

Reconstructing the Phylogeny of the Human Chromosome 4 Synteny using Comparative Karyology and Genomic Data Analysis

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Abstract — This work focuses on the evolution of the architecture of human chromosome 4 (HSA4) through the analysis of chromosomal regions that have been conserved over time, and the comparison of regions that have been involved in different rearrangements in placental lineages. As with most elements of the human genome, HSA4 is considered to be evolutionarily stable. A more detailed analysis indicates that the syntenic association has been reshuffled by a series of rearrangements, yielding different chromosomes in various taxa. In its ancestral eutherian state, HSA4 has a syntenic association with HSA8p. We investigated the complex origin of this human chromosome using three different approaches, including: the analysis of chromosome painting features among 157 mammalian species gleaned from published data; the analysis of conserved syntenic orthologous blocks derived from the Ensembl dataset (www.ensembl.org); and the reconstruction of the orthologues of HSA4 in various species, using a maximum parsimony (MP) analysis of evolutionary breakpoints. The phylogenetic pattern recovered shows four discrete chromosomal regions have primarily been implicated in chromosomal rearrangement: 4p15.3, 4p16.1, 4q12 and 4q31.1. Our results demonstrate that chromosome painting and ancestral chromosome reconstructions can elucidate the diverse structural rearrangements that characterize different evolutionary lineages.

Key words: comparative chromosome painting, comparative genomics, evolutionary breakpoints, human evolution.

INTRODUCTION

The use of ZOO-fluorescence *in situ* hybridization (ZOO-FISH) has facilitated large-scale genome-wide comparisons across different taxa. Using human/non-human DNA sequences as probes it is possible to identify regions of chromosomal homology between species with a precision that exceeds conventional cytogenetic approaches (SCHERTHAN *et al.* 1994; MULLER *et al.* 1999). This has allowed molecular cytogeneticists to compare hundreds of species from almost all extant orders of mammals, and to recognize that many chromosomal regions or whole hu-

man syntenies have been highly conserved over extensive evolutionary periods. From a theoretical viewpoint, a chromosomal rearrangement is considered a valuable phylogenetic marker, less prone to convergence than other, less complex mutations; this allows the application of cladistic analysis to chromosomal homologies. The application of cladistic procedures has aided the understanding of genomic evolution in animals (MULLER *et al.* 2000; FROENICKE 2005) by identifying plesiomorphic (ancestral) and apomorphic (derived) character states, which can be used to reconstruct hypothetical ancestral karyotypes (YANG *et al.* 2003; FERGUSON-SMITH and TRIFONOV 2007).

The catalyst behind the recent acceleration in knowledge of human syntenies, and the theoretical advances contingent on this knowledge (PEVZNER 2003; MURPHY *et al.* 2005; BOURQUE *et al.* 2005; MA *et al.* 2006), is the Human Genome Project (HGP). Even if the primary goal of the

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project was the identification of human sequence autapomorphies, or sequences and markers associated with genetic diseases, it yielded insights into the architecture of the human genome and its fine-scale organization, and the evolution of homologous chromosomes or chromosomal regions (CARBONE *et al.* 2006; COLLINS *et al.* 2003; HARDISON *et al.* 2003; WEISE *et al.* 2005; WIENBERG *et al.* 2004; 2005).

Large-scale genome sequencing has also begun to generate powerful resources for evolutionary biologists. Partial and complete sequences from a wide diversity of animals are now available to researchers (www.genome.ucsc.edu; www.ensembl.org). These resources, taken together with cytogenetic data, have greatly enhanced the scope of comparative genomic analysis, providing a means for exploring the nature and timing of changes in chromosomal architectures; changes that are not always detectable using chromosome painting.

Human Chromosome 4 - HSA4 is one of the last chromosomes to have attracted the attention of researchers (HILLIER *et al.* 2005; WIENBERG 2005). It is a large "B group" autosome. According to the Ensembl data set (www.ensembl.org), it consists of 191,154,276 base pairs, and is characterized by 905 known protein-coding genes, 146 pseudogenes, and the largest "gene desert" of the human genome. HSA4 further displays a low percentage of duplicated sequences (2.3%) with respect to the genome average (5.2%), and is relatively devoid of segmental duplications.

Since the localization of the gene for Huntington's disease on HSA4p16 (WALKER 2007), other disease-related genes, like those responsible for Wolf-Hirschhorn Syndrome (ZOLLINO *et al.* 2004; GANDELMAN *et al.* 1992) and Fascioscapulohumeral Muscular Dystrophy (FSHD) (BODEGA *et al.* 2007), have been linked to HSA4. The chromosome is increasingly implicated in hypertension, rheumatoid arthritis, dentinogenesis imperfecta, and renal carcinoma (MCKNITE *et al.* 2008; ANEAS *et al.* 2009; KOJIMA *et al.* 2009; KLATTE *et al.* 2009). Chromosomal physiology and comparative studies indicate that the HSA4 synteny includes bands that are prone to breakage. These bands contain fragile sites of uncertain significance (RUIZ-HERRERA *et al.* 2002) or hotspots with possible evolutionary consequences (FINELLI *et al.* 1999; BREEN *et al.* 1999; MULLER *et al.* 1999; YANG *et al.* 2000; YANG *et al.* 2003; KEMKEMER *et al.* 2009). Furthermore, a variety of "cancer-associated" genes potentially reside at or near these breakpoints (RUIZ-HERRERA *et al.*

2005, 2006). None of the breakpoints appear to be specific to the primate lineage.

The HSA4 syntenic assemblage exists as a 4/8p ancestral synteny in boreoeutherians (RICHARD *et al.* 2003a; SVARTMAN *et al.* 2004; WIENBERG *et al.* 2004). This association has apparently undergone a series of diverse rearrangements in different lineages. In this study, we reconstructed the evolution of the HSA4 syntenic association using three different approaches. First, we compiled published chromosome painting results for 157 mammalian species (Table 1). Second, we identified and compared orthologous syntenic blocks taken from the Ensembl dataset (www.ensembl.org), and constructed the most plausible representation of the ancestral vertebrate chromosome 4, followed by an in-depth analysis of the dynamics of HSA4 within the order Primates. The final analysis consisted of an architectural reconstruction of HSA4 homologs in various species, with the aim of relating these rearrangements to the evolutionary breakpoints that have been identified on HSA4.

MATERIAL AND METHODS

Data sources - We analysed published chromosome painting data from 157 species (Table 1) classified under 16 mammalian orders. This comparative analysis was used to build a phylogenetic hypothesis of the evolutionary dynamics of the HSA4 synteny within the taxa. Twelve species for which both reciprocal chromosome painting and whole genomic comparative data were available, were used for the construction of a phylogenetic tree for the syntenic association. These species represented 12 orders. The opossum (*Monodelphis domestica*) and the chicken (*Gallus gallus*) were included as outgroups. A phylogenetic tree for primates was reconstructed on the basis of chromosomal data for 80 species.

Phylogenetic analysis - On the base of reciprocal painting and sequence data (www.ensembl.org), a comparative table of breakpoints was compiled (Table 2), and a data matrix was assembled based on four potential character states for each breakpoint: 0=absent (chromosomal bands are not involved in breakpoints and/or associated with human syntenies); 1=presence of an evolutionary breakpoint localized in a specific band; 2=presence of chromosomal bands orthologous to HSA4 associated with human chromosome 8; 3=presence of chromosomal bands

TABLE 1 — List of species analysed by chromosomal painting using human paints and/or comparison of whole genome assemblies. The number of human chromosome 4 orthologous blocks (OB) is indicated for each species. CP: chromosomae painting; RP: reciprocal painting data.

| Order | Species | OB | References | Methods |
|-------------------------------|----------------------------------|------------------------------|---------------------------------|------------------------------|
| Scandentia | <i>Tupaia belangeri</i> | 4 | Muller <i>et al.</i> 1999 | RP |
| | | | Stanyon, personal communication | RP |
| Dermoptera | <i>Galeopterus variegatus</i> | 3 | Nie <i>et al.</i> 2008 | RP |
| Rodentia | <i>Sciurus carolinensis</i> | 2 | Li <i>et al.</i> 2004 | RP |
| | <i>Petaurista albiventer</i> | 2 | Li <i>et al.</i> 2004 | CP |
| | <i>Callosciurus erythracheus</i> | 2 | Li <i>et al.</i> 2004 | CP |
| | <i>Tamias sibiricus</i> | 2 | Li <i>et al.</i> 2004 | CP |
| | <i>Menetes berdmorei</i> | 2 | Richard <i>et al.</i> 2003b | CP |
| | <i>Graphiurus ocularis</i> | 2 | Picone, unpublished data | RP |
| | <i>Graphiurus platyops</i> | 2 | Picone, unpublished data | RP |
| | <i>Castor fiber</i> | 3 | Graphodatsky <i>et al.</i> 2008 | CP |
| | <i>Sicista betulina</i> | 3 | Graphodatsky <i>et al.</i> 2008 | CP |
| | <i>Pedetes capensis</i> | 3 | Graphodatsky <i>et al.</i> 2008 | CP |
| | <i>Mus musculus</i> | 4 | www.ensembl.org | |
| | <i>Rattus norvegicus</i> | 9 | www.ensembl.org | |
| | Lagomorpha | <i>Oryctolagus cuniculus</i> | 2 | Korstanje <i>et al.</i> 1999 |
| Hayes <i>et al.</i> 2002 | | | | RP |
| Pholidota | <i>Manis javanica</i> | 2 | Yang <i>et al.</i> 2006 | CP |
| Eulipotyphla | <i>Talpa europea</i> | 4 | Volleth and Muller 2006 | ZOO-FISH |
| | <i>Sorex araneus</i> | 2 | Ye <i>et al.</i> 2006 | CP |
| | <i>Neotetrarcus sinensis</i> | 4 | Ye <i>et al.</i> 2006 | CP |
| | <i>Blarinella griselda</i> | 4 | Ye <i>et al.</i> 2006 | CP |
| | <i>Hemiechinus auritus</i> | 5 | Yang <i>et al.</i> 2006 | CP |
| Cetartiodactyla | <i>Camel dromedarius</i> | 2 | Balmus <i>et al.</i> 2007 | RP |
| | <i>Sus scrofa</i> | 2 | Balmus <i>et al.</i> 2007 | RP |
| | | | Froenicke and Wienberg 2001 | CP |
| | <i>Bos taurus</i> | 4 | Balmus <i>et al.</i> 2007 | RP |
| | | | www.ensembl.org | |
| | <i>Ovis aries</i> | 2 | Froenicke and Wienberg 2001 | CP |
| | | | Iannuzzi <i>et al.</i> 1999 | Comparative genomics |
| | | | Schibler <i>et al.</i> 1998 | Gene mapping |
| | <i>Giraffa camelopardalis</i> | 2 | Huang <i>et al.</i> 2008 | CP |
| | | | Kulemzina <i>et al.</i> 2009 | ZOO-FISH |
| | <i>Muntiacus muntjak vag.</i> | 2 | Froenicke <i>et al.</i> 1997a | CP |
| | | | Yang <i>et al.</i> 1997a | CP |
| | <i>Globicefala melas</i> | 2 | Kulemzina <i>et al.</i> 2009 | ZOO-FISH |
| <i>Hippopotamus amphibius</i> | 3 | Kulemzina <i>et al.</i> 2009 | ZOO-FISH | |
| <i>Okapia johnstoni</i> | 3 | Kulemzina <i>et al.</i> 2009 | ZOO-FISH | |
| <i>Moschus moschifera</i> | 3 | Kulemzina <i>et al.</i> 2009 | ZOO-FISH | |
| Perissodactyla | <i>Equus zebra</i> | 3 | Richard <i>et al.</i> 2001 | CP |
| | | | Trifonov <i>et al.</i> 2008 | ZOO-FISH |
| | <i>Equus caballus</i> | 3 | Yang <i>et al.</i> 2004 | RP |
| | | | www.ensembl.org | |
| | <i>Equus asinus</i> | 3 | Trifonov <i>et al.</i> 2008 | ZOO-FISH |
| | <i>Equus grevyi</i> | 3 | Trifonov <i>et al.</i> 2008 | ZOO-FISH |
| | <i>Equus burcellii</i> | 3 | Yang <i>et al.</i> 2004 | RP |
| | <i>Equus zebra harmannae</i> | 3 | Trifonov <i>et al.</i> 2008 | ZOO-FISH |
| | <i>Equus hemionus onager</i> | 3 | Trifonov <i>et al.</i> 2008 | ZOO-FISH |
| | <i>Equus przewalskii</i> | 3 | Trifonov <i>et al.</i> 2008 | ZOO-FISH |
| | <i>Ceratotherium simum</i> | 3 | Trifonov <i>et al.</i> 2008 | ZOO-FISH |

TABLE 1 — Condt.

