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Chromosomal evolution in Rattini (Muridae, Rodentia)

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Abstract The Rattini (Muridae, Murinae) includes the biologically important model species Rattus norvegicus (RNO) and represents a group of rodents that are of clinical, agricultural and epidemiological importance. We present a comparative molecular cytogenetic investigation of ten Rattini species representative of the genera Maxomys, Leopoldamys, Niviventer, Berylmys, Bandicota and Rattus using chromosome banding, cross-species painting (Zoo-fluorescent in situ hybridization or FISH) and BAC-FISH mapping. Our results show that these taxa are characterised by slow to moderate rates of chromosome evolution that contrasts with the extensive chromosome restructuring identified in most other murid rodents, particularly the mouse lineage. This extends to genomic features such as NOR location (for example, NORs on RNO 3 are present on the corresponding chromosomes in all species except Bandicota savilei and Niviventer fulvescens, and the NORs on RNO 10 are conserved in all Rattini with the exception of Rattus). The satellite I DNA family detected and characterised herein appears to be taxon

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(Rattus) specific, and of recent origin (consistent with a feedback model of satellite evolution). BAC-mapping using clones that span regions responsible for the morphological variability exhibited by RNO 1, 12 and 13 (acrocentric/submetacentric) and their orthologues in Rattus species, demonstrated that the differences are most likely due to pericentric inversions as exemplified by data on Rattus tanezumi. Chromosomal characters detected using R. norvegicus and Maxomys surifer whole chromosome painting probes were mapped to a consensus sequence-based phylogenetic tree thus allowing an objective assessment of ancestral states for the reconstruction of the putative Rattini ancestral karyotype. This is thought to have comprised 46 chromosomes that, with the exception of a single pair of metacentric autosomes, were acrocentric in morphology.

Keywords Chromosome painting · karyotype evolution · ancestral karyotype · satellite DNA · BAC-FISH · Rattini

Abbreviations

BAC	Bacterial artificial chromosome
BBE	Berylmys berdmorei
BBO	Berylmys bowersi
BSA	Bandicota savilei
COI	Cytochrome C oxidase I
DAPI	4′, 6-Diamidino-2-phenylindole
dist	Distal
FISH	Fluorescent in situ hybridisation
IHB	Interstitial heterochromatic block
IRBP	Interphotoreceptor retinoid-binding protein
LED	Leopoldamys edwardsi
LINE-1	Long interspersed element-1
MSU	Maxomys surifer
mya	Million years ago
my	Million years
NFU	Niviventer fulvescens
NOR	Nucleolar organizer region
prox	Proximal
RAK	Rattini ancestral karyotype
Rb	Robertsonian
rDNA	Ribosomal DNA
REX	Rattus exulans
RLO	Rattus losea
RNO	Rattus norvegicus
RRA	Rattus rattus
RTA	Rattus tanezumi

Introduction

Rodents are highly valued as model organisms in physiology and biomedical research (Wilson and Reeder 2005). In addition, their role as reservoirs and vectors of human pathogens (see Meerburg et al. 2009 for recent review) has led to increased interest and recognition of the importance of understanding their systematics, ecology and evolution (Jansa and Weksler 2004). This is enhanced by the commensal nature of many species (Wolfe et al. 2007). For example, Rattus rattus, Rattus norvegicus and Rattus exulans colonized along the routes of human migration throughout the Indo-Pacific region, a pattern that has been elegantly illustrated for R. exulans, a species that is often found in close association with human settlements (Matisoo-Smith and Robins 2004). Moreover, many taxa are highly invasive in new areas of colonization—for example Rattus tanezumi in South Africa (Taylor et al. 2008; Bastos et al. 2011).

Rattini, the murid tribe that forms the focus of our investigation, comprises >167 species distributed among 34 genera (Wilson and Reeder 2005; see Table 2 in Lecompte et al. 2008). It harbours the biologically important model species, the Norway rat, R. norvegicus (Aplin et al. 2003), for which a vast amount of data, both genomic and immunogenetic, have been generated (see for example Aitman et al. 2008; Twigger et al. 2008). The other Rattini species have, however, attracted less attention. In particular, comparative studies of their karyotypes remain scarce. Although the relationship between mouse and rat genomes has been extensively investigated by chromosome painting (Grutzner et al. 1999; Guilly et al. 1999; Stanyon et al. 1999; Helou et al. 2001; Nilsson et al. 2001; Cavagna et al. 2002), somewhat surprisingly, no comparisons have been conducted between Rattus species and their allies.

The paucity of chromosomal data for the Rattini highlights a critical need for more extensive systematic research on this important group. The present study provides the first assessment of the mode and tempo of genome organization within these rodents. We present data showing: (1) the distribution of rat-specific centromeric repeats, telomeres and NORs in ten species representative of the genera Maxomys, Leopoldamys, Niviventer, Berylmys, Bandicota and Rattus, (2) the results of multidirectional chromosome painting summarized in comparative chromosome homology maps for these species and (3) the outcome of a bacterial artificial chromosome-fluorescent in situ hybridisation

(BAC-FISH) analysis of the heteromorphic chromosomes RNO 1, 12–13 and their orthologues in R. tanezumi. These chromosomes correspond to pairs 1, 9 and 13 of Yosida and colleagues' Rattus karyotypic descriptions (Yosida et al. 1971a, b; Yosida 1976, 1977). Their variability in Rattus (specifically within R. rattus, R. norvegicus, Rattus losea, R. tanezumi, R. annadalei and R. muelleli) has been attributed to either short-arm heterochromatic amplification or pericentric inversions (Yosida et al. 1971a, b; Yosida and Sagai 1975; Yosida 1976, 1977).

Materials and methods

Tissue samples, cell culture, chromosome preparation, G-bands and Ag-NORs

We studied ten species representative of six of the 34 recognized genera (Table 1). Other than R. norvegicus, which was obtained commercially, all specimens originated from Thailand in the vicinity of the settlements Loei (17°29′N, 101°43′E), Kalasin (16°49′N, 103°53′E) and Phrae (18°09′N, 100°08′E). Importantly, the species-specific status of each specimen was unambiguously assessed using molecular typing (Pagès personal communication) as described in Pagès et al. (2010). This is particularly important in Rattini where some aspects of the taxonomy are still unclear. Our nomenclature follows the most recent revision of the group (Pagès et al. 2010).

Chromosome preparations were made from bone marrow after yeast stimulation (Lee and Elder 1980) or from fibroblast cell-cultures established from intercostal tissue following standard procedures. Chromosomes were identified by G-banding (Seabright 1971). The distribution of NORs was investigated using silver staining (Goodpasture and Bloom 1975; Barch 1997) on previously DAPI-banded slides.

Flow sorting, generation of chromosome-specific painting probes and Zoo-FISH experiments

Chromosome-specific painting probes were generated at the Cambridge Resource Centre for Comparative Genomics, UK, from flow-sorted R. norvegicus (RNO, Stanyon et al. 1999) and Maxomys surifer fibroblasts (MSU, present study) on AT/GC ratio and size. The DNA was amplified using 6-MW primers by degenerate oligonucleotide primed PCR (DOP-PCR; Telenius et al. 1992) and fluorescently labelled with biotin and/or digoxigen-dUTP (Roche). Flow-sorted peaks were assigned by hybridizing each fluorescently labelled flow-sort to DAPI-banded metaphase chromosome spreads of the species of origin. Interspecific Zoo-FISH experiments were conducted in order to establish comparative chromosomal maps between the Rattini species. This involved reciprocal painting between MSU and RNO and unidirectional painting experiments using RNO and MSU paints to detect regions of synteny between these species and R. exulans (REX), R. tanezumi (RTA), R. losea (RLO), Bandicota savilei (BSA), Berylmys berdmorei (BBE), Berylmys bowersi (BBO), Leopoldamys edwardsi (LED) and Niviventer fulvescens (NFU). Hybridization of chromosome paints followed Gilbert et al. (2006) except that chromosome preparations were denatured by incubation in 70% formamide/0.6× SSC solution at 65°C for 30 s–1 min (depending on the probe/target species used) rather than the 30–45 s at 70°C as originally described.

