Chromosome Research (2008) 16:261–274
DOI: 10.1007/s10577-007-1191-5

Tracking genome organization in rodents by Zoo-FISH

Alexander S. Graphodatsky[1]*, Fengtang Yang[2], Gauthier Dobigny[3], Svetlana A. Romanenko[1], Larisa S. Biltueva[1], Polina L. Perelman[1], Violetta R. Beklemisheva[1], Elena Z. Alkalaeva[1], Natalya A. Serdukova[1], Malcolm A. Ferguson-Smith[4], William J. Murphy[5] & Terence J. Robinson[6]
[1]*Institute of Cytology and Genetics, SB RAS 6300090, Novosibirsk, Russia; E-mail: graf@bionet.nsc.ru;* [2]*The Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, CB10 1SA, UK;* [3]*Institut de Recherche pour le Développement, Centre de Biologie et Gestion des Populations, Campus International de Baillarguet, CS30016, 34988, Montferrier-sur-Lez, France;* [4]*Cambridge Resource Centre for Comparative Genomics, Department of Veterinary Medicine, University of Cambridge, Madingley Road, Cambridge, CB3 OES, UK;* [5]*Department of Veterinary Integrative Biosciences, Texas A&M University, College Station, TX, 77843, USA;* [6]*Evolutionary Genomics Group, Department of Botany and Zoology, University of Stellenbosch, Matieland, 7602, South Africa*
* *Correspondence*

Received 5 August 2007. Received in revised form and accepted for publication by Herbert Macgregor 20 November 2007

Key words: ancestral karyotype, chromosome painting, comparative cytogenetics, Rodentia

Abstract

The number of rodent species examined by modern comparative genomic approaches, particularly chromosome painting, is limited. The use of human whole-chromosome painting probes to detect regions of homology in the karyotypes of the rodent index species, the mouse and rat, has been hindered by the highly rearranged nature of their genomes. In contrast, recent studies have demonstrated that non-murid rodents display more conserved genomes, underscoring their suitability for comparative genomic and higher-order systematic studies. Here we provide the first comparative chromosome maps between human and representative rodents of three major rodent lineages Castoridae, Pedetidae and Dipodidae. A comprehensive analysis of these data and those published for Sciuridae show (1) that Castoridae, Pedetidae and Dipodidae form a monophyletic group, and (2) that the European beaver *Castor fiber* (Castoridae) and the birch mouse *Sicista betulina* (Dipodidae) are sister species to the exclusion of the springhare *Pedetes capensis* (Pedetidae), thus resolving an enduring trifurcation in rodent higher-level systematics. Our results together with published data on the Sciuridae allow the formulation of a putative rodent ancestral karyotype (2n=50) that is thought to comprise the following 26 human chromosomal segments and/or segmental associations: HSA1pq, 1q/10p, 2pq, 2q, 3a, 3b/19p, 3c/21, 4b, 5, 6, 7a, 7b/16p, 8p/4a/8p, 8q, 9/11, 10q, 12a/22a, 12b/22b, 13, 14/15, 16q/19q, 17, 18, 20, X and Y. These findings provide insights into the likely composition of the ancestral rodent karyotype and an improved understanding of placental genome evolution.

Electronic supplementary material
The online version of this article (doi:10.1007/s10577-007-1191-5) contains supplementary material, which is available to authorized users.

Introduction

Rodentia, the largest order in mammals encompassing at least 40% of mammalian species diversity (reviewed in Wilson & Reeder 2005), includes index species such as mouse (*Mus domesticus*) and rat (*Rattus norvegicus*) which are widely used for fundamental and applied research in medicine and genetics. The order is also noteworthy for the inclusion of several species that are agricultural pests or vectors of human pathogens. Although whole-genome sequences are available for both mouse and rat (Mouse Genome Sequencing Consortium 2002, Rat Genome Sequencing Project Consortium 2004), phylogenetic relationships among the major lineages of rodents remain equivocal, a situation that is compounded by the fact that comparative genomic data are lacking for the great majority of species.

Rodentia is considered to comprise five monophyletic suborders: (i) Ctenohystrica (gundis, porcupines, mole-rats, guinea-pigs, chinchillas, tuco-tucos and capybaras, among others; Huchon *et al*. 2002); (ii) Sciuromorpha (Gliridae=dormice+Aplodontidae—with a single species the mountain beaver—and Sciuridae=squirrels); (iii) Myomorpha (Muridae=mice, rats and allies and Dipodidae=gerboas); (iv) Anomaluromorpha (Anomaluridae=scaly-tailed squirrels and Pedetidae=springhares); and (v) Castorimorpha (Castoridae=beavers, Heteromyidae=pocket and kangaroo mice and Geomyidae=pocket gophers; see Murphy *et al*. 2001a, Huchon *et al*. 2002, Adkins *et al*. 2003, DeBry 2003; Carleton & Musser 2006). Given issues of saturation, non-independent substitution and the functional constraints that are often associated with sequence data, the use of alternative phylogenomic characters to resolve problematic nodes has gained momentum. One category of so-called rare genomic change (RGC, Rokas & Holland 2000) entails the modification of chromosomal architecture. These structural changes can alter ancestral syntenic associations that can be detected using multispecies genome map alignments based on complete genome sequences and radiation hybrid maps (Murphy *et al*. 2004 and references therein), as well as by cross-species chromosome painting (Zoo-FISH Scherthan *et al*. 1994, reviewed in Rens *et al*. 2006 and references therein). The number of rodent species examined by any of these approaches is limited but particularly so in the case of chromosome painting. The main reason is that the rodent index species *M. domesticus* and *R. norvegicus* possess highly rearranged genomes making homologies hard to assess by Zoo-FISH. Recently the use of human whole-chromosome painting probes to detect regions of homology with squirrels (Richard *et al*. 2003a, Stanyon *et al*. 2003, Li *et al*. 2004, 2006) has demonstrated that non-murid rodents display more conserved genomes, underscoring earlier conclusions based on comparative banding analysis (Petit *et al*. 1984; Viegas-Pequignot *et al*. 1986). These findings suggest that the use of non-murids for higher-order systematic and comparative genomic studies will be fruitful, but at this point the extent of the retention of a 'conserved genome' in other rodent lineages is unknown.

