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# Autosome and sex chromosome diversity among the African pygmy mice, subgenus *Nannomys* (Murinae; *Mus*)

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**Abstract**

The African pygmy mice, subgenus *Nannomys*, constitute the most speciose lineage of the genus *Mus* with 19 recognized species. Although morphologically very similar, they exhibit considerable chromosomal diversity which is here confirmed and extended by the G-banding analysis of 65 mice from West and South Africa. On the basis of their karyotype and distribution area, the specimens were assigned to at least five species. Extensive differentiation both within and between species was observed that involved almost exclusively Robertsonian translocations, 23 of which are newly described. Two of the rearrangements were sex chromosome-autosome translocations, associated in some cases with partial deletions of the X or Y chromosomes. Several authors have predicted that the highly deleterious effect of this rearrangement would be reduced if the sex and autosomal segments were insulated by a block of centromeric heterochromatin. The C-banding analyses performed showed that among the species carrying X-autosome translocations, one followed the expected pattern, while the other did not. In this case, functional isolation of the sex and autosome compartments must involve other repetitive sequences or genomic traits that require further molecular characterization. Such studies will provide insight into the causes and consequences of the high diversity of sex chromosome rearrangements in this subgenus.

## Introduction

The Murinae, the rats and mice of the Old World, comprise almost a quarter of rodent species biodiversity (Musser & Carleton 1993) and occupy a diversity of ecological niches. However, contrary to the famous adaptive radiations of the Galapagos finches (Grant 1981, Sato *et al.* 1999) or *Anolis* lizards in the Caribbean (Losos *et al.* 1998), the evolutionary success of Murinae has not been accompanied by spectacular morphological modifications, making species identification difficult. Cytogenetic studies exemplified by the pioneering work of Matthey (1952, 1959, 1964, 1965, 1966) have revealed extensive inter- and intra-specific karyotypic diversity in rodents

which, in turn, provides an extremely useful tool for identifying and discriminating sibling species. Indeed, in some instances, the taxonomic status of karyologically differentiated populations can be inferred from the level of reproductive isolation predicted by the karyotypes, since chromosomal changes may contribute to the rapid development of an efficient reproductive barrier between taxa (e.g. Britton-Davidian *et al.* 2000, Dobigny *et al.* 2002, Delneri *et al.* 2003, reviews by King 1993, Searle 1993 and Rieseberg 2001).

The African pygmy mice, *Nannomys*, an African subgenus of *Mus* sensu lato (Muridae, Murinae), constitute a group of small-sized rodents (<12 g) that are widespread throughout sub-Saharan Africa (Catzeflis & Denys 1992). They are a complex of morphologically very similar species (Petter 1963, 1981, Macholan 2001), which has led to the description of 5 to 30 species (see Marshall 1981). Most recently, Musser and Carleton (1993) recognized 19 species, making *Nannomys* the most speciose subgenus of *Mus*. In contrast with the highly conserved morphology, chromosomal studies have uncovered extensive karyotypic diversity within this group that includes Robertsonian (Rb) translocations between autosomes, between sex chromosomes and autosomes, tandem fusions, pericentric inversions, heterochromatin additions and even deletion of the sex chromosomes (Matthey 1966, 1970, Jotterand 1970, Jotterand-Bellomo 1984, 1986, 1988). Paradoxically, in spite of this high chromosomal diversity, few G-banding studies have been performed to determine the patterns of chromosomal evolution within this group (Jotterand-Bellomo 1984, 1986, 1988, Aniskin *et al.* 1998, Castiglia *et al.* 2002).