BAC clone selection, preparation and BAC-FISH

BAC clones (Table 2) were selected from the Wellcome Trust Sanger Institute Ensembl contigs (http://www.ensembl.org) and were obtained from the Children's Hospital Oakland-BACPAC Resources, Oakland, CA, USA (http://www.bacpac.chori.org). The BACs were selected to span the pericentromeric regions of chromosomes RNO 1, 12–13 that varied with respect the morphology of the orthologues in R. rattus, R. losea, R. tanezumi, R. annadalei and R. muelleli. The R. tanezumi specimen analysed here was heteromorphic (i.e. possessing an acrocentric and a submetacentric morph) for the three variable orthologous pairs. This allowed the rapid comparison of the two chromosomal morphs (acrocentric vs. submetacentric), and whether the orientation of the BAC clones was altered (i.e. due to an inversion) or retained (i.e. the chromosomal variation is the result of heterochromatic variation and/or centromeric shifts). BAC clones were received as bacterial LB agar stab cultures and were handled according to the supplier's instructions (http://www.bacpac.chori.org/vectorsdet.htm). DNA (~1 µg) was extracted from each BAC clone using Wizard Plus SV Miniprep DNA purification system (Promega) and labelled by standard nick translation with either biotin- or digoxigenin-dUTP (Roche). BAC-FISH followed Gilbert et al. (2006) except that the chromosome preparations together with the probe-mix were denatured on a hot plate at 65°C for 3 min, and hybridization took place

overnight in a humid chamber at 37°C. Post-hybridization washes consisted of a first wash in 0.4× SSC/0.3% Tween 20 for 5 min at 60°C, followed by second wash in 2× SSC/0.1% Tween 20 for 1 min at room temperature. The remainder of the detection protocol was carried out as previously described. Images were captured with a CCD camera coupled to an Olympus BX60 fluorescence microscope and analysed using Genus Imaging Software (Applied Imaging). Signals were assigned to specific chromosomes according to morphology, size and DAPI banding.

Detection of telomeres by fluorescent in situ hybridization

A telomeric probe containing the repeat motif $(TTAGGG)_n$ was constructed and biotin-labelled by PCR as described by Ijdo et al. (1991). This probe was hybridized to metaphase chromosomes of nine species (see Table 1) within Rattini following the protocol described below for satellite DNA (satDNA).

DNA extraction, Satellite I DNA probe isolation and sequencing

Genomic DNA (gDNA) was extracted from tissue samples and/or pelleted fibroblasts of ten species (see Table 1) using the QIAamp DNA Micro kit (Qiagen) following manufacturers' instructions. PCR primers (forward: 5′-TCCCAGTAGCCTGCTCTTGT-3′ and reverse: 5′-TCAGTTCGTTAAAACGTTGCTC -3′) were designed according to the R. norvegicus satellite I DNA (sat I DNA) sequences available in the NCBI database (acc. no: V01570 J00784). PCR amplification was performed using 50 µl reaction mixture that contained 100–300 ng gDNA, 10× buffer, 25 mM $MgCl_2$, 2.5 mM dNTPs, 10 µM of each forward and reverse primer, and 5 U Taq. Cycling parameters entailed an initial denaturing step of 94°C for 3 min followed by 25 cycles at 94°C for 45 s, 55/62°C for 45 s and 72°C for 1 min 30 s. A final extension of 72°C for 10 min completed the programme. PCR products were electrophoresised in 1% agarose gels, excised and purified using the Wizard SV Gel and PCR Clean-up System (Promega) following the manufacturer's recommendations. Cycle-sequencing reactions were performed using BigDye Chemistry and products were analysed on an automated sequencer (AB 3100, Applied Biosystems). Nucleotide sequences were edited and aligned using ClustalW Multiple Alignment in Bioedit v. 7.0.9.0 (Hall 1999). DNA sequences were compared to those in the EMBL and Genbank database using BLASTN searches. Size of the repeats and their substructure was established using Tandem Repeat Finder (TRF) v 4.00 (Benson 1999).

Labelling of the PCR products for FISH was with either digoxigen-11-dUTP (Roche) or biotin-16-dUTP (Roche) using 5 µl buffer (10×), 2 µl $MgCl_2$ (25 mM), 5 µl dACG (2.5 mM), 1.5 µl dUTP-biotin or DIG (1 mM), 5 µl of each forward and reverse primer (10 µM), 1 µl Taq (5 U), 5 µl DNA (PCR product of the first amplification). This was made up to a final volume of 50 µl with dH_2O. The PCR programme selected was the same as for the first round of amplification specified above. FISH of satellite I DNA probes followed Chaves et al. (2002, 2003a, b) with the exception that the probe mixtures were denatured at 80°C for 10 min and cooled on ice rather than the 65°C specified in these publications.

Mapping the chromosome rearrangements onto a consensus molecular tree

A robust consensus phylogenetic tree for intergeneric relationships within Rattini was derived from published molecular studies that utilize different DNA markers including LINE-1 insertion sites (Verneau et al. 1997, 1998), mitochondrial (cytochrome b and CO1) as well as nuclear (IRBP, syndecan-4) genes (Lecompte et al. 2008; Pagès et al. 2010; Badenhorst 2011). These show (1) Maxomys as a basal lineage within Rattini followed by (2) a Niviventer+Leopoldamys clade, then (3) a sister grouping of Berylmys and Bandicota+Rattus (Fig. 1).

Chromosomal differences (characters) inferred from the cross-species chromosome painting results were mapped to this topology. Published data on R. rattus (Cavagna et al. 2002) was included in the analysis. To facilitate the comparisons of banding patterns among taxa only intrachromosomal rearrangements involving segments that were conserved as whole syntenies/ chromosomes in Rattini were included. This allowed us to determine the polarity as well as the mode and tempo of karyotypic evolution in the various Rattini lineages. In terms of the latter, the rate of change was calculated using the ratio between the number of chromosome rearrangements and time of divergence— the latter inferred from molecular analyses that included

representatives of each murine genus investigated in this study (Lecompte et al. 2008; Robins et al. 2008). The mapping of chromosomal characters onto the consensus tree permitted the identification of autapomorphies, synapomorphies, hemiplasies and homoplasies associated with the grouping of various species. Finally, the topology was used to reconstruct ancestral characters at each node thus providing insights to the Rattini ancestral karyotype (RAK).

Results

G-banded karyotypes and Ag-NORs

The G-banded karyotypes of the Rattini species included in this study have previously been described (Badenhorst et al. 2009). Diploid numbers vary from 40 to 52 (i.e. B. bowersi 2n=40, B. berdmorei 2n=41, B. savilei 2n=43, N. fulvescens 2n=46, M. surifer 2n=52) with four of the species (R. exulans, R. tanezumi, R. losea and L. edwardsi) characterised by an invariant 2n=42, a diploid number identical to that of R. norvegicus (Hamta et al. 2006; Badenhorst et al. 2009).

Silver staining detected a variable number of NOR-bearing autosomal chromosome pairs (Table 3 and supplementary material S1). For example, two NOR pairs were identified in B. savilei and eight in M. surifer. N. fulvescens and L. edwardsi both show five NOR-bearing chromosome pairs, and both B. bowersi and B. berdmorei are characterised by four pairs. Rattus exulans, R. losea and R. tanezumi all contained three NOR-bearing chromosome pairs, a finding that is consistent with data on R. norvegicus (Szabo et al. 1978; Kodama et al. 1980; Sasaki et al. 1986; Cavagna et al. 2002; see Table 3 present study). There is strong conservation of NOR location among species. For example, the NORs on RNO 3 are present on the corresponding chromosomes in all species except B. savilei and N. fulvescens, and the NORs on RNO 10 are conserved in all Rattini with the exception of Rattus. The remaining NOR loci are present in a minimum of two species, while those on RNO 5 are autapomorphic in M. surifer. The NOR locus on RNO 11 appears to be characteristic of the Rattus group but, importantly, is absent in R. rattus.