| Order | Species | OB | References | Methods | | |
|-------------------------------|-----------------------------------|---------------------------|---|--|-------------------------------|----------|
| Carnivora | <i>Diceros bicornis</i> | 3 | Trifonov <i>et al.</i> 2008 | ZOO-FISH | | |
| | <i>Tapirus indicus</i> | 3 | Trifonov <i>et al.</i> 2008 | ZOO-FISH | | |
| | <i>Tapirus terrestris</i> | 3 | Trifonov <i>et al.</i> 2008 | ZOO-FISH | | |
| | <i>Tapirus bairdii</i> | 3 | Trifonov <i>et al.</i> 2008 | ZOO-FISH | | |
| | <i>Tapirus pinchaque</i> | 3 | Trifonov <i>et al.</i> 2008 | ZOO-FISH | | |
| | <i>Felis catus</i> | 2 | Wienberg <i>et al.</i> 1997 Yang <i>et al.</i> 2000 Murphy <i>et al.</i> 2007 | Comparative genomics CP RH map | | |
| | <i>Canis familiaris</i> | 6 | Breen <i>et al.</i> 1999 Yang <i>et al.</i> 2000 Graphodatsky <i>et al.</i> 2000a Graphodatsky <i>et al.</i> 2008 www.ensembl.org | RP CP CP ZOO-FISH | | |
| | | <i>Mustela vison</i> | 2 | Hameister <i>et al.</i> 1997 Graphodatsky <i>et al.</i> 2001 Graphodatsky <i>et al.</i> 2002 | ZOO-FISH CP RP | |
| | | | <i>Mustela putorius furo</i> | 2 | Cavagna <i>et al.</i> 2000 | CP |
| | | | <i>Phoca vitulina</i> | 2 | Froenicke <i>et al.</i> 1997b | ZOO-FISH |
| | | <i>Tremarctos ornatus</i> | 2 | Tian <i>et al.</i> 2004 | RP | |
| | | Chiroptera | <i>Ailuropoda melanoleuca</i> | 2 | Nash <i>et al.</i> 1998 | CP |
| | <i>Eonycteris spelea</i> | | 3 | Volleth <i>et al.</i> 2002 | ZOO-FISH | |
| | <i>Glossophaga soricina</i> | | 2 | Volleth <i>et al.</i> 2002 | ZOO-FISH | |
| <i>Myotis myotis</i> | 3 | | Volleth <i>et al.</i> 2002 | ZOO-FISH | | |
| <i>Mormopterus planiceps</i> | 2 | | Volleth <i>et al.</i> 2002 | ZOO-FISH | | |
| <i>Hipposideros larvatus</i> | 2 | | Volleth <i>et al.</i> 2002 Mao <i>et al.</i> 2007 | ZOO-FISH CP | | |
| <i>Rhinolophus mehelyi</i> | 3 | | Volleth <i>et al.</i> 2002 | ZOO-FISH | | |
| <i>Aselliscus stoliccanus</i> | 3 | | Mao <i>et al.</i> 2007 | CP | | |
| <i>Megaderma spasma</i> | 3 | | Mao <i>et al.</i> 2008 | CP | | |
| <i>Taphozus melanopogon</i> | 3 | | Mao <i>et al.</i> 2008 | CP | | |
| Proboscidea | <i>Loxodonta africana</i> | 3 | Yang <i>et al.</i> 2003 | RP | | |
| | <i>Elephas maximus</i> | 3 | Yang <i>et al.</i> 2003 | CP | | |
| Tubulidentata | <i>Orycteropus afer</i> | 1 | Robinson <i>et al.</i> 2004 | RP | | |
| Afrosoricida | <i>Chrysochloris asiatica</i> | 1 | Robinson <i>et al.</i> 2004 | CP | | |
| Macroscelidea | <i>Elephantulus rupestris</i> | 1 | Robinson <i>et al.</i> 2004 | CP | | |
| | <i>Macroscidelis proboscideus</i> | 1 | Svartman <i>et al.</i> 2004 | CP | | |
| Sirenia | <i>Trichechus manatus</i> | 1 | Kellogg <i>et al.</i> 2007 | CP | | |
| Pilosa | <i>Choloepus didactylus</i> | 2 | Yang <i>et al.</i> 2006 | CP | | |
| | <i>Tamandua tetradactyla</i> | 5 | Yang <i>et al.</i> 2006 Svartman <i>et al.</i> 2006 | CP CP | | |
| Cingulata | <i>Choloepus hoffmannii</i> | 1 | Svartman <i>et al.</i> 2006 | CP | | |
| | <i>Dasyurus novemcintus</i> | 2 | Svartman <i>et al.</i> 2006 | CP | | |
| Primates | | | | | | |
| Strepsirhini | | | | | | |
| Lorisiformes | <i>Nycticebus coucang</i> | 2 | Nie <i>et al.</i> 2006 Stanyon <i>et al.</i> 2006 | RP RP | | |
| | <i>Otolemur garnettii</i> | 2 | Stanyon <i>et al.</i> 2006 | RP | | |
| | <i>Otolemur crassicaudatus</i> | 2 | Stanyon <i>et al.</i> 2002 | CP | | |
| | <i>Galago moholi</i> | 2 | Stanyon <i>et al.</i> 2002 Nie <i>et al.</i> 2006 | CP RP | | |
| Lemuriformes | <i>Lemur catta</i> | 5 | Cardone <i>et al.</i> 2002 Warter <i>et al.</i> 2005 | BAC/ PAC ZOO-FISH | | |

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| Order | Species | OB | References | Methods |
|--------------|--------------------------------------|----|--------------------------------|----------------------|
| | <i>Eulemur macaco macaco</i> | 5 | Cardone <i>et al.</i> 2002 | BAC/PAC |
| | <i>Eulemur fulvus mayottensis</i> | 5 | Richard <i>et al.</i> 2000 | CP |
| | | | Warter <i>et al.</i> 2005 | ZOO-FISH |
| | <i>Daubentonia madagascariensis</i> | 1 | Warter <i>et al.</i> 2005 | ZOO-FISH |
| | <i>Microcebus murinus</i> | 5 | Warter <i>et al.</i> 2005 | ZOO-FISH |
| | <i>Avahi laniger</i> | 5 | Warter <i>et al.</i> 2005 | ZOO-FISH |
| | <i>Propithecus verreauxi</i> | 5 | Warter <i>et al.</i> 2005 | ZOO-FISH |
| | | | Warter <i>et al.</i> 2005 | ZOO-FISH |
| | <i>Indri indri</i> | 5 | Warter <i>et al.</i> 2005 | ZOO-FISH |
| | <i>Lepilemur mustelinus</i> | 5 | Warter <i>et al.</i> 2005 | ZOO-FISH |
| | | | Rumpler <i>et al.</i> 2008 | ZOO-FISH/RB |
| | <i>Lepilemur jamesi</i> | 5 | Warter <i>et al.</i> 2005 | ZOO-FISH |
| | | | Rumpler <i>et al.</i> 2008 | ZOO-FISH/RB |
| | <i>Lepilemur leucopus</i> | 5 | Warter <i>et al.</i> 2005 | ZOO-FISH |
| | | | Rumpler <i>et al.</i> 2008 | ZOO-FISH/RB |
| | <i>Lepilemur ruficaudatus</i> | 5 | Warter <i>et al.</i> 2005 | ZOO-FISH |
| | | | Rumpler <i>et al.</i> 2008 | ZOO-FISH/RB |
| | <i>Lepilemur edwardisi</i> | 5 | Warter <i>et al.</i> 2005 | ZOO-FISH |
| | | | Rumpler <i>et al.</i> 2008 | ZOO-FISH/RB |
| | <i>Lepilemur microdon</i> | 5 | Warter <i>et al.</i> 2005 | ZOO-FISH |
| | | | Rumpler <i>et al.</i> 2008 | ZOO-FISH/RB |
| Haplorhini | | | | |
| Platirrhinae | <i>Ateles geoffroy</i> | 3 | Morescalchi <i>et al.</i> 1997 | CP |
| | <i>Ateles paniscus chamek</i> | 3 | Seuanez <i>et al.</i> 2001 | Comparative genomics |
| | <i>Ateles belzebuth marginatus</i> | 3 | de Oliveira <i>et al.</i> 2005 | CP |
| | <i>Ateles belzebuth</i> | 3 | Garcia <i>et al.</i> 2002 | ZOO-FISH |
| | <i>Brachyteles arachnoides</i> | 3 | de Oliveira <i>et al.</i> 2005 | CP |
| | <i>Lagothrix lagotricha</i> | 3 | Stanyon <i>et al.</i> 2001 | RP |
| | <i>Alouatta belzebuth</i> | 3 | Consigliere <i>et al.</i> 1998 | CP |
| | <i>Alouatta caraya</i> | 3 | de Oliveira <i>et al.</i> 2002 | CP |
| | <i>Alouatta fusca clamitans</i> | 2 | de Oliveira <i>et al.</i> 2002 | CP |
| | <i>Alouatta fusca fusca</i> | 2 | de Oliveira <i>et al.</i> 2002 | CP |
| | <i>Alouatta seniculus macconelli</i> | 4 | de Oliveira <i>et al.</i> 2002 | CP |
| | <i>Alouatta s. sara</i> | 2 | Consigliere <i>et al.</i> 1998 | CP |
| | <i>Alouatta s. arctoidea</i> | 3 | Consigliere <i>et al.</i> 1998 | CP |
| | <i>Callicebus moloch</i> | 1 | Stanyon <i>et al.</i> 2000 | CP |
| | <i>Callicebus lugens</i> | 1 | Stanyon <i>et al.</i> 2003 | RP |
| | <i>Callicebus donacophilus p.</i> | 2 | Barros <i>et al.</i> 2003 | CP |
| | <i>Callicebus cupreus</i> | 2 | Dumas <i>et al.</i> 2005 | RP |
| | <i>Cebus nigrivittatus</i> | 1 | Garcia <i>et al.</i> 2002 | ZOO-FISH |
| | <i>Cebus capucinus</i> | 1 | Richard <i>et al.</i> 1996 | ZOO-FISH |
| | <i>Cebus albifrons</i> | 1 | Garcia <i>et al.</i> 2002 | ZOO-FISH |
| | <i>Cebus apella</i> | 1 | Garcia <i>et al.</i> 2002 | ZOO-FISH |
| | <i>Cebus a. olivaceus</i> | 1 | Amaral <i>et al.</i> 2008 | CP |
| | <i>Cebus a. robustus</i> | 1 | Amaral <i>et al.</i> 2008 | CP |
| | <i>Cebus a. paraguayanus</i> | 1 | Armal <i>et al.</i> 2008 | CP |
| | <i>Saimiri sciureus</i> | 1 | Stanyon <i>et al.</i> 2000 | CP |
| | | | Dumas <i>et al.</i> 2007 | RP |
| | <i>Saguinus oedipus</i> | 1 | Muller <i>et al.</i> 2001a | CP |
| | | | Neusser <i>et al.</i> 2001 | CP |
| | <i>Cebuella pygmaea</i> | 1 | Neusser <i>et al.</i> 2001 | CP |
| | | | Dumas <i>et al.</i> 2007 | RP |

TABLE 1 — Condt.

| Order | Species | OB | References | Methods |
|-----------------|------------------------------------|----|---------------------------------|-------------------|
| | <i>Callithrix jacchus</i> | 1 | Neusser <i>et al.</i> 2001 | CP |
| | <i>Callithrix argentata</i> | 1 | Neusser <i>et al.</i> 2001 | CP |
| | | | Dumas <i>et al.</i> 2007 | RP |
| | <i>Callimico goeldii</i> | 1 | Neusser <i>et al.</i> 2001 | CP |
| | | | Dumas <i>et al.</i> 2007 | RP |
| | <i>Chiropotes utabicki</i> | 1 | Stanyon <i>et al.</i> 2004 | CP |
| | <i>Leontopithecus crysomelas</i> | 1 | Serreau <i>et al.</i> 2004 | CP |
| | <i>Aotus nancymae</i> | 2 | Ruiz-Herrera <i>et al.</i> 2005 | CP |
| Catarrhinae | | | | |
| | <i>Macaca fascicularis</i> | 1 | Ruiz-Herrera <i>et al.</i> 2002 | ZOO-FISH |
| | <i>Macaca arctoides</i> | 1 | Ruiz-Herrera <i>et al.</i> 2002 | ZOO-FISH |
| | <i>Mandrillus sphinx</i> | 1 | Ruiz-Herrera <i>et al.</i> 2002 | ZOO-FISH |
| | <i>Macaca silvana</i> | 1 | Wienberg <i>et al.</i> 1992 | CP |
| | <i>Macaca fuscata</i> | 1 | Wienberg <i>et al.</i> 1992 | CP |
| | <i>Macaca nemestrina</i> | 1 | Muller and Wienberg 2001b | CP |
| | <i>Macaca mulatta</i> | 1 | www.ensembl.org | |
| | <i>Colobus guereza</i> | 1 | Bigoni <i>et al.</i> 1997a | CP |
| | <i>Trachipithecus cristatus</i> | 1 | Bigoni <i>et al.</i> 1997b | CP |
| | <i>Trachipithecus obscurus</i> | 1 | Ponsà <i>et al.</i> 1983 | CB |
| | <i>Pygathrix namaeus</i> | 1 | Bigoni <i>et al.</i> 2004 | CP |
| | <i>Nasalis larvatus</i> | 1 | Bigoni <i>et al.</i> 2003 | CP |
| | <i>Semnopithecus francoisi</i> | 1 | Nie <i>et al.</i> 1998 | CP |
| | <i>Semnopithecus phayrei</i> | 1 | Nie <i>et al.</i> 1998 | CP |
| | <i>Allenopithecus nigroviridis</i> | 1 | Clemente <i>et al.</i> 1990 | CB |
| | <i>Erythrocebus patas</i> | 1 | Clemente <i>et al.</i> 1990 | CB |
| | <i>Miopithecus talapoin</i> | 1 | Clemente <i>et al.</i> 1990 | CB |
| | <i>Chlorocebus aethiops</i> | 2 | Finelli <i>et al.</i> 1999 | CP |
| | <i>Cercopithecus l'hoesti</i> | 1 | Stanyon and Sineo 1983 | CB |
| | | | Moulin <i>et al.</i> 2008 | ZOO-FISH |
| | <i>Cercopithecus ascanius</i> | 1 | Stanyon and Sineo 1983 | CB |
| | <i>Cercopithecus neglectus</i> | 1 | Stanyon and Sineo 1983 | CB |
| | | | Stanyon <i>et al.</i> 2005 | RP |
| | <i>Cercopithecus bamlymi</i> | 1 | Moulin <i>et al.</i> 2008 | ZOO-FISH |
| | <i>Cercopithecus mona</i> | 1 | Clemente <i>et al.</i> 1990 | CB |
| | | | Moulin <i>et al.</i> 2008 | ZOO-FISH |
| | <i>Hylobates hoolock</i> | 2 | Nie <i>et al.</i> 2001 | CP |
| | | | Muller <i>et al.</i> 2003 | ZOO-FISH |
| | <i>Hylobates lar</i> | 3 | Jauch <i>et al.</i> 1992 | CP |
| | | | Misceo <i>et al.</i> 2008 | BAC hybridization |
| | <i>Hylobates syndactylus</i> | 3 | Koehler <i>et al.</i> 1995 | CP |
| | | | Muller and Wienberg 2001 | CP |
| | <i>Nomascus concolor</i> | 4 | Koehler <i>et al.</i> 1995 | CP |
| | | | Muller and Wienberg 2001 | CP |
| | <i>Nomascus leucogenis</i> | 5 | Nie <i>et al.</i> 2001 | CP |
| | | | Roberto <i>et al.</i> 2008 | CP |
| | | | Carbone <i>et al.</i> 2006 | BAC hybridization |
| | <i>Symphalangus syndactylus</i> | 5 | Muller and Wienberg 2001 | CP |
| | | | Muller <i>et al.</i> 2003 | ZOO-FISH |
| | <i>Pongo pygmaeus</i> | 1 | www.ensembl.org | |
| | <i>Gorilla gorilla</i> | 1 | www.ensembl.org | |
| | <i>Pan troglodytes</i> | 1 | www.ensembl.org | |
| Didelphimorphia | <i>Monodelphis domestica</i> | 2 | www.ensembl.org | |
| Galliformes | <i>Gallus gallus</i> | 1 | www.ensembl.org | |