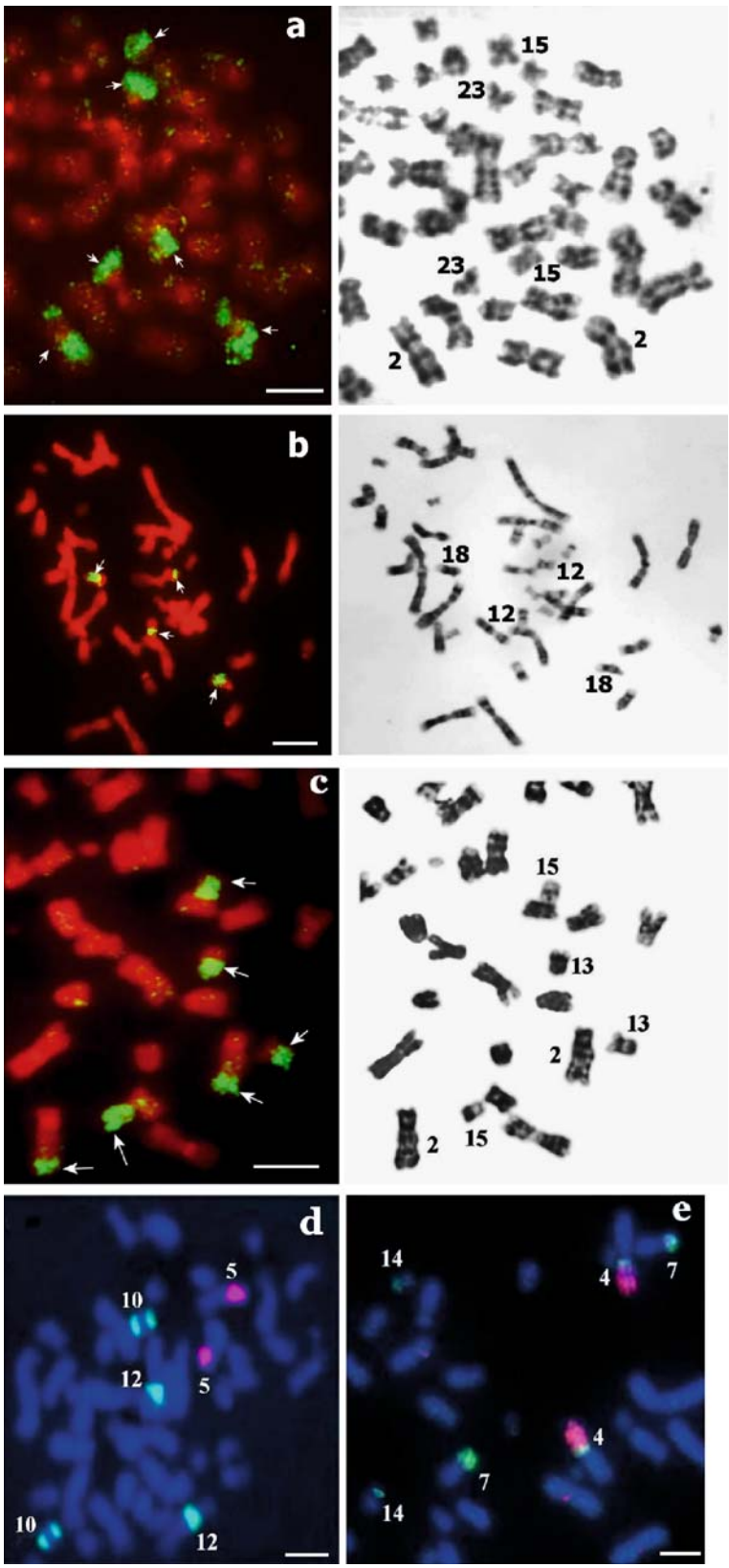
In an attempt at redress we have performed cross-species painting experiments using human chromosome painting probes on representatives of three main non-murid lineages represented by Pedetidae, Castoridae, and Dipodidae. This allows a direct comparison with previously published data on Sciuridae and expands the taxonomic coverage to include representatives of four of the five major evolutionary lineages recognized in Rodentia. Our aims were twofold: first to clarify phylogenetic relationships among Sciuridae, Pedetidae, Castoridae and Dipodidae (i.e., the Rodentia suborders) and, secondly, to provide insights into the putative ancestral karyotype of Rodentia, thus facilitating comparative genomic studies among placentals.

Materials and methods

Metaphase preparations, G-banding and chromosome identification

Fibroblast cell lines were established from skin biopsies from one male European beaver, *Castor*

Figure 1. Examples of fluorescence *in situ* hybridization. **(a)** Localization of HSA3 probe on three pairs of *Castor fiber* chromosomes. **(b)** Localization of HSA11 probe on two pairs of *Pedetes capensis* chromosomes. **(c)** Localization of HSA1 probe on three pairs of *Sicista betulina* chromosomes. Images on the right of panels (a–c) show the G-banded metaphases, while those on the left show the same cells after FISH. Arrows indicate the sites of hybridization; chromosome numbers correspond to those in Figures 2–4. **(d, e)** Localization of HSA14 (red) and HSA15 (green) on *C. fiber* and *P. capensis* chromosomes respectively; chromosome numbers corresponding to those in Figures 2 and 3.


a
15
23
23
15
2
2
b
18
12
12
18
c
15
13
13
2
2
15
d
5
10
12
5
10
12
e
14
4
7
4
7
14

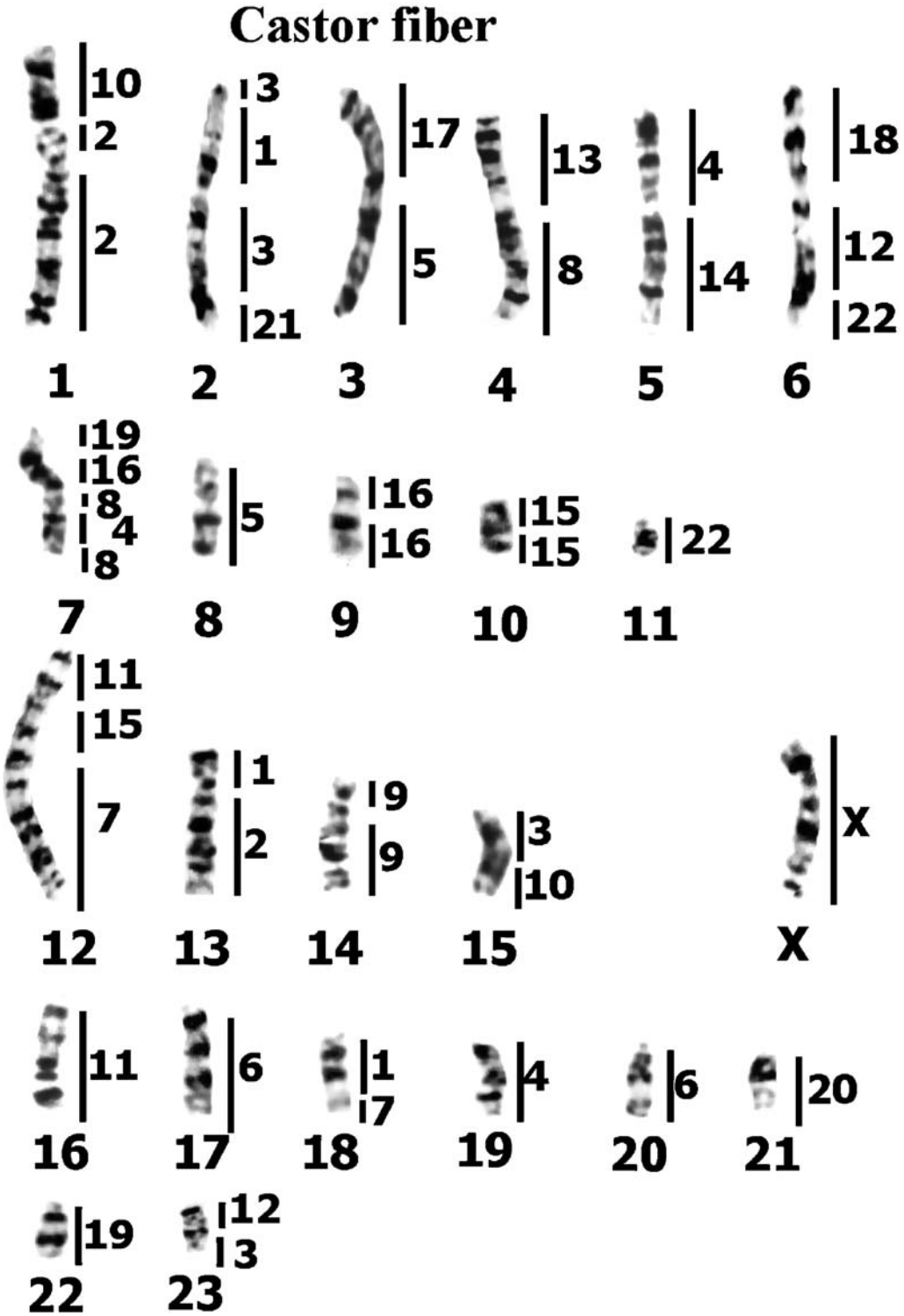


Figure 2. The haploid G-banded karyotype of the European beaver (*Castor fiber*, CFI) showing genome-wide chromosomal correspondence with human (HSA). Regions of conserved synteny are shown to the right of each beaver chromosome.

fiber (Castoridae), one male birch mouse, *Sicista betulina* (Dipodidae), and one female springhare, *Pedetes capensis* (Pedetidae). Cell lines for *Sicista* and *Castor* are available at the Institute of Cytology and Genetics, SB RAS, Novosibirsk, and for *Pedetes* at the University of Stellenbosch. Cell culture, metaphase preparations and G-banding were carried out following conventional methods.