Detection of telomeres by fluorescent in situ hybridization

As expected the telomeric $(TTAGGG)_n$ probe hybridized to the termini of all the chromosomes in the species investigated (supplementary material S2), an observation that is consistent with reports that these structures are crucial for maintaining the stability of chromosomes (Bolzan and Bianchi 2006, among others). In only one instance was a non-terminal TTAGGG signal detected—at the centromeric region of pair 4 of M. surifer (supplementary material S2). There was no evidence to suggest its involvement with any of the chromosomal rearrangements identified in this study (see below). Additionally, interstitial telomeric signals were not detected at the B. savilei X-autosome translocation junction, or at the sites of the head-to-head (Robertsonian, Rb) fusion chromosomes in B. bowersi and B. berdmorei (supplementary material S2).

Satellite I DNA analysis

Sequences were obtained from the amplified PCR products for all species of Rattus (i.e. R. exulans, R. losea and R. tanezumi) as well as for N. fulvescens, B. savilei and B. berdmorei. However, sequencing of the PCR products from M. surifer, L. edwardsi and B. bowersi proved problematic and these species had to be excluded from further sequence analysis. Although clear sequences could be obtained for B. savilei, N. fulvescens and B. berdmorei, no homology could be found between them and sat I DNA of R. norvegicus following BLASTN analysis, and no internal sub- structure was identified using TRF (data not shown). Moreover pair-wise comparisons between these non-Rattus species similarly showed no meaningful homology, and the characteristics of these sequences remain to be elucidated.

There was, however, significant homology between the R. norvegicus sat I DNA sequence available on Genbank and those obtained for R. exulans (97%), R. losea (91%) and R. tanezumi (90%) in the present study (Fig. 2). In addition, a largely conserved 92-bp reiterated subunit was identified in the three Rattus species investigated here through the TRF analysis (supplementary material S3). Pair-wise comparisons between the R. norvegicus 92-bp repeat subunit and R. exulans, R. losea and R. tanezumi 92-bp subunits, indicated 83% nucleotide similarity with R. exulans, 77% similarity with R. losea and 75% sequence similarity with R. tanezumi. Similarly, pair-wise comparisons between R. losea with R. exulans and with R. tanezumi indicated 89% and 86% nucleotide similarity, respectively. Pair-wise comparison between R. exulans and R. tanezumi showed 86% sequence similarity.

Physical mapping of the labelled PCR products generated by the sat I DNA primers was conducted using FISH. The isolated non-Rattus sequences (B. savilei, B. berdmorei and N. fulvescens) resulted in an interspersed FISH pattern in both heterologous (i.e. between species) and autologous (i.e. within species) painting experiments (supplement file S2) emphasizing the lack of co-localization with evolutionary breakpoints identified through Zoo-FISH analysis using RNO and MSU paints. In addition, no clear hybridization signal could be obtained in heterologous painting experiments using Rattus PCR products against the chromosomes of the non-Rattus species (B. savilei, B. bowersi, B. berdmorei, N. fulvescens, L. edwardsi and M. surifer).

In sharp contrast, hybridization patterns of sequences isolated from R. exulans, R. losea and R. tanezumi were invariably centromeric following autologous painting experiments. Similar patterns (centromeric) were identified in heterologous painting schemes using R. losea isolated satellite sequence as representative of the Rattus group against chromosomes of Rattus (supplementary material S2).

FISH with BACs

The order of BAC clones located on the q arm of RNO is unchanged in the acrocentric and submetacentric variants of RTA 1, 12 and 13 (that correspond to RNO 1, 12 and 13) indicating they fall outside of the region responsible for the differing morphologies of these chromosomes in R. tanezumi. Importantly, however, the order of the BAC clones selected for their positions on the submetacentric form of RNO 1, 12 and 13 are inverted in the acrocentric form of the heteromorphic pairs RTA 1, 12 and 13 of R. tanezumi (Fig. 3). For example, the order of the BAC clones on the p arm (from the terminal segment to the proximal segment of the submetacentric RNO 12) is: BAC clone 5 (12p12-Cy3) followed by BAC clone 6 (12p11-FITC), whereas the order is inverted in the R. tanezumi acrocentric morph (Fig. 3a). This pattern clearly confirms that the changes in morphology of these chromosomes are due to pericentric inversions in the three pairs and are not attributable to centromeric shifts, transposition or heterochromatic arm variability in these Rattus species (Fig. 3a–c). In addition, these data permit a correction to the Ensembl database which gives the position of R. norvegicus BAC clone 2 at RNO 1p11 in contradiction to RNO 1q11 as evidenced by our FISH mapping data (Fig. 3d), raising the possibility that the sequenced individual may have had a structural polymorphism.

Flow-sorting and characterisation of painting probes

The M. surifer karyotype (2n = 52, XY) resolved into 20 peaks (Fig. 4). Twelve contained a single chromosome (MSU 1, 2, 4, 7, 14, 17, 19–21, 24, Y) with MSU 4 identified in two separate peaks. This most likely reflects differing amounts of heterochromatin between the two homologs, although this was not readily apparent on C-band analysis. Seven peaks contained a mix of two different chromosomes (MSU X + 4, 5 + 6, 8 + 9, 11 + 13, 10 + 15, 22 + 25 and 3 + 12), and one peak contained a mix of three chromosomes (MSU 16 + 18 + 23). The assignment of one of the seven peaks containing two different chromosomes by FISH was unsuccessful as this peak failed to hybridize. It is assumed that MSU 3 and 12, which were not present in any of the other flow sorts, were present in this peak. We were consequently unable to clarify breakpoints identified by R. norvegicus Zoo-FISH that involved orthologues of MSU 3 and 12 (see below), but we were able to resolve these ambiguities using G-band comparisons.

As detailed by Stanyon et al. (1999), the R. norvegicus (2n = 42) flow karyotype comprised 21 individual peaks of which two contained more than one chromosome—specifically RNO 11 + 15 and RNO 13 + 14 + 15. Importantly, RNO 15 is common to both peaks and therefore two-colour FISH can be used for differentiating between non-pure sorts involving RNO 15 from RNO 11, 13 and 14. The identification of RNO 13 and 14 could not be unambiguously clarified using either M. surifer chromosome paints or dual-colour FISH since their orthologues were contained in non-pure flow sorts. Fortunately, however, both chromosomes are easily distinguished on G-band patterns.

Reciprocal chromosome painting between MSU and RNO

The results of the cross-species chromosome painting of R. norvegicus chromosome paints onto M. surifer chromosomes are shown in Fig. 5. Sixteen R. norvegicus chromosomes (RNO 3, 7–20 and X) are conserved in toto in the M. surifer karyotype. Five R. norvegicus chromosomes each produced two signals (RNO 1, 2, 4, 5 and 6).

The reciprocal analysis of M. surifer whole chromosome paints to R. norvegicus chromosomes confirmed the unidirectional assignments based on

painting R. norvegicus probes to M. surifer (Fig. 5) permitting a more precise delimitation of subchromosomal homologies in M. surifer. Importantly, the M. surifer chromosome paints proved useful for delimiting most breakpoints identified by the R. norvegicus Zoo-FISH analysis. The only exception was the disruption of RNO 2, since MSU 3 is presumed to be present in a peak that failed to hybridize when conducting the flow-sort assignments (see above). Thus, cross-hybridization to delimit breakpoint junctions in RNO 2 was only possible using the M. surifer probe corresponding to MSU 4. It seems reasonable to assume based on G-band comparisons and hybridisation of MSU 4, however, that the breakpoints are identical in all three genera (M. surifer, N. fulvescens and B. savilei), at least at the level of resolution permitted by FISH.

Chromosome painting using MSU and RNO probes onto other Rattini species

Cross-species chromosome painting using RNO and MSU probes was successfully performed on R. exulans, R. losea, R. tanezumi, B. savilei, B. berdmorei, B. bowersi, L. edwardsi and N. fulvescens. The half-karyotype comparisons of G-banded chromosomes of the nine species under investigation in this study compared to that of R. norvegicus is presented in Fig. 6, and examples of Zoo-FISH among the different species using R. norvegicus chromosomes are provided in supplementary material S4. The hybridization results are summarized in Table 4. Briefly, 16 of 21 pairs of chromosomes are conserved in toto in the nine Rattini genomes investigated in the present study (i.e. pairs orthologous to RNO 3, 7–20 and X). However, comparisons of the G-banding patterns showed a number of intrachromosomal misalignments that most probably reflect pericentric inversion differences between the karyotypes investigated in comparison to their R. norvegicus orthologues.

