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Phylogenomics of the genus *Mus* (Rodentia; Muridae): extensive genome repatterning is not restricted to the house mouse

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The house mouse (*Mus musculus*) is universally adopted as the mammalian laboratory model, and it is involved in most studies of large-scale comparative genomics. Paradoxically, this taxon is rarely the index species for evolutionary analyses of genome architecture owing to its highly rearranged karyotype. To unravel the origin and nature of this extensive repatterning genome, we performed a multidirectional chromosome painting study of representative species within the genus *Mus*. However, the latter includes four extant subgenera (*Mus*, *Coelomys*, *Nannomys* and *Pyromys*) between which the phylogenetic relationships remain elusive despite the numerous molecular studies. Comparative genomic maps were established using chromosome-specific painting probes of the laboratory mouse and *Nannomys minutoides*. Hence, by integrating closely related species within *Mus*, this study allowed us to: (i) unambiguously resolve for the first time the long-standing controversial phylogeny, (ii) trace the evolution of genome organization in the house mouse, (iii) track rearrangements that necessitated new centromere locations, i.e. formation of neocentromere or reactivation of latent centromeres, (iv) reveal an extremely high rate of karyotypic evolution, with a 10- to 30-fold acceleration which was coincidental with subgeneric cladogenesis and (v) highlight genomic areas of interest for high-resolution studies on neocentromere formation and synteny breakpoints.

Keywords: fluorescence *in situ* hybridization; phylogenomics; *Mus*; *Nannomys*; *Coelomys*; neocentromere

1. INTRODUCTION

In the last decade, considerable advances have been made in understanding mammalian genomic architecture through genome-sequencing initiatives and cross-species chromosome painting. Large-scale comparative mapping analyses have focused primarily on the three mammalian species for which the most complete genomic data are available: human, mouse and rat. The investigations have shown that (i) the random breakage model of genome evolution (Nadeau & Taylor 1984) is flawed, since extensive breakpoint reuse is apparent (Pevzner & Tesler 2003; Bailey *et al.* 2004; Zhao *et al.* 2004), (ii) centromeric shifts and/or neocentromere formation are relatively common (Ventura *et al.* 2001, 2003, 2004; Murphy *et al.* 2005) and (iii) rates of chromosomal repatterning show considerable variation among lineages (O'Brien *et al.* 1999; Murphy *et al.* 2005). However, bioinformatic approaches are limited by the availability of sequenced genomes. In contrast, cross-species chromosome painting, i.e. zoo-fluorescence *in situ* hybridization (FISH) analyses (flow-sorted fluorescent chromosome probes hybridized *in situ* to chromosomes of another target species) have provided genomic information, albeit at a far lower resolution, on species spanning most of the 20 modern orders of mammals. Most comparisons of genome organization have involved human chromosome-specific paints, while those using the house mouse as the index species have been relatively scarce despite the biomedical and genomic importance of this mammalian laboratory model, and have only recently started accumulating. The lack of interest in this rodent is attributed to its highly fragmented and rearranged karyotype compared to that of the human and other mammals (Stanyon *et al.* 1999; Nilsson *et al.* 2001; Gregory *et al.* 2002). Several zoo-FISH comparisons between *Mus* and other rodent genera have been undertaken (e.g. Yang *et al.* 2000; Cavagna *et al.* 2002; Rambau & Robinson 2003; Matsubara *et al.* 2004; Engelbrecht *et al.* 2006) and have shed some insight into

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our understanding of the origin and nature of the extensive repatterning in the house mouse genome. However, the investigations have not been extended to closely related species within the genus *Mus*. The only exception to this has been *Mus platythrix*, which falls within the subgenus *Pyromys* (Matsubara *et al.* 2003).

The genus *Mus* (Rodentia, Muridae and Murinae) is a highly speciose murid genus, which exhibits extensive chromosomal evolution (e.g. Britton-Davidian *et al.* 2000; Veyrunes *et al.* 2004; Piàlek *et al.* 2005). This genus encompasses at least 40 species divided into four subgenera: *Mus sensu stricto*, *Nannomys*, *Coelomys* and *Pyromys* (Musser & Carleton 1993). The Eurasian subgenus *Mus* is by far the most extensively studied, and consists of 11 species to which a new species from the island of Cyprus has recently been added (Cucchi *et al.* 2006). The three other subgenera are less well known. The subgenus *Nannomys*, the African pygmy mice, has a sub-Saharan distribution and comprises 19 recognized species. The two last subgenera are restricted to the Indian subcontinent and southeastern Asia: *Pyromys* with five species and *Coelomys* with four species (Musser & Carleton 1993). The *Mus* genus has been the focus of a plethora of phylogenetic studies (e.g. Bonhomme 1986, 1992; Jouvin-Marche *et al.* 1988; She *et al.* 1990; Catzeflis & Denys 1992; Boursot *et al.* 1993; Sourrouille *et al.* 1995; Lundrigan *et al.* 2002; Chevret *et al.* 2003, 2005; Suzuki *et al.* 2004; Veyrunes *et al.* 2005). However, while the monophyly of the genus and of each of the four subgenera are clearly established, the relationships between them are still unresolved, despite the large variety of molecular markers used. Even the sequencing of not less than six paternally, maternally and biparentally inherited genes failed to provide strong support for the intersubgeneric relationships (Lundrigan *et al.* 2002). This lack of resolution most likely reflects the rapid radiation of these four clades, which is thought to have occurred within 1 Myr (e.g. Chevret *et al.* 2005; Veyrunes *et al.* 2005). Thus, new genetic markers are required to resolve these phylogenetic uncertainties. Chromosomal rearrangements appear to be ideal candidates as they are considered to be rare genomic changes *sensu* Rokas & Holland (2000), and provide cladistic signatures with very low levels of homoplasy (e.g. Murphy *et al.* 2004; Wienberg 2004). In effect, zoo-FISH comparative chromosome painting constitutes a powerful and elegant method for both detecting chromosome homologies between species and resolving long-standing phylogenetic controversies such as within the Carnivora, Rodentia or Primate orders (de Oliveira *et al.* 2002; Nie *et al.* 2002; Muller *et al.* 2003; Li *et al.* 2004).