The nomenclature for the European beaver and springhare chromosomes follows the Atlas of Mammalian Karyotypes (Biltueva *et al*. 2006; O'Brien *et al*. 2006, selected lists: Graphodatsky 2006). The chromosomes of the birch mouse were arranged according to relative chromosomal size. The chromosomes of human, mouse, rat and rabbit conformed to the standard nomenclature for these species (see the Atlas of Mammalian Chromosomes).

Zoo-FISH using human chromosome-specific paint probes

Human chromosome-specific painting probes were made by degenerate oligonucleotide primed PCR (DOP-PCR) amplification of flow-sorted chromosomes (Telenius *et al*. 1992) and these were used to delimit homologous chromosomes or chromosomal segments in the genomes of the European beaver, birch mouse and springhare following Yang *et al*. (1997, 1999).

Phylogenetic analysis

Human/rodent comparative genomic maps were established on the basis of our chromosome painting results (see Figures 1–4). The comparable data on the squirrels *Menetes berdmorei*, *Petaurista albiventer* and *Sciurus carolinensis* (Rodentia, Sciuridae) were obtained from Richard *et al*. (2003a) and Li *et al*. (2004), and those for the rabbit, *Oryctolagus cuniculus*, from Korstanje *et al*. (1999) and Chantry-Darmon *et al*. (2005).

Binary cytogenomic characters were coded in accordance with cladistic principles and methods following Dobigny *et al*. (2004) in which chromosome changes were used as characters and their 'presence/absence' as character states (Supplementary Table S1). The rabbit (Lagomorpha) was used as outgroup. Note that no *a priori* polarization (e.g., segmental association resulting from fusion vs. synteny breakage resulting from fission) was assumed and that character state changes were all strictly inferred through outgroup comparisons thus allowing *a posteriori* reconstruction of chromosome evolution. In addition, the inversion of a block corresponding to two associated HSA segments (e.g., inv(1/22) in *Sicista betulina*) was coded as '?' for species in which the segmental associations was not observed (e.g., HSA1/22 in *C. fiber*). Although we are aware that unidirectional painting does not always allow a complete assessment of primary homology (even when supplemented with good G-bands), the chromosomal repatterning in the species studied here was fortunately limited making it possible to determine that most of HSA segmental associations identified were unique. Phylogenetic reconstruction relied upon maximum parsimony exhaustive searches (ACCTRAN option) using PAUP v.4.0b (Swofford 1998). The robustness of the most parsimonious topologies retrieved was explored through consistency index (CI) and retention index (RI), as well as by bootstrap support (10 000 replicates).

Results

The complete set of human chromosomal probes (excluding the Y) was successfully hybridized to G-banded metaphase chromosome spreads of the three rodent species examined herein. Examples of fluorescence *in situ* hybridization are shown in Figure 1 and in Supplementary Figures S1, S2 and S3.

Castor fiber (CFI) has a 2n=48 karyotype with NFa=66. The human painting probes (HSA 1–22+X) each mapped to one, two or three CFI chromosomes respectively (Figure 2 and Table 1), and delineated a total of 43 homologous segments among these species. Twelve CFI chromosomes were homologous to the following human segmental associations: HSA2/10, 3/1/3/21, 17/5, 13/8, 4/14, 18/12/22, 19/16/8/4/8, 11/15/7, 1/2, 3/10, 1/7 and 12/3. The 12 remaining CFI chromosomes each corresponded to either one single complete (HSA9, 20 and X) or partial human chromosome (HSA4, 5, 6, 15, 16, 19, 20 and 22) (Figure 2). In addition, most of the *Castor* autosomes display large pericentromeric C-positive blocks (Ward *et al*. 1991) which remained unpainted by the human painting probes (e.g., see HSA15 on CFI10 on Figure 1). This observation is not

Table 1. Chromosomal correspondence between human and Glires (Lagomorpha+Rodentia). Correspondence between human, rat and mice chromosomes are based on the mapping data of Murphy *et al*. (2005). The revised standardization of the rabbit karyotype have been used (Hayes *et al*. 2002). We have included only one representative of the conservative Sciuridae, the chipmunk (Li *et al*. 2004)

Human 2n=46	Rabbit 2n=44	Chipmunk 2n=38	Beaver 2n=48	Springhare 2n=38	Birch mouse 2n=32	Mouse 2n=40	Rat 2n=42
1	13, 16	3, 8	2, 13, 18	2, 15, 16	2,13,15,15	1,3,3,4,5,8,13	2,2,5,13,14,17,19
2	2, 7	4, 13	1, 13	1, 5	3,4,7	1,1,2,5,6,11,12,17	3,4,6,9,13,14
3	9, 14	7, 14, 17	2,2,15, 23	2,2, 7, 11, 12	4,5,9,9,11	3,3,6,9,16	2,2,4,8,11,11
4	2, 15	2, 11	5, 7, 19	5, 7, 13	1,2,5	3,3,3,5,6,8,8	2,2,2,4,14,16,19
5	3, 11	5	3, 8	1, 15	3,4,6,	11,13,15,18	2,10,17,18
6	12	6	17, 20	3,14	3,9,13	1,4,9,10,13,17,17	1,5,8,9,17,20,20
7	6, 7, 10	12, 18	12, 18	4, 16	2,11	5,5,6,13	4,12,17
8	2, 3	2,2, 8	4, 7,7	7,7, 8	5,11	1,3,4,8,14,15	2,5,7,15,16
9	1	1	14	17	1	2,4	3,5
10	16, 18	3, 10	1, 15	9,10	1,1,3,11	6,7,14,19	1,1,4,16,
11	1	1	12, 16	12, 18	1,8,8	2,7,7,9,9,19	1,1,1,3,8,8
12	4, 8, 21	2, 12	6, 23	3,6	2,5,10	5,6,10,15	4,7,7,7,12
13	8	10	4	6	1,9	3,5,8,14,14	2,12,15,15,16
14	17, 20	9	5	4	6,10	12,14	6,15
15	17,17	9	10, 12	4,7,14	3,8,11	2,7,9	1,3,8
16	5, 6	16, 18	7, 9	9, 13	8,12	7,8,16,17	1,10,19,19,19
17	19	4	3	3	3,4	11	10
18	9	15	6	10	7,14	1,17,18,18	9,13,18,18
19	5, 10	16, 17	7, 22	8,11	2,3,8,9,10	7,8,8,8,9,10	1,7,8,12,16,19
20	4	9	21	1	3	2	3
21	14	7	2	2,2	4,6	16,17	11,20
22	4, 21	2, 12	6, 11	3,6	2,12,14,15	8,11,15	7,14,19
X	X	X	X	X	X	X	X
Total number of segments	39	36	43	46	62	96	95

surprising since constitutive heterochromatin usually comprises highly repetitive DNA sequences that are known to undergo high rates of sequence evolution and consequently escape detection by cross-species FISH. The disruption of a chromosome-specific, euchromatic signal by intervening C-bands was considered as a single syntenic block in *C. fiber*, as well as in the other species included in our study.