Six interchromosomal rearrangements (two segmental associations, three disruptions and one sex-autosome translocation) were unambiguously identified through Zoo-FISH. These include the segmental associations detected in B. berdmorei and B. bowersi (i.e. syntenic association of RNO 9 and 11), as well as disruptions of R. norvegicus chromosomes in N. fulvescens (i.e. RNO 1 and 2) and B. savilei (i.e. RNO 2). A translocation involving RNO X and 11 was identified demonstrating the presence of a XY1Y2 sex chromosome system in the latter species. This was confirmed by the analysis of a B. savilei male specimen where Y1 represents the original Y, and Y2 represents the unfused autosome (homolog of RNO 11); the other autosomal RNO 11 homolog is fused with the original X (supplementary material S4). All interchromosomal rearrangements were confirmed through the hybridization of M. surifer paints (supplementary material S1), except for the disruption of RNO 2 (previously addressed).

Mapping the chromosomal rearrangements onto a Rattini consensus molecular tree

The mapping of G-banding and Zoo-FISH data to the Rattini consensus tree permitted the identification of ten synapomorphies, seven autapomorphies and three potentially homoplasic characters. Two synapomorphies were retrieved that unite Rattini, namely the segmental association of RNO 7prox + 7dist (RNO 7) and RNO 16prox + 16dist (RNO 16) (Fig. 1). Inversions of RNO 19 and 20 unite the Rattini representatives to the exclusion of M. surifer. Six synapomorphies were retrieved supporting the grouping of Rattus (R. losea; R. tanezumi and R. exulans), B. savilei, B. bowersi and B. berdmorei (inversions of RNO 11 and RNO 14–18). Five autapomorphies were identified in M. surifer (fissions of RNO 4 to 6, inversions of RNO 10 and 16) and a single autapomorphy was present in R. rattus (RRA), Rb 5;7. The remaining autapomorphy involved a sex-autosome translocation in B. savilei comprising the R. norvegicus orthologues RNO X and 11. Close scrutiny of the three potentially homoplasic rearrangements identified by FISH analysis (the fusion of 1p/1q prox + 1q dist, 2prox + 2dist and Rb 9;11) suggests that two are hemiplasic (Avise and Robinson 2008) and one, the Rb 9;11 fusion is a true homoplasy.

Discussion

Cross-species painting using R. norvegicus whole chromosome paints together with reciprocal painting between R. norvegicus and M. surifer and comparisons of the various G-banding patterns facilitated the generation of genome-wide comparative maps among nine Rattini species (Fig. 1). In total, six different murine genera were investigated in this study—Bandicota, Leopoldamys, Rattus, Berylmys, Niviventer and Maxomys. These comparative maps, together with published data (from R. rattus and representatives of Murini, Arvicanthini and

Apodemini), allowed the tracking of chromosomal rear- rangements that have occurred during the their usefulness in discriminating phylogenetic evolution of Rattini. This permitted an assessment of relationships among species and the construction of the putative RAK.

Mode and tempo of chromosomal evolution within Rattini

Karyotypes may differ by many chromosome rearrangements, often evident among species within the same family (for example the Muridae). However, although at least 19 chromosome rearrangements (fusions, fissions and pericentric inversions) have occurred within Rattini, the variation is somewhat muted when one takes each species/lineage separately into account (Fig. 1). Although the rate of chromosomal change is low in most Rattini branches (≤0.6 rearrangements per million years, represented hereafter as R/my), this is far from even. For example, there is evidence of an elevated rate on the branch leading to the common ancestor of Berylmys, Bandicota and Rattus assemblage (node B) that entails six pericentric inversions that were fixed within a short time period (~1.8 my). This rapid rate of change (3.33 my^{-1}) was followed by a period of stasis, the only exceptions being a single fusion shared by B. bowersi and B. berdmorei, the fixation of fission and the origin of a sex/autosome fusion in B. savilei and two fusions which restructured the R. rattus karyotype. As a consequence, the Rattini appear to exhibit a high degree of genomic conservation and, if inversions were excluded, L. edwardsi and Rattus (R. losea; R. tanezumi and R. exulans) have retained largely invariant karyotypes since their last common ancestor ~7 million years ago (mya). This is in sharp contrast to some other murids—for example, Nannomys, Mus, Coelomys and Pyromys (Veyrunes et al. 2006)—emphasizing that chromosomal rearrangements in Rattini do not closely track speciation events.

A more detailed comparison of the slow rate of chromosomal change in Rattini with other rodents is informative. For example, the four subgenera of Mus that diverged within ~1 my (between 7.5 and 6.5 mya) are represented by a rate of 13 mutations per million years (Veyrunes et al. 2006). Moreover, 16 Rb fusions became fixed in the M. minutoides clade in less than 1 million years (Veyrunes et al. 2010). Similarly, the recently diverged West African gerbilline Taterillus species have undergone extremely rapid rates of chromosome evolution being characterised by a rate of 45 changes per my (Dobigny et al. 2002a; 2005). This contrasts with non-murid rodents such as squirrels (Sciuridae), where karyotypes (i.e. Menetes berdmorei, Sciurus carolinensis and Callosciurus erythraeus; Richard et al. 2003; Stanyon et al. 2003; Li et al. 2004, 2006) show extensive conservation with that of human (i.e. ~0.3 R/my). This underscores the well-established observation that chromosomal evolution can vary between lineages (Rattini, conserved vs. Murini, rearranged). The pattern of slow chromosomal evolution in Rattini is also reflected in the analysis of repetitive elements (rat sat I DNA, telomeres and NORs) which were found to be largely conserved with respect to distribution and location (see above).

Although our investigation identified two types of rearrangements that are common in mammals (fusions and fissions), it was in fact changes to the short arms of many autosomes (considered to be pericentric inversions) that predominate in the karyotypic evolution of Rattini, echoing earlier observations by Gadi and Sharma (1983). Pericentric inversions were unambiguously confirmed by BAC-mapping in R. tanezumi, one of the species which exhibits short-arm variability in chromosomes orthologous to RNO 1, 12 and 13, and it seems reasonable to assume that this similarly applies to other short-arm variants in Rattus (other species that show these heteromorphisms are R. rattus, R. norvegicus, R. exulans, R. losea, R. annadalei and R. muelleli). The most obvious explanation for this is that the inversions were polymorphic in their last common ancestor (the deepest divergence in Rattus is dated at ~3.5 mya, and that of R. norvegicus, the most basal of the species analysed in our study, at ~2.9 mya, Robins et al. 2008), and have since persisted as polymorphisms in each lineage (i.e. reflecting incomplete lineage sorting; see discussion on hemiplasic characters below). Alternative explanations involving independently acquired inversions (or centromeric shifts and transpositions, rearrangements that could similarly account for the observed morphological differences) in species other than R. tanezumi are considered unlikely on grounds of parsimony. Should this hypothesis hold, it may be that these inversions have an adaptive role promoting rapid genetic diversification among populations through repressed recombination thus preventing admixture

of the newly evolved combinations of alleles (Noor et al. 2001; Hoffman et al. 2004; Ayala and Coluzzi 2005; Brown and O'Neill 2010 and references therein). It has been postulated that these processes may explain the association of balanced inversion clines along adap- tive gradients (e.g. Bonvicino et al. 2001; Noor et al. 2001; Coluzzi et al. 2002; Stefansson et al. 2005). Interestingly Yosida (1980) postulated that a pericen- tric inversion in the acrocentric pair 1 of an insular Asian black rat population in Southeast Asia, most likely R. tanezumi (2n = 42), appeared to confer a selective advantage to the survival of carriers in warmer climates. Although appealing, interpretations of the geographic patterns and the possible adaptive value of inversion polymorphisms in these rodents are problematic, in part due to questionable taxonomic divisions in several species, and the confounding effects of human transportation of Rattus species (i.e. R. exulans) across geographic boundaries.