In the present study, a multidirectional chromosome painting analysis is performed between representative species of three subgenera of *Mus* (*Nannomys*, *Coelomys* and *Mus*). By including published data for the fourth subgenus (*Pyromys*; Matsubara *et al.* 2003), and using available rodent species as outgroups, a chromosomal phylogeny is reconstructed following three aims: (i) to test the performance of chromosomal rearrangements in resolving the phylogenetic relationships between the subgenera of *Mus*; (ii) to infer the ancestral karyotype of the genus *Mus* for use in future comparisons with other taxa and finally (iii) to gain insight into patterns and processes of genome organization and evolution leading to the house mouse karyotype.

2. MATERIAL AND METHODS

(a) *Animals, chromosome preparation and identification*

In order to avoid nomenclatural ambiguities, we refer to subgenera to distinguish lineages (i.e. *Nannomys*, *Coelomys*, *Mus* and *Pyromys*) and not to the genus name (*Mus*).

The female *Nannomys mattheyi*, male *Nannomys minutoides*, female *Coelomys pahari* and male *Mus musculus* specimens used in this study originated, respectively, from Samaya in Mali, Stellenbosch in South Africa, India (precise locality unknown) and Clapiers in France. The chromosome preparations were made either from bone marrow of yeast-stimulated animals (*N. mattheyi*, *C. pahari* and *M. musculus*) or fibroblast cell-cultures established from skin biopsy following the standard procedures (*N. minutoides*). The identification of chromosomes was also accomplished by G- and DAPI banding concurrently with *in situ* hybridization.

(b) *Flow sorting and chromosome-specific painting probes preparation*

As cell cultures of *N. mattheyi* were not available, flow sorting was performed for *N. minutoides*. The chromosomes were prepared for sorting as described previously (Yang *et al.* 1997). The stained chromosome preparations were sorted on a dual laser cell sorter (FAC-Star Plus, Becton Dickinson). Flow-sorted chromosomes were used as templates for amplification by degenerate oligonucleotide-primed PCR (DOP-PCR) using 6 MW primers (Telenius *et al.* 1992). Primary DOP-PCR products were used as a source of template for the incorporation of biotin-16-dUTP (Boehringer).

(c) *Fluorescence* in situ *hybridization*

The complete set of commercial chromosome-specific painting probes from the house mouse *M. musculus* (Cambio) and those made from *N. minutoides* were hybridized across representative species of the other subgenera i.e. *Nannomys*, *Coelomys* and *Mus*, respectively. Hybridization and detection were carried out following the procedure described in Robinson *et al.* (2004). Biotin-labelled probes were visualized using Cy3-avidin (1 : 500 dilution, Amersham). Slides were then mounted in Vectashield mounting medium with DAPI (Vector Laboratories). Images were captured using the Genus software (Applied Imaging). The hybridization signals were assigned to specific chromosomal regions identified by DAPI staining.

(d) *Phylogenetic analysis*

The phylogenetic analysis was performed using the comparative chromosomal maps of *Mus* versus *Nannomys* and *Coelomys*, and published data on *Pyromys* (Matsubara *et al.* 2003). Three additional Murinae species were included as outgroups: *Rattus rattus* (Cavagna *et al.* 2002), *Rhabdomys pumilio* (Rambau & Robinson 2003) and *Apodemus sylvaticus* (Matsubara *et al.* 2004). A subsequent zoo-FISH analysis of *A. sylvaticus* by Stanyon *et al.* (2004) revealed several discrepancies between the two studies. We chose the former as it included one synteny in common with our analysis, which was overlooked in the latter. Contiguous chromosomal segment associations (syntenies) were used as characters to establish a binary data matrix (electronic supplementary material) following the procedure for encoding chromosomal data reviewed in Dobigny *et al.* (2004). We chose to exclude from the matrix the pericentromeric material homologous to

Mus chromosome 14 on *Pyromys* chromosomes 5, 8 and 12, which may consist of duplications of 18S–28S ribosomal RNA genes (Matsubara *et al.* 2003; see also Thomas *et al.* 2003). In the absence of accurate information, we postulated that all characters had the same weight (i.e. same probability of appearance/fixation). The most parsimonious phylogenetic tree was obtained using an exhaustive search in PAUP v. 4.0b10 (Swofford 1999). The robustness of each node was assessed by bootstrap estimates after 1000 iterations.