TABLE 2 — List of evolutionary chromosomal bands involved in breakpoints in primates and others mammals (Black spot). Black cross indicate the chromosomal bands homologous to HSA4 associated with the ancestral HSA8. Grey boxes chromosomal bands associated with other human synteny. Asterix indicate the contemporary presence of human fragile (Fs).

| Chromosomal bands | Species | | | | | | | | | | | | | | | | | | | | | | | |
|-------------------|---------|-----|-----|-----|-----|-----|---------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|----|
| | MMU | MFA | PPY | PTR | GGO | NLE | GMO/NCO | LCA | LLA | TBE | GVA | OCU | MMU | RNO | SCA | LAF | OAF | BTA | SSC | EQU | CEA | MDO | GGA | |
| 4p16.3 | | | | | | + | | | | | | + | | | | | + | | | | | | + | |
| 4p16.2 | | | | | | + | | | | | | + | | | | | + | | | | | | + | |
| 4p16.1 | | | | | | + | | | | | | + | *• | | | | + | *• | | | | | + | • |
| 4p15.33 | | | | | | + | | | | | | + | | | | | + | | | | | | + | |
| 4p15.32 | | | | | | + | | | | | | + | | | | | + | | | | | | + | |
| 4p15.31 | | | | | | + | | | | | | + | | | | | + | | | | | | + | |
| 4p15.3 | *• | | | | | + | | | | | | + | | | | | + | *• | | | | | + | |
| 4p15.2 | | | | | | + | | | | | | + | | | | | + | | | | | | + | |
| 4p15.1 | | | | | | + | | | | | | + | | | | | + | | | | | | + | |
| 4p15 | | | | | | + | | | | | | + | | | | | + | | | | | | + | |
| 4p14 | | | | | | + | | | | | | + | | | | | + | | | | | | + | |
| 4p13 | | | • | | | + | | | | | | + | | | | | + | | | | | | + | |
| 4p12 | | | | • | | + | | | | | | + | | | | | + | | | | | | + | |
| 4p11 | | | | | | + | | | | | | + | | | | | + | | | | | | + | |
| CEN | | | | | | + | | | | | | + | | | | | + | | | | | | + | |
| 4q11 | | | | | | + | | | | | | + | | | | | + | | | | | | + | |
| 4q12 | • | | • | | | + | | | | | | + | | | | | + | | | | | | + | *• |
| 4q13 | • | | | | | + | | | | | | + | | | | | + | | | | | | + | |
| 4q13.1 | | | | | | + | | | | | | + | | | | | + | | | | | | + | |
| 4q13.2 | | | | | | + | | | | | | + | | | | | + | | | | | | + | |
| 4q13.3 | | | | | | + | | | | | | + | | | | | + | | | | | | + | |
| 4q21.1 | • | | • | | | + | | | | | | + | | | | | + | | | | | | + | • |
| 4q21.21 | | | | | | + | | | | | | + | | | | | + | | | | | | + | |
| 4q21.22 | | | | | | + | | | | | | + | | | | | + | | | | | | + | |
| 4q21.23 | | | | | | + | | | | | | + | | | | | + | | | | | | + | |
| 4q21.3 | | | | | | + | | | | | | + | | | | | + | | | | | | + | |
| 4q22.1 | | | | | | + | | | | | | + | | | | | + | | | | | | + | |
| 4q22.2 | | | | | | + | | | | | | + | | | | | + | | | | | | + | • |
| 4q22.3 | | | | | | + | | | | | | + | | | | | + | | | | | | + | |
| 4q22 | | | | | | + | | | | | | + | | | | | + | | | | | | + | • |
| 4q23 | | | | | | + | | | | | | + | | | | | + | | | | | | + | • |
| 4q24 | | | | | | + | | | | | | + | | | | | + | | | | | | + | |

TABLE 2 — Condt.

| Chromosomal bands | Species | | | | | | | | | | | | | | | | | | | | | | | | |
|-------------------|---------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|---|
| | MMU | MFA | PPY | PTR | GGO | NLE | GMO | NCO | LCA | LLA | TBE | GVA | OCU | MMU | RNO | SCA | LAF | OAF | BTA | SSC | EQU | CFA | MDO | GGA | |
| 4q25 | | | | | | • | + | + | + | + | | | | | | | | + | | | + | | | | |
| 4q26 | | | | | • | • | + | + | + | + | | | | • | • | | | + | • | | + | | + | | |
| 4q27 | * | * | | | | + | + | + | + | + | | | | • | • | | | + | • | | + | | + | | |
| 4q28 | | | | | | + | + | + | + | + | | | | | | | | + | | | + | | | | • |
| 4q28.1 | | | | | | | + | + | + | + | | | | | | | | + | | | + | | + | | |
| 4q28.2 | | | | | | | + | + | + | + | | | | | | | | + | | | + | | + | | |
| 4q28.3 | | | | | | + | + | + | + | + | | | | | | | | + | | | + | | + | | |
| 4q3.1.1 | *• | *• | • | • | • | • | + | + | + | + | | | | *• | *• | | | + | | | + | | + | | |
| 4q3.1.2 | | | | | | + | + | + | + | • | | | | • | • | | | + | | | + | | • | | |
| 4q3.1.21 | | | | | | + | + | + | + | + | | | | • | • | | | + | | | + | | | | + |
| 4q3.1.22 | | | | | | + | + | + | + | + | | | | • | • | | | + | | | + | | | | + |
| 4q3.1.23 | | | | | | + | + | + | + | + | | | | • | • | | | + | | | + | | | | + |
| 4q3.1.3 | | | | | | + | + | + | + | + | | | | • | • | | | + | | | + | | | | • |
| 4q32 | | | | | | + | + | + | + | + | | | | • | • | | | + | | | + | | | | |
| 4q32.1 | | | | | | + | + | + | + | + | | | | • | • | | | + | | | + | | | | |
| 4q32.2 | | | | | | + | + | + | + | + | | | + | | | | | + | | | + | | | | |
| 4q32.3 | | | | | | + | + | + | + | + | | | + | | | | | + | | | + | | | | |
| 4q33 | | | | | | + | + | + | + | + | | | | • | • | | | + | | | + | | | | • |
| 4q34 | | | | | | + | + | + | + | + | | | | • | • | | | + | | | + | | | | • |
| 4q34.1 | | | | | | + | + | + | + | + | | | | • | • | | | + | | | + | | | | • |
| 4q34.2 | | | | | | + | + | + | + | + | | | | • | • | | | + | | | + | | | | • |
| 4q34.3 | | | | | | + | + | + | + | + | | | | • | • | | | + | | | + | | | | • |
| 4q35 | | | | | | + | + | + | + | + | | | | • | • | | | + | | | + | | | | • |
| 4q35.1 | | | | | | + | + | + | + | + | | | | • | • | | | + | | | + | | | | • |
| 4q35.2 | | | | | | + | + | + | + | + | | | | • | • | | | + | | | + | | | | • |

MMU= Macaca mulatta; MFA= Macaca fascicularis; PPY= Pongo pygmaeus; PTR= Pan troglodytes; GGO= Gorilla gorilla; NLE= Nomascus leucogenis; GMO= Galago moholi; NCO= Nycticebus coucang; LCA= Lemur catta; LLA= Lagotherix lagotricha; TBE= Tupia belangeri; GVA= Galeopterus variegatus; OCU= Oryctolagus cuniculus; MMU= Mus musculus; RNO= Rattus norvegicus; SCA= Scurus carolinensis; LAF= Loxodonta africana; OAF= Orycteropus afer; BTA= Bos taurus; SSC= Sus scrofa; EQU= Equus caballus; CFA= Canis Familiaris; MDO= Monodelphis domestica; GGA= Gallus gallus.

orthologous to HSA4 associated to other human syntenies (Table 3). The data were subjected to an unweighted maximum parsimony (MP) analysis using PAUP 4.0 software (SWOFFORD 1998) and a heuristic search with 100 random sequence additions and TBR branch swapping. The strict consensus tree is shown in Figure 2. Table 2 summarizes the chromosomal bands involved in the breakpoints, and their presence or absence in the eutherian species investigated, in the opossum (Metatheria), and in the chicken (Aves).

RESULTS

Comparative analysis of chromosomal data from 157 mammalian species gave us a window into the evolutionary dynamics of HSA4, into its ancestral constitution, and the various reorganization events that marked the emergence and diversification of different mammal clades. A MP

analysis was conducted on those taxa for which both reciprocal chromosome painting and genomic data were available at the time of the analysis. Application of both analytical approaches allowed us to generate a phylogenetic tree (Figure 1a) summarizing the evolution of the ancestral chromosome 4 in boreoeutherian, eutherian, therian, and amniote ancestors. Because convincing evidence exists of an ancestral 4/8p synteny in boreoeutherians (RICHARD *et al.* 2003a; SVARTMAN *et al.* 2004; WIENBERG *et al.* 2004), our tree includes data on chromosome 8. The ancestral status of the HSA4/8p association is confirmed in our analysis. The identification of 4q/8p/4p in the outgroups *Gallus* and *Monodelphis* supports the presence of the segmental association, not only in the ancestral eutherian karyotype, but also in the common ancestor of the Amniota (ROBINSON and RUIZ-HERRERA 2008).