Pedetes capensis (PCA) has a 2n=38 karyotype with NFa=68. The HSA painting probes hybridized to 46 different regions in the springhare, with each probe showing homology to one, two, three or four PCA chromosomes and/or chromosomal fragments respectively (Figure 3 and Table 1). Sixteen HSA segmental associations each corresponding to a single springhare chromosome were detected: HSA2/20/5, 1/3/21/3/21, 17/12/22/6, 14/15/7, 2/4, 13/12/22, 8/4/8/3/15, 19/8, 16/10, 10/18, 19/3, 11/3, 16/4, 15/6, 1/5 and 7/1. The regions corresponding to HSA9, part of HSA11, and the X were homologous to the entire PCA17, 18 and X respectively (Figure 3).

Finally, the 23 human probes detected 62 homologous regions in *S. betulina* (SBE; 2n=32, NFa=58). All SBE autosomes were homologous to multiple HSA segments: SBE 1=HSA13/4/10/11/9/10, SBE 2=4/22/12/19/7/1, SBE 3=19/10/17/2/5/20/6/15, SBE 4=17/5/2/3/21, SBE 5=8/4/3/12, SBE 6=14/21/5, SBE 7=18/2, SBE 8=19/16/11/15/11, SBE 9=3/6/3/13/19, SBE 10=14/19/12, SBE 11=3/10/7/8/15, SBE 12=22/16, SBE 13=6/1, SBE 14=1/22/1, and SBE 15=22/18 (Figure 4).

A total of 81 binary characters could be identified on the basis of these comparative maps as well as those available for the rabbit (Korstanje *et al*. 1999, Hayes *et al*. 2002) and three squirrel species (Richard *et al*. 2003a, Li *et al*. 2004) (Supplementary Table S1). Among these, 74 correspond to associations of HSA segments that most probably arose due to fusion/fission events (for example 'HSA2/10', see

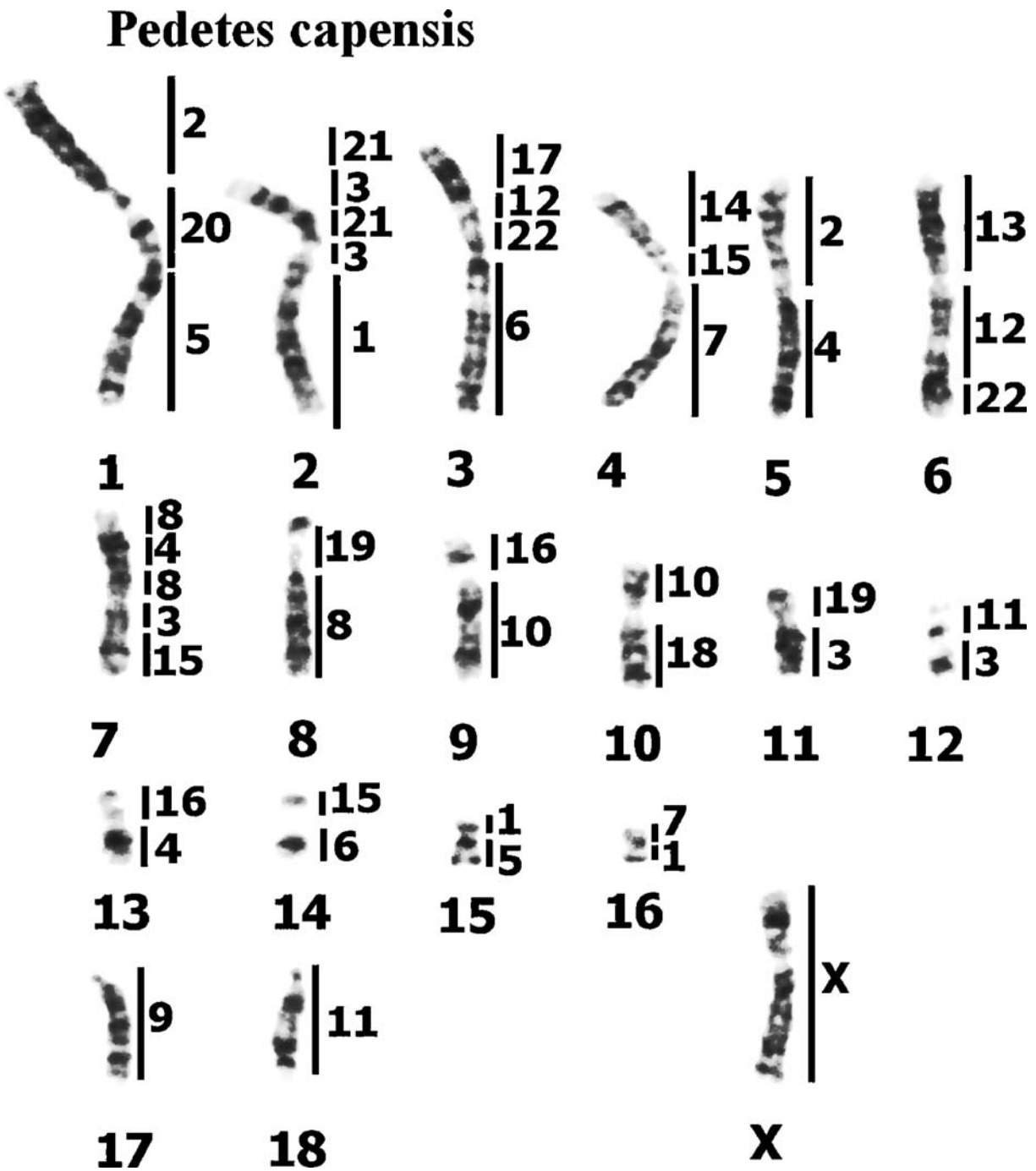


Figure 3. The haploid G-banded karyotype of the springhare (*Pedetes capensis*, PCA) showing genome-wide chromosomal correspondence with human (HSA). Regions of conserved synteny are shown to the right of each springhare chromosome.

Supplementary Table S1). The remaining seven binary characters were interpreted as probable inversions (noted as 'inv' in the table). We are aware that other inversions within segments have most probably remained undetected (a limitation of FISH with whole-chromosome painting probes) thus escaping