3. RESULTS

(a) *G-banded karyotypes*

The G-banded karyotypes of the *Nannomys* species have already been described in Veyrunes *et al.* (2004): *N. mattheyi* has an all-acrocentric $2n=36$ karyotype and *N. minutoides* has a diploid number of $2n=18$ with all chromosomes being biarmed. The G-banding analyses (Veyrunes *et al.* 2004) indicate that the lower diploid number in the latter species resulted exclusively from Robertsonian (Rb) fusions; in particular, both sex chromosomes are involved in Rb fusions with pair 1 to form the chromosomes Rb(X.1) and Rb(Y.1). The G-banded karyotype of *C. pahari* represents the first ever published for this subgenus, and is composed of 48 acrocentric chromosomes.

(b) *Flow-sorted karyotype of* N. minutoides

Figure 1 shows the flow karyotype of *N. minutoides*. Chromosome suspensions were sorted on base pair composition and chromosomal size. This resulted in 10 peaks which represent the eight autosomal pairs, and the Rb(X.1) and Rb(Y.1). Identification of the different peaks was achieved by hybridizing DOP-PCR generated probes onto DAPI-banded *N. minutoides* metaphases.

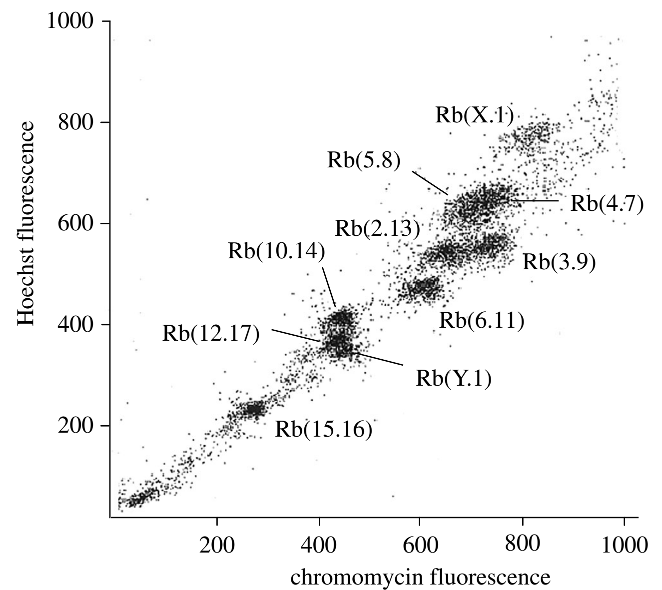

Figure 1. Flow karyotype of a male *Nannomys minutoides* ($2n=18$) resolving 10 peaks, each containing an Rb fusion chromosome pair.

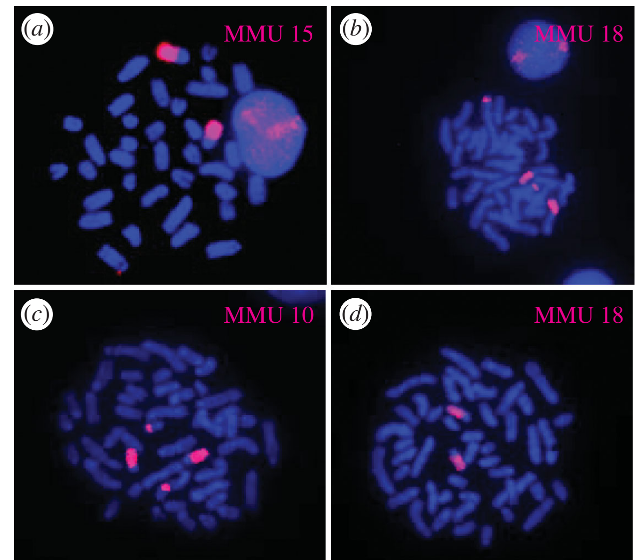

Figure 2. Examples of hybridization using mouse chromosome paints (MMU) to (*a*,*b*) *Nannomys mattheyi* and (*c*,*d*) *Coelomys pahari* metaphase spreads counterstained with DAPI.

(c) *Reciprocal chromosome painting between* Mus *and* Nannomys

All the house mouse chromosome-specific probes successfully hybridized to the euchromatic regions of the pygmy mouse chromosomes (see figure 2 for examples). The 19 autosomal and X paints defined 27 segments of homology between *Mus* and *Nannomys*. Regions of homology are indicated on the G-banded karyotype of *N. mattheyi* (figure 3*a*). Fourteen *Mus* chromosomes (2–4, 6–8, 10–12, 14–16, 19 and X) were each retained as a single conserved block, five *Mus* probes (1, 9, 13, 17 and 18) each produced two signals, and finally the *Mus* chromosome 5 probe hybridized to three regions in the *Nannomys* karyotype.

Reciprocal chromosome painting (*Nannomys* paints onto the *Mus* karyotype) was used to define precisely the genome-wide homologies between these two subgenera. Twenty-six homologous segments were detected and summarized in table 1. In addition to confirming the painting results of the *Mus* probes, this procedure allowed us to assign subchromosomal homologies between the *Mus* and *Nannomys* chromosomes. The only exception to this was chromosome 9 of *Mus*, which is homologous to parts of chromosomes 2 and 13 of *N. mattheyi*, but are present as a single fused chromosome in the *N. minutoides*-derived painting probe, Rb(2.13).