Primates show a complex evolutionary scenario (Figure 1b). The HSA4 synteny appears

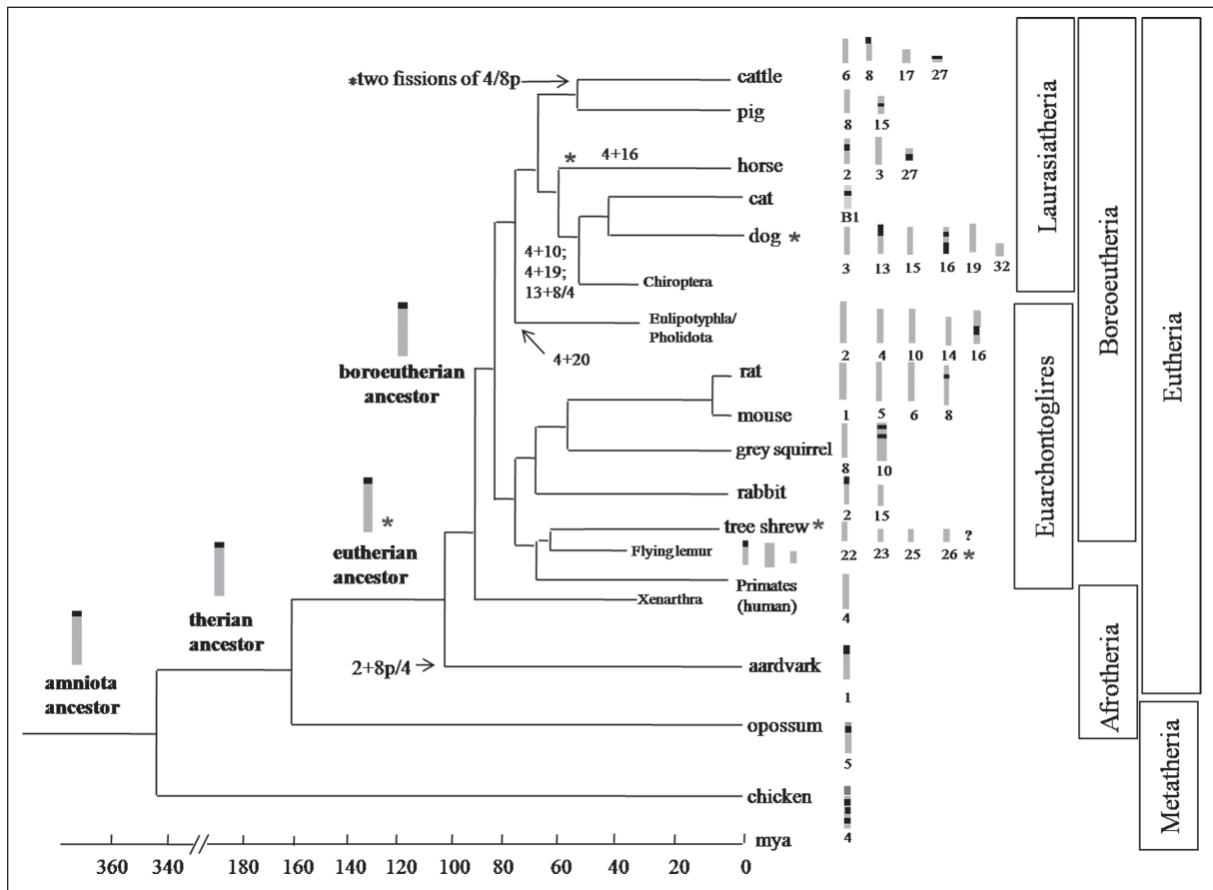


Fig. 1a — Fourteen species assigned to 16 orders were included in the sample to track the evolution of the ancestral chromosome 4 using phylogenetic reconstruction. The choice of species was influenced by the availability of reciprocal painting data and whole genome comparisons. Grey segments represent homologs to HSA4, whereas black segments are homologs to HSA8. The opossum (Metatheria) and chicken (Aves) are outgroups.

to have been present in the ancestral primate karyotype. Conversely, our study revealed a remarkable diversity in the number of orthologous blocks corresponding to HSA4 in the different primate lineages, and in the number of apomorphies identified among Strepsirhini and in Haplorhini (Figure 1b). The most derived groups are the Strepsirhini and the Platyrrhini, where the number of segments homologous to HSA4 varies from 1-5 and 1-3, respectively. Lorisiformes are characterized by a single block containing only sequences orthologous to HSA4, and another chromosome normally associated with diverse human segments. The lemuriform genera show great variability in levels of chromosomal reorganization. The basal divergence is occupied by the aye-aye, *Daubentonia madagascariensis*, where the orthologous to HSA4 is entirely conserved in one chromosome. By contrast, *Lepilemur* is the genus that shows the greatest complexity in the number of blocks orthologous to HSA4. Within Platyrrhini, Cebidae and Pitheciidae show that HSA4 has been conserved entirely. By contrast, Atelidae appears to have the most derived form of HSA4; in almost all species studied, the HSA4 synteny is split into three segments associated

with other human synteny. In the ancestral karyotype of Catarrhini, the HSA4 homolog is conserved entirely as a single entity. Cercopitheciidae and Hominiidae are grouped by the retention of HSA4 in a single syntenic block. Lesser apes (Hylobatidae), however, display different degrees of HSA4 disruption and association.

Figure 2 shows the Maximum Parsimony consensus tree (L = 198, CI = 0.70, RI = 0.80, excluding non-informative characters) for evolutionary breakpoints mapped on to HSA4. The topology we recovered indicates that some HSA4 bands involved in evolutionary breakpoints are shared by phylogenetically diverse lineages. Our reconstruction indicates four main results. First, within Primates, the split between Hominiidae (*Pongo*, *Pan* and *Gorilla*) and Cercopitheciidae (*Macaca*) is unresolved as a result of the taxa sharing a unique breakpoint located at HSA4q31.1. HSA4p16.1 represents a breakpoint that has occurred in three different lineages [Euarchontoglires (*Mus musculus* and *Rattus norvegicus*); Laurasiatheria (*Bos taurus*, *Sus scrofa* and *Canis familiaris*) and Galliformes (*G. gallus*)]. Additionally, the chromosomal bands extending from HSA4q32.1 to HSA4q35.2 have been retained in

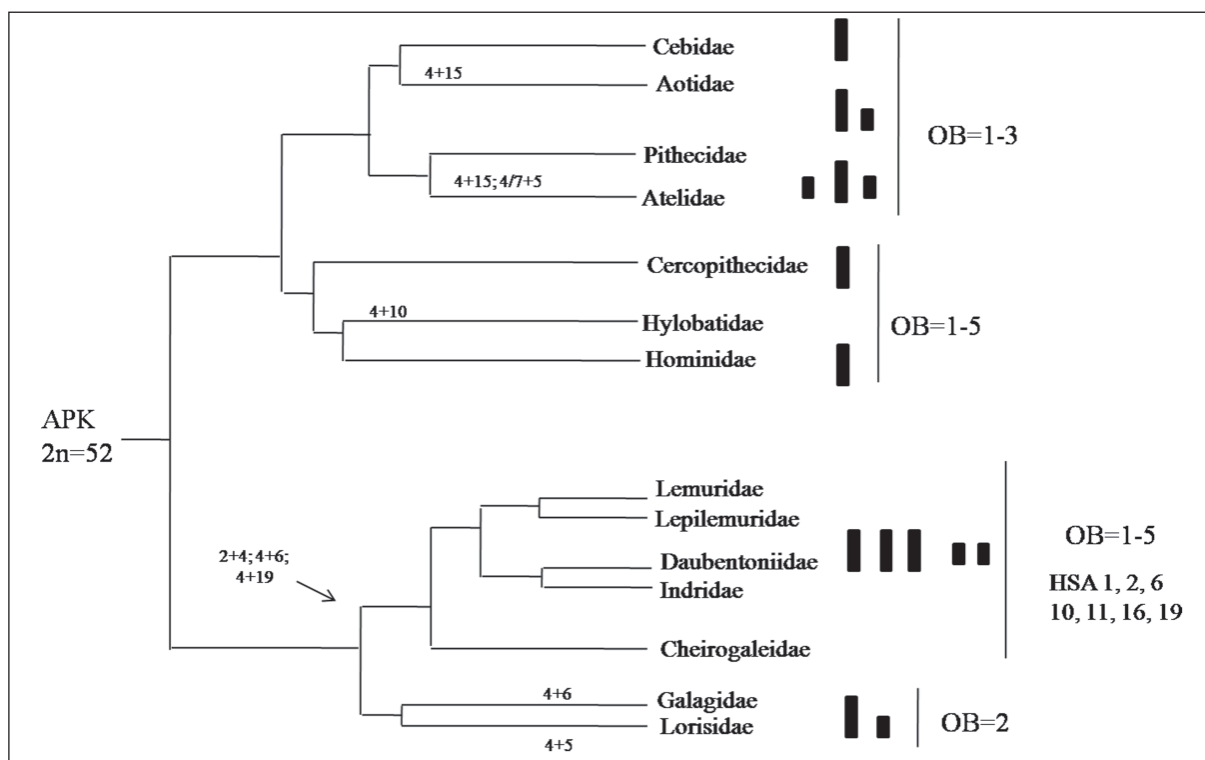


Fig. 1b — Primate phylogenetic tree showing the different orthologous blocks (OB) corresponding to human chromosome 4 in Haplorhini and Strepsirhini.

Tupaia belangeri, *Galeopterus variegatus*, *Sciurus carolinensis* and *Equus caballus*. Strepsirhines, *Lagothrix lagotricha* and *Loxodonta africana* are linked by sharing a range of chromosomal bands (from 4q31.1 to 4q35.2), homologous to HSA4 and other associated human synteny. An unexpected relationship indicated between *Monodelphis domestica* (Metatheria) and *Orycteropus afer* (Afrotheria), and is moderately supported (CI=0.6). This association is probably the result of a sympleiomorphic arrangement of chromosomal bands homologous to HSA4 that have been retained in the two lineages. Muridae (*Mus* and *Rattus*) share the highest number of breakpoints (CI = 1.0), a clearly reflection of the extensive common phylogenetic history of mice and rats, which only diverged 16-23 Mya (SPRINGER *et al.* 2003).

DISCUSSION

The evolution of 4/8 organization in eutherian mammals - Our analysis of all available data from reciprocal chromosome painting in placental mammals, together with some whole genomic comparisons, indicated that the chromosomal regions comprising HSA4 have been assembled in remarkably diverse ways over the course of their 340 million years of evolution (Figure 1a). Estimated divergence times in the tree follow SPRINGER *et al.* (2003), and VAN RHEEDE *et al.* (2006).

Human/mouse hybridization patterns identify the regions involved in the ancestral association as 4q32.2-ter and 8p22-ter (Ensemble.org). Human sequence data indicate that 4qter is integrated into a region homologous to 8p.

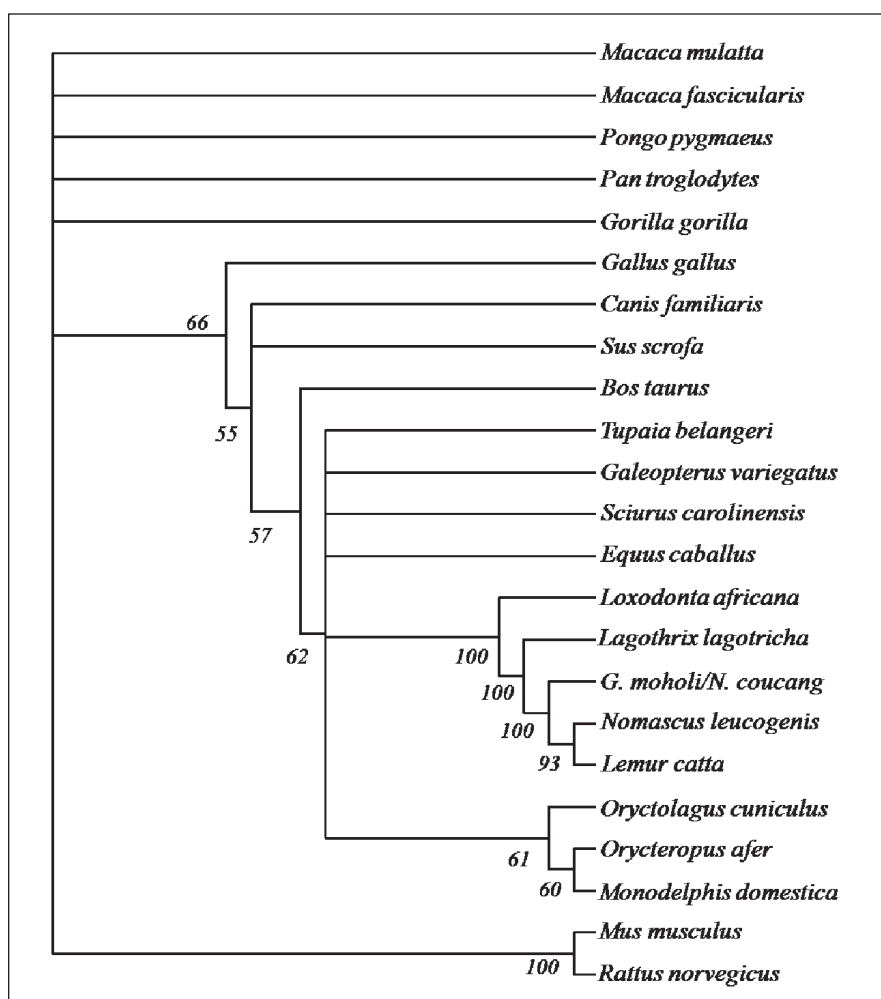


Fig. 2 — The tree displays plausible relationships between different mammalian species according to common evolutionary breakpoints localized on Human chromosome 4 in this study. Numbers at nodes indicate support values obtained from 1000 bootstrap replicates.

The genesis of this syntenic association has two alternative explanations: a parsimonious 4/8 association (as in Tubulidentata and Felidae), or a complex tandem association of 8/4ter/8/4pq (hypothetically present in the Euarchontoglires ancestor). The model of association is far from clear, as it is possible that breakpoints have been “reused” (PEVZNER and TESLER, 2003), limiting the reliability of both models, although the 4/8 association does appear in a variety of extant taxa (FROENICKE 2005; FERGUSON-SMITH *et al.* 2007) [i.e. *Trichecus manatus latirostris*, *Loxodonta africana* (Afrotheria, see YANG *et al.* 2003; KELLOGG *et al.* 2007); *Blarinella griselda* (Eulipotyphla, YE *et al.* 2006); *Muntiacus muntiac vaginalis* (Cetartiodactyla, FROENICKE *et al.* 1997a); *Mormopterus planiceps*, *Hipposiderus larvatus*, *Glossophaga soricina* (Chiroptera, VOLLETH *et al.* 1999, 2002)].

