Mammalian karyotype evolution

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Abstract | The chromosome complements (karyotypes) of animals display a great diversity in number and morphology. Against this background, the genomes of all species are remarkably conserved, not only in transcribed sequences, but also in some chromosome-specific non-coding sequences and in gene order. A close examination with chromosome painting shows that this conservation can be resolved into small numbers of large chromosomal segments. Rearrangement of these segments into different combinations explains much of the observed diversity in species karyotypes. Here we discuss how these rearrangements come about, and show how their analysis can determine the evolutionary relationships of all mammals and their descent from a common ancestor.

The Origin of Species (1859) introduced biologists to the concept that allied species are descended from a common ancestor and, indeed, that all forms of life arise from early progenitors. Darwin saw the Natural System as a genealogical arrangement with various grades of difference marked by the terms varieties, species, genera, families and so on. He realized that in order to understand the relationships between species it was necessary to determine "the lines of descent by the most permanent characters whatever they may be"[1]. He followed the classifications that were introduced by the *Systema Naturae* of Linnaeus (Tenth edition, 1758 (REF. 2)), which were mostly based on anatomical features of living organisms and their fossil progenitors. But using morphological characters as the basis for classification does not always lead to dependable results as they are often far removed from the genotype.

Fortunately, comparative genomics now provides Darwin's successors with the more appropriate DNA-based characters with which to investigate these relationships. The most productive genetic techniques, in order of increasing resolution, have been chromosome analysis, gene mapping and gene sequencing. In recent years, modern cytogenetics has contributed a large share of information about evolutionary relationships between a great number of mammalian species[3]. Each species has a characteristic chromosome complement — the species karyotype — that consists of pairs of chromosomes that can be arranged in order of size. One pair are the sex chromosomes, XY in male mammals and XX in females; the remaining pairs are autosomes. By comparing the chromosomes of mammalian species, much can be learned not only about karyotype evolution, but also about the mechanisms involved and their significance for speciation and, indeed, about some of the genetic factors that distinguish between closely related organisms.

Here we focus on the mechanisms and events that have contributed to mammalian karyotype evolution, and the insights that this examination provides into evolutionary relationships among mammals. We begin this overview by describing the extent of genome conservation and how genome variation within this conserved structure can lead to the modifications in development that are observed in all organisms. We briefly introduce the modern molecular cytogenetic methods that are used in evolutionary studies. These methods are comparatively recent, and their use in phylogenomics is not always appreciated by evolutionary biologists[3]. We describe chromosome homology mapping on which evolutionary trees of the main groups of mammals can be based. Finally, we show how such studies have led to the derivation of ancestral karyotypes of extinct species. The results presented here complement and extend phylogenies based on gene sequencing. A full discussion of the latter is outside the scope of this Review, and the reader is referred to the literature for further details[4].

Genome conservation

Genome sequencing of an increasing number of organisms reveals that the transcribed sequences of genomes of all species are highly conserved. This conservation is seen at several levels and includes homology of genetic linkage groups, and even large regions of chromosomes. Groups of genes that are linked together and in similar order can be found in species as disparate

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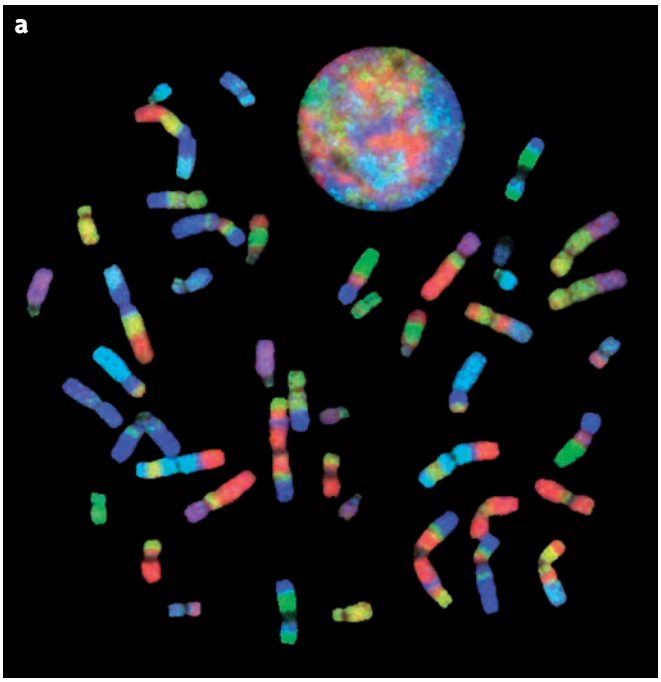
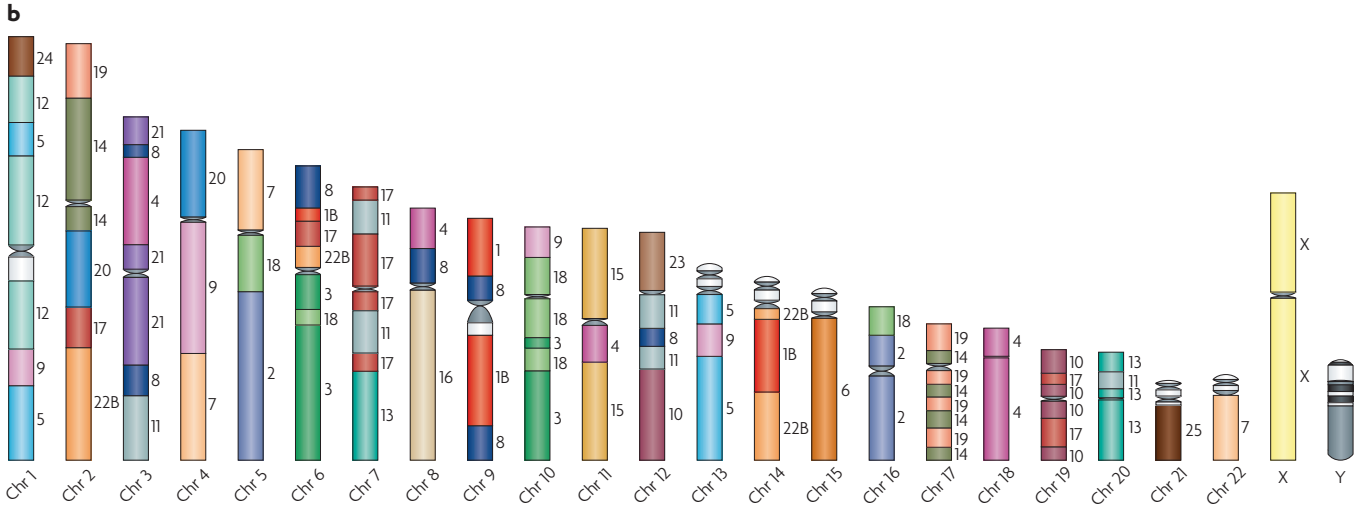
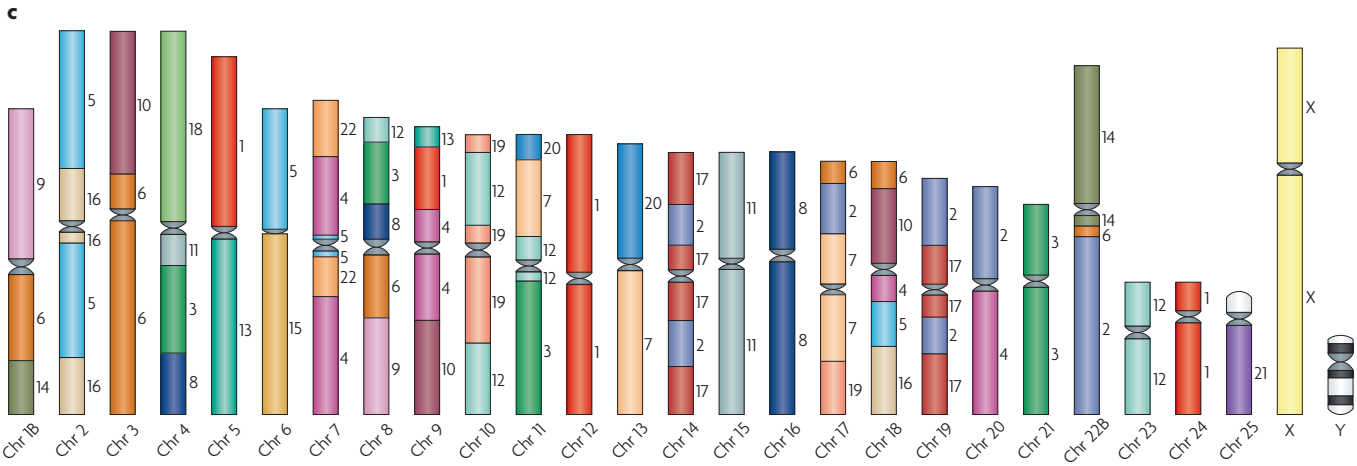
Box 1 | **How chromosomes are painted**