Sex chromosome-autosome translocations are of particular interest in *Nannomys*, since three of them have so far been identified that involve either the X, the Y or both chromosomes in different species (Matthey 1966, Jotterand-Bellomo 1986, 1988). Such diversity is uncommon in mammals, as when X-autosome tranlocations are present the same one is usually retained by all species within a lineage. This is the case in ruminants, e.g. genus *Gazella* and other *Antilopinae* (Vassart *et al.* 1995), *Muntiacus* (Neitzel 1987, Yang *et al.* 1997), bats of the Phyllostomidae family (Tucker 1986, Rodrigues Noronha *et al.* 2001), insectivores, e.g. *Sorex* (Pack *et al.* 1993), carnivores, e.g. *Herpestes* (Fredga 1965, 1972), and rodents, e.g. *Taterillus* (Volobouev & Granjon 1996, Dobigny *et al.* 2002) or *Gerbillus* (Viegas-Péquignot *et al.* 1982, Ratomponirina *et al.* 1986). The low frequency of X-autosome translocations in mammals is associated with their highly deleterious effects on gene expression and gametogenesis due to conflicting inactivation and replication requirements of the sex chromosome and autosome components (King 1993, Ashley 2002, Dobigny *et al.* submitted). To account for the fixation of these unusual chromosomal rearrangements, several authors (Viegas-Péquignot *et al.* 1982, Ratomponirina *et al.* 1986, Jaafar *et al.* 1993, review in Dobigny *et al.* submitted) have proposed that the accumulation of repetitive sequences between the sex and autosomal segments may serve to insulate the two chromosomal arms, thus reducing the disruptive effects of these types of rearrangements.

The aim of this study was to identify the pattern of chromosomal diversity in *Nannomys* from two extremes of their distribution range (West and South Africa) by G-banding analyses. In addition, the distribution of C-bands was studied to test for the presence of intercalary heterochromatic blocks between the sex and autosomal arms in taxa carrying sex-autosome translocations. These data are compared to those previously published on this subgenus as well as other *Mus* species, thus providing insights into the patterns of chromosomal evolution within *Nannomys*.

## Material and methods

A total of 65 specimens of *Nannomys* were karyotyped, all of which were collected by hand after excavation or night drives, or using baited Sherman traps. Sampled localities included agricultural (e.g. bean, rice and potato fields), commensal (e.g. village) or natural savannah and grassland habitats. The origin, sex, diploid and fundamental numbers, morphology of the sex chromosomes and type of chromosomal analysis performed are provided for each individual in Table 1. The geographical distribution of the samples are indicated in Figure 1.

Metaphase spreads were obtained using the air-drying technique (Evans *et al.* 1964) from either bone marrow cells of yeast-stimulated individuals

*Table 1.* Origin, karyotypic data, chromosomal analysis and species assignment of samples.

| Species | Country | Locality | Number and Sex | 2n | FN | Morphology of X | Chromosomal analysis |
|---|---|---|---|---|---|---|---|
| *M. mattheyi* | Burkina Faso | Nazinga, 1 | 4 M | 36 | 36 | acro | st, GBG, CGB |
| | Burkina Faso | Nazinon, 2 | 1 F | 36 | 36 | acro | st, GBG |
| | Burkina Faso | Oursi, 3 | 2 ? | 36 | 36 | acro | st |
| | Mali | Balamansala, 4 | 1 F | 36 | 36 | acro | st |
| | Mali | Farabana, 5 | 5 M, 1 F | 36 | 36 | acro | st, GBG |
| | Mali | Kabakoro, 6 | 1 F | 36 | 36 | acro | st |
| | Mali | Kabalabougou, 7 | 5 M, 1 F | 36 | 36 | acro | st, GBG |
| | Mali | Samaya, 8 | 6 M, 3 F | 36 | 36 | acro | st, GBG, CBG |
| | Mali | Sibi Sibi, 9 | 1 F | 36 | 36 | acro | st, GBG |
| | Senegal | Bandia, 10 | 1 M | 36 | 36 | acro | st, GBG, CGB |
| *M. indutus* | South Africa | Tussen die reviere, 11 | 2 F | 36 | 36 | acro | st, GBG, CBG |
| *M. haussa* | Chad | Karal, 12 | 1 F | 30 | 38 | acro | st |
| | Mali | Ménaka, 13 | 5 M, 1 F | 32–33 | 38 | acro | st, GBG |
| | Niger | Babangata, 14 | 1 F | 32 | 38 | acro | st |
| | Niger | Guileyni, 15 | 1 M, 1 F | 33–34 | 38 | acro | st |
| | Niger | Kollo, 16 | 3 M, 2 F | 31–33 | 38 | acro | st |
| | Senegal | Thiès, 17 | 2 M, 2 F | 28 | 38 | acro | st, GBG, CGB |
| *M. musculoides* | Mali | Diaban, 18