Cytogenetic signatures for Rattini

1. Autosomal characters: Cytogenetic signatures that support some of the natural groupings at higher systematic levels were retrieved in this study. For example, the syntenic associations 7prox + 7dist and 16a + 16b that gave rise to RNO 7 and RNO 16, respectively, were identified as potentially defining Rattini. However, the 16a + 16b association should be considered provisional until reciprocal painting in a wider range of species is completed.

   The monophyly of B. bowersi, B. berdmorei, B. savilei and Rattus (Fig. 1) is supported by six pericentric inversions. This is consistent with an earlier report that posits pericentric inversions played a key role in the karyotype evolution of Rattus and Bandicota (Gadi and Sharma 1983). In contrast, the sister grouping of N. fulvescens + L. edwardsi suggested by sequence data, was not supported by chromosomal characters (Fig. 1). This gives credence to an earlier report that L. edwardsi is chromosomally more closely related to Berylmys, Bandicota and Rattus, than it is to Niviventer (Gadi and Sharma 1983).

   Of the three potentially homoplasic characters identified in our study (the fusion of 1p/1q prox + 1q dist, 2prox+ 2dist and Rb 9; 11), the most parsimo- nious explanation based on the dates and patterns of occurrence on the tree is that two are hemiplasies (the 1p/1q prox+ 1q dist and 2prox+ 2dist syntenies) and one (Rb 9;11) a true homoplasy (Fig. 1). Hemiplasy (Avise and Robinson 2008) is consid- ered likely when the internodes are short and the polymorphism persists for relatively short time periods (~3 my; Stefansson et al. 2005, Robinson et al. 2008, Robinson and Ropiquet 2011 and MacEachern et al. 2009 suggest 5–8 my for the lineage sorting of polymorphic sites in autosomal genes). Under hemiplasy, both the 1p/1q prox + 1q dist and 2prox + 2dist syntenies were present as polymorphic rearrangements at node A and the alternative forms were fixed idiosyncratically in different species. Based on their distribution on the tree (Fig. 1) the 1p/1q prox+ 1q dist fusion/fission polymorphism would be required to minimally persist for 1.5 my (~7.2–5.7 my), and the 2prox + 2dist fusion/fission polymorphism for 3.2 my (~7.2–4.0 my). In contrast, the fusion 9 + 11 is considered a true homoplasy (i.e. it arose conver- gently in the common ancestor to B. bowersi + B. berdmorei, and in R. rattus) given the protracted period required for its persistence (~5 my).
2. Gonosomal characters: An X chromosome-autosome translocation was identified in B. savilei involving a fusion of RNO X and 11. This type of rearrangement is generally considered to be highly deleterious due to the effects of X-inactivation on the autosomal component of the fusion chromosome and the differing replication times of the two (Dobigny et al. 2004b). It has been postulated that an interstitial heterochromatic block (IHB) between the X chromosome and the autosomal segment can act as a barrier thus preventing the spread of inactivation across the X/autosomal boundary. Interstitial heterochromatic blocks are consequently thought to represent a "regulatory superstructure" for the differential timing of replication (Dobigny et al. 2004b) and, given the presence of the IHB detected in the B. savilei X chromosome (see Badenhorst et al. 2009), this epigenomic hypothesis would apply.
3. Nucleolar organizer regions: The contention that NORs are useful phylogenetic markers for species delimitation (see for example Matsubara et al. 2004; Nguyen et al. 2008; Wang et al. 2009) does not hold in the Rattini since the NORs were conserved across divergent species (i.e. principally on the orthologues of RNO 3, 4, 6, 8, 9, 10 and 12, Table 3). Even the NORs on the orthologues of RNO 11 that appeared to unite R. exulans, R.

losea, R. tanezumi and R. norvegicus to the exclusion of the other taxa proved unsatisfactory in a broader phylogenetic context following the inclusion of R. rattus (Table 3). This highlights several important considerations when using these chromosomal landmarks as cytogenetic markers for species discrimination (reviewed in Dobigny et al. 2004a). The first is that intraspecific NOR variation, a well-known phenomenon in mammals, necessitates adequate sample sizes and geographically representative sampling before solid conclusions on number and location can be reached. This is particularly relevant to the present study since Yosida (1979), Sasaki et al. (1986) and Wang et al. (2003) have documented intraspecific variation in NOR number and position in both Rattus and Niviventer. Secondly, silver staining (used here) will only detect active NORs and may also reveal non-ribosomal loci (see examples for hedgehogs, Sanchez et al. 1995, and gerbils, Dobigny et al. 2002b). Analysis by FISH, which would address the non-specificity of silver staining (Sanchez et al. 1995), was attempted (5S rRNA and 28S rRNA genes isolated from gDNA) but was unsuccessful. Nonetheless, even with the use of single specimens and the limitations associated with silver staining, our results show a surprising conservation of NORs among Rattini species for which comparable data exist (i.e. Szabo et al. 1978; Kodama et al. 1980; Sasaki et al. 1986; Cavagna et al. 2002) and provide novel baseline information for taxa where these data are lacking (M. surifer, L. edwardsi, N. fulvescens, B. savilei, B. berdmorei and B. bowersi).

4. Satellite I DNA: Analysis (physical mapping and molecular analyses) of another repetitive element, the rat sat I DNA family, demonstrated a complete lack of homology between sequences isolated from non-Rattus species (N. fulvescens, B. savilei and B. berdmorei) and R. norvegicus sat I DNA. We interpret the interspersed pattern detected in the non-Rattus taxa as reflecting either a technical artefact (i.e. mispriming) or, alternatively, that the sat I primers used amplified long interspersed nuclear elements (LINE-1), or something that might cross hybridize to LINE-1 (see Waters et al. 2004). The rat sat I DNA family was, however, highly conserved within Rattus (R. exulans, R. losea and R. tanezumi; >90% sequence similarity), which was corroborated by TRF analysis (tandem repeated 92 bp subunit, >75% pair-wise sequence similarity, corresponding to the previously described 92 bp repeat subunit for R. norvegicus sat I DNA by Pech et al. 1979). Interestingly, although the rat sat I DNA family is reported to concentrate in the centromeric DNA of R. norvegicus (see Sternes and Vig, 1995; see also supplement material S2—present study), there is variability in its location in Rattus (i.e. all R. norvegicus chromosomes carry centromeric sat I DNA, while only eight pairs of chromosomes in R. tanezumi show this; supplementary material S2). Additionally, satDNA hybridization signals were undetected on chromosomes 12 and 13 in R. losea and chromosome 1 in R. exulans. The variable distribution and signal intensity of satDNA in Rattus is not unique since satDNA has been found to vary with regards to its abundance, sequence and chromosomal distribution between other closely related species (Adega et al. 2008; Louzada et al. 2008; Acosta et al. 2010).

Although some studies have suggested that Bandicota may be congeneric with Rattus (see Verneau et al. 1997, 1998), the analysis of repetitive elements discussed above does not support this. For example the B. savilei sequence showed no meaningful homology with rat sat I DNA following physical mapping and molecular analyses. These findings, together with the conservation of satellite sequence within Rattus suggest rather that the rat sat I DNA family is Rattus-specific. If so it provides an opportunity to date the appearance of the repeat in this group of rodents. Molecular clock estimates place the deepest divergence within Rattus at ~3.5 mya and R. norvegicus is thought to have diverged ~2.9 mya (Robins et al. 2008). It seems likely, therefore, that its presence in the common ancestor to Rattus at ~3.5 mya reflects the minimum age of this satellite family, and its localization at the centromeres in species of Rattus and its absence in taxa outside of this assemblage may be consistent with a feedback model of satellite evolution (Nijman and Lenstra 2001; reviewed in Slamovits and Rossi 2002). Based on this model, the new rat sat I DNA family is considered to be undergoing the initial phase of its evolutionary history as the three Rattus satDNA sequences (R. tanezumi, R. losea and R. exulans) currently display a high degree of conservation (>90% sequence similarity) with R. norvegicus. (Each phase is characterised by different levels of sequence identity where the initial phase is favoured by sequence homogeneity).