(d) *Chromosome painting of* Mus *and* Nannomys *probes onto* Coelomys *chromosomes*

The 19 house mouse autosomal probes and the X paint delineated 35 homologous segments in the *C. pahari* genome (figure 3*b* and also figure 2 for examples). Nine *Mus* chromosomes (3, 6, 9, 12, 14, 16, 18, 19 and X) showed complete conservation of synteny (i.e. retained as single sites of hybridization), seven (1, 2, 4, 10, 11, 15 and 17) each painted two chromosomal regions and four (5, 7, 8 and 13) detected three segments in the *Coelomys* karyotype.

The hybridization of *Nannomys* probes onto *Coelomys* chromosomes (not shown) revealed 30 homologous segments in perfect concordance with the preceding results and allowed us to assign several subchromosomal homologies between *Mus* and *Coelomys* (table 1) via the *Nannomys* karyotype.

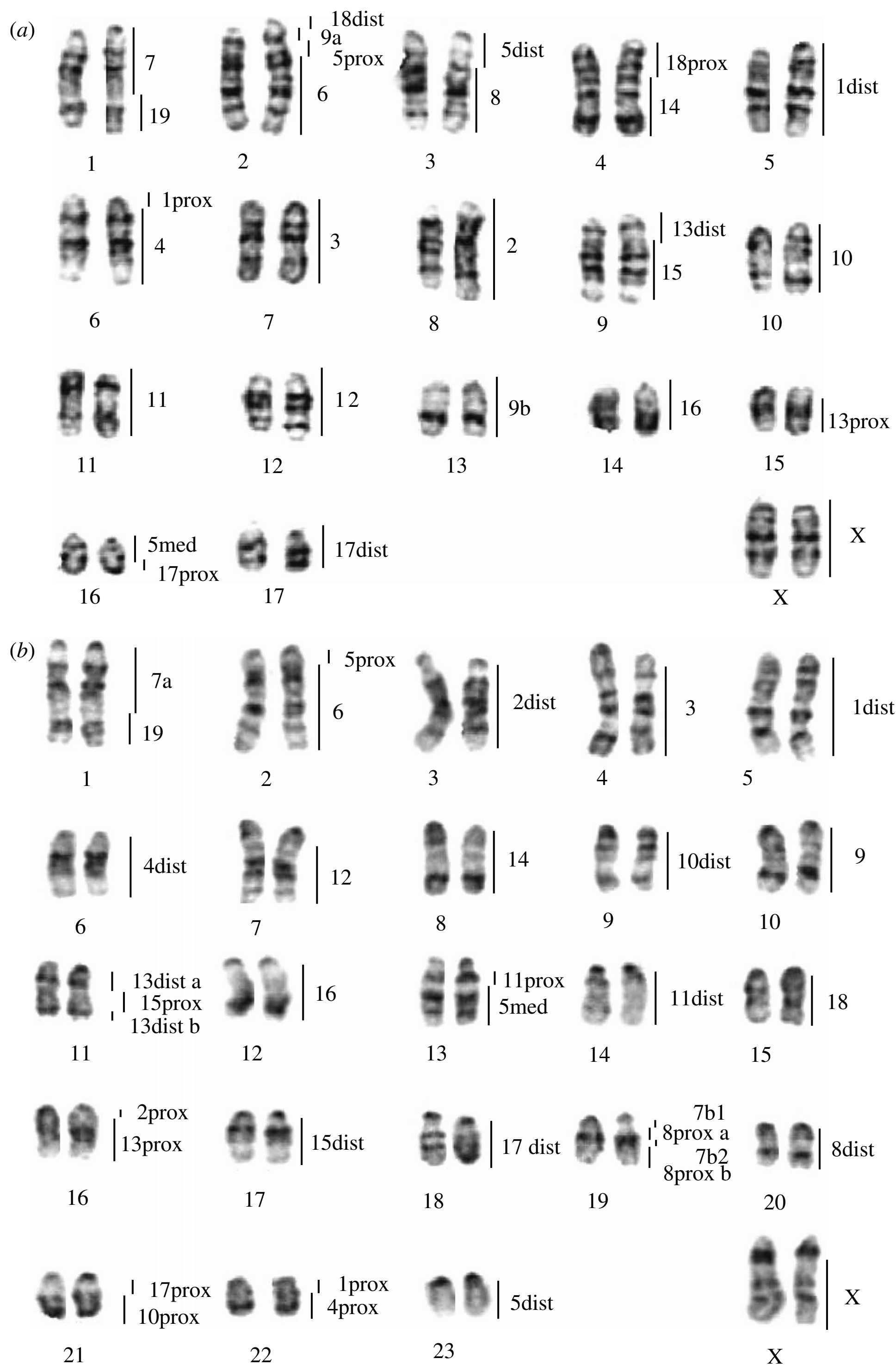

Figure 3. G-banded karyotypes of (*a*) *Nannomys mattheyi* and (*b*) *Coelomys pahari* with the assignments of *Mus musculus* homologous segments (chromosome pair numbers on the right) revealed by the mouse probes. 'dist', 'med' and 'prox' refer to the distal, median and proximal segment of the chromosome, respectively, and a and b refer to unidentified subchromosomal segments.