The HSA4 configuration has been retained as an intact orthologous block, without disruption, in some afrotherian and carnivoran species, as well as in a variety of primates (MULLER *et al.* 1999; YANG *et al.* 2003; ROBINSON *et al.* 2004; FERGUSON-SMITH and TRIFONOV 2007; ROBINSON and RUIZ-HERRERA, 2008). MULLER *et al.* (1999) described a translocation in *Tupaia* separating the ancestral HSA4/8 association, and supporting a Scandentia-Primates sister-group (MARTIN 1993). However, a re-analysis of *Tupaia* using reciprocal chromosome painting reveals the HSA4/8 association as present (R. Stanyon, personal communication), and supporting a sister-clade relationship between Scandentia and Dermoptera (NIE *et al.* 2008). In Rodentia and Lagomorpha, HSA4 is fragmented in rabbit and squirrel, while in the rat, nine different orthologous blocks are detectable, and the HSA4/8p (4qter-8p) association has also been conserved.

The Xenarthra, Afrotheria and Laurasiatheria clades are characterized by other, apparently ancestral associations. The HSA2/8p/4 association is a cytogenetic signature that subtends the Afrotherian grouping (ROBINSON *et al.* 2004). A more complex scenario has been maintained by individual lineages within the Laurasiatheria. Two fission events involving the ancestral 4/8p association characterize different species (i.e. cattle, horse, dog - Figure 1a); further, a significant number of inter-chromosomal rearrangements involve blocks orthologous to human chromosome 4 and other human chromosome segments, in several orders. The HSA4/20 association is a synapomorphy that links the Eu-

lipotyphla and Pholidota (YANG *et al.* 2006; YE *et al.* 2006). A translocation between HSA4 and HSA12 is a cytogenetic signature for Artiodactyla (YANG *et al.* 1997a), within which 4/12/22 is a special marker for Ruminantia (HUANG *et al.* 2008). Finally, the segmental associations of HSA4/10 and HSA4/19p are considered synapomorphic, supporting chiropteran monophyly (VOLLETH *et al.* 1999; 2002).

Reconstruction of HSA4 ancestral bands in eutherians and an evolutionary breakpoint framework - Although the ancestral HSA4/8 association is present in a great variety of mammalian species, it is necessary to study the HSA4 architecture in these species to verify whether the ancestral organization is really conserved. Extensive comparative chromosome painting data show that the entire HSA4 and the HSA8p segment constitute a single chromosome in the ancestors of Amniota (therians and eutherians) (ROBINSON and RUIZ-HERRERA 2008; FERGUSON-SMITH *et al.* 2007), which underwent several rearrangements during the course of evolution. Starting with the ancestral association, different chromosomal bands can be shown to be distributed among the various orders. The first node, which subtends the divergence of the outgroup *Gallus gallus*, is characterized by a 4/8 tandem translocation of the association (chromosome 5) unique to this lineage that leads to a 4/8/4/8/4/8 (4q31.21-31.3/8p12/4q21.21-23/8p23.1/4q35.1-2/8p22) sequence, never described before. In *Monodelphis domestica* (opossum), the association 4/8 is present and conserves the ancestral structure, but in this lineage an inversion has generated a 4/8/4 (4q26-4q28.1/8p12/4q13.3-pter) autapomorphy.

The proposed ancestral eutherian arrangement can be seen in the armadillo, *Oryzomys azer* (OAF, Tubulidentata). Chromosome 1 of OAF comprises the entire ancestral HSA4/8 association, which is associated with HSA2p (YANG *et al.* 2003). The same association has been revealed in other afrotherian species by unidirectional chromosomal painting (YANG *et al.* 2003; ROBINSON *et al.* 2004). The African elephant (*Loxodonta africana*) presents an exception, where the ancestral association is not present, and furthermore, the HSA4 synteny is disrupted into three blocks: HSA4p16-4q31.2 (LAF5), HSA 4q31.2-q32/15 (LAF17) and HSA 4q32-q35/16/19 (LAF20). A different scenario has been proposed in Euarchontoglires. The Dermoptera, Scandentia and Rodentia

TABLE 3 — The data matrix subjected to PAUP. Four different codes have been established. 0= absent ; code 1= presence of an evolutionary breakpoint localized in a specific band; 2= presence of chromosomal bands orthologous to HSA4 associated with the human chromosome 8; 3=presence of chromosomal bands orthologues to HSA4 associated to other human syntenies.

| No | Character mutata | Macaca fascicularis | Macaca pygmaea | Pongo rogoldites | Pan gorilla | Gorilla leucogenis | Nomascus N. couang | G. moholi/ catta | Lemur leptoticha | Lagothrix belangeri | Tupaia variegatus | Galepterus cucullus | Oryzologus musculus | Mus norvegicus | Rattus caolinensis | Sciurus africana | Loxodonta afer | Oryzoperus taurus | Bos familiaris | Canis caullus | Equus scroia | Sus gallus | Gallus domestica | Monodelphis |
|----|---------------------|------------------------|-------------------|---------------------|----------------|-----------------------|-----------------------|---------------------|---------------------|------------------------|----------------------|------------------------|------------------------|-------------------|-----------------------|---------------------|-------------------|----------------------|-------------------|------------------|-----------------|---------------|---------------------|-------------|
| 1 | 4p16.3 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 3 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 2 | 0 | 1 | 0 | 0 | 0 | 2 |
| 2 | 4p16.2 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 2 |
| 3 | 4p16.1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 1 | 1 | 0 | 0 | 2 | 1 | 1 | 0 | 1 | 1 | 2 |
| 4 | 4p15.33 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 2 |
| 5 | 4p15.32 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 2 |
| 6 | 4p15.31 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 2 |
| 7 | 4p15.3 | 1 | 1 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 2 | 1 | 1 | 0 | 0 | 0 | 2 |
| 8 | 4p15.2 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 2 |
| 9 | 4p15.1 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 2 |
| 10 | 4p15 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 3 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 2 |
| 11 | 4p14 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 3 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 2 |
| 12 | 4p13 | 0 | 0 | 0 | 1 | 0 | 3 | 0 | 3 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 2 | 0 | 1 | 0 | 0 | 0 | 2 |
| 13 | 4p12 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 3 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 2 | 0 | 1 | 0 | 0 | 0 | 2 |
| 14 | 4p11 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 2 |
| 15 | 4q11 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 2 |
| 16 | 4q12 | 1 | 0 | 1 | 0 | 0 | 3 | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 1 | 0 | 1 | 1 | 0 |
| 17 | 4q13 | 1 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 |
| 18 | 4q13.1 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 2 | 0 | 0 | 0 | 2 |
| 19 | 4q13.2 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 2 | 0 | 0 | 0 | 2 |
| 20 | 4q13.3 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 1 | 0 | 0 | 0 | 2 |
| 21 | 4q21.1 | 1 | 1 | 0 | 1 | 0 | 3 | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 1 | 0 | 1 | 1 | 1 |
| 22 | 4q21.21 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| 23 | 4q21.22 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| 24 | 4q21.23 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| 25 | 4q21.3 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| 26 | 4q22.1 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| 27 | 4q22.2 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| 28 | 4q22.3 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| 29 | 4q22 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| 30 | 4q23 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 1 | 3 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| 31 | 4q24 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 2 | 0 | 0 | 0 | 0 |
| 32 | 4q25 | 0 | 0 | 0 | 0 | 0 | 1 | 3 | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| 33 | 4q26 | 0 | 0 | 0 | 0 | 0 | 1 | 3 | 3 | 3 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 2 | 1 | 0 | 2 | 0 | 0 | 2 |
| 34 | 4q27 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 3 | 3 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 2 | 1 | 1 | 2 | 0 | 0 | 2 |
| 35 | 4q28 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 2 |
| 36 | 4q28.1 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 2 |
| 37 | 4q28.2 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 2 |
| 38 | 4q28.3 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 2 |
| 39 | 4q31.1 | 1 | 1 | 1 | 1 | 1 | 1 | 3 | 3 | 3 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 2 |

TABLE 3 — Condt.

| No | Character mutata | Macaca fascicularis | Macaca pygmaeus | Pongo troglodites | Pan gorilla | Gorilla leucogenis | Nomascus N. coucang | G. moholi/ catta | Lemur leopitricha | Lagothrix belangeri | Tupaia variegatus | Galopterus cuniculus | Oryzologus musculus | Mus norvegicus | Rattus canoninus | Sciurus africana | Loxodonta afer | Oryzeteropus taurus | Bos familiaris | Canis caullus | Equus scrofa | Sus gallus | Callus domestica | Monodelphis |
|----|---------------------|------------------------|--------------------|----------------------|----------------|-----------------------|------------------------|---------------------|----------------------|------------------------|----------------------|-------------------------|------------------------|-------------------|---------------------|---------------------|-------------------|------------------------|-------------------|------------------|-----------------|---------------|---------------------|-------------|
| 40 | 4q31.2 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 3 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 3 | 2 | 0 | 2 | 0 | 0 | 0 | |
| 41 | 4q31.21 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 2 | 0 | 2 | 0 | 2 | 0 | |
| 42 | 4q31.22 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 2 | 0 | 2 | 0 | 2 | 0 | |
| 43 | 4q31.23 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 2 | 0 | 2 | 0 | 2 | 0 | |
| 44 | 4q31.3 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 2 | 0 | 2 | 0 | 2 | 0 | |
| 45 | 4q32 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 3 | 3 | 0 | 0 | 0 | 1 | 1 | 0 | 3 | 2 | 1 | 2 | 1 | 0 | 0 | |
| 46 | 4q32.1 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 2 | 0 | 2 | 0 | 2 | 0 | |
| 47 | 4q32.2 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 3 | 3 | 2 | 2 | 2 | 0 | 0 | 2 | 3 | 2 | 0 | 2 | 0 | 2 | 0 | |
| 48 | 4q32.3 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 3 | 3 | 2 | 2 | 2 | 0 | 0 | 2 | 3 | 2 | 0 | 2 | 0 | 2 | 0 | |
| 49 | 4q33 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 3 | 3 | 2 | 2 | 2 | 0 | 0 | 2 | 3 | 2 | 0 | 2 | 0 | 2 | 0 | |
| 50 | 4q34 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 3 | 3 | 2 | 2 | 2 | 0 | 0 | 2 | 3 | 2 | 1 | 2 | 1 | 1 | 0 | |
| 51 | 4q34.1 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 3 | 3 | 2 | 2 | 2 | 0 | 0 | 2 | 3 | 2 | 0 | 2 | 0 | 2 | 0 | |
| 52 | 4q34.2 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 3 | 3 | 2 | 2 | 2 | 0 | 0 | 2 | 3 | 2 | 2 | 2 | 2 | 2 | 0 | |
| 53 | 4q34.3 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 3 | 3 | 2 | 2 | 2 | 0 | 0 | 2 | 3 | 2 | 2 | 2 | 2 | 2 | 0 | |
| 54 | 4q35 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 3 | 3 | 2 | 2 | 2 | 0 | 0 | 2 | 3 | 2 | 2 | 2 | 2 | 2 | 0 | |
| 55 | 4q35.1 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 3 | 3 | 2 | 2 | 2 | 0 | 0 | 2 | 3 | 2 | 2 | 2 | 2 | 2 | 1 | |
| 56 | 4q35.2 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 3 | 3 | 2 | 2 | 2 | 0 | 0 | 2 | 3 | 2 | 2 | 2 | 2 | 2 | 0 | |

are linked by a particular HSA4/8p association involving the chromosomal regions extending from 4q32.2 to the 4q35 band and the entire p arm of HSA8 or, alternatively, the single band 8p22ter (our study).

In *Galeopterus variegatus* (GVA - Dermaptera) chromosome 4 is in three parts. The 4/8 ancestral synteny is maintained for the portion HSA4q32.2/4qter, in association with HSA8p22-ter (NIE *et al.* 2008). The G-banding comparison between GVA and TBE (*Tupaia belangeri*, Scandentia) and the analysis of the reciprocal HSA/TBE painting (MÜLLER *et al.* 1999) indicate that the region homologous with 4q32.2-4q35 is localized on TBE22. Conversely, within Rodentia there is a different HSA4/8p organization. Mouse and rat show the ancestral 4q32.2-4qter /8p22-ter association, but are characterized by the highest number of rearrangements involving the ancestral synteny, leading to diverse human syntenic associations. In the sciurid group the association is a 8/4/8 synapomorphy, and involves the entire HSA 4q32.2-4qter/8p. In Lagomorpha the association 4/8 is present on *Oryzologus cuniculus* chromosome 2 and is formed by 4p and 4q32.2-4ter/8p (KORSTANJE *et al.* 1999; HAYES *et al.* 2002).

