. Montemurro, L. Carbone, P.J. de Jong, R. Stanyon, P. D’Addabbo, N. Archidiacono, X. She, E.E. Eichler, P.E. Warburton, M. Rocchi, Independent centromere formation in a capricious, gene-free domain of chromosome 13q21 in Old World monkeys and pigs, Genome Biol. 7 (2006) R91.

[18] D.M. Larkin, A. Everts-van der Wind, M. Rebeiz, P.A. Schweitzer, S. Bachman, C. Green, C.L. Wright, E.J. Campos, L.D. Benson, J. Edwards, L. Liu, K. Osoegawa, J.E. Womack, P.J. de Jong, H.A. Lewin, A cattle–human comparative map built with cattle BAC-ends and human genome sequence, Genome Res. 13 (2003) 1972–1996.

[19] L. Carbone, S.G. Nergadze, E. Magnani, D. Misceo, M. Francesca Cardone, R. Roberto, L. Bertoni, C. Attolini, M. Francesca Piras, P. de Jong, T. Raudsepp, B.P. Chowdhary, G. Guerin, N. Archidiacono, M. Rocchi, E. Giulotto, Evolutionary movement of centromeres in horse, donkey, and zebra, Genomics 87 (2006) 777–782.

[20] A. Everts-Van Der Wind, S.R. Kata, M.R. Band, M. Rebeiz, D.M. Larkin, R.E. Everts, C.A. Green, L. Liu, S. Natarajan, T. Goldammer, J.H. Lee, S. McKay, J.E. Womack, H.A. Lewin, A 1463 gene cattle–human comparative map with anchor points defined by human genome sequence coordinates, Genome Res. 14 (2004) 1424–1437.

[21] T. Itoh, T. Watanabe, N. Ihara, P. Mariani, C.W. Beattie, Y. Sugimoto, A.