(e) *Phylogenetic analysis*

The reciprocal painting results, in combination with G-banding comparison, allowed us to identify most of the sub-regional homologies between the representative karyotypes of the three subgenera (table 1). The homologous adjacent segments (syntenic associations) identified between these species and those published for *Pyromys* and the outgroups were translated into 67 chromosomal characters (electronic supplementary material). The maximum parsimony analysis resulted in only one most parsimonious tree (74-steps long, consistency index = 0.91, retention index = 0.79, homoplasy index = 0.09; figure 4). The first subgenus to diverge is *Coelomys*, followed by *Nannomys*, then *Mus* and *Pyromys*. There is no synapomorphy that supports the monophyly of the genus, resulting in the lowest bootstrap value (equal to 75) for this node. The two other nodes are much more robust (equal to 97 and 98) and are supported by several chromosomal changes, i.e. four translocations, three fusions and one inversion all provide strong support for the *Nannomys*/*Mus*/*Pyromys* cluster; four translocations and one fusion characterize the *Mus*/*Pyromys* clade (figure 4).

Table 1. Homologies between *Mus musculus*, *Nannomys mattheyi* and *Coelomys pahari* chromosomes, inferred from reciprocal and cross-species chromosome painting and G-banded patterns. ('dist', 'med' and 'prox' refer to the distal, median and proximal segment of the chromosome, respectively. 'tot' refers to entire chromosome; a, b refer to subsegments of chromosome.)

Mus	*Nannomys*	*Coelomys*
1prox	6prox	22prox
1dist	5tot	5tot
2prox	8prox	16prox
2dist	8dist	3tot
3tot	7tot	4tot
4prox	6med	22dist
4dist	6dist	6tot
5prox	2med b	2prox
5med	16prox	13dist
5dist	3prox	23tot
6tot	2dist	2dist
7tot	1prox	1prox+19prox+med b
8prox	3med	19med a+dist
8med	3dist	20tot
9tot	13tot+2med a	10tot
10prox	10prox	21dist
10dist	10dist	9tot
11prox	11prox	13prox
11dist	11dist	14tot
12tot	12tot	7tot
13prox	15dist	16dist
13dist	9prox	11prox+dist
14tot	4dist	8tot
15prox	9med	11med
15dist	9dist	17tot
16tot	14tot	12tot
17prox	16dist	21prox
17dist	17tot	18tot
18prox	4prox	15prox
18dist	2prox	15dist
19tot	1dist	1dist
Xtot	Xtot	Xtot

Semantically, we considered 'translocation', the transfer of a fragment of one chromosome onto another chromosome, and 'fusion and/or fission', events involving two entire chromosomes.

4. DISCUSSION

(a) *Phylogenetic relationships*

Despite the number of studies involved and the variety of molecular markers used (see §1), an unambiguous phylogenetic tree for the genus *Mus* has remained elusive. In such a context, subgeneric relationships within *Mus* were investigated using chromosomal rearrangements. They constitute alternative genetic markers with low levels of convergence, and are highly informative as being under-dominant mutations, they are fixed (or lost) rapidly in the populations, contrary to genes which may remain polymorphic for long periods of time.

Our phylogenomic analysis yielded a single most parsimonious tree in which the subgeneric relationships were resolved for the first time (figure 4). The nodes were well supported by the standard method of bootstrap, and more importantly, each one was supported by several unique non-ambiguously identified chromosomal rearrangements providing strong confidence to the topology retrieved. The first subgenus to diverge is *Coelomys*, followed by *Nannomys* at the base of a *Mus*–*Pyromys* clade. This topology was previously suggested by Lundrigan *et al.* (2002), but was not supported by high bootstrap values (except for one nuclear marker *Tcp-1*). Other studies have also clustered *Mus* with *Pyromys*, although with similarly weak support (e.g. Catzeflis & Denys 1992; Chevret *et al.* 2005). Curiously, the lowest bootstrap value in our study (equal to 75) defines the monophyly of the genus, a node consensually supported by a variety of molecular datasets (e.g. Lundrigan *et al.* 2002; Chevret *et al.* 2003, 2005; Suzuki *et al.* 2004; Veyrunes *et al.* 2005).

Palaeontological and molecular data indicate that the genus *Mus* originated in Asia, with the oldest true-*Mus* fossil reported from Pakistan from the Late Miocene (e.g. Suzuki *et al.* 2004; Chevret *et al.* 2005). The resolved cytogenetically based phylogenetic tree allowed us to order the dispersal events that took place during the evolution of the genus into three successive bursts of differentiation that occurred approximately 7 Myr ago (Chevret *et al.* 2005; Veyrunes *et al.* 2005), the first one involving *Coelomys* in southeastern Asia, followed by *Nannomys* with the colonization of Africa via the Middle East, then *Mus* in Eurasia and *Pyromys* in Southeast Asia and the Indian subcontinent.