In Primates there is no trace of the ancestral eutherian 4/8p association. The human chromosome 4 syntenic order is present in the APK (Ancestral Primate Karyotype), in the AAP (Ancestral Ape Karyotype) and in the APLK (Ancestral Platyrrhini Karyotype) without any trace of the ancestral eutherian 4/8p association (STANYON *et al.* 2008). In Lorisiformes (Galagidae and Lorisidae) the chromosomes on which human syntenic associations HSA6+4 and HSA5+4 are present, contain the chromosomal bands from 4q12 to 4q35, while the rest of HSA4 is represented by the 4p16-4p12 bands. The Lemuriformes genome is characterized by a series of associations, including 4/5 and 4/6, but with the involvement of different breakpoints (CARDONE *et al.* 2002). We assume that HSA4q22 and HSA4q25 are the breakpoints involved respectively in the 4/5 and 4/6 associations. The Homiidae (humans and great apes) and Old World monkeys have karyotypes closely resembling the APK (STANYON *et al.* 2008). The chromosome IV elements in the great ape (PTR, PPY, GGO) lineages differ from the human homolog by distinctive pericentric inversions (MARZELLA *et al.* 2000). A pericentric inversion involving two breakpoints at the HSA4p13 and HSA4q21 bands is the main difference between PTR (*Pan*

troglydytes) and humans; in PPY (*Pongo pygmaeus*) the breakpoint is close to the centromere in the HSA4q12 homologues region; in GGO (*Gorilla gorilla*) a pericentric inversion is present in the HSA4p13 region. Among Cercopithecidae, in MMU (*Macaca mulatta*) a breakpoint was identified only on the region homologous to human HSA4q21, and two inversions related to the human regions HSA4q12-13 (see also KÄRERE *et al.* 2008; ROBERTO *et al.* 2008). *Chlorocebus aethiops* (CAE) is the only species in which HSA4 is present as two distinct orthologous blocks: HSA4q (CAE7) and HSA4p (CAE 27). Hylobatid karyotypes appear highly derived and rearranged (JAUCH *et al.* 1992; KOELHER *et al.* 1995). Our analysis indicates the number of syntenic blocks corresponding to the human genome as ranging from 2 to 5. In New World monkeys, HSA4 is conserved in a single orthologous block in Cebidae, while Atelidae shows the greatest number of rearrangements. Comparative analysis allowed us to identify three fragments: 4a (4q31.3ter), 4b(4q23-q31.2)/15 and 4c(HSA4pter-q22). Our analysis of the reciprocal chromosome painting on *Lagothrix lagotricha* indicates the occurrence of two breakpoints (HSA4q23 and HSA4q31.2) in the association 4/15, that may constitute a synapomorphy linking all atelid genera. In the Aotidae, *Aotus nancymae* displays a different syntenic association: HSA4pter-q22 and HSA4q31.3-q35/15.

Among Laurasiatheria the association 4/8 exists in two forms. In the dog (*Canis familiaris*) one of the ancestral segments identified with HSA8p is localized on CFA13 (HSA4p13-p12; HSA4q12-q13.1/q13.3), while the other is on CFA16 (HSA4q34.2-qter), while in the cat (*Felis catus*) the block orthologous to the ancestral eutherian state is fully conserved in a single chromosome (B1). In the horse (*Equus caballus*) the ancestral bands orthologous to HSA4 occupy HSA4q24 to HSA4q34.1 on ECA2, and HSA4q34.2-q35 on ECA27. In cattle (*Bos taurus*), chromosome 8 carries the chromosomal bands homologous to HSA4q32.3-34.1 and HSA4q34.2-q35 (BTA27), and in the pig (*Sus scrofa*), HSA4q34.2-q35 is found on chromosome 15.

In conclusion evolutionary breakpoints are not uniformly distributed along the length of the human chromosomes, and are often conserved during chromosomal evolution. The identification of the breakpoints in HSA4 reveals complex and diverse scenarios of reorganization of the synteny from its ancestral state. Our analysis

provides evidence of the following:

The Afrotheria, where the 4/8 association is conserved; the Euarchontoglires, where the bands ranging from 4q32 to 4q35 appear to be the boundaries of the 4/8 mammalian ancestral association, variably distributed in the five orders; the Laurasiatheria where the 4/8 association is variably reassembled.

There is an association between fragile sites and evolutionary breakpoints corresponding to human bands 4p15.3, 4p16.1, 4q12, and 4q31.1. The matrix in Table 3 indicates that the bands 4p15.3 and 4p16.1 house fragile site and evolutionary breakpoint events; the band 4q12 houses two fragile sites and five evolutionary breakpoint events; and the band 4q31.1 houses four fragile sites and eight evolutionary breakpoint events.

Evolutionary interpretations of chromosome painting results and ancestral chromosome reconstructions must be considered in the light of real structural rearrangements that have taken place in different evolutionary lineages.

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REFERENCES

- AMARAL P.J.S., FINOTELO L.F.M., DE OLIVEIRA E.H.C., PISSINATI A., NAGAMACHI C.Y. and PIEZARCKA J.C., 2008 — *Phylogenetic studies of the genus Cebus (Cebidae-Primates) using chromosome painting and G-banding*. BMC Evolutionary Biology, 8: 169-178.
- ANEAS I., RODRIGUES M.V., PAULETTI B.A., SILVA G.J., CARMONA R., CARDOSO L., KWITEK A.E., JACOB H.J., SOLER J.M. and KRIEG J.E., 2009 — *Congenic strains provide evidence that four mapped loci in chromosomes 2, 4, and 16 influence hypertension in the SHR*. Physiological Genomics, 3: 52-57.
- BALMUS G., TRIFONOV V.A., BILTUEVA L.S., O'BRIEN P.C.R., ALKALAEVA E.S., FU B., SKIDMORE J.A., ALLEN T., GRAPHODATDSKY A.S. and YANG F., *et al.* 2007 — *Cross-species chromosome painting among camel, cattle pig and human: further insights into the putative Cetartiodactyla ancestral karyotype*. Chromosome Research, 15: 499-514.

- BARROS R.M., NAGAMACHI C.Y., PIECZARCA J.C., RODRIGUES L.R.R., NEUSSER M., DE OLIVEIRA E.H.C., WIENBERG J., MUNIZ J.A.P.C., RISSINO D.J. and MULLER S., 2003 — *Chromosomal studies in Callicebus donacophilus pallescens, with classic and molecular cytogenetic approaches: multicolour FISH using human and Saguinus oedipus painting probes*. Chromosome Research, 11: 327-334.
- BIGONI F., KOEHLER U., STANYON R., ISHIDA T. and WIENBERG J., 1997a — *Fluorescence in situ hybridization establishes homology between human and silvered leaf monkey chromosomes, reveals reciprocal translocations between chromosomes homologous to human Y/5, 1/9, and 6/16, and delineates an X1X2Y1Y2/ X1X1X2X2 sex-chromosome system*. American Journal of Physical Anthropology, 102: 315-327.
- BIGONI F., KOEHLER U., STANYON R., MORESCALCHI A.M. and WIENBERG J., 1997b — *Mapping homology between human and black and white colobine monkey chromosomes by fluorescent in situ hybridization*. American Journal of Primatology, 42: 289-298.
- BIGONI F., STANYON R., WIMMER R. and SCHEMP W., 2003 — *Chromosome painting shows that the proboscis monkey (Nasalis larvatus) has a derived karyotype and is phylogenetically nested within Asian Colobines*. American Journal of Primatology, 60: 85-93.
- BIGONI F., HOUCK M.L., RYDER O.A., WIENBERG J. and STANYON R., 2004 — *Chromosome painting shows that Pygathrix nemaeus has the most basal karyotype among Asian Colobinae*. International Journal of Primatology, 25: 679-688.
- BODEGA B., CARDONE M.F., MÜLLER S., NEUSSER M., ORZAN F., ROSSI E., BATTAGLIOLI E., MAROZZI A., RIVA P. and ROCCHI M., et al. 2007 — *Evolutionary genomic remodelling of the human 4q subtelomere (4q35.2)*. BMC Evolutionary Biology, 7: 39-52.
- BOURQUE G., ZDOBNOV E.M., BORK P., PEVZER P.A. and TESLER G., 2005 — *Comparative architectures of mammalian chromosomes and chicken genomes reveal highly variable rates of genomic rearrangements across different lineages*. Genome Research, 15: 98-110.
- BREEN M., THOMAS R., BINNS M.M., CARTER N.P. and LANGFORD C.F., 1999 — *Reciprocal chromosome painting reveals detailed regions of conserved synteny between the karyotypes of the domestic dog (Canis familiaris) and human*. Genomics, 61: 145-155.
- CARBONE L., VESSERE G.M., TEN HALLERS B.F.H., ZHU B., OSOEGAWA K., MOOTNICK A., KOFLER A., WIENBERG J., ROGERS J. and HUMPHRAY S., et al. 2006 — *A high-resolution map of synteny disruptions in gibbon and human genomes*. PLoS Genetics, 2 (12):2162-2175.
- CARDONE M.F., VENTURA M., TEMPESTA S., ROCCHI M. and ARCHIDIACONO N., 2002 — *Analysis of chromosome conservation in Lemur catta studied by chromosome paints and BAC/PAC probes*. Chromosoma, 111: 267-272.
- CAVAGNA P., MENOTTI A. and STANYON R., 2000 — *Zooming in on the human-mouse comparative map: genome conservation re-examined on a high-resolution scale*. Genome Research, 7(12): 1123-1137.
- CLEMENTE I.C., PONSÁ M., GARCIA M. and EGOZCUE J., 1990 — *Evolution of the Simiiformes and the phylogeny of human chromosomes*. Human Genetics, 84: 493-506.
- COLLINS F.S., GREEN E.D., GUTTMACHER A.E. and GUYER M.S., 2003 — *A vision for the future of genomics research*. Nature, 422: 835-847.
- CONSIGLIERE S., STANYON R., KOELHER U., ARNOLD N. and WIENBERG J., 1998 — *In situ hybridization (FISH) maps chromosomal homologies between Alouatta belzebul (Platyrrhini, Cebidae) and other primates and reveals extensive interchromosomal rearrangements between howler monkey genomes*. American Journal of Primatology, 46 (2): 119-133.
- DE OLIVEIRA E.H.C., NEUSSER M., FIGUEIREIDO W.B., NAGAMACHI C., PIECZARCA J.C., SBALQUIERO I.J., WIENBERG J. and MULLER S., 2002 — *The phylogeny of howler monkeys (Alouatta, Platyrrhini): reconstruction by multicolor cross-species chromosome painting*. Chromosome Research, 10 (8): 669-683.
- DE OLIVEIRA E.H.C., NEUSSER M., NAGAMACHI C., PIECZARCA J.C., SBALQUIERO I.J. and MULLER S., 2005 — *Phylogenetic inferences of Atelinae (Platyrrhini) based on multi-directional chromosome painting in Brachyteles arachnoides, Ateles paniscus paniscus and Ateles b. marginatus*. Cytogenetic and Genome Research, 108 (1-3): 183-190.
- DUMAS F., BIGONI F., STONE G., SINEO L. and STANYON R., 2005 — *Mapping genomic rearrangements in titi monkeys by chromosome flow sorting and multidirectional in-situ hybridization*. Chromosome Research, 13 (1): 85-96.
- DUMAS F., BIGONI F., STONE G., SINEO L. and STANYON R., 2007 — *Phylogenomics of species from four genera of New World monkeys by flow sorting and reciprocal chromosome painting*. BMC Evolutionary Biology, 7 (Suppl 2): 11-25.
- FERGUSON-SMITH M.A. and TRIFONOV V., 2007 — *Mammalian karyotype evolution*. Nature Reviews Genetics, 8: 950-962.
- FINELLI P., STANYON R., PLESKER R., FERGUSON-SMITH M.A., O'BRIEN P.C.M. and WIENBERG J., 1999 — *Reciprocal chromosome painting shows that the great difference in diploid number between human and African green monkey is mostly due to non-Robertsonian fissions*. Mammalian Genome, 10: 713-718.
- FROENICKE F. and SCHERTHAN H., 1997a — *Zoo-fluorescence in situ hybridization analysis of human and Indian muntjac karyotypes (Muntiacus muntjak vaginalis) reveals satellite DNA clusters at the margins of conserved syntenic segments*. Chromosome Research, 5: 251-261.
- FROENICKE F., MULLER S., NAVIA J., ROMANAKIS K. and SCHERTHAN H., 1997b — *Chromosomal homologies between human, harbor seal (Phoca vitulina)*