The Rattini ancestral karyotype

The mapping of polarized chromosomal characters allowed an objective assessment of ancestral states for the reconstruction of the putative RAK (Fig. 7). The analyses presented herein suggest that the RAK had 2n = 46 and this comprised, with one exception (a single small metacentric pair), only acrocentric chromosomes. Moreover, it shares 16 autosomal pairs that were conserved in toto (entire block or syntenic segment: MMU 7/19, 10prox/17prox, 13dist/15prox, 3, 2dist, 5prox/6, 1prox/4, 9, 16dist, 5dist, 1dist, 5med/11prox, 14dist, 13prox/2prox, 18 and 8 dist) with the 2n = 46 acrocentric ancestral Mus karyotype proposed by Veyrunes et al. (2006).

Concluding comments

This study presents a karyotypic investigation of Rattini that includes species that are representative of six of the 34 recognized genera. The data show that chromosome evolution in these rodents was driven largely by pericentric inversions. We also report a novel X-autosome translocation in B. savilei, a rearrangement hitherto undetected within Rattini. The overwhelming pattern of chromosomal evolution in these rodents is one of constrained change. This, together with the suggestion that the rat genome is structurally closer to that of human than is the mouse (see Zhao et al. 2004), underscores the usefulness of the rat as a model species in some comparative genomic studies. Consequently, the Rattini ancestral karyotype presented here (the first comprehensive hypothesis of the putative RAK) may prove useful in directing the selection of rodent species for future large-scale investigations of genome organization.

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Table 1 List of species and specimens included in the present investigation for both chromosome painting and repeat element (satellite I DNA, telomeres and NORs) analysis; sampling localities and grid references are provided for each locality

Species	Specimen	Sex	Origin	Material	2n	Zoo-FISH analysis	BAC-mapping	Telomeric repeats	Satellite DNA analysis	Ag-NORs
Rattus losea	R4724	F	Loei	cc	42 X			X	X	X
Rattus tanezumi	R4003	F	Kalasin	bm	42 X		X			
	R4182	F	Phrae	bm	42 X			X	X	X
Rattus exulans	R4033	F	Phrae	bm	42 X			X	X	X
	R 4035	F	Phrae	bm	42 X					
Rattus norvegicus[a]	/	F	South Africa	cc	42		X		X	
Bandicota savilei	R4143	M	Phrae	bm	45 X					
	R4408	F	Loei	cc	43 X			X	X	X
Berylmys berdmorei	R4406	F	Loei	cc	41 X			X	X	X
Berylmys bowersi	R4400	M	Loei	cc	40 X			X	X	X
Leopoldamys edwardsi	R5239	M	Loei	cc	42 X			X	X	X
Niviventer fulvescens	R4519	F	Loei	bm	46 X			X	X	X
Maxomys surifer[b]	R4404	M	Loei	cc	52 X			X	X	X

Specimen numbers refer to the CBGP Asian rodent collection (Montpellier, France). Diploid (2n) number and type of biological material used (bm bone marrow; cc cell culture) are indicated for each specimen

[a] The R. norvegicus specimen was obtained commercially and its precise origin is unknown

[b] The M. surifer specimen used for the production and characterisation of flow-sorted chromosome paints

(/) not applicable

Table 2 BAC clones used in the study with their positions on the rat ideogram, clone names and relevant accession numbers

	Number	Chromosome position	Clone ID	Accession number	End accession number	
					SP6	T7
RNO 1	1	1p13	CH230-347 K17	AC135531	BZ154483	BZ154484
	2	1p11	CH230-99D20	AC109433	BH314535	BH314564
	3	1q11	CH230-365B17	/	BZ116707	BZ116708
	4	1q12	CH230-447 J17	/	BZ248996	BZ248997
RNO 12	5	12p12	CH230-30 M1	/	BH293553	BH293555
	6	12p11	CH230-70E3	/	BH273680	BH273682
	7	12q11	CH230-IJ13	AC095368	BH297088	BH297089
	8	12q12	CH230-204 G18	AC123562	BH366346	BH366347
RNO 13	9	13p13	CH230-52 N12	AC103093	BH318049	BH318051
	10	13p11	CH230-231 G7	/	BZ113582	BZ113584
	11	13q11	CH230-221 F10	/	BH353297	BZ094368
	12	13q12	CH230-128D5	AC106070	BH279605	BH279609

The map positions were confirmed by both NCBI and UCSC genome browsers

(/) These BAC clones have not yet been fully sequenced, however, all BAC clones have had both ends sequenced (SP6 and T7)

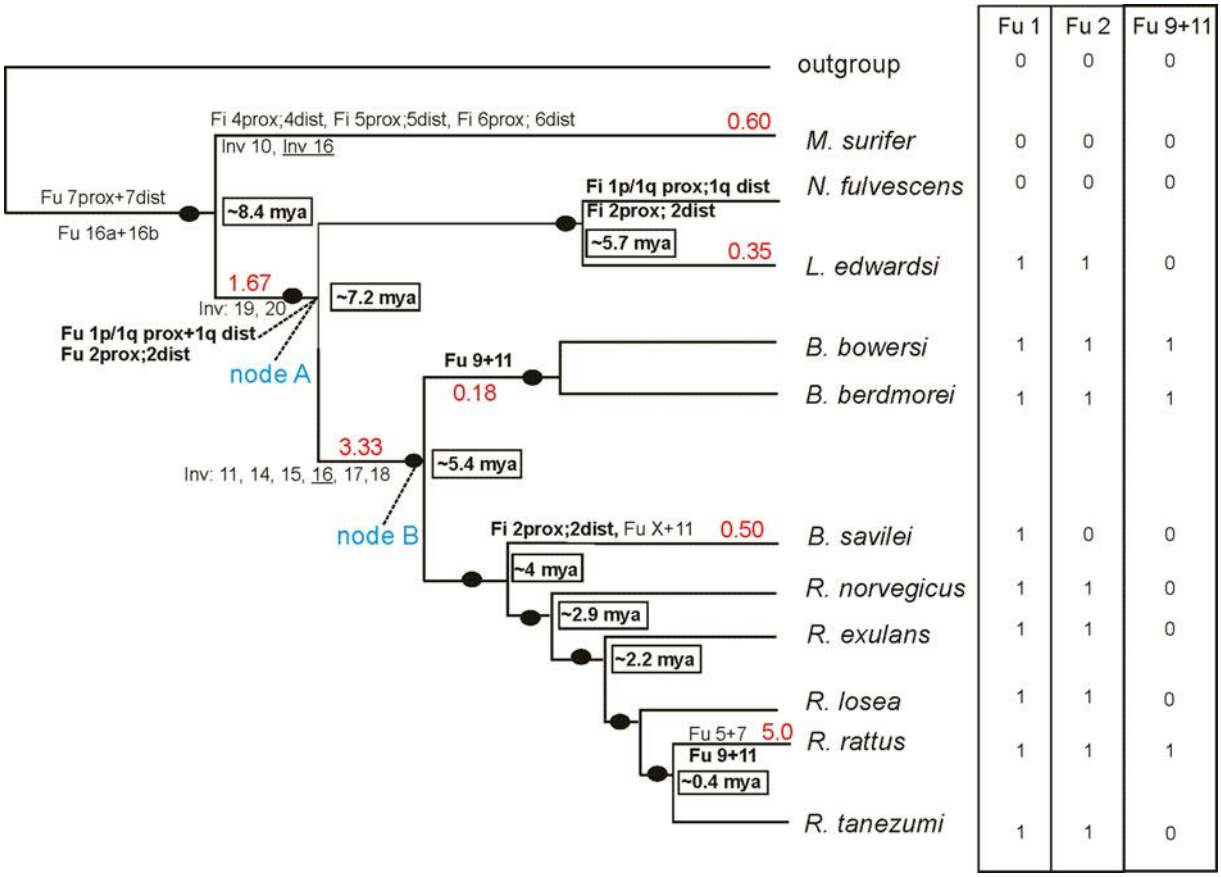

Fig. 1 Mapping of the chromosome changes (as inferred from Zoo-FISH experiments) to the Rattini molecular phylogenetic tree (modified from Verneau et al. 1997; Lecompte et al. 2008; Pagès et al. 2010; Badenhorst 2011) and the rates of chromosome evolution against divergence times. The numbering of rearrangements corresponds to the chromosomes of R. norvegicus. Numbers in squares indicate molecular divergence estimates in million years (mya) as inferred by Lecompte et al. (2008) and Robins et al. (2008). Numbers in red represent the average rates of rearrangement per million years. Fu fusion, Fi fission, Inv pericentric inversion, dist distal, prox proximal, a and b refer to unidentifiable chromosomal segments resulting from the uncertain location of the breakpoint. Black ovals indicate strong nodal support (BI > 0.95; BP > 95). Underlined chromosome numbers indicates two different breakpoints involving the inversion of the RNO 16 ortholog and homoplasic characters are denoted in bold. Columns on right demonstrate the presence (1) or absence (0) of the Fu 1, Fu 2 and Rb 9+11 rearrangements