(b) *Genome comparison and ancestral* Mus *karyotype*

Although the house mouse is perhaps the most widely studied mammal in terms of chromosomal evolution (e.g. Britton-Davidian *et al.* 2000; Capanna & Castiglia 2004; Piàlek *et al.* 2005), comparisons between subgenera within the genus *Mus* are very scarce. Thus, our cross-species multidirectional chromosome painting involving one representative species belonging to all four subgenera represents the first attempt to establish genome-wide comparative chromosome maps in the genus. Moreover, comparison of these data with those for five other murid genera (Yang *et al.* 2000; Cavagna *et al.* 2002; Rambau & Robinson 2003; Matsubara *et al.* 2004; Engelbrecht *et al.* 2006) has shed light on shared primitive and derived chromosomal syntenies in the murid lineage. The results reveal that drastic genome shuffles have occurred in the genus *Mus*. Several *Mus* autosomes are retained as complete chromosomes or chromosome blocks (e.g. 3, 12, 16 and 19), whereas others have undergone considerable disruption (e.g. 5 and 17). The small-sized chromosome 17 shows extensive fragmentation in all karyotypes, hybridizing to nine regions in the Chinese hamster chromosomes, eight in *R. pumilio*, four in *Otomys irroratus*, five in the black rat (*Rattus rattus*) and six in *Apodemus*. These results suggest that the synteny of mouse chromosome 17 evolved recently. Typically, the X chromosome is conserved across all taxa (e.g. Graves *et al.* 2002). Although intrachromosomal rearrangements usually escape detection by chromosome painting, the pattern shown by several syntenic associations allowed us to detect two inversion events. Thus, the 8/7/8/7 synteny on chromosome 19 of *C. pahari* provides evidence that a paracentric inversion occurred (figure 3*b*). In the same way, the combination 13a/15/13b on chromosome 11 in this same species, which is also present in the *Apodemus*

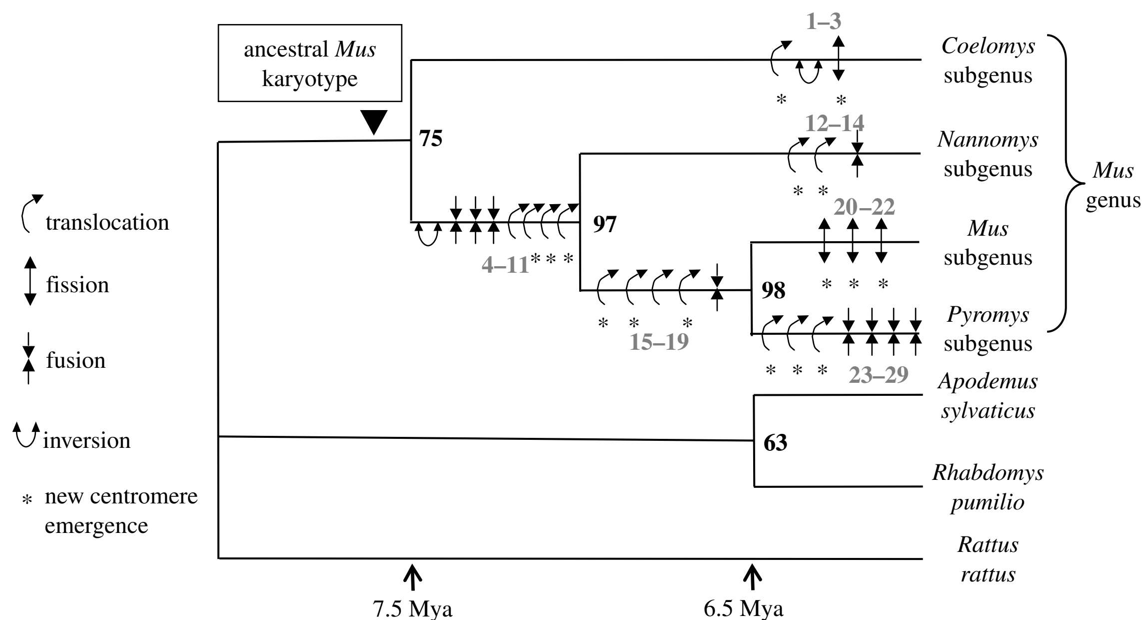

Figure 4. Most parsimonious phylogeny using PAUP, based on the 67 chromosomal characters (electronic supplementary material). Bootstrap values supporting each clade are indicated in bold on nodes. The chromosomal rearrangements, which have occurred within the genus *Mus* are mapped onto the tree and are numbered in grey. Each rearrangement is coded as follows: transl, translocation; inv, inversion; fiss, fission; fus, fusion followed by the character numbers of the table in the electronic supplementary material. Asterisk indicates the emergence of a new centromere. Rearrangements 1–3: transl* + inv 52–53; fiss* 3—rearrangements 4–11: inv 14; fus 16; fus 8; fus 58; transl 11; transl* 60; transl* 10; transl* 2—rearrangements 12–14: transl* 57; transl* 56; fus 59—rearrangements 15–19: transl* 1; transl* 18; transl 5; transl* 4; fus 13—rearrangements 20–22: fiss* 25; fiss* 26; fiss* 58—rearrangements 23–29: transl* 66; transl* 65; transl* 67; fus 64; fus 62; fus 61; fus 63. The divergence times mentioned follow Chevret *et al.* (2005) and Veyrunes *et al.* (2005).