Takasuga, A comprehensive radiation hybrid map of the bovine genome comprising 5593 loci, Genomics 85 (2005) 413–424.
[22] B.P. Chowdhary, T. Raudsepp, S.R. Kata, G. Goh, L.V. Millon, V. Allan, F. Piumi, G. Guerin, J. Swinburne, M. Binns, T.L. Lear, J. Mickelson, J. Murray, D.F. Antczak, J.E. Womack, L.C. Skow, The first-generation whole-genome radiation hybrid map in the horse identifies conserved segments in human and mouse genomes, Genome Res. 13 (2003) 742–751.
[23] T. Leeb, C. Vogl, B. Zhu, P.J. de Jong, M.M. Binns, B.P. Chowdhary, M. Scharfe, M. Jarek, G. Nordsiek, F. Schrader, H. Blocker, A human–horse comparative map based on equine BAC end sequences, Genomics 87 (2006) 772–776.
[24] M. Menotti-Raymond, V.A. David, Z.Q. Chen, K.A. Menotti, S. Sun, A.A. Schaffer, R. Agarwala, J.F. Tomlin, S.J. O'Brien, W.J. Murphy, Second-generation integrated genetic linkage/radiation hybrid maps of the domestic cat (Felis catus), J. Hered. 94 (2003) 95–106.
[25] M. Yerle, P. Pinton, C. Delcros, N. Arnal, D. Milan, A. Robic, Generation and characterization of a 12,000-rad radiation hybrid panel for fine mapping in pig, Cytogenet. Genome Res. 97 (2002) 219–228.
[26] S.N. Meyers, M.B. Rogatcheva, D.M. Larkin, M. Yerle, D. Milan, R.J. Hawken, L.B. Schook, J.E. Beever, Piggy-BACing the human genome. II. A high-resolution, physically anchored, comparative map of the porcine autosomes, Genomics 86 (2005) 739–752.
[27] A. Everts-van der Wind, D.M. Larkin, C.A. Green, J.S. Elliott, C.A. Olmstead, R. Chiu, J.E. Schein, M.A. Marra, J.E. Womack, H.A. Lewin, A high-resolution whole-genome cattle–human comparative map reveals details of mammalian chromosome evolution, Proc. Natl. Acad. Sci. USA 102 (2005) 18526–18531.
[28] J. Zhang, S. Qin, S.N. Sait, L.L. Haley, W.M. Henry, M.J. Higgins, N.J. Nowak, T.B. Shows, D.S. Gerhard, The pericentromeric region of human chromosome 11: evidence for a chromosome-specific duplication, Cytogenet. Cell Genet. 94 (2001) 137–141.
[29] S. Kurtz, J.V. Choudhuri, E. Ohlebusch, C. Schleiermacher, J. Stoye, R. Giegerich, REPuter: the manifold applications of repeat analysis on a genomic scale, Nucleic Acids Res. 29 (2001) 4633–4642.
[30] T.D. Taylor, H. Noguchi, Y. Totoki, A. Toyoda, Y. Kuroki, K. Dewar, C. Lloyd, T. Itoh, T. Takeda, D.W. Kim, X. She, K.F. Barlow, T. Bloom, E. Bruford, J.L. Chang, C.A. Cuomo, E. Eichler, M.G. Fitzgerald, D.B. Jaffe, K. Labutti, R. Nicol, H.S. Park, C. Seaman, C. Sougnez, X. Yang, A.R. Zimmer, M.C. Zody, B.W. Birren, C. Nusbaum, A. Fujiyama, M. Hattori, J. Rogers, E.S. Lander, Y. Sakaki, Human chromosome 11 DNA sequence and analysis including novel gene identification, Nature 440 (2006) 497–500.
[31] An international system for human cytogenetic nomenclature (1985) ISCN 1985, Report of the Standing Committee on Human Cytogenetic Nomenclature, Birth Defects Orig. Artic. Ser. 21 (1985) 1–117.
[32] G.C. Ferreri, D.M. Liscinsky, J.A. Mack, M.D. Eldridge, R.J. O'Neill, Retention of latent centromeres in the mammalian genome, J. Hered. 96 (2005) 217–224.
[33] A. Alonso, R. Mahmood, S. Li, F. Cheung, K. Yoda, P.E. Warburton, Genomic microarray analysis reveals distinct locations for the CENP-A binding domains in three human chromosome 13q32 neocentromeres, Hum. Mol. Genet. 12 (2003) 2711–2721.
[34] K.H.A. Choo, Centromere DNA dynamics: latent centromeres and neocentromere formation, Am. J. Hum. Genet. 61 (1997) 1225–1233.
[35] P. Pevzner, G. Tesler, Human and mouse genomic sequences reveal extensive breakpoint reuse in mammalian evolution, Proc. Natl. Acad. Sci. USA 100 (2003) 7672–7677.
[36] J.A. Bailey, Z. Gu, R.A. Clark, K. Reinert, R.V. Samonte, S. Schwartz, M.D. Adams, E.W. Myers, P.W. Li, E.E. Eichler, Recent segmental duplications in the human genome, Science 297 (2002) 1003–1007.
[37] P. Stankiewicz, S.S. Park, K. Inoue, J.R. Lupski, The evolutionary chromosome translocation 4;19 in Gorilla gorilla is associated with microduplication of the chromosome fragment syntenic to sequences surrounding the human proximal CMT1A-REP, Genome Res. 11 (2001) 1205–1210.
[38] M. Ventura, M. Boniotto, M.F. Cardone, L. Fulizio, N. Archidiacono, M. Rocchi, S. Crovella, Characterization of a highly repeated DNA sequence family in five species of the genus Eulemur, Gene 275 (2001) 305–310.
[39] P. Lichter, C.-J. Tang Chang, K. Call, G. Hermanson, G.A. Evans, D. Housman, D.C. Ward, High resolution mapping of human chromosomes 11 by in situ hybridization with cosmid clones, Science 247 (1990) 64–69.
[40] H. Nishihara, M. Hasegawa, N. Okada, Pegasoferae, an unexpected mammalian clade revealed by tracking ancient retroposon insertions, Proc. Natl. Acad. Sci. USA 103 (2006) 9929–9934.
[41] M. Goodman, The genomic record of humankind's evolutionary roots, Am. J. Hum. Genet. 64 (1999) 31–39.
[42] J. Rogers, R. Garcia, W. Shelledy, J. Kaplan, A. Arya, Z. Johnson, M. Bergstrom, L. Novakowski, P. Nair, A. Vinson, D. Newman, G. Heckman, J. Cameron, An initial genetic linkage map of the rhesus macaque (*Macaca mulatta*) genome using human microsatellite loci, Genomics 87 (2006) 30–38.