- and the putative ancestral carnivore karyotype revealed by Zoo-FISH. *Chromosoma*, 106: 108-113.
- FROENICKE L. AND WIENBERG J., 2001 — *Comparative chromosome painting defines the high rate of karyotype changes between pigs and bovids*. *Mammalian Genome*, 12:442-449.
- FROENICKE L., 2005 — *Origins of primate chromosomes – as delineated by Zoo-FISH and alignments of human and mouse draft genome sequences*. *Cytogenetic and Genome Research*, 108 (1-3):122-138.
- GANDELMAN K.Y., GIBSON L., MEYN M.S. and YANG-FENG T.L., 1992 — *Molecular definition of the smallest region of deletion overlap in the Wolf-Hirschhorn syndrome*. *American Journal of Human Genetics*, 51: 571– 578.
- GARCIA F., RUIZ-HERRERA A., EGZOCUE M., PONSÁ M. and GARCIA M., 2002 — *Chromosomal homologies between Cebus and Ateles (Primates) based on ZOO-FISH and G-banding comparison*. *American Journal of Primatology*, 57: 177-188.
- GRAPHODATSKY A.S., YANG F., O'BRIEN P.C.M., SERDUKOVA N., MILNE B.S., TRIFONOV V. and FERGUSON-SMITH M.A., 2000a — *A comparative chromosome map of the Arctic fox, red fox and dog defined by chromosome painting and high resolution G-banding*. *Chromosome Research*, 8: 253-263.
- GRAPHODATSKY A.S., YANG F., O'BRIEN P.C.M., PERELMAN P., MILNE B.S., SERDUKOVA N., KAWADA S.I. and FERGUSON-SMITH M.A., 2001 — *Phylogenetic implications of the 38 putative ancestral chromosome segments for four canid species*. *Cytogenetics and Cell Genetics*, 92: 243-247.
- GRAPHODATSKY A.S., YANG F., PERELMAN P., O'BRIEN P.C.M., SERDUKOVA N., MILNE B.S., BILTUEVA L.S., FU B., VOROBIEVA N.V. and KAWADA S.I., et al. 2002 — *Comparative molecular cytogenetic studies in the order Carnivora: mapping chromosomal rearrangements onto the phylogenetic tree*. *Cytogenetic and Genome Research*, 96: 137-145.
- GRAPHODATSKY A.S., YANG F., DOBIGNY G., ROMANENKO S.A., BILTUEVA L.S., PERELMAN P.L., BEKLESHIEVA V.R., ALKALAEVA E.Z., SERDUKOVA N.A. and FERGUSON-SMITH M.A., et al. 2008 — *Tracking the evolution of genome organization in rodents by ZOO-FISH*. *Chromosome Research*, 16: 261-274.
- HAMEISTER H., KLETT C., BRUCH J., DIXKENS C., VOGEL W. and CHRISTENSEN K., 1997 — *Zoo-FISH analysis: the American mink (Mustela vison) closely resembles the cat karyotype*. *Chromosome Research*, 5: 5-11.
- HARDISON R.C., ROSKIN K., YANG S., DIEKHANS M., KENT W.J., WEBER R., ELNITSKI L., LI J., O'CONNOR M. and KOLBE D., et al. 2003 — *Covariation in frequencies of substitution, deletion, transposition, and recombination during eutherian evolution*. *Genome Research*, 13: 13-26.
- HAYES H., ROGEL-GAILLARD C., ZIJLSTRA C., DE HAAN N.A., URIEN C., BOURGEOUX N., BERTAUD N. and BOSMA A.A., 2002 — *Establishment of an R-banded rabbit karyotype nomenclature by FISH localization of 23 chromosome-specific genes on both G- and R-banded chromosomes*. *Cytogenetic and Genome Research*, 98: 199-205.
- HILLIER L.W., GRAVES T.A., FULTON R.S., FULTON L.A., PEPIN K.H., MINX P., WAGNER-MCPHERSON C., LAYMAN D., WYLIE K. and SEKHON M., et al. 2005 — *Generation and annotation of the DNA sequences of human chromosomes 2 and 4*. *Nature*, 434 (7034): 724-731.
- HUANG L., NESTERENKO A., NIE W., WANG J., SU W., GRAPHODATSKY A.S. and YANG F., 2008 — *Karyotype evolution of giraffes (Giraffa camelopardalis) revealed by cross-species chromosome painting with Chinese muntjac (Muntiacus reevesi) and human (Homo sapiens) paints*. *Cytogenetic and Genome Research*, 122: 132-138.
- IANNUZZI L., DI MEO G.P., PERUCCATI A. and INCARNATO D., 1999 — *Comparison of the human with the sheep genomes by use of human chromosome-specific painting probes*. *Mammalian Genome*, 10: 719-723.
- JAUCH A., WIENBERG J., STANYON R., ARNOLD N., TOFANELLI S., ISHIDA T. and CREMER T., 1992 — *Reconstruction of genomic rearrangements in great apes and gibbons by chromosome painting*. *Proceedings of the National Academy of Sciences of the United States of America*, 89: 8611-8615.
- KARERE G.M., FROENICKE L., MILLION L., WOMACK J.E. and LYONS L.A., 2008 — *A high-resolution radiation hybrid map of rhesus macaque chromosome 5 identifies rearrangements in the genome assembly*. *Genomics*, 92: 210-218.
- KELLOGG M., BURKETT S., DENNIS T.R., STONE G., GRAY B.A., MCGUIRE P.M., ZORI R.T. and STANYON R., 2007 — *Chromosome painting in the manatee supports Afrotheria and Paenungulata*. *BMC Evolutionary Biology*, 7: 6-13.
- KEMKEMER C., KOHN M., COOPER D.N., FROENICKE L., HÜGEL J., HAMAISTER H. and KEHER-SAWATZKI H., 2009 — *Gene synteny comparisons between different vertebrates provide new insights into breakage and fusion events during mammalian karyotype evolution*. *BMC Evolutionary Biology*, 9: 84-108.
- KLATTE T., SEITZ C., WALDERT M., DE MARTINO M., KIKC Z., BOHMING G.A., HAITEL A., SCHMIDBAUER J., MARBERGER M. and REMZI M., 2009 — *Features and outcomes of renal cell carcinoma of native kidneys in renal transplant recipients*. *British Journal of Urology International*, 105 (9): 1260-1265.
- KOELHER U., ARNOLD N., WIENBERG J., TOFANELLI S. and STANYON R., 1995 — *Genomic reorganization and disrupted chromosomal synteny in the siamang (Hylobates syndactylus) revealed by fluorescence in situ hybridization*. *American Journal of Physical Anthropology*, 97(1): 37-47.
- KOJIMA T., SHIMAZUI T., HINOTSU S., JORAKU A., OIKAWA T., KAWAI K., HORIE R., SUZUKI H., NAGASHIMA R. and YOSHIKAWA K., et al. 2009 — *Decreased expression of CXXC4 promotes a malignant phenotype in renal cell carcinoma by activating Wnt signaling*. *Oncogene*, 28: 297-305.
- KORSTANJE R., O'BRIEN P.C.M., YANG F., RENS W.,

- BOSMA A.A., VAN LITH H.A., VAN ZUPTHEN L.F.M. and FERGUSON-SMITH M.A., 1999 — *Complete homology maps of the rabbit (Oryctolagus cuniculus) and human by reciprocal chromosome painting*. Cytogenetics and Cell Genetics, 86: 317-322.
- KULEMZINA A.I., TRIFONOV V.A., PERELMAN P.L., RUBTSOVA N.V., VOLOBUEV V., FERGUSON-SMITH M.A., STANYON R., YANG F. and GRAPHODATSKY A.S., 2009 — *Cross-species chromosome painting in Cetartiodactyla: Reconstructing the karyotype evolution in key phylogenetic lineage*. Chromosome Research, 17: 419-436.
- LI T., O'BRIEN P.C.M., BILTUEVA L., FU B., WANG J., NIE W., FERGUSON-SMITH M.A., GRAPHODATSKY A.S. and YANG F., 2004 — *Evolution of genome organizations of squirrels (Sciuridae) revealed by cross-species chromosome painting*. Chromosome Research, 12: 317-335.
- MA J., ZHANG L., SUH B.B., RANEY B.J., BURTHANS R.C., KENT W.J., BLANCHETTE M., HAUSLERR D. and MILLER W., 2006 — *Reconstructing contiguous regions of an ancestral genome*. Genome Research, 16: 1557-1565.
- MCKNIGHT D.A., SIMMER J.P., HART P.S., HART T.C. and FISHER L.W., 2008 — *Overlapping DSPP Mutations Cause Dentin Dysplasia and Dentinogenesis Imperfecta*. Journal of Dental Research, 87(12): 1108-1111.
- MAO X., NIE W., WANG J., SU W., FENG Q., WANG Y., VOLLETH M. and YANG F., 2007 — *Karyotype evolution in Rhinolophus bats (Rhinolophidae, Chiroptera) illuminated by cross-species chromosome painting and G-banding comparison*. Chromosome Research, 15: 835-847.
- MAO X., NIE W., WANG J., SU W., FENG Q., WANG Y., VOLLETH M., DOBIGNY G. and YANG F., 2008 — *Comparative cytogenetics of bats (Chiroptera): The prevalence of Robertsonian translocations limits the power of chromosomal characters in resolving interfamilial phylogenetic relationships*. Chromosome Research, 16: 155-170.
- MARTIN R.D., 1993 — *Primate origins: plugging the gaps*. Nature, 363: 223-234.
- MARZELLA R., VIGGIANO L., MIOLLA V., STORLAZZI C.T., RICCO A., GENTILE E., ROBERTO R., SURACE C., FRATELLO A. and MANCINI M., et al. 2000 — *Molecular cytogenetic resources for chromosome 4 and comparative analysis of Phylogenetic Chromosome IV in Great Apes*. Genomics, 63: 307-313.
- MISCEO D., CAPOZZI O., ROBERTO R., DELL'OGGIO M.P., ROCCHI M., STANYON R. and ARCHIDIACONO N., 2008 — *Tracking the complex flow of chromosome rearrangements from the Hominoidea Ancestor to extant Hylobates and Nomascus Gibbons by high-resolution synteny mapping*. Genome Research, 18(9): 1530-1537.
- MORESCALCHI M.A., SCHEMPF W., CONSIGLIERE S., BIGONI F., WIENBERG J. and STANYON R., 1997 — *Mapping chromosomal homology between humans and the black-handed spider monkey by fluorescence in situ hybridization*. Chromosome Research, 5: 527-536.
- MOULIN S., GERBAULT-SEUREAU M., DUTRILLUAUX B. and RICHARD F.A., 2008 — *Phylogenomics of African guenons*. Chromosome Research, 16 (5): 783-99.
- MÜLLER S., STANYON R., O'BRIEN P.C.M., FERGUSON-SMITH M.A., PLESKER R. and WIENBERG J., 1999 — *Defining the ancestral karyotype of all primates by multidirectional chromosome painting between tree shrews, lemurs and humans*. Chromosoma, 108: 393-400.
- MÜLLER S., STANYON R., FINELLI P., ARCHIDIACONO N. and WIENBERG J., 2000 — *Molecular dissection of human chromosome 3 and 21 evolution*. Proceedings of the National Academy of Sciences of the United States of America, 97: 206-211.
- MÜLLER S., NEUSSER M., O'BRIEN P.C.M. and WIENBERG J., 2001a — *Molecular cytogenetic characterization of the EBV-producing cell line B 95-8 (S. oedipus, Platyrrhini) by chromosome sorting and painting*. Chromosome Research, 9: 689-693.
- MÜLLER S. and WIENBERG J., 2001b — *"Bar-coding" primate chromosomes: molecular cytogenetic screening for the ancestral hominoid karyotype*. Human Genetics, 109 (1): 85-94.
- MÜLLER S., HOLLAZ M. and WIENBERG J., 2003 — *Chromosomal phylogeny and evolution of gibbons (Hylobatidae)*. Human Genetics, 113: 493-501.
- MURPHY W.J., LARKIN D.M., EVERTS-VAN DER WIND A., BOURQUE G., TESLER G., AUVIL L., BEEVER J.E., CHOWDHARY B.P., GALIBERT F. and GATZE L., et al. 2005 — *Dynamics of mammalian chromosome evolution inferred from multispecies comparative maps*. Science, 309: 613-617.
- MURPHY W.J., DAVIS B., DAVID D.A., AARWALA R., SCHALFER A.A., PEARKS WILKERSON A.J., NEELAM B., O'BRIEN S.J. and MENOTTI-RAYMOND M., 2007 — *A 1.5Mb-resolution radiation hybrid map of the cat genome and comparative analysis with the canine and human genome*. Genomics, 89: 189-196.
- NASH W.G., WIENBERG J., FERGUSON-SMITH M.A., MENNINER J.C. and O'BRIEN S.J., 1998 — *Comparative genomics: tracking chromosome evolution in the family Ursidae using reciprocal chromosome painting*. Cytogenetics and Cell Genetics, 83: 182-192.
- NEUSSER M., STANYON R., BIGONI F., WIENBERG J. and MULLER S., 2001 — *Molecular cytogenomics of New World monkeys (Platyrrhini) a comparative analysis of five species by multi-color chromosome painting gives evidence for a classification of Callimico goeldii within the family of Callitrichidae*. Cytogenetics and Cell Genetics, 94: 206-215.
- NIE W., LIU R., CHEN Y., WANG J. and YANG F., 1998 — *Mapping chromosomal homologies between humans and two langurs (Semnopithecus francoisi and S. phayrei) by chromosome painting*. Chromosome Research, 6 (6): 447-453.
- NIE W., RENS W., WANG J. and YANG F., 2001 — *Conserved chromosome segments in Hylobates hoolock revealed by human and H. leucogenys paint probes*. Cytogenetics and Cell Genetics, 92(3-4):248-53.
- NIE W., O'BRIEN P.C.M., FU B., WANG J., SU W.,