karyotype (Matsubara *et al.* 2004, but not detected in Stanyon *et al.* 2004), suggests that it is the ancestral state, and was subsequently modified by an inversion (13a+b/15) in the lineage leading to three other *Mus* subgenera. The data allow us to reconstruct the likely ancestral karyotype of the genus *Mus*. This was done by mapping changes along the phylogenetic tree (figure 4) and inferring ancestral character states at the different nodes by listing shared syntenies between all ingroup species, or between at least one ingroup and an outgroup. Examples include the widespread associations 7/19, 10/17 and 13/15 found in species of all the investigated genera, the synteny 5/6 also observed in *Rattus* and *Apodemus* and 2/13 in *Cricetulus* and *Rattus*. Finally, the subgenera *Coelomys* and *Nannomys* share synteny 1/4 suggesting that it was present in the recent ancestor of the genus *Mus*. In summary, the ancestral *Mus* karyotype is thought to consist of $2n=46$ acrocentric chromosomes (figure 5). It shares 13 autosomal pairs conserved *in toto* (block or synteny 7/19, 2, 3, 14, 10, 9, 11/5, 18, 2/13, 15, 8, 8, 17/10) with the $2n=54$ ancestral murid karyotype proposed by Stanyon *et al.* (2004). The extensive repatterning of the house mouse karyotype has often led to its exclusion from most interspecific genomic comparisons (e.g. Richard *et al.* 2003; Stanyon *et al.* 2003). In contrast, the more representative *Mus* ancestral karyotype may be a helpful substitute for large-scale comparisons of genome organization.

(c) *Rates of genome reorganization in the genus* Mus

Our analyses provide insight into rates of chromosomal evolution in the genus *Mus*. The chromosomal phylogeny identified 29 rearrangements that have been fixed during the diversification of the genus *Mus*. The subgenus *Coelomys* has a conserved karyotype, which differs from the ancestral one by only three rearrangements, whereas the others have undergone greater genome shuffles, with 11 rearrangements in *Nannomys*, 16 in *Mus* and 20 in *Pyromys* (figure 4). This analysis clearly shows that extensive genome repatterning is not unique to the house mouse karyotype, since only three rearrangements are autapomorphic, but is in fact a characteristic of the *Mus* lineage within the Muridae. In addition, the rearrangements are not randomly distributed along the branches (figure 4). The four subgenera of *Mus* diverged nearly simultaneously within 1 Myr during which nearly half of the rearrangements occurred, representing a rate of 13 mutations per million years. In contrast, as few as 3–7 were subsequently fixed in the terminal branches leading to the four subgenera during the last 6–7 Myr (Chevret *et al.* 2005; Veyrunes *et al.* 2005), yielding a rate range between 0.4 and 1.2 Myr^{-1}. Thus, the pattern of karyotypic evolution exhibits a short phase of intensive diversification followed by a stage with a lower rate of chromosomal change. In *Mus*, this rate acceleration is concomitant with cladogenetic events, i.e. the separation of the four subgenera. Hence, we are tempted to correlate the karyotypic diversification with the speciation events on the basis that such an accumulation of rearrangements may lead to reproductive isolation (e.g. King 1993; Rieseberg 2001; Delneri *et al.* 2003; Olmo 2005). Such data provide additional support for higher rates of chromosomal reorganization in murids compared to other mammalian lineages, which generally display a low rate of chromosome exchange, of the order of 0.1–0.2 mutations per million years, although drastic karyotype reshuffling has also been evidenced in several lineages

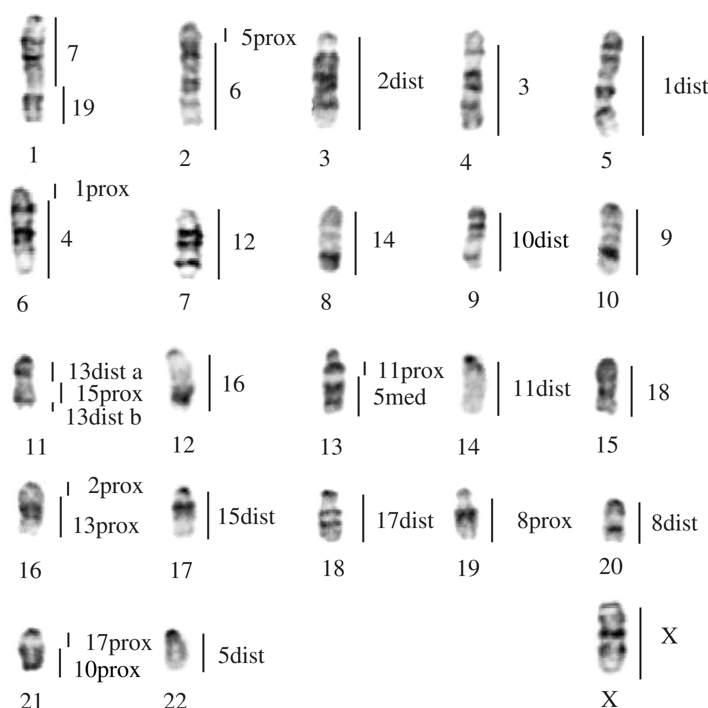

Figure 5. Inferred haploid set of the ancestral karyotype of the genus *Mus* ($2n=46$) reconstructed with *Coelomys*, *Nannomys* and *Mus* G-banded chromosomes. Homology to *Mus* chromosomes is indicated to the right of each putative ancestral chromosome. 'dist', 'med' and 'prox' refer to the distal, median and proximal segment of the chromosome, respectively, and a and b refer to unidentified subchromosomal segments.