Table 3 Number of chromosome pairs showing nucleolar organizer regions (NORs) in the nine species analysed in the present study (numbering according to their respective G-banded karyotypes; Badenhorst et al. 2009), including R. norvegicus and R. rattus from published data (Cavagna et al. 2002)

	No. NORs pairs	Chromosome no.	Respective R. norvegicus ortholog[a]
R. exulans (2n = 42, XX)	3	REX 3, 11 and 12	3, 11 and 12
R. losea (2n = 42, XX)	3	RLO 3, 11 and 12	3, 11 and 12
R. tanezumi (2n = 42, XX)	3	RTA 3, 11 and 12	3, 11 and 12
B. savilei (2n = 43, XX)	2	BSA 10 and 20	10 and 12
B. bowersi (2n = 40, XY)	4	BBO 4, 7, 10 and 9	3, 6, 8 and 10
B. berdmorei (2n = 41, XX)	4	BBE 4, 7, 10 and 9	3, 6, 8 and 10
L. edwardsi (2n = 42, XY)	5	LED 2, 3, 4, 10 and 20	1, 4, 3, 10 and 12
N. fulvescens (2n = 46, XX)	5	NFU 1, 6, 8, 9 and 10	1q dist, 6, 8, 9 and 10
M. surifer (2n = 52, XY)	8	MSU 1, 2, 4, 6, 7, 14, 17 and 19	1q dist, 3, 2 dist, 8, 9, 10, 5 dist and 6 prox
R. norvegicus (2n = 42)[b]	3	RNO 3, 11 and 12	/
R. rattus (2n = 38)[b]	2	RRA 5, 8 and 16	RNO 3, 8 and 12

[a] Correspondence to R. norvegicus orthologous chromosomes identified by cross-species chromosome painting using R. norvegicus paints

[b] Based on silver staining

(/) not applicable

Fig. 2 Multiple alignment of four Rattus sat I DNA sequences (238 bp) displaying significant homology by ClustalW. Dots indicate identity with the R. norvegicus sat I DNA sequence

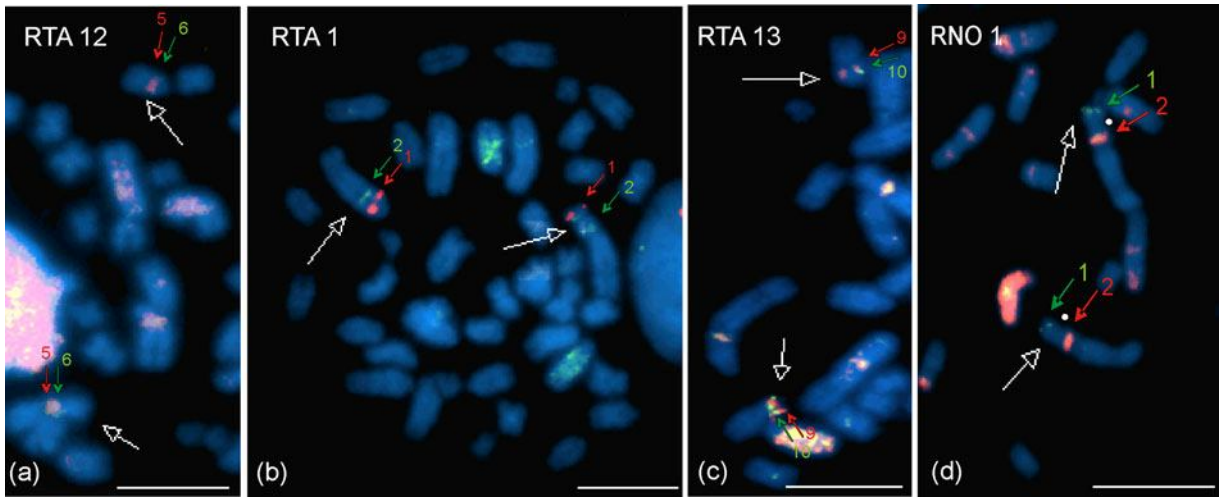

Fig. 3 a, b and c Representative metaphase spreads from a R. tanezumi specimen that is heterozygous for the acrocentric and submetacentric morphs of chromosome orthologous to RNO 1, 12, 13 (white arrows). Green and red arrows indicate the localization of BAC clones that map to R. norvegicus (RNO) 12p (BAC clone 5 red, BAC clone 6 green) (a); RNO 1p (BAC clone 1 red, BAC clone 2 green) (b); RNO 13p (BAC clone 9 red, BAC clone 10 green) (c). d Representative metaphase spread of R. norvegicus (RNO) showing localization of BAC clones on chromosome pair 1 (white arrow), centromere position indicated by white oval. The BAC clone selected for 1p13 (BAC clone 1, green) confirms the assignment given in Ensembl database while BAC clone 2, reportedly mapping to RNO1p11 by Ensembl, is located on the q arm (approximately 1q11) in both R. norvegicus and R. tanezumi. Scale bar = 10 μm. See text for details

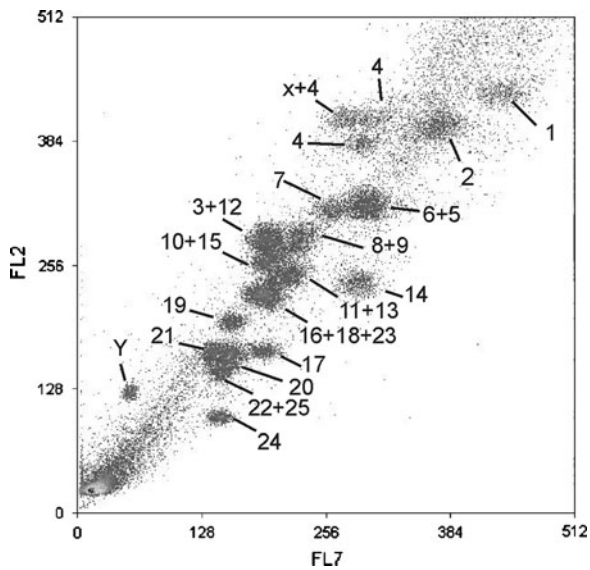

Fig. 4 Flow-sorted karyotypes of M. surifer (MSU, 2n = 52, XY) showing the flow-peaks and their correspondence with the respective chromosomes (see text for details)