- FERGUSON-SMITH M.A., ROBINSON T.J. and YANG F., 2006 — *Chromosome painting between human and lorisiform prosimians: evidence for the HSA 7/16 synteny in the primate ancestral karyotype*. American Journal of Physical Anthropology, 129 (2): 250-259.
- NIE W., FU B., O'BRIEN P.C.M., WANG J., SU W., TANOMTONG A., VOLOBOUEV V., FERGUSON-SMITH M.A. and YANG F., 2008 — *Flying lemurs - The 'flying tree shrews'? Molecular cytogenetic evidence for a Scandentia-Dermoptera sister clade*. BMC Biology, 6: 18-28.
- PONSA' F.M., DE BOER L.E.M. and EGOZCUE J., 1983 — *Banding patterns of the chromosomes of Presbytis cristatus purrbus and P. obscura*. American Journal of Primatology, 4:165-169.
- PEVZENER P.A. and TESLER G., 2003 — *Human and mouse genomic sequences reveal extensive breakpoint reuse in mammalian evolution*. Proceedings of the National Academy of Sciences of the United States of America, 100: 7672-7677.
- RICHARD F., LOMBARD M. and DUTRILLAUX B., 1996 — *ZOO-FISH suggests a complete homology between human and capuchin monkey (Platyrrhini) euchromatin*. Genomics, 36: 417-423.
- RICHARD F., LOMBARD M. and DUTRILLAUX B., 2000 — *Phylogenetic origin of human chromosomes 7, 16, and 19 and their homologs in placental mammals*. Genome Research, 10(5): 644-651.
- RICHARD F., MESSAOUDI C., LOMBARD M. and DUTRILLAUX B., 2001 — *Chromosome homologies between man and mountain zebra (Equus zebra hartmannae) and description of a new ancestral synteny involving sequences homologous to human chromosome 4 and 8*. Cytogenetics and Cell Genetics, 93: 291-296.
- RICHARD F., LOMBARD M. and DUTRILLAUX B., 2003a — *Reconstruction of the ancestral karyotype of eutherian mammals*. Chromosome Research, 11: 605-618.
- RICHARD F., MESSAOUDI C., BONNET-GARNIER A., LOMBARD M. and DUTRILLAUX B., 2003b — *Highly conserved chromosomes in an Asian squirrel (Menetes berdmorei, Rodentia: Sciuridae) as demonstrated by ZOO-FISH with human probes*. Chromosome Research, 11: 597-603.
- ROBERTO R., CAPOZZI O., WILSON R.K., MARDIS E.R., LOMIENTO M., TUZUN E., CHENG Z., MOOTNICK A.R., ARCHIDIACONO N. and ROCCHI M., et al. 2008 — *Molecular refinement of gibbon genome rearrangements*. Genome Research, 17: 249-257.
- ROBERTO R., MISCEO D., D'ADDABBO P., ARCHIDIACONO N. and ROCCHI M., 2008 — *Refinement of macaque synteny arrangement with respect to the official rhesus macaque sequence assembly*. Chromosome Research, 16: 977-985.
- ROBINSON T.J., FU B., FERGUSON-SMITH M.A. and YANG F., 2004 — *Cross-species chromosome painting in the golden mole and elephant shrew: support for the mammalian clades Afrotheria and Afroinsectiphilia but not Afroinsectivora*. Proceedings of the Royal Society of London B, Biological Sciences, 271 (1547): 1477-1484.
- ROBINSON T.J. and RUIZ-HERRERA A., 2008 — *Defining the ancestral eutherian karyotype: A cladistic interpretation of chromosome painting and genome sequence assembly data*. Chromosome Research, 16: 1133-1141.
- RUIZ-HERRERA A., GARCIA F., AZZALIN C., GIULIOTTO E., EGOZCUE J., PONSA' F.M. and GARCIA M., 2002 — *Distribution of intrachromosomal telomeric sequences (ITS) on Macaca fascicularis (Primates) chromosome and their implication for chromosome evolution*. Human Genetics, 110: 578-586.
- RUIZ-HERRERA A., GARCIA F., AGUILERA M., GARCIA M. and PONSA' F.M., 2005 — *Comparative chromosome painting in Aotus reveals a highly derived evolution*. American Journal of Primatology, 65: 173-185.
- RUIZ-HERRERA A., CASTRESANA J. and ROBINSON T.J., 2006 — *Is mammalian chromosomal evolution driven by regions of genome fragility?* Genome Biology, 7 (12): 115-131.
- RUMPLER Y., WARTER S., HAUWY M., FAUSSER J.L., ROOS C. and ZINNER D., 2008 — *Comparing chromosomal and mitochondrial phylogenies of sportive lemurs (genus Lepilemur, Primates)*. Chromosome Research, 16: 1143-1158.
- SCHERTHAN H., CREMER T., ARNASSON U., WEIER H.U., LIMA-DE-FARIA A. and FRONICKE L., 1994 — *Comparative chromosome painting studies discloses homologous segments in distantly related mammals*. Nature Genetics, 6: 342-347.
- SCHIBLER L., VAIMAN D., OUSTRY A., GIRAUD-DELVILLE C. and CRIBIU E.P., 1998 — *Comparative gene mapping: a fine-scale survey of chromosome rearrangements between ruminants and humans*. Genome Research, 8:901-915.
- SERREAU M., BONNET-GARNIER A., RICHARD F. and DUTRILLAUX B., 2004 — *Chromosome painting comparison of Leontopithecus chrysomelas (Callitrichinae, Platyrrhini) with man and its phylogenetic position*. Chromosome Research, 12(7): 691-701.
- SEUANEZ H.N., LIMA C.R., LEMOS B., BONVICINO C.R., MOREIRA M.A. and CANAVEZ F.C., 2001 — *Gene assignment in Ateles paniscus chamek (Platyrrhini, Primates). At location of 18 markers of human syntentic groups 1,2,7,14,15, 17 and 22*. Chromosome Research, 9(8): 631-639.
- SPRINGER M.S., MURPHY W.J., EIRIZIK E. and O'BRIEN S.J., 2003 — *Placental mammal diversification and the Cretaceous-Tertiary boundary*. Proceedings of the National Academy of Sciences of the United States of America, 100: 1056-1061.
- STANYON R. and SINEO L., 1983 — *Citotassonomia e filogenesi del genere cercopithecus*. Antropologia Contemporanea, 6 (3): 237-252.
- STANYON R., CONSIGLIERE S., MULLER S., MORESCALCHI A., NEUSSER M. and WIENBERG J., 2000 — *Fluorescence in situ hybridization (FISH) maps chromosomal homologies between the dusky titi and squirrel monkey*. American Journal of Primatology, 50: 95-107.

- STANYON R., CONSIGLIERE S., BIGONI F., FERGUSON-SMITH M., O'BRIEN P.C.M. and WIENBERG J., 2001 — *Reciprocal chromosome painting between a New World primate, the woolly monkey, and humans*. *Chromosome Research*, 9: 97-106.
- STANYON R., KOELHER U. and CONSIGLIERE S., 2002 — *Chromosome painting reveals that galagos have highly derived karyotypes*. *American Journal of Physical Anthropology*, 117: 319-326.
- STANYON R., BONVICINO C.R., SVARTMAN M. and SEUANEZ H.N., 2003 — *Chromosome painting in Callicebus lugens, the species with the lowest diploid number (2n=16) known in primates*. *Chromosoma*, 112: 201-206.
- STANYON R., BIGONI F., SLABY T., MULLER S., STONE G., BONVICINO C.R., NEUSSER M. and SEUANEZ H.N., 2004 — *Multi-directional chromosome painting maps homologies between species belonging to three genera of New World monkeys and humans*. *Chromosoma*, 113: 305-315.
- STANYON R., BRUENING R., STONE G., SHEARIN A. and BIGONI F., 2005 — *Reciprocal painting between humans, De Brazza's and patas monkeys reveals a major bifurcation in the Cercopithecini phylogenetic tree*. *Cytogenetic and Genome Research*, 108: 175-182.
- STANYON R., DUMAS F., STONE G. and BIGONI F., 2006 — *Multidirectional chromosome painting reveals a remarkable syntenic homology between the greater galagos and the slow loris*. *American Journal of Primatology*, 68: 349-359.
- STANYON R., ROCCHI M., CAPOZZI O., ROBERTO R., MISCEO D., VENTURA M., CARDONE M.F., BIGONI F. and ARCHIDIACONO N., 2008 — *Primate chromosome evolution: Ancestral karyotypes, marker order and neocentromeres*. *Chromosome Research*, 16: 17-39.
- SVARTMAN M., STONE G., PAGE J.E. and STANYON R., 2004 — *A chromosome painting test of the basal eutherian karyotype*. *Chromosome Research*, 12: 45-53.
- SWOFFORD D.L., 1988 — *Phylogenetic Analysis Using Parsimony, version 4.0*. Sunderland, MA: Sinauer Associated.
- TIAN Y., NIE W., WANG J., FERGUSON-SMITH M.A. and YANG F., 2004 — *Chromosome evolution in bears: reconstructing phylogenetic relationships by cross species chromosome painting*. *Chromosome Research*, 12: 55-63.
- TRIFONOV V.A., STANYON R., NESTERENKO A.I., FU B., PERELMAN P.L., O'BRIEN P.C.M., STONE G., RUNTSOVA N.V., HOUCK M.L. and ROBINSON T.J., et al. 2008 — *Multidirectional cross-species painting illuminates the history of karyotypic evolution in Perissodactyla*. *Chromosome Research*, 16: 89-107.
- VAN RHEEDE T., BASTIAANS T., BOONE D.N., HEDGES S.B., DE JONG W.W. and MADSEN O., 2006 — *The platypus is in its place; nuclear genes and indels confirm the sister group relation of monotremes and therians*. *Molecular Biology and Evolution*, 23: 587-597.
- VOLLETH M., KLETT C., KOLLAK A., DIXKENS C., WINTER Y., JUST W., VOGEL W. and HAMAISTER H., 1999 — *ZOO-FISH analysis in a species of the order Chiroptera: Glossophaga soricina (Phyllostomidae)*. *Chromosome Research*, 7: 57-64.
- VOLLETH M., HELLER K.G., PFEIFFER R.A. and HAMASTEIR H., 2002 — *A comparative ZOO-FISH analysis in bats elucidates the phylogenetic relationships between Megachiroptera and five microchiroptera families*. *Chromosome Research*, 10: 477-497.
- VOLLETH M. and MÜLLER S., 2006 — *Zoo-FISH in the European mole (Talpa europaea) detects all ancestral Boreo-Eutherian human homologous chromosome associations*. *Cytogenetic and Genome Research*, 115: 154-157.
- ZOLLINO M., LECCE R., SELICORNI A., MURDOLO M., MANCUSO I., MARANGI G., ZAMPINO G., GARAVELLI L., FERRARINI A. and ROCCHI M., et al. 2004 — *A double cryptic chromosome imbalance is an important factor to explain phenotypic variability in Wolf-Hirschhorn syndrome European*. *Journal of Human Genetics*, 12: 797-804.
- WALKER F.O., 2007 — "Huntington's disease". *Lancet*, 369 (9557): 218-228.
- WARTER S., HAUWY M., DUTRILLAUX B. and RUMPLER Y., 2005 — *Application of molecular cytogenetics for chromosomal evolution of the Lemuriformes (Prosimians)*. *Cytogenetic and Genome Research*, 108: 197-203.
- WEISE A., STARKE H., MRASEK K., CLAUSSEN U. and LIEHR T., 2005 — *New insights into the evolution of chromosome 1*. *Cytogenetic and Genome Research*, 108: 217-222.
- WIENBERG J., STANYON R., JAUCH A. and CREMER T., 1992 — *Homologies in human and Macaca fuscata chromosomes revealed by in situ suppression hybridization with human chromosome-specific DNA libraries*. *Chromosoma*, 101: 265-270.
- WIENBERG J., STANYON R., NASH W.G., O'BRIEN P.C.M., YANG F., O'BRIEN S.J. and FERGUSON-SMITH M.A., 1997 — *Conservation of human vs. feline genome organization revealed by reciprocal chromosome painting*. *Cytogenetics and Cell Genetics*, 77: 211-217.
- WIENBERG J., 2004 — *The evolution of eutherian chromosomes*. *Current Opinion in Genetics and Development*, 14: 657-666.
- WIENBERG J., 2005 — *Fluorescence in situ hybridization to chromosomes as a tool to understand human and primate genome evolution*. *Cytogenetic and Genome Research*, 108: 139-160.
- YANG F., MULLER S., JUST R., FERGUSON-SMITH M.A. and WIENBERG J., 1997a — *Comparative chromosome painting in mammals: Human and the Indian muntjac (Muntiacus muntjak vaginalis)*. *Genomics*, 39 (3): 396-401.
- YANG F., GRAPHODATSKY A.S., O'BRIEN P.C.M., COLABELLA A., SOLANKY N., SQUIRE M., SARGAN D.R. and FERGUSON-SMITH M.A., 2000 — *Reciprocal chromosome painting illuminates the history of genome evolution of the domestic cat, dog and human*. *Chromosome Research*, 8: 393-404.

- YANG F., ALKALAEVA E.Z., PERELMAN P.L., PARDINI A.T., HARRISON W.R., O'BRIEN P.C.M., FU B., GRAPHODATSKY A.S., FERGUSON-SMITH M.A. and ROBINSON T.J., 2003 — *Reciprocal chromosome painting among human, armadillo, and elephant (superorder Afrotheria) reveals the likely eutherian ancestral karyotype*. Proceedings of the National Academy of Sciences of the United States of America, 100: 1062-1066.
- YANG F., FU B., O'BRIEN P.C.M., NIE W., RYDER O.A. and FERGUSON-SMITH M.A., 2004 — *Refined genome-wide comparative map of the domestic horse, donkey and human based on cross-species chromosome painting: insight into the occasional fertility of mules*. Chromosome Research, 12: 65-76.
- YANG F., GRAPHODATSKY A.S., LI T., FU B., DOBIGNY G., WANG J., PERELMAN P.L., SERDUKOVA N.A., SU W. and O'BRIEN P.C.M., *et al.* 2006 — *Comparative genome maps of the pangolin, hedgehog, sloth, anteater and human revealed by cross-species chromosome painting: further insight into the ancestral karyotype and genome evolution of eutherian mammals*. Chromosome Research, 14: 283-296.
- YE J., BILTUEVA L., HUANG L., NIE W., WANG J., JING M., SU W., VOROBIEVA N.V., JIANG X. and GRAPHODATSKY A.S., *et al.* 2006 — *Cross-species chromosome painting unveils cytogenetic signatures for the Eulipotyphla and evidence for the polyphyly of Insectivora*. Chromosome Research, 14: 151-15.

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