Chromosome painting is a form of fluorescence *in situ* hybridization (FISH) that has been highly productive in the construction of chromosome homology maps. The technique is described here using a gibbon–human comparison. A human metaphase and interphase nucleus is shown in panel **a** after hybridization with a chromosome-specific paint probe set that was derived from gibbon chromosomes. The probe set was made from a fluid suspension of gibbon chromosomes that were sorted and separated in a dual laser flow cytometer[27]. Several hundred of each pair in the karyotype were collected in separate tubes. DNA in each tube was amplified by random-primed PCR[26] and labelled with a combination of five fluorochromes so that each chromosome-specific DNA had a unique colour combination[24,27,65]. A mixture of the complete set of labelled DNA probes was then hybridized in annealing conditions to denatured human metaphases that were fixed and air-dried onto microscope slides. Under these conditions, the gibbon paint probes anneal to complementary DNA sequences on human chromosomes, and the result (as shown in panel **a**) can be observed by digital fluorescence microscopy. The homology map of gibbon chromosomal segments on human chromosomes that is derived from this painting experiment is shown in panel **b**. The reciprocal exercise using human paints on gibbon (shown in panel **c**) serves to identify those parts of each gibbon chromosome that are homologous to each human chromosome. Panels **b** and **c** are taken from the CHROMHOME database, which provides many useful chromosome homology maps between species, and is compiled by the Cambridge Resource Centre for Comparative Genomics.







Box 2 | **Comparative mapping using BAC clones.**

The procedure for making genomic libraries for whole-genome mapping and sequencing involves cloning of fragments of genomic DNA of about 150 kb into BACs. Individual BAC clones can be used as positional markers along the length of the chromosome. When they include fragments of a gene, they can help to assign the locus of that gene to its chromosome region by fluorescence *in situ* hybridization (FISH). BACs from species A can be used to screen genomic libraries of species B to isolate BACs that contain homologous fragments. FISH mapping of the homologous BACs from species B to species B chromosomes thus identifies the region of homology with species A. For example, comparative BAC mapping successfully demonstrates homology between chicken Z sequences and sequences on four of the five platypus X chromosomes, indicating their possible origin from a reptilian ancestor (see text).

as humans, chickens, flies, worms and sea anemones[5]. Among mammals, large blocks of DNA (syntenic blocks) revealed by cytogenetic methods, often amounting to whole chromosomes or chromosome arms, can be shared by distantly related species. Although species might differ in chromosome number and chromosome morphology[6], the differences are due to these syntenic blocks being assembled in different combinations. Blocks that are fused together in one species can be separated on different chromosomes in another. Chromosome numbers can increase or decrease by chromosome fission or fusion, respectively[7]. Segments within blocks can be inverted and centromeres repositioned. In themselves, these positional changes have little or no phenotypic effect, as shown by geographically separate populations of *Mus musculus* with karyotypes that differ in number and form, largely owing to chromosome fusion[8,9]. However, these changes sometimes preclude successful reproduction between populations and can be a factor in the emergence of new species.

The new combinations of syntenic blocks result from illegitimate meiotic recombination (non-allelic homologous recombination) events that occurred during the mammalian radiation[10]. This mechanism can lead to both genetically balanced and unbalanced products, but the latter are normally eliminated by natural selection and only those that are genetically balanced are passed in the germ line to future generations. The genetic duplications and deletions that occasionally result from these rearrangements are well-known causes of human constitutional chromosome abnormalities in which the phenotype can be severely affected and procreation precluded. The number of these rearrangements — the most common of which are centric fusion (Robertsonian) translocations — that have become fixed in the evolutionary history of mammals is surprisingly small.

The total number of rearranged blocks per haploid autosomal set, as revealed by cytogenetics, provides in many cases a measure of relationship between species. When compared with humans, most eutherians have 30 to 40 separate blocks of homology with the human genome. Some species are exceptional, such as dogs and gibbons, and have about twice as many conserved blocks[11,12]. The mouse is unique in having well over 200 blocks. There have been many more illegitimate recombinations in these species during their evolution, but the reasons for these differences in rate are as yet unknown. Superimposed on this conservation of syntenic blocks is variation at the DNA sequence level. Some of this variation consists of point mutations that may or may not be functional. This type of change occurs at an approximately constant rate and is caused by errors of replication or repair, or by environmental mutagenic agents.

Another source of variation comes from mobile element insertions, short tandem duplications and deletions that result from unequal crossing over during meiosis. Retrotransposon insertions are particularly useful in phylogenetic studies, because they represent non-homologous markers (the chance of convergent insertion of the same element is low)[13]. Copy-number variations[14], similar in origin to low-copy-number repeats and segmental duplications[15,16], often seem to have no phenotypic effect when observed between individuals within a species. At present, these changes have been described in hominids and rodents although they are expected to occur more widely. More extensive variation of this type could be an important factor in speciation and, for example, could contribute to some of the phenotypic differences that have occurred following the divergence of humans and chimpanzees[16,17]. Mobile element insertion and copy-number variation do not affect the identification of syntenic blocks, which were first described using conventional chromosome analysis and are now pursued by the advanced techniques of molecular cytogenetics.

Developments in karyotype analysis

The emergence of modern cytogenetics can be traced to the discovery by Tjio and Levan in 1956 of the correct chromosome number of 2n = 46 in humans[18,19]. Shortly afterwards, chromosome numbers were studied in primates, and later in many other mammalian species by Hsu and Benirschke[20], and others. The chromosome number and morphology were noted to be different and characteristic for most species, although some closely related species (for example, Felidae) were shown to have very similar karyotypes[21]. Distinct regions of homology between species became more apparent with the introduction of chromosome banding in the 1970s[22,23]. Chromosome-specific DNA probes labelled with fluorescence dyes, first introduced in 1988 (REF. 24), provided even greater resolution by revealing regions of homology through cross-species fluorescence *in situ* hybridization (FISH)[11,12,24–28]. In this technique, known as chromosome painting, chromosome-specific DNA is prepared from chromosomes that have been sorted by flow cytometry, or by microdissection, and amplified

Eutherians
Placental mammals (Placentalia).

Segmental duplication
Duplicated blocks of genomic DNA sequence that account for 5–10% of the human genome. Illegitimate recombination between such repeats on different chromosomes can lead to chromosome rearrangements.

Chromosome banding
Chromosome preparations are stained to reveal horizontal light and dark bands across the chromosome arms that serve to identify each chromosome.