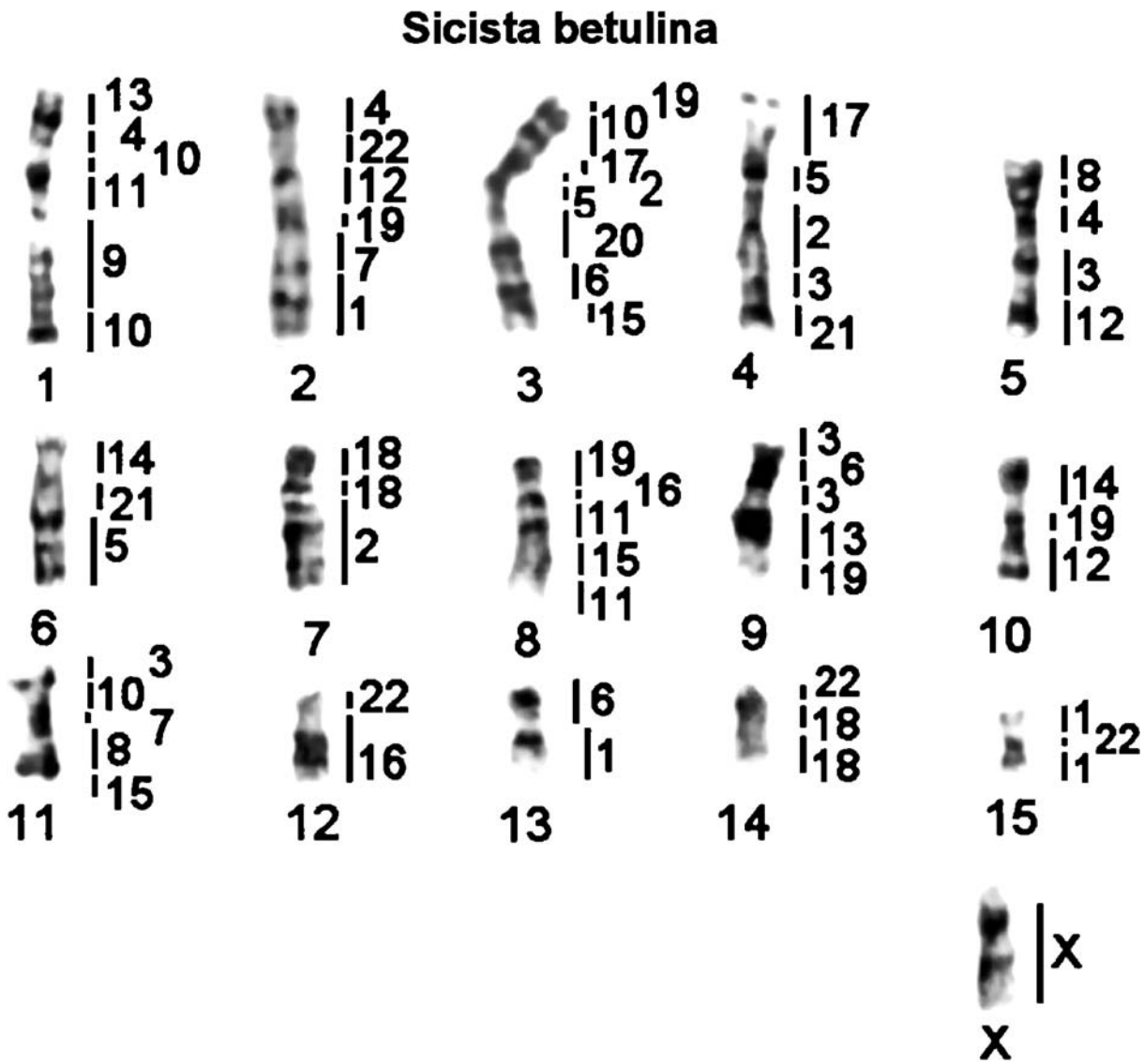


Figure 4. The haploid G-banded karyotype of the birch mouse (*Sicista betulina*, SBE) showing genome-wide chromosomal correspondence with human (HSA). Regions of conserved synteny are shown to the right of each birch mouse chromosome.

our inventory of potentially informative characters. Nevertheless, and in the absence of independent data (such as reciprocal painting, BAC mapping, or interpretable comparisons of G-banded patterns), we chose to include only those inversions that had two overlapping HSA segments as revealed by Zoo-FISH (e.g., HSA1/22/1 corresponding to SBE15 on Figure 4, and coded as 'inv(1/22)' in Supplementary Table S1).

Two equally most parsimonious trees (L=91, CI=0.87, RI=0.67) were obtained (see the strict consensus of these in Figure 5). Within the ingroup, 49 autapomorphic, 24 homoplasic and 13 phylogenetically informative character changes were retrieved. The topology shows a basal position for sciurids (here represented by *Petaurista*, *Sciurus* and *Menetes*), and a close relationship between Castoridae and Dipodidae with Pedetidae as their sister lineage. Nodes were moderately supported by bootstrap values (BP=58–98), a result that is not so surprising given the generally low number of informative character changes inherent in

cytogenomic analyses (see Dobigny *et al*. 2004). More convincingly, each node was strongly supported by 3 to 6 unambiguous, non-homoplasic genome repatterning events (i.e., rare genomic changes). The unambiguous and *a posteriori* polarized chromosomal changes were mapped directly to the consensus topology (Figure 5), showing that at least five inversions, 66 segmental associations and eight synteny disruption events have been fixed during the course of evolution of the ingroup taxa. Importantly the *Pedetes*+*Castor*+*Sicista* clade was supported by one segmental association (HSA1/7) and two synteny disruptions (HSA1/10 and HSA7/16); the *Castor*+*Sicista* clade was characterized by four segmental associations (HSA3/10, 3/12, 5/17 and 11/15) and one synteny disruption (HSA14/15). The resulting topology was consistent with that presented herein, although the branching of the *Pedetes* lineage could not be solved. The fall-off in resolution probably reflects incorrect assessments of primary homologies in the absence of reciprocal painting data (see Materials and Methods). Importantly, however, the *Castor*+*Sicista* association was retrieved.

Discussion

Higher-level systematics in Rodentia

Rodents represent the most speciose mammalian order and resolution of their taxonomy and systematic relationships has greatly benefited from the increasing number of molecular studies involving large concatenations of DNA sequences. However several nodes remain problematic, most probably as a result of rapid cladogenesis. For example, the diversification of rodent suborders occurred in a relatively narrow window between 85 and 95 Mya (see Table 3 in Adkins *et al*. 2003), thus limiting the usefulness of sequence-based characters for detecting significant phylogenetic support. Chromosomal

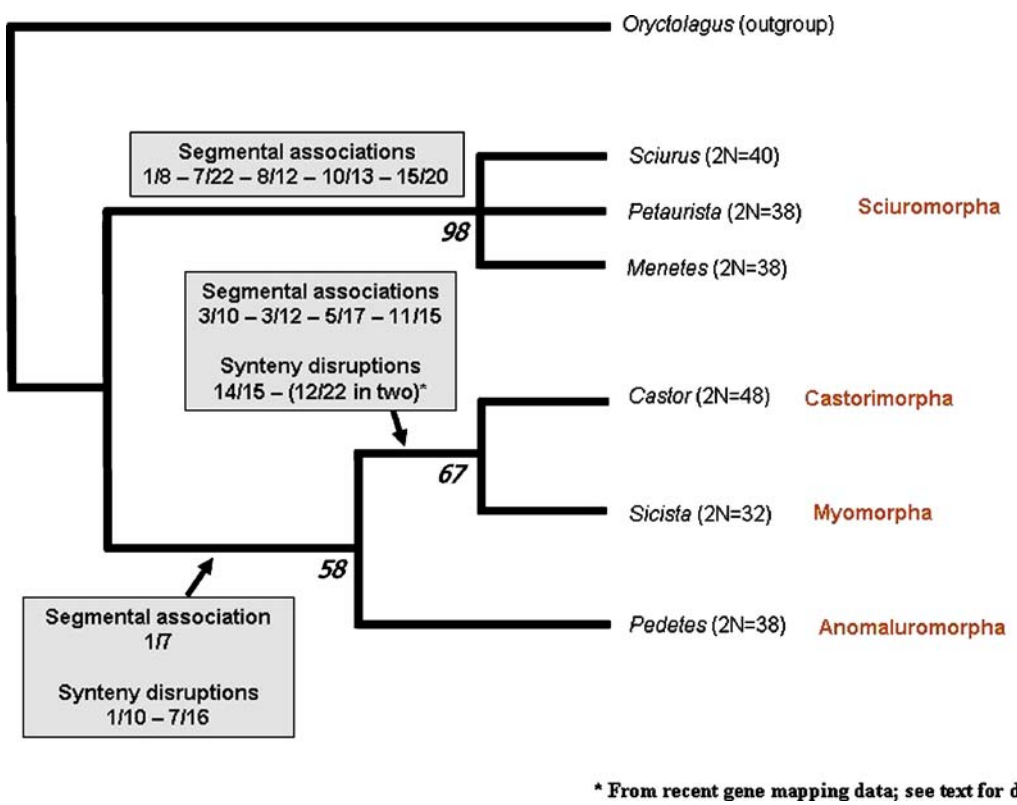


Figure 5. The strict consensus of the two most parsimonious trees retrieved from cladistic analysis of chromosomal characters identified in our study. Bootstrap values are indicated in italics.