(O'Brien *et al.* 1999; Wienberg 2004). However, even within the murids, the evolution of genome structure in the genus *Mus* is remarkably extensive. For example, the 20 mouse paints revealed 37 homologous segments in *R. rattus* (Cavagna *et al.* 2002), which is only slightly higher than in *Coelomys* (equal to 35), but the divergence *Mus*/*Rattus* occurred 11–12 Myr ago, which is twice that estimated between the two subgenera of *Mus* (Chevret *et al.* 2005). Moreover, a chromosome painting survey in *Apodemus*, the only other Eurasian murine genus that matches *Mus* in terms of species diversity and geographic range, reveals the presence of only one translocation plus a few inversions among species belonging to the four major clades (Matsubara *et al.* 2004), even though their diversification was estimated to have occurred prior to the radiation of *Mus* (Michaux *et al.* 2002).

(d) *Modes of genome reorganization in the genus* Mus

The chromosomal changes that have occurred during the *Mus* radiation are mapped onto the branches of the phylogeny (figure 4), and therefore, are *a posteriori* polarized (e.g. fusion versus fission) using the outgroup criterion. Among the 29 rearrangements identified in the genus, the majority are translocations (14), followed by fusions (9) and fissions (4). Very few inversions were identified (2) which is likely due to the painting protocol used (discussed earlier) and would require more refined approaches to be identified (Zhao *et al.* 2004). Although we cannot assess the frequency of inversions in these species, the observed preponderance of translocations is in agreement with recent genome sequence comparisons between human, mouse and/or rat, which indicate that interchromosomal versus intrachromosomal rearrangements are much more frequent in the mouse lineage than in that of the human (Friedman & Hughes 2004) or the rat (Zhao *et al.* 2004). All fusion events identified are tandem and not Robertsonian (i.e. centromere–telomere instead of centromere–centromere fusion), whereas curiously, the *Nannomys* subgenus, and even more so the house mouse are taxa prone to the accumulation of Rb fusions (e.g. Veyrunes *et al.* 2004; Piàlek *et al.* 2005). This suggests that changes in the structure or nature of the centromere may have recently occurred in these lineages allowing a greater frequency of Rb fusions (Redi *et al.* 1990). Segmental translocations and particularly fissions necessitate the appearance of centromeres at new locations (figure 4). The process of emergence of new centromeres remains unclear, and may in fact involve different independent mechanisms, such as reactivation of ancestral latent centromeres, chromosome healing by telomere sequence seeding, or prior segmental duplications of pericentromeric or other sequences (Choo 1997; du Sart *et al.* 1997; Ventura *et al.* 2001, 2003, 2004; Amor *et al.* 2004; Nergadze *et al.* 2004). By tracking chromosomal segments throughout the phylogeny, the nature of these new centromeres can be ascertained. Thus, a minimum of two involve previous centromere locations, and 14 require possible *de novo* acquisition of a centromere (i.e. neocentromerization). Thus, neocentromere formation is apparently a recurrent event during the evolution of *Mus*, mirroring the situation in primates and marsupials (Ventura *et al.* 2004; Ferreri *et al.* 2005). One of the neocentromeres, which was identified, appeared following the break of synteny 5prox/6, the flanking regions of which have been studied by comparative cytogenomic mapping (Walentinsson *et al.* 2001; Thomas *et al.* 2003). Thomas *et al.* (2003) uncovered pericentromeric duplications at this breakpoint, the sequence divergence of which allowed them to date the event at 3–7 Myr ago. By including close relatives of the house mouse in our phylogenetic

framework, we are able to more accurately time the occurrence of this event (i.e. break of the synteny 5prox/6 by the translocation of 5prox onto 5med (character 4; electronic supplementary material); it occurred before the split of the subgenera *Mus* and *Pyromys* dated at 7 Myr (Chevret *et al*. 2005; Veyrunes *et al*. 2005). The two rearrangements involving latent centromere location correspond to: (i) the synteny 1prox/4prox (character 55) which appeared along the branch leading to the genus *Mus*, and was subsequently broken along the branch leading to the *Mus*/*Pyromys* clade (character 1) and (ii) the synteny 5dist/8 (character 58) with fusion along the branch leading to the *Nannomys*/*Mus*/*Pyromys* cluster, and fission in the *Mus* lineage (figure 4). These events are homoplasic, both involving a synteny formation followed by a break further along the tree. These reversals (fusion then fission) suggest that 'fossil' (i.e. latent) centromeres may be a hotspot for breakpoints and centromere reactivation.

For the first time, this study using cross-species chromosome painting allows to resolve the long-standing controversial phylogeny of the genus *Mus*. Conversely, this phylogenetic analysis provides a more accurate assessment of chromosome evolution in the genus. In addition, we highlight chromosomal genomic areas of interest for higher resolution studies (such as gene-mapping, FISH with BACs or *in silico* genome exploration) on sequence composition of neocentromeres and synteny breakpoints and their involvement in restructuring the mouse genome. The advantage of this phylogenetic framework, involving closely related species within *Mus*, is the shorter evolutionary timescale than the human– and/or rat–mouse split, allowing us to trace ancestral sequences at breakpoints, and date the rearrangements and associated segmental duplications more precisely.

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