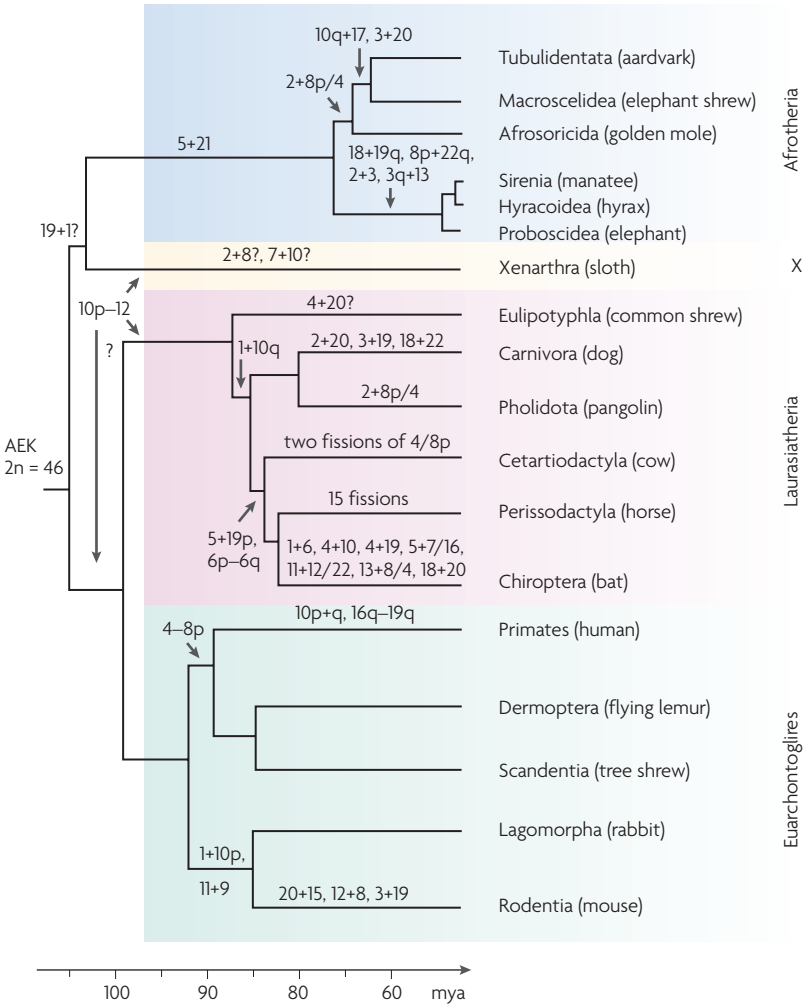


Figure 1 | **The mammalian evolutionary tree.** The tree is based on genetic, morphological and fossil data. The mammalian orders are linked by syntenic associations and show the likely genealogy against a geological timescale of millions of years (mya). Coloured areas represent the superordinal clades, where X is Xenarthra. Representative species studied by chromosome painting are indicated in brackets for each order. AEK indicates the ancestral eutherian karyotype (2n = 46). Plus and minus signs indicate characteristic fusions and fissions of corresponding human segments, respectively. Forward slashes indicate characteristic syntenies for the eutherian ancestor. Question marks indicate putative syntenies that need to be verified. Modified with permission from REF. 50 © (2005) S. Karger, and REF. 95 © (2006) John Wiley & Sons.

and labelled by PCR using different fluorochromes. When hybridized to air-dried metaphase preparations *in situ*, the probes anneal to complementary sequences along the length of the chromosome, thus revealing by fluorescence microscopy distinct coloured regions of chromosome homology (BOX 1). This technique has been highly productive in the construction of comparative chromosome homology maps (as seen in the CHROMHOME database).

Reciprocal cross-species painting, whereby chromosome-specific fluorescent probes (paints) from one species are hybridized to another and vice versa, allows the more exact identification of homologous chromosome segments. However, it is not possible to determine the orientation of each conserved block within a chromosome unless the procedure is coupled with FISH mapping of single-copy sequence probes that mark the ends of the block. Likewise, it is not possible by chromosome painting to identify most intrachromosomal rearrangements, such as an inverted segment or an interstitial insertion, without using the additional FISH method of BAC mapping (BOX 2). Moreover, cross-species painting is not successful between groups of animals whose DNA has diverged for more than 105 million years. Despite these limitations, chromosome painting probes satisfy the Darwinian criterion for 'permanent characters' for determining lines of descent within, if not between, the major classes of animals.

Mechanisms of karyotype evolution

Non-allelic homologous recombination at meiosis is the basis by which conserved segments of the genome are separated and fused in different combinations[10,17,29]. These changes can occur during the emergence of a new species, although many related species have apparently identical karyotypes. The breakpoints of these rearrangements have been found at sites of segmental chromosome duplication, between interspersed repetitive elements (such as retrotransposons), between low-copy repeats and between different members of the same gene family located on different chromosomes[17,29]. In other words, interchromosomal rearrangements are the result of accidental crossing over between homologous segments on non-homologous chromosomes (as described for the origin of some human chromosome aberrations[10,29]). Only a brief mention of what is now known about the mechanisms involved in these rearrangements is possible here. They may be promoted by a chromosomal inversion within one of the segments. The breakpoint sites tend to lie near telomeres and centromeres. The same sites can be re-used in other lineages[6]. Non-homologous end-joining may be another mechanism responsible for non-recurrent rearrangements[29]. Many of the breakpoint sites are associated with the formation of acrocentric (apparently single-armed) chromosomes in some species and metacentric (bi-armed) chromosomes in others. Metacentric chromosomes are often the product of fusion between two acrocentric chromosomes, and acrocentrics can result from fission of a metacentric. Both are illegitimate events that occur during meiosis and both are common mechanisms in karyotype evolution. For example, the domestic dog has 39 pairs of chromosomes, all of which are acrocentric except for the pair of sex chromosomes. Another canid, the red fox, has 16 pairs of autosomes, all of which are metacentric. Eight of the fox metacentric chromosomes can be interpreted as fusions of two dog acrocentric chromosomes, seven by fusion of three dog chromosomes and the remainder by more complex rearrangements[11]. All but four of the dog chromosome pairs are retained intact in the fox karyotype, and all rearrangements can be classed as either centromeric (centric) or tandem fusions.

Flow cytometry
A procedure whereby cells or chromosomes are measured and sorted in a fluid suspension.

Interstitial insertion
A chromosome rearrangement in which a segment is excised from one region and inserted into another.

It has been postulated that the non-random segregation of centric fusion translocations in female meiosis can lead to a bias towards either acrocentric or metacentric heterozygotes among offspring[30]. If true, this could be the mechanism that leads to the occurrence of different kinds of karyotypes, some with mostly acrocentric chromosomes and a high diploid number (dog-like), and others with mostly metacentric chromosomes and a low diploid number (fox-like). Simultaneous fission of all bi-armed chromosomes is another mechanism

Table 1 | **Syntenic associations revealed by chromosome painting with human-chromosome-specific probes**

Superorder/ order	Species	Syntenic associations																							
		3/21	4/8p	7/16	12/22	14/15	16/19	10p/12	19p/1	5/21	2/8/4	3/20	18/19	2/8	7/10	4/20	1q/10q	2/20	3/19p	18/22	5/19p	11/19	19p/q	1/10p	20/15	12/8
Afrotheria	*Aardvark*	•	•	•	•	•	•	•	•	•	•	•														
	Elephant shrew	•	•	•	•	•	•	•	•	•	•	•														
	African elephant	•		•	•	•	•	•	•	•			•													
	Golden mole	•	•	•	•	•	•	•	•	•	•															
	Manatee	•		•	•	•	•	•	•	•			•													
Xenarthra	*Two-toed sloth*	•	•	•	•	•								•	•											
	Anteater	•	•	•	•	•	•		•					•	•											
Eulipotyphla	*Shrew-hedgehog*	•	•	•	•	•		•	•					•		•										
	Common shrew	•	•	•	•	•	•									•			•							
Carnivora	*Cat*	•	•	•	•	•	•	•										•	•	•						
	Hyena	•	•	•	•	•	•	•										•	•	•						
	Dog	•	•	•	•	•											•		•							
	Mink	•	•	•	•	•	•	•										•	•	•						
	Giant panda	•	•		•		•												•							
Pholidota	*Pangolin*	•	•	•	•	•	•				•			•		•										
Cetartiodactyla	*Dolphin*	•		•	•	•	•													•	•					
	Indian muntjac	•		•	•	•	•		•											•	•					
	Pig	•	•	•	•	•	•														•	•				
	Camel	•	•	•	•	•	•														•					
	Cow	•	•	•	•	•	•										•				•					
Perissodactyla	*Rhinoceros*	•	•	•	•	•											•				•					
	Zebra	•	•	•	•	•	•										•				•	•				
	Horse	•	•	•	•	•											•				•	•				
Scandentia	*Tree shrew*	•			•	•	•																			
Chiroptera	*Bat*	•	•	•	•	•	•																			
Primates	*Howler monkey*	•				•												•					•			
	Slow loris	•		•	•	•																				
Lagomorpha	*Rabbit*	•	•	•	•	•	•																	•		
Rodentia	*Gray squirrel*	•	•	•	•	•	•												•					•	•	•
	*Mouse**	•	•	•	•	•	•												•					•	•	•

*Human associations on mouse were transferred from the mouse sequencing data at the Ensembl Mouse web site.