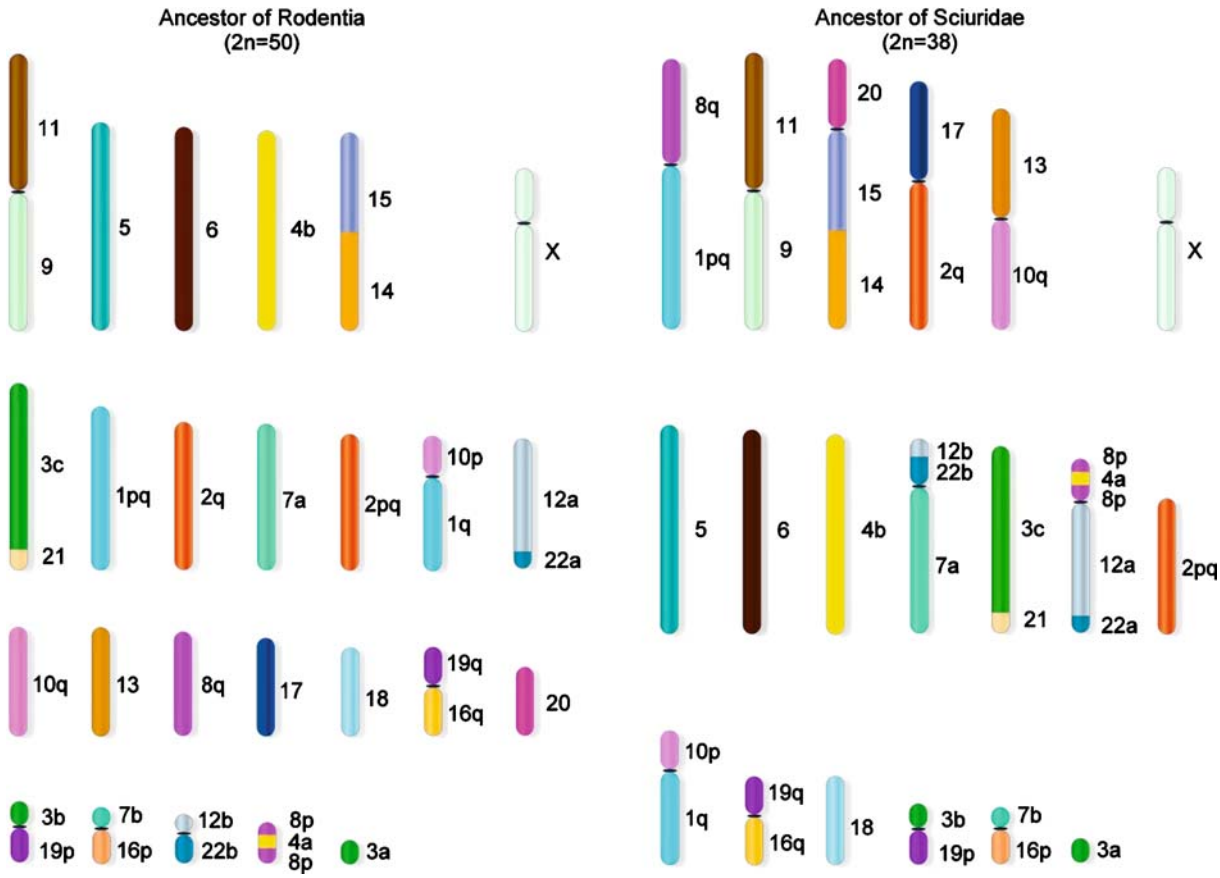


Figure 6. Ancestral karyotypes of the Rodentia (left) and the Sciuridae (right) reconstructed from cross-species chromosome painting results from representatives of four (out of five) main rodent lineages from this and previously published studies (Richard *et al*. 2003a, Stanyon *et al*. 2003, Li *et al*. 2004, 2006). Each ancestral chromosome is numbered according to its homologous segments in the human genome. The homologies of individual conserved segments to human chromosomes are identified by 23 different colours and numbered to the right of each schematic.

changes, on the other hand, may provide phylogenetic resolution since they are sometimes involved in the speciation process itself (reviewed in King 1993), and may be fixed within a very short time (e.g. Britton-Davidian *et al*. 2000, Wang & Lan 2000, Dobigny *et al*. 2005, Aniskin *et al*. 2006). Consequently they can provide signatures of events that occurred within a brief evolutionary period, our findings here being a case in point.

First, our analysis retrieved one segmental association (HSA1/7) and two synteny disruptions (HSA1/10 and 7/16) that provide good support for the recognition of a Castoridae+Pedetidae+Dipodidae clade. Secondly, despite the use of various nuclear genes and long stretches of sequences (3600–9800 bp), no consensus has yet emerged on the evolutionary relationships of the three lineages within this clade. In fact all possible combinations have been proposed, often with only moderate to weak support for each hypothesis (Huchon *et al*. 2002, Adkins *et al*. 2003, DeBry 2003): Pedetidae+Castoridae (DeBry 2003), Pedetidae+Dipodidae (ML analysis of GHR gene, as well as MP analysis of *BRCA1* gene, in Adkins *et al*. 2003; see also the Muridae+Pedetidae association in Murphy *et al*. 2001a), and Castoridae+Dipodidae (ML analysis of *BRCA1* gene, in Adkins *et al*. 2003). In this context, our findings are clearly of significance. We have identified four segmental associations (HSA3/10, 3/12, 5/17 and 11/15) and one synteny disruption (HSA14/15) as synapomorphies for Castoridae+Dipodidae. Furthermore, our painting results show that the HSA12/22 segmental association is present as a single fragment in the *C. fiber* and *S. betulina*

genomes, whereas it is present as two fragments in *P. capensis* and the squirrels and is consequently homoplasic in our cladistic analysis (see Table 1). Importantly, gene mapping data (Chantry-Darmon *et al*. 2005) show two fragments of HSA12/22 in the rabbit (our outgroup species), suggesting that the fused state is in fact a derived condition and therefore an additional valid synapomorphy for *Castor*+*Sicista*. Consequently, although some characters were found to be homoplasic within Castoridae, Pedetidae and Dipodidae (four shared by *Castor* and *Pedetes*, two by *Sicista* and *Pedetes*; data not shown, but see our matrix in Supplementary Table S1), our phylogenomic survey of the main rodent lineages clearly shows that Castorimorpha (represented by *Castor*) and Myomorpha (represented by *Sicista*) are closer to each other than they are to Anomaluromorpha (represented by *Pedetes*).

Genome architecture and cytogenetic signatures in placentals

Chromosomal similarities detected among distantly related mammalian species using banding led to early attempts at defining hypothetical ancestral karyotypes for Primates and Carnivora (Dutrillaux & Couturier 1983, Couturier & Dutrillaux 1986). Moreover, Dutrillaux *et al*. (1980), Petit *et al*. (1984) and Viegas-Pequignot *et al*. (1986) used R-banding patterns to compare the karyotypes of primates, carnivores and sciurids, as well as human and rabbit. They found a high degree of chromosomal homology between selected chromosomes of these species, thereby providing a platform for the more detailed molecular dissection of karyotypes (including those that are significantly rearranged) through Zoo-FISH. In fact, a substantial number of segmental associations or synteny disruptions of human chromosomes have subsequently been proposed as cytogenetic signatures that underpin the recognition of different placental orders (Volleth *et al*. 2002, Richard *et al*. 2003b, Yang *et al*. 2003, 2006, Murphy *et al*. 2004, Robinson *et al*. 2004, reviewed in Froenicke 2005) including Rodentia, as evidenced in the present investigation.

We and others (Richard *et al*. 2003a, Stanyon *et al*. 2003, Li *et al*. 2004) have identified several human syntenies (HSA8/4/8/12/22, 20/15/14, 7/22/12, 2/17, 10/13, 1/8, 3/19) and possibly the disruption of HSA3 into three independent fragments as potential signatures for Sciuridae. Most of these are consistent with our human vs. *Castor*, *Sicista* and *Pedetes* hybridization results, the exceptions being HSA2/17 (present in *Sicista*) and the HSA3 disruption (into three fragments, though inverted, in *Sicista* and *Castor*). The former character, namely HSA2/17, could thus be an ancestral feature of rodents rather than an autapomorphy for Sciuridae and suggests a useful line of investigation in the last, as yet unexplored rodent lineage, the Hystricomorpha.