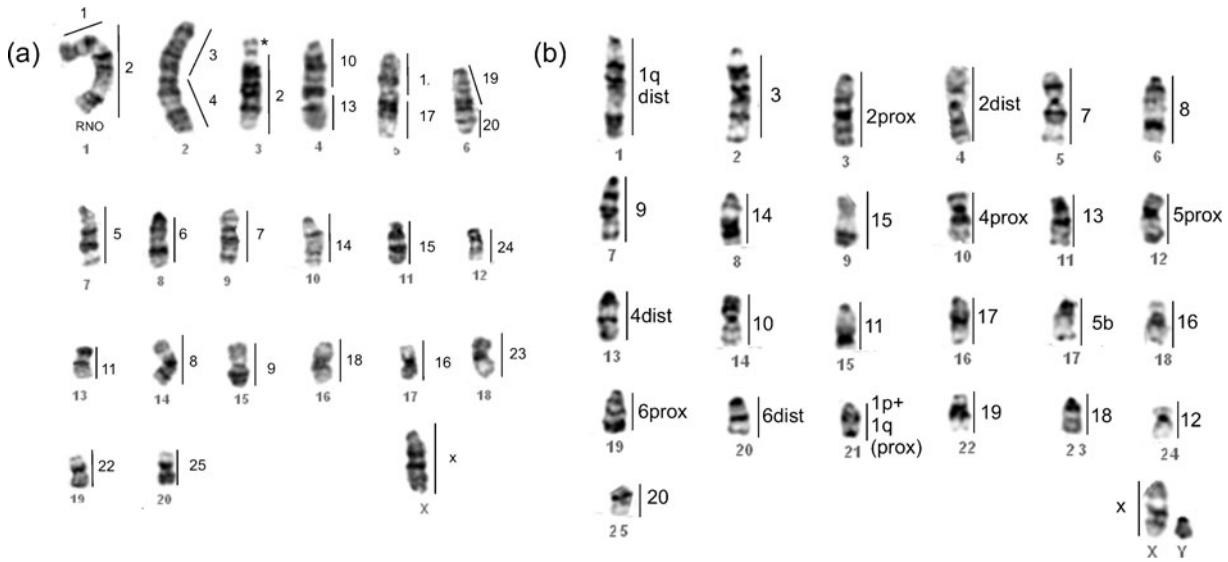

Fig. 5 a Regions of orthology between R. norvegicus (2n = 42), and M. surifer (2n = 52) chromosomes based on reciprocal chromosome painting and mapped to the R. norvegicus G- banded half-karyotype (2n = 42). Segments orthologous to M. surifer are shown on the right. b G-banded half-karyotype of male M. surifer (2n = 52) with regions of orthology to R. norvegicus (numbered on the right, except for X) as determined by cross-species chromosome painting. Asterisk indicates blocks that were not hybridized by any of the chromosome paints and which correspond to heterochromatic regions identified through C-banding (Badenhorst et al. 2009)

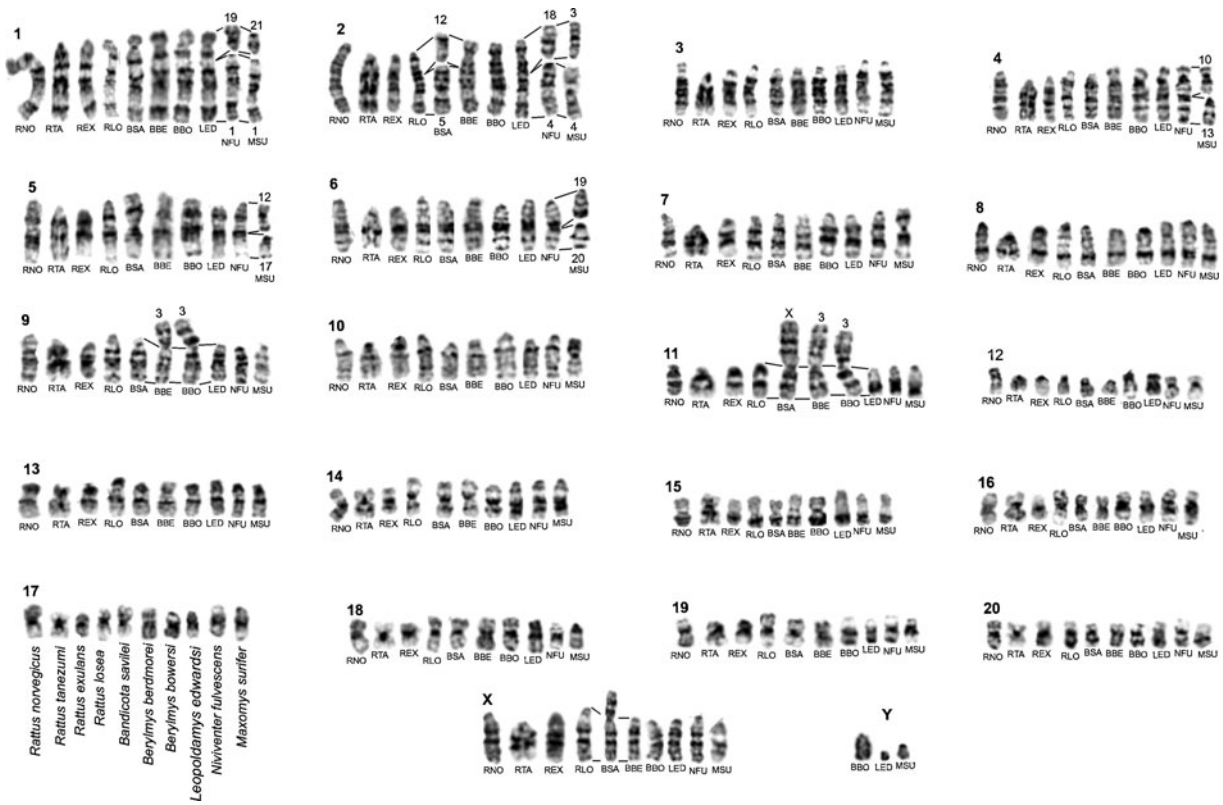

Fig. 6 G-banded half-karyotype comparison between R. norvegicus and the nine species analysed in this study showing genome-wide chromosomal correspondence defined by paint- ing (using R. norvegicus and M. surifer chromosome paints) and banding homologies. Karyotypes were arranged according to R. norvegicus standard karyotype (committee for a standard- ized karyotype of R. norvegicus, 1973)

Table 4 Conserved chromosomal regions detected in Rattini species using RNO painting probes

R. norvegicus	R. losea	R. tanezumi	R. exulans	B. bowersi	B. berdmorei	B. savilei	L. edwardsi	N. fulvescens	M. surifer
1	1	1	1	1	1	1	2	19, 1	21, 1
2	2	2	2	2+	2+	5+, 12	1+	18, 4	3, 4+
3	3	3	3	4	4	4	4	3	2
4	4	4	4	6+	6+	2	3+	2+	10+, 13
5	5	5	5	5+	5+	3+	5	5	12+, 17
6	6	6	6	7	7	6	6	6	19, 20
7	7	7	7	8	8	7	7	7	5+
8	8	8	8	10	10	8	8	8	6
9	9	9	9	3	3	9+	13	9	7
10	10	10	10	9	9	10	10	10	14+
11	11	11	11	3	3	X	17+	16+	15+
12	12	12	12	19	19	20	20	22	12
13	13	13	13	11	11	11	11	11	11
14	14	14	14	12	12	13	9+	12+	14+
15	15	15	15	13	13	15	12+	13+	15+
16	16	16	16	16	15	14	14+	14+	16+
17	17	17	17	15	16	16	15+	15+	17+
18	18	18	18	14	14	17	16+	17+	18+
19	19	19	19	17	17	18	18	20	22+
20	20	20	20	18	18	19	19	21	20+
X	XX	XX	XX	X	X	X	X	XX	X
Y				Y			Y		Y

Intrachromosomal rearrangements identified following comparisons with their respective R. norvegicus orthologues are denoted by +

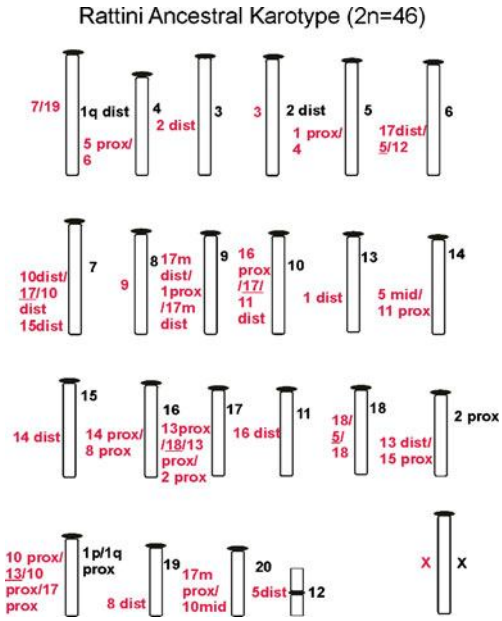

Fig. 7 Putative ancestral karyotype of Rattini with R. norve- gicus and M. musculus homologies numbered on the right (black) and left (red), respectively. Underlined M. musculus segments represent small, generally undetected conserved segments between mouse and rat. dist, mid, prox, m prox refer to the distal, middle, proximal and mid proximal segments of the chromosomes, respectively