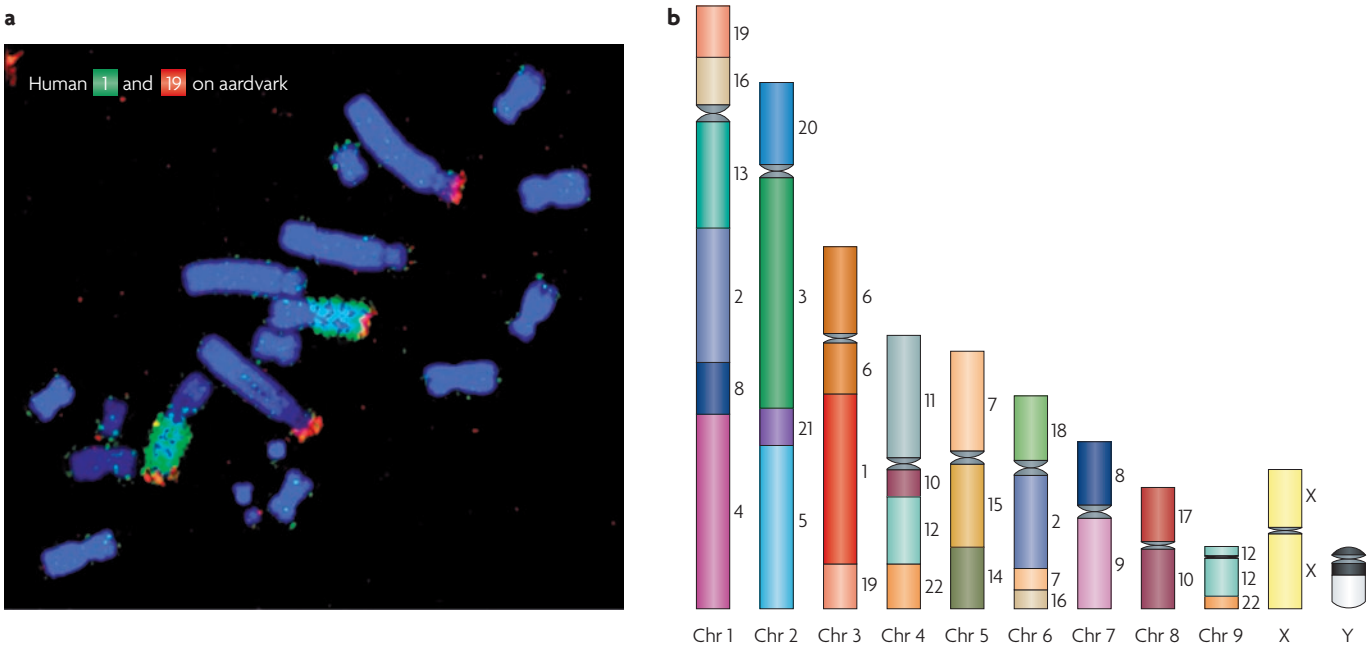


Figure 2 | **Mapping human homologies on aardvark chromosomes. a** | Paint probes that are specific for human chromosomes 1 and 19 hybridize to aardvark chromosomes 1p and 3q. **b** | A complete map of the human homologies on aardvark chromosomes. Image in part **a** courtesy of F. Yang, The Wellcome Trust Sanger Institute, Cambridge, UK.

that is proposed to explain the occurrence of karyotypes that are characterized by many acrocentrics[31]. Interestingly, the number of chromosomes seems to be irrelevant in terms of the phenotype. For example, among deer, Indian and Chinese muntjacs look almost identical, yet the former has 6 chromosomes and the latter has 46 (REF. 32). Among mammals, numbers vary from 2n = 6 in the Indian muntjac to 2n = 102 in the viscacha rat, but it is not known what governs the optimal number for any given species. Nonetheless, there appears to be a limit to chromosomal size: in species such as the field vole (*Microtus agrestis*), the exceptionally large X chromosome can bulge out of the interphase nucleus and this can sometimes disrupt normal cell division[33].

Overview of karyotype evolution in mammals

One recent view of the relationships of the 18 orders of placental mammals is shown in FIG. 1, as studies now suggest that divergence times of early eutherians can be placed at around 93 million years ago (mya), that is, long before the Cretaceous–Tertiary boundary[34]. Representative species within each of the mammalian orders have now been studied by cross-species chromosome painting, and separate trees (phylogenies) have been constructed showing the relationship between species, genera and families in each order. A comparison between orders leads to the construction of the most likely ancestral eutherian karyotype (AEK). In most cases, comparisons have been made with reference to the human genome so that conserved syntenic blocks defined by homology with human chromosomes can be recognized in all species.

When comparative chromosome maps were first drawn outlining the blocks of conserved homology between human and other species, it was observed that certain blocks that mapped to different human chromosomes tended to be fused together in other species. Compared with distantly related groups (outgroups, that is, species belonging to a different taxon), some of these associations can be classed as shared ancestral characters because they are found in all species of a particular taxon. Others are classed as common derived characters because they have arisen in a known common ancestor. As a chromosome fission can occur more than once at the same site, as a result of the reuse of an evolutionary breakpoint, care is needed in distinguishing *de novo* events (due to convergence) masquerading as ancestral characters. For instance, centromeric fusions and fissions are highly prone to convergence. Other apparent examples of the convergence of similar syntenic associations have been shown to have different breakpoints when studied by sequencing across the breakpoint. Common syntenic associations are thus more useful than common fissions in phylogenetic studies. With these provisos, data have been accumulated for all orders of mammals, except for some rodent groups, on associations of conserved syntenic blocks in which each block is identified on the basis of its location on a human (HSA) chromosome. TABLE 1 lists syntenic associations in a range of animals on the basis of homologies with human chromosomes. For example, an association between parts of HSA 3 and 21 is found in the karyotype of all animals listed, indicating that it is a shared ancestral character. Conversely, an HSA 5/21 synteny is found only in the aardvark, elephant shrew, African

Synteny
DNA sequences that are located on the same chromosome are syntenic.

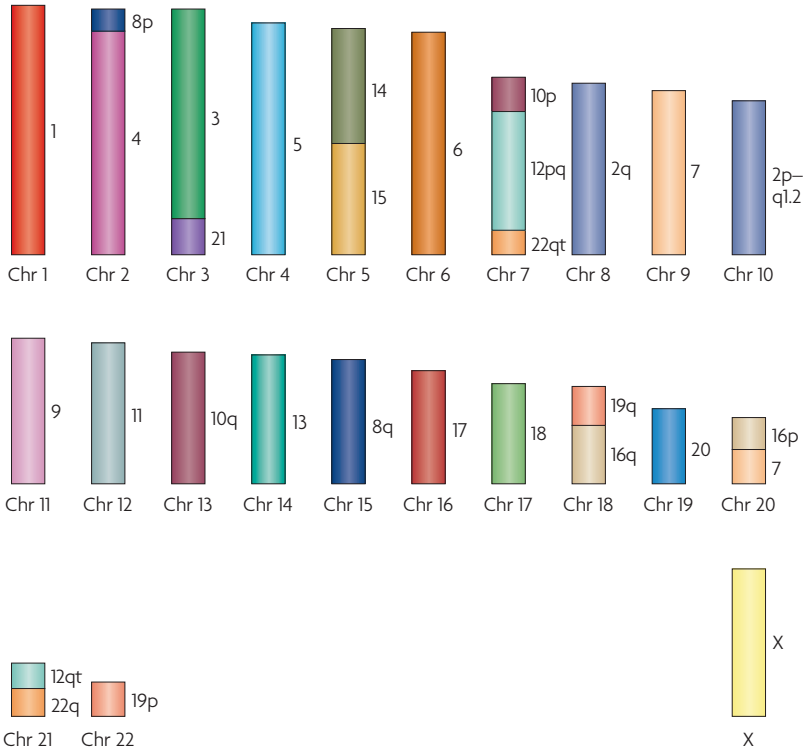


Figure 3 | **Ancestral eutherian karyotype.** The ancestral eutherian karyotype is based on syntenic associations that are shared by all orders, including the most basal clades in TABLE 1. Homologies to human chromosomes are indicated to the right of each chromosome. Modified with permission from REF. 50 © (2005) Karger.

elephant, golden mole and manatee[35,36]. This indicates that these species share a derived character that is distinct from outgroup species, and one that has arisen in an immediate ancestor of the group. These findings provide evidence for the inclusion of these species in the Afrotherian superorder[37,38]. By contrast, some very similar associations or convergences are found in different groups that have evolved independently. For example, the association HSA 2/8/4 that is observed in the pangolin[39] is very similar to the HSA 2/8/4 synteny in the aardvark (FIG. 1; TABLE 1), but reciprocal painting experiments have shown the segments of HSA 2 in pangolin and afrotherian species are different[39].

Whereas most of the phylogenies based on karyotype evolution have used chromosome-specific paints prepared from human chromosomes, chromosome homology maps of higher resolution can be prepared from chromosome-specific paints from animals with greater chromosome numbers and more highly rearranged karyotypes. Thus, phylogenies within some orders have been constructed using chromosome paints from the domestic dog (2n = 78)[40–42]. Among marsupials, most of whose chromosome numbers vary from 14 to 22, the rufous bettong (*Aepyprymnus rufescens,* 2n = 32) has been useful in comparing relationships within the order[43]. Because of the number of rearrangements in muroid rodents, conserved syntenies are defined in terms of their location in mice or hamsters rather than in human chromosomes.

From homology maps to karyotype evolution

We have chosen the hybridization of human-chromosome-specific paint probes to a metaphase from the aardvark (2n = 20) to describe how cross-species chromosome painting is used to construct chromosome homology maps[35]. An example of the homology that is revealed by two human paints, HSA 1 and HSA 19, on aardvark chromosomes is shown in FIG. 2a; FIG. 2b is a diagrammatic compilation of the complete homology map of human on aardvark. The latter reveals a number of syntenic associations that are common to many species. Note especially the syntenic associations that correspond to HSA 3/21 (chromosome 2), 4/8 (chromosome 1), 7/16 (chromosome 6), 12/22 (chromosomes 4 and 9), 14/15 (chromosome 5), 16/19 (chromosome 1) and 10/12 (chromosome 4). These associations are common to a wide range of species (TABLE 1) and, in fact, all studies to date suggest that they must be represented in the AEK (FIG. 3). Other syntenic associations are specific to each order and superorder, and some are characteristic of particular families. The patterns of associations that are revealed by chromosome painting therefore provide information on karyotype evolution and on the phylogenetic relationships of species and groups of species.

Superordinal karyotypes. The mammalian orders are grouped into a series of six superordinal clades and, with the exception of the monotremes and the American and Australian marsupials, the superorders can be linked successfully into a common pedigree using chromosome painting (FIG. 1). Afrotheria, comprising aardvarks, elephant shrews, African elephants, hyraxes, tenrecs, golden moles and manatees, are believed to be the most basal clade, arising approximately 105 mya[37,38]. The syntenic associations HSA 5/21 and 1/19 (FIG. 2; TABLE 1) are the characteristic signatures of this clade[35,36,44–46]. Xenarthra is another ancient clade, arising some 100 mya[36,39,47], including the South American armadillos, anteaters and two sloth species. Although cross-species painting is at an early stage, HSA 2/8 and HSA 7/10 seem to be characteristic of this clade[39,48]. HSA 19p/1 might be a characteristic trait that is common to Afrotheria and Xenarthra (which were recently suggested to be sister groups[49]), although at present we cannot exclude that this character is a result of convergence, or that it is ancestral and was disrupted in all other mammalian suborders.

The two other mammalian superorders are Euarchontoglires (representing primates, tree shrews, flying lemurs, rabbits and rodents) and the Laurasiatheria (pangolins, carnivores, horses and tapirs, ungulates, bats and insectivores). Cytogenetic signatures for these clades have not yet been identified, probably because their early divergence was not accompanied by major chromosomal rearrangements.

An AEK composed of 46 chromosomes (FIG. 3) can be postulated with some confidence on the basis of the information in TABLE 1 (REFS 50,51). By coincidence, the number of AEK chromosomes is identical to the human chromosome number. The human karyotype

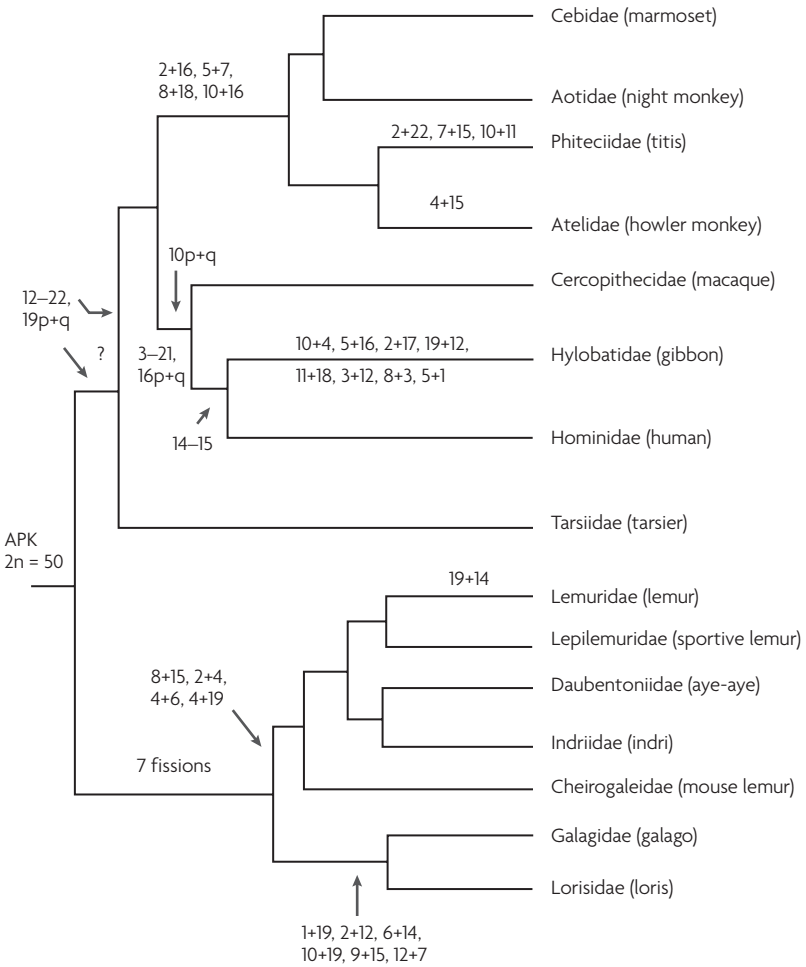


Figure 4 | **Primate evolutionary tree.** The ancestral primate karyotype can be constructed from 14 of the 15 primate families (here, excluding Tarsiidae). Representative species of each primate family are linked to the putative ancestral primate karyotype (APK) by patterns of syntenic associations based on chromosome painting with human probes. Plus and minus signs indicate characteristic fusions and fissions of corresponding human segments, respectively. Question marks indicate putative syntenies that need to be verified. Composed from various data from REFS 50,51,57–65.

shows many features of the AEK, notably entire human chromosomes 1, 5, 6, 9, 11, 13, 17, 18, 20 and X are conserved intact in the AEK.

Using a similar strategy, it is possible to construct phylogenetic trees composed of families and genera within each order. Below we present selected examples with references, but the same exercise has been published for all orders except Dermoptera, which is currently in the process of being assembled and verified by our group.

Primates. Chromosome painting between humans and the great apes shows, with two exceptions, that each human chromosome is homologous to a single ape chromosome. The centric fusion of the two ape chromosomes to form HSA 2 (REFS 52,53) (by illegitimate recombination between duplicated segments on each) and the reciprocal translocation between two chromosomes in the gorilla that are homologous to HSA 5 and 17, are the two exceptions[54–55]. G-banding, gene-mapping and colour-banding studies in some of the human–ape homologues reveal intrachromosomal inversions and neocentromeres[56]. The results of these studies help to determine the order of evolution from the ancestral great ape karyotype (2n = 48) to the human karyotype (2n = 46) (reviewed in REF. 50). They show that the lineage of the orangutan diverged earlier than the gorilla and chimpanzee lineages, which share inversions of HSA 3, 7 and 11. The gorilla lineage diverged next, and three subsequent inversions of HSA 7, 9 and 10 in the chimpanzee–human lineage occurred before their separation. Thereafter, the human line acquired the additional inversions within chromosomes 4, 17 and 18 and reduced the ancestral chromosome number to 46 by the fusion of the two acrocentric chromosomes into HSA 2.

The ancestral karyotype of all primates, including prosimians (lemurs and lorises)[57–59], New World monkeys (including Cebidae and Atelidae)[60,61], Old World Monkeys[62,63], gibbons[64,65], great apes and humans[50,63,65] has been determined by identifying syntenic associations relative to human chromosomes (FIG. 4). The key associations that are found in the ancestral primate are HSA 3/21, 14/15, 12a/22b, 12b/22a and 7b/16p; single ancestral chromosomes are represented by HSA 19p, 19q, 16q, 2q, 2p and 7a[50]. For example, one of the characteristics of prosimians is the presence of HSA 19p and 19q as separate chromosomes, which become fused in New World monkeys and other primate groups. Similarly, HSA 16p and 16q are separate in New World monkeys, but are fused in Old World monkeys and apes. The HSA 14/15 association is present in all primates except apes. Chromosome fission after the divergence of Old World monkeys leads to the separation of HSA 14 and 15.

Carnivores. Resolution of 8 of the 14 carnivore families into a phylogenetic tree has also been accomplished by chromosome painting using human-chromosome-specific probes (FIG. 5). Traditionally, the order has been divided into two monophyletic groups: Feliformia (cats, mongooses, hyenas and civets) and Caniformia (dog, bears, raccoons, mustelids and pinnipeds)[66]. The results of chromosome painting strongly support the previously suggested phylogeny[67–71]. The ancestral carnivore karyotype (ACK) is characterized by the associations HSA 3/19p, 18/22 and 2/20. Within the eight families, additional information on the phylogenetic relationships of subfamilies has been contributed by cross-species painting with cat, dog, mink and stone martin probes[67–72]. Although the cat, mink and stone martin probes are simpler to construct because they are separated more readily by flow cytometry, they have less resolution than the more numerous dog probes, which have been particularly informative in revealing intrachromosomal rearrangements[40]. Notable findings include further evidence for placing the red panda among the Musteloidea and the giant panda among bears[68,71].

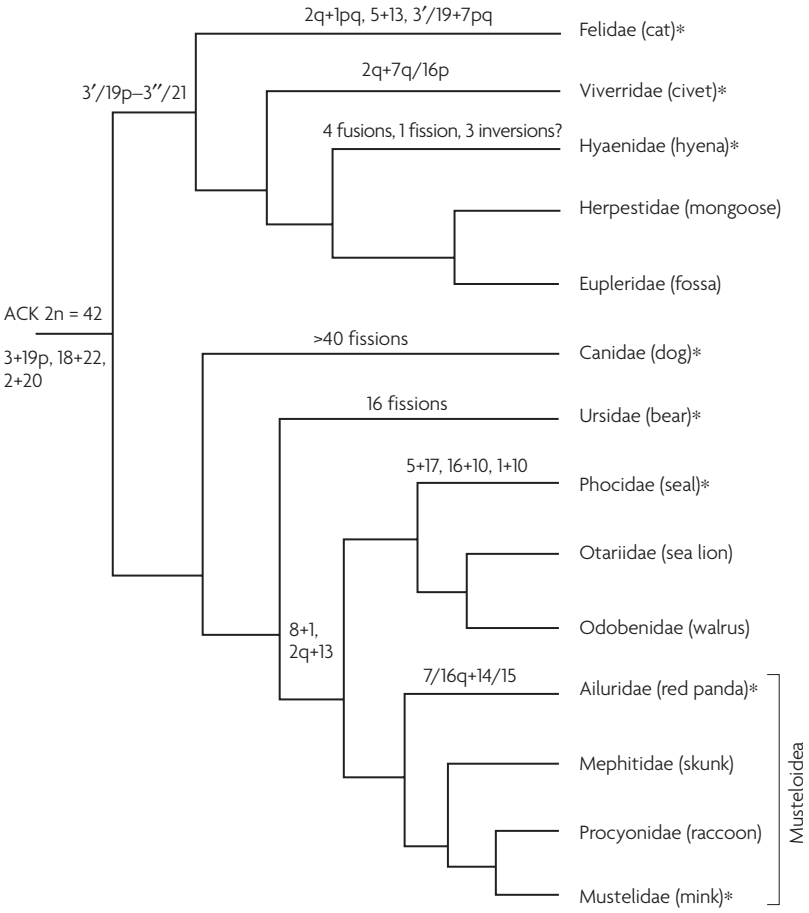


Figure 5 | **Carnivore evolutionary tree.** Of the 14 carnivore families, 8 (indicated by an asterisk) are arranged into a phylogeny using human-chromosome-specific probes from which a putative ancestral carnivore karyotype (ACK) can be resolved. Plus and minus signs indicate characteristic fusions and fissions of corresponding human segments, respectively. Question marks indicate putative syntenies that need to be verified. Composed from various data from REFS 66–75.

The Felidae family shows a low rate of karyotype evolution with almost all species having a 2n = 38 karyotype similar to the ACK, with the notable exceptions of the ocelot and marguay, both of which have 36 chromosomes[21,73]. Very similar karyotypes were found in the hyena and civet, confirming the extensive chromosomal conservation that is characteristic for most feliforms[70]. This is in contrast to the Canidae family, which has extensively rearranged karyotypes[11,74]. A rather high rate of karyotype evolution has also occurred among bears, accompanied by multiple fissions of ancestral carnivore elements[69,71]. Chromosome painting in 12 species from the Mustelidae family (European minks, steppe polecats, forest polecats including its albino form, the domestic ferret, striped polecats, least weasels, mountain weasels, Japanese sables, stone martens, yellow-throated martens, American minks, ferret badgers and Old World badgers) revealed that members of this family have highly conserved karyotypes that are similar to the ACK[67,68,72]. Together with pinnipeds[75] and red pandas, mustelids represent the lowest rate of chromosomal evolution within Caniformia (see below).

Cetartiodactyls. Cattle, sheep, deer, giraffes, pigs, camels and whales belong to the order Cetartiodactyla, in which the ancestral karyotype is predicted to have 52 chromosomes. The cytogenetic signature that is common to the order is HSA 5/19p, and fission HSA 6p–q is another characteristic trait. Camels seem to be the most primitive group within this taxon[76], whereas cetaceans might have the most conserved karyotype[77]. Both HSA 5/19p and disruption of the HSA 6p/6q association might have occurred in the common ancestor of the cetartiodactyls and the perissodactyls (horses, asses, zebras, rhinoceroses and tapirs)[76,78]. In bats (Chiroptera), a HSA 4/19p association is present in all species studied[79]. In only two bat families is this association found to be fused with a HSA 5 homologous element, thus forming a HSA 4/19p/5 association. This is a good example of a derived character. Extensive rearrangements have occurred during the divergence of the groups represented by cattle, pigs, camels and whales (FIG. 6). Chromosome painting of representatives of other artiodactyl families (dolphins, giraffes, hippopotamuses, pronghorns and mouse deer) will help to refine the position of rearrangements that are noted on the phylogenetic tree, and may lead to the discovery of new cytogenetic signatures.

Rodents. Rodents (FIG. 7a) represent the largest mammalian order with over 2,000 species, comprising 40% of all mammalian species. Chromosome painting has been particularly productive in the analysis of non-muroid families, such as the Sciuridae (squirrels), the karyotypes of which are highly conserved and retain many ancestral syntenies[80–83]. Syntenic associations, such as HSA 1/10p, 20/15, 12/8, 3/19 and 11/9 have been found in most of the rodent species studied to date. These form part of the predicted ancestral rodent karyotype. The HSA 1/10p and 11/9 associations are also present in the rabbit[80,84] confirming that the rodents and lagomorphs are sister groups within the Orchontoglires.

The superfamily of muroid rodents, including the important laboratory animals — mice, hamsters and rats — has highly rearranged karyotypes in comparison with humans, and cross-species painting with human probes can sometimes be difficult to interpret. Fortunately, human and mouse genome sequences are available for comparative studies of mouse and human chromosomes, and human homologies can be inferred from cross-species painting between mice and other muroids using mouse and hamster probes[85]. Several studies have used painting probes from the mouse, the hamster and the vole to resolve some of these complex phylogenies[85–87]. Figure 7b shows how the ancestral muroid karyotype has been derived from 20 species representing extant genera and families. The HSA 3/19p association has been found in rodents[80–83] and carnivores[67,68,72], and is probably a result of convergence, which can sometimes confuse the construction of phylogenetic trees.

Rates of chromosomal rearrangement. The chromosome painting data that are now available for many species from different orders help to estimate the

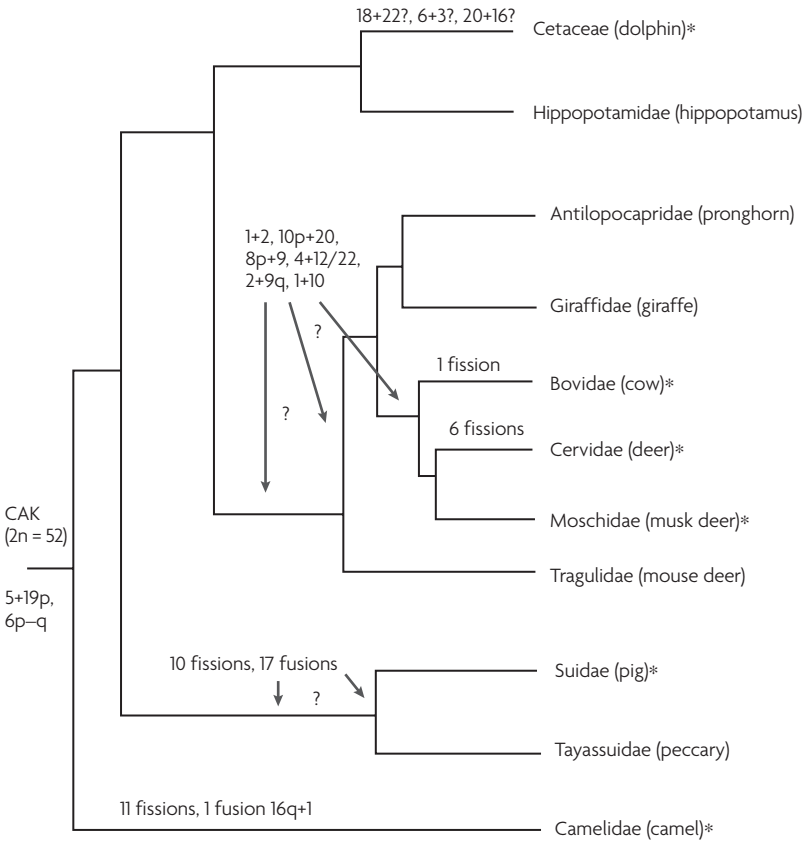


Figure 6 | **Cetartiodactyl evolutionary tree.** Of the 11 cetartiodactyl families, 6 (indicated by an asterisk) are arranged into a phylogeny using human-chromosome-specific probes. For details of the extensive rearrangements in this phylogeny, see references in the text. Plus and minus signs indicate characteristic fusions and fissions of corresponding human segments, respectively. Question marks indicate putative syntenies that need to be verified. CAK, Cetartiodactyl ancestral karyotype. Composed from data from REFS 76,77.

average rate of evolutionary rearrangements during different periods in different lineages. Murphy *et al.*[88] propose two main modes of karyotype evolution rate: an ancestral slow rate (one or less exchange per 10 mya) and higher rates. However, closer investigation within groups has suggested that at different times the rate of evolution, as well as the prevailing type of rearrangement, has changed greatly. For example, for 50 million years in the lineage that extends from the eutherian ancestor to the primate ancestor, only three rearrangements took place[51]. Thereafter, a sudden karyotype diversification occurred in the gibbon lineage, with 24 rearrangements leading to the common gibbon ancestor and then multiple rearrangements subsequently leading to the karyotypes of extant species[63,64]. During the same period, karyotype evolution within the great apes was extremely slow.

Record high rates of karyotype evolution are found in muroid rodents[85–87], canids[11,40], gibbons[64] and equids[78,89]. However, each of these mammalian orders contains taxa with the slow rates of chromosomal rearrangement (Sciuridae family among rodents[80–83], Felidae and Phocidae among carnivora[70,75], apes among primates[50], rhinoceroses among perissodactyls[90]). There is therefore no default rate in mammalian karyotype evolution and the variation in rates remains unexplained. Environmental effects, overall mutation rates, population size and the activation of mobile elements and retroviruses are among the possible contributory factors.

Ancestral vertebrate karyotype. Cross-species chromosome painting is not possible between placental mammals (eutherians) and monotremes, marsupials and the other major vertebrate classes of birds, reptiles, amphibians and fish, owing to the extent of chromosomal DNA divergence. However, chromosome painting works well within each of these groups, and this, together with BAC mapping (BOX 2), means that studies of karyotype evolution can be extended to most of them. For instance, BAC mapping has been used to link the platypus X chromosomes with various regions of homology in human and chicken chromosomes. The platypus has five pairs of X chromosomes in the female and five Xs and five Ys in the male[91], none of which share homology with either the marsupial or eutherian XX–XY system. However, BAC mapping reveals that several platypus orthologues of genes on the chicken Z chromosome, and their homologues on human autosomes 5 and 9, map to platypus X_1, X_2, X_3 and X_5, and their XY pairing regions on Y_1 and Y_2 (REF. 92). Orthologues of human X-linked genes map to platypus autosome 6 (P. Waters & F. Veyrunes, personal communication), demonstrating that platypus sex chromosomes are unrelated to those of therians and have evolved from different ancestral chromosomes.

With the increasing availability of DNA sequence data from representative species, comparisons can be made with the complementary sequences in the human genome database. Chromosome homology maps of human and non-mammalian species can be inferred from these sequence comparisons. Such *in silico* analyses have enabled Kohn *et al.*[93] to compare human, chicken, zebrafish and pufferfish chromosomes and determine conserved homologies. These authors reconstructed an ancestral vertebrate karyotype (AVK) comprising eleven protochromosomes. The AVK seems to be highly conserved in birds and fish, but the AEK is extensively rearranged in comparison. This means that a great number of evolutionary rearrangements must have occurred in the therian lineage after the divergence of birds approximately 300 mya and in the lineage leading to monotremes, marsupials and eutherians. Similar comparisons with the recently published opossum sequence[94] reveal that the opossum chromosomes are similar to those of chickens but are greatly rearranged by comparison with those of humans. Thus, chicken chromosome 1 is homologous to only two opossum chromosomes (numbers 4 and 8) while sharing homology with parts of 12 human chromosomes; these correspond to ten segments of homology within the AEK. This places the period of intense evolutionary change between the divergence of marsupials (180 mya) and the

Therians
Marsupials (metatherians) and eutherians (monotremes are prototherians).

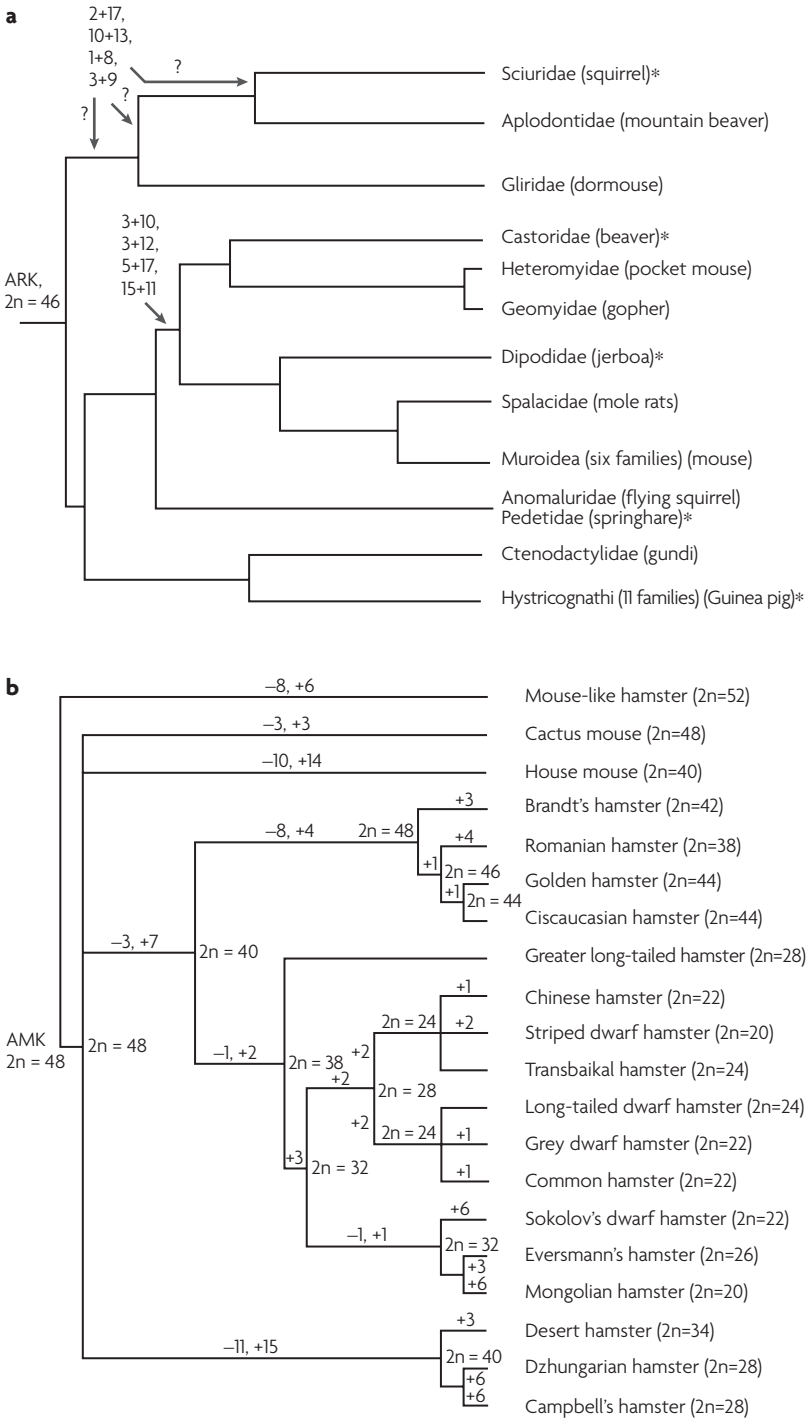


Figure 7 | **Rodent evolutionary tree. a** | The ancestral rodent karyotype (ARK) can be constructed from 5 (indicated by an asterisk) of the 28 rodent families using chromosome painting with human probes. The remaining relationships are derived largely from molecular data[96,97]. Data on families Castoridae and Dipodidae, as well as on superfamily Hystricognathi, were provided by A. Graphodatsky (personal communication). The place of the Muroidea within Rodentia is shown. Plus and minus signs indicate characteristic fusions and fissions of corresponding human segments, respectively. Question marks indicate putative syntenies that need to be verified. **b** | Representative species from 20 genera within the muroid family are shown with their karyotypic relationship to the putative ancestral muroid karyotype (AMK) based on banding patterns and cross-species painting with various rodent probes. Presumptive ancestral diploid numbers are shown for each node[86], with loss or gain of chromosomes as shown. Modified with permission from REF. 86 © (2007) Springer Netherlands.

emergence of eutherians (100 mya). Karyotypes in most eutherian orders remained relatively stable thereafter as confirmed by the presence of the low numbers of conserved syntenic associations that are evident from chromosome painting.

Remarkably, conserved linkage groups representing the ancient eumetazoan chromosomes have been found by comparing the human and the recently sequenced sea anemone (Cnidaria) genomes. Despite 700 million years of evolution, 40 large homologous segments were identified, demonstrating conserved synteny between human and sea anemone chromosomes[5]. This long-term conservation of large chromosomal segments suggests selection against mutations that disrupt higher level chromosomal organization and gene regulation[5].

Conclusions

Cross-species chromosome painting is an excellent method for identifying conserved blocks of chromosome homology between species and discovering combinations that reveal their evolutionary relationships. The technique is simple and reliable, and the results are immediately visible by fluorescence microscopy. The resolution is sufficient to enable the construction of pedigrees that show the relationships between species, families and orders, and the lines of descent from common ancestors as postulated in *The Origin of Species* more than 150 years ago. The study of karyotype evolution complements and extends other evolutionary studies that are based on the fossil record, morphological features and molecular sequence data. Nevertheless, chromosome painting has its limitations when it comes to tracing links between eutherians, monotremes and marsupials, owing to the extensive divergence of non-coding chromosome-specific DNA in these lineages over time. The challenge in the future is to find effective means to bridge these evolutionary gaps. At present, the only available course is to extend comparative gene mapping and DNA sequencing, both of which are labour-intensive and costly. Progress with more distantly related vertebrates will depend on complete genome sequencing of additional representative species of reptiles, amphibians and fish. Meanwhile, the pursuit of karyotype evolution within mammalian orders will help to solve unanswered biological questions, such as the evolution of sex determination and genomic imprinting, and the mechanism of dosage compensation of sex-linked genes in monotremes and marsupials. The function of much of the non-transcribed DNA, which is chromosome-specific and conserved across species, is poorly understood, and comparative studies may shed light on what was previously considered to be junk DNA. The findings discussed here are not only relevant to our understanding of the mechanisms of chromosome fusion and fission during species divergence, and in revealing the importance of segmental duplication in the occurrence of illegitimate meiotic recombination, but they also attest to the power of comparative genomics in the investigation of biological processes.

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