Secondly, HSA8/12 has been proposed as a possible ancestral character in both murid and sciurid rodents. Although convergence of this character cannot be excluded (Murphy *et al*. 2001b), we found no evidence of this segmental association in any of the three rodent lineages investigated here. No definitive conclusion can presently be reached and we must defer to future investigations involving hystricomorph species to resolve this. In the same manner the HSA1/10 synteny has been proposed as a signature for Glires (Rodentia+Lagomorpha) monophyly since it is shared by rabbit, squirrel, mouse and rat (Stanyon *et al*. 2003). However, none of the springhare, the beaver, and the gerboa display this segmental association. Finally, a significant number of eutherian ancestral syntenies and/or chromosomes have been identified (see Yang *et al*. 2006, and references therein), most of which have also been retrieved in squirrels (such as HSA3/21, 4/8, 7/16, 12/22, 14/15, 16/19; Richard *et al*. 2003a, Stanyon *et al*. 2003, Li *et al*. 2004, 2006), the only other rodents investigated with human painting probes. The Zoo-FISH results obtained in our non-sciurid species are in good agreement with these results as we are able to detect HSA3/21, 4/8 and 12/22 in all three species, HSA16/19 in *Sicista* and *Castor* and HSA14/15 in *Pedetes*. In contrast the HSA7/16 synteny was not observed in any of the non-sciurid species analysed here, suggesting its disruption in their common ancestor. Importantly, the association is also absent in the mouse and rat genomes (Murphy *et al*. 2005), strongly supporting our own results. Finally, the hybridization of the chipmunk chromosome 18 (homologous to HSA7/16) to beaver further confirmed the breakage of this conserved synteny in the latter species (see Supplementary Figure S3). Although one would intuitively anticipate that a reversal such as this would be unlikely, requiring as it does the disassociation of a specific breakpoint junction, similar disruptions of some ancestral

syntenic associations, including HSA7/16, have also occurred in hominoid primates (see Wienberg 2005, and references therein).

Recently, Froenicke and colleagues (2006) have proposed a 2n=46 ancestral karyotype for Rodentia that includes the syntenic associations HSA20/15/14, 11/9, 3/21, 8/4/8/12/22, 1/10, 16/19, 3/19, 7/16 and 12/22 based mainly on the published Zoo-FISH results in Sciuridae, as well as on alignments of mouse, rat and human genome sequences. Our results demonstrate the conservation of many of these proposed ancestral syntenic associations in the three other rodent lineages and these findings, together with those from published data, allow the first formulation of a likely ancestral karyotype of Myomorpha, Sciuromorpha, Castorimorpha and Anomaluromorpha. By extension these results provide a more comprehensive glimpse of the rodent ancestral karyotype since only the Hystricomorpha is missing from our dataset. The data suggest a 2n=50 chromosomal complement at the base of Rodentia that is likely to have comprised the following human chromosomal segments and segmental associations: HSA1pq, 1q/10p, 2pq, 2q, 3a, 3b/19p, 3c/21, 4b, 5, 6, 7a, 7b/16p, 8p/4a/8p, 8q, 9/11, 10q, 12a/22a, 12b/22b, 13, 14/15, 16q/19q, 17, 18, 20, X and Y (Figure 6). In contrast, the ancestral Sciuridae had 2n=38 (Stanyon *et al*. 2003, Li *et al*. 2004) consisting of HSA1pq/8q, 1q/10p, 2pq, 2q/17, 3a, 3b/19p, 3c/21, 4b, 5, 6, 7a/22b/ 12b, 7b/16p, 9/11, 10q/13, 8p/4a/8p/12a/22a, 14/15/20, 16q/19q, 18, X and Y (Figure 6). It is noteworthy, however, that of the 10 adjacent syntenies identified in our rodent ancestor (i.e., 11/9, 15/14, 3/21, 10/1, 12/22×2, 19/16, 3/19, 7/16, 4/8) only five are present in the murid ancestor (i.e., 3/21, 3/19, 12/22, 4/8, 19/16, see supplementary material in Murphy *et al*. 2005). Although the Murphy *et al*. (2005) study and the present investigation are focused at different taxonomic levels, the paucity of ancestral syntenies in the Murphy *et al*. (2005) murid ancestor is striking. The most likely explanation for this is that their reconstruction has been biased through the use of the highly rearranged mouse and rat genomes. Given the evidence of ancestral syntenic disruptions referred to above, as well as the differences in methodology in retrieving conserved syntenies (Bourque *et al*. 2006, Froenicke *et al*. 2006 and Robinson *et al*. 2006), the relatively poor correspondence between the two studies is less problematic than it might seem.

In conclusion, our results have led to improved resolution of several important phylogenetic nodes within Rodentia, thereby providing insights to the genomic architecture of the common ancestor of these lineages and the likely composition of the ancestral rodent karyotype. The latter now constitutes a working hypothesis that may serve to stimulate comparative genomic investigations in other rodents and thus aid the search for a more definitive interpretation of the evolution of genome organization in Placentalia.

Acknowledgements

This study was funded in part by the research grants of the Russian Fund for Basic Research, programs of the Russian Academy of Science: MCB, BOE, and Integration program of the Siberian Branch of the Russian Academy of Science (A.S.G.) and a Wellcome Trust grant to M.A.F.-S.

References

Adkins RM, Walton AH, Honeycutt RL (2003) Higher-level systematics of rodents and divergence time estimates based on two congruent nuclear genes. *Mol Phylogenet Evol* **26**: 409–420.

Aniskin VM, Benazzou T, Biltueva L, Dobigny G, Granjon L, Volobouev V (2006) Unusually extensive karyotype reorganization in four congeneric *Gerbillus* species (Muridae: Gerbillinae). *Cytogenet Genome Res* **112**: 131–140.

Biltueva LS, Graphodatsky AS, Robinson TJ (2006) *Pedetes capensis*. In: O'Brien SJ, Nash WG, Menninger JC, eds. *Atlas of Mammalian Karyotypes*. New Jersey: Wiley, p. 310.

Britton-Davidian J, Catalan J, da Graca Ramalhinho M *et al*. (2000) Rapid chromosomal evolution in island mice. *Nature* **403**: 158.

Bourque G, Tesler G, Pevzner PA (2006) The convergence of cytogenetics and rearrangement-based models for ancestral genome reconstruction. *Genome Res* **16**: 311–313.

Carleton MD, Musser GG (2006) Order Rodentia. In: Wilson DE, Reeder DA, ed. *Mammal Species of the World: A Taxonomic and Geographic Reference*, 3rd edn. Baltimore, MD: The Johns Hopkins University Press, pp. 745–1600.

Chantry-Darmon C, Bertaud M, Urien C *et al*. (2005) Expanded comparative mapping between man and rabbit and detection of a new conserved segment between HSA22 and OCU4. *Cytogenet Genome Res* **111**: 134–139.

Couturier J, Dutrillaux B (1986). Chromosomal evolution in Carnivora. *Mammalia* **50**: 124–163.

DeBry RW (2003) Identifying conflicting signal in a multigene analysis reveals a highly resolved tree: the phylogeny of Rodentia (Mammalia). *Syst Biol* **52**: 604–617.

Dobigny G, Ducroz JF, Robinson TJ, Volobouev V (2004) Cytogenetics and cladistics. *Syst Biol* **53**: 470–484.

Dobigny G, Aniskin V, Granjon L, Cornette R, Volobouev V (2005) Recent radiation in West African Taterillus (Rodentia, Gerbillinae): the concerted role of chromosome and climatic changes. *Heredity* **95**: 358–368.

Dutrillaux B, Couturier J (1983) The ancestral karyotype of Carnivora: comparison with that of platyrrhine monkeys. *Cytogenet Cell Genet* **35**: 200–208.

Dutrillaux B, Viegas-Pequignot E, Couturier J (1980) Tres grande analogie de marquage chromosomique entre le lapin (*Oryctolagus cunuculus*) et les promates dont l'homme. *Ann Genet* **23**: 22–25.

Froenicke L (2005) Origins of primate chromosomes—as delineated by Zoo-FISH and alignments of human and mouse draft genome sequences. *Cytogenet Genome Res* **108**: 122–138.

Froenicke L, Caldes MG, Graphodatsky A *et al*. (2006) Are molecular cytogenetics and bioinformatics suggesting contradictory models of ancestral mammalian genomes? *Genome Res* **16**: 306–310.

Graphodatsky AS (2006) *Castor fiber*. In: O'Brien SJ, Nash WG, Menninger JC, eds. *Atlas of Mammalian Karyotypes*. New Jersey: Wiley, p. 182.

Hayes H, Rogel-Gaillard C, Zijlstra C *et al*. (2002) Establishment of an R-banded rabbit karyotype nomenclature by FISH localization of 23 chromosme-specific genes on both G- and R-banded chromosomes. *Cytogenet Genome Res* **98**: 199–205.

Huchon D, Madsen O, Sibbald MJJB *et al*. (2002) Rodent phylogeny and a timescale for the evolution of Glires: evidence from an extensive taxon sampling using three nuclear genes. *Mol Biol Evol* **19**: 1053–1065.

King M (1993) *Chromosomal Speciation Revisited (Again). Species Evolution. The Role of Chromosome Change*. Cambridge, UK: Cambridge University Press.

Korstanje R, O'Brien PC, Yang F *et al*. (1999) Complete homology maps of the rabbit (*Oryctolagus cuniculus*) and human by reciprocal chromosome painting. *Cytogenet Cell Genet* **86**: 317–322.

Li T, O'Brien PC, Biltueva L, Fu B *et al*. (2004) Evolution of genome organizations of squirrels (Sciuridae) revealed by cross-species chromosome painting. *Chromosome Res* **12**: 317–333.

Li T, Wang J, Su W, Nie W, Yang F (2006) Karyotypic evolution of the family Sciuridae: inferences from the genome organizations of ground squirrels. *Cytogenet Genome Res* **112**: 270–276.

Mouse Genome Sequencing Consortium (2002) Initial sequencing and comparative analysis of the mouse genome. *Nature* **420**: 520–562.

Murphy WJ, Eizirik E, Johnson WE, Ya PZ, Ryder OA, O'Brien SJ (2001a) Molecular phylogenetics and the origins of placental mammals. *Nature* **409**: 614–618.

Murphy WJ, Stanyon R, O'Brien SJ (2001b) Evolution of mammalian genome organization inferred from comparative gene mapping. *Genome Biol* **2**: 0005.1-0005.8.

Murphy WJ, Pevzner PA, O'Brien SJ (2004) Mammalian phylogenomics comes of age. *Trends Genet* **20**: 631–639.

Murphy WJ, Larkin DM, Everts-van der Wind A *et al*. (2005) Dynamics of mammalian chromosome evolution inferred from multispecies comparative maps. *Science* **309**: 613–617.

O'Brien SJ, Menniger JC, Nash WG (2006) *Atlas of Mammalian Chromosomes*. New Jersey: Wiley.

Petit D, Couturier J, Viegas-Pequignot E, Lombard M, Dutrillaux B (1984) Great degree of homoeology between the ancestral karyotype of squirrels (Rodentia) and that of Primates and Carnivora. *Ann Genet* **27**: 201–212.

Rat Genome Sequencing Project Consortium (2004) Genome sequence of the Brown Norway rat yields insights into mammalian evolution. *Nature* **428**: 493–521.

Rens W, Fu B, O'Brien PCM, Ferguson-Smith MA (2006) Cross-species chromosom painting. *Nat Protoc* **1**: 783–790.

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

Richard F, Lombard M, Dutrillaux B (2003b) Reconstruction of the ancestral karyotype of eutherian mammals. *Chromosome Res* **11**: 605–618.

Robinson TJ, Fu B, Ferguson-Smith MA, Yang F (2004) Cross-species chromosome painting in the golden mole and elephant shrew: support for the mammalian clades Afrotheria and Afroinsectiphillia but not Afroinsectivora. *Proc R Soc Lond B Biol Sci* **271**: 1477–1484.

Robinson TJ, Ruiz-Herrera A, Froenicke L (2006) Dissecting the mammalian genome—new insights into chromosomal evolution. *Trends Genet* **22**: 297–301.

Rokas A, Holland PWH (2000) Rare genomic changes as a tool for phylogenetics. *Trends Ecol Evol* **15**: 454–459.

Scherthan H, Cremer T, Arnason U, Weier HU, Lima-de-Faria A, Frönicke L (1994) Comparative chromosome painting discloses homologous segments in distantly related mammals. *Nat Genet* **6**: 342–347.

Stanyon R, Stone G, Garcia M, Froenicke L (2003) Reciprocal chromosome painting shows that squirrels, unlike murid rodents, have a highly conserved genome organization. *Genomics* **82**: 245–249.

Swofford DL (1998) *Phylogenetic Analysis Using Parsimony*, version 4.0. Sunderland, MA: Sinauer Associates.

Telenius H, Pelmear AH, Tunnacliffe A *et al*. (1992) Cytogenetic analysis by chromosome painting using DOP-PCR amplified flow-sorted chromosomes. *Genes Chromosomes Cancer* **4**: 257–263.

Volleth M, Heller KG, Pfeiffer RA, Hameister H (2002) A comparative ZOO-FISH analysis in bats elucidates the phylogenetic relationships between Megachiroptera and five microchiropteran families. *Chromosome Res* **10**: 477–497.

Viegas-Pequignot E, Petit D, Benazzou T *et al*. (1986) Chromosomal evolution in Rodents. *Mammalia* **50**: 164–202.

Ward OG, Graphodatsky AS, Wurster-Hill DH, Eremina (Beklemisheva) VR, Park JP, Yu Q (1991) Cytogenetics of beavers: a case of monobrachial fusions. *Genome* **34**: 324–328.

Wang W, Lan H (2000) Rapid and parallel chromosomal number reductions in muntjac deer inferred from mitochondrial DNA phylogeny. *Mol Biol Evol* **17**: 1326–1333.

Wienberg J (2005) Fluorescence in situ hybridization to chromosomes as a tool to understand human and primate genome evolution. *Cytogenet Genome Res* **108**: 139–160.

Wilson DE, Reeder DM (2005) *Mammal Species of the World: a Taxonomic and Geographic Reference*. Baltimore, MD: The John Hopkins University Press.

Yang F, Müller S, Just R, Ferguson-Smith MA, Wienberg J (1997) Comparative chromosome painting in mammals: human and the Indian muntjac (*Muntiacus muntjak vaginalis*). *Genomics* **39**: 396–401.

Yang F, O'Brien PCM, Milne BS *et al*. (1999) A complete comparative chromosome map for the dog, red fox and human and its integration with canine genetic maps. *Genomics* **62**: 189–202.

Yang F, Alkalaeva EZ, Perelman PL *et al*. (2003) Reciprocal chromosome painting among human, aardvark, and elephant (superorder Afrotheria) reveals the likely eutherian ancestral karyotype. *Proc Natl Acad Sci USA* **100**: 1062–1066.

Yang F, Graphodatsky AS, Li T *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 Res* **14**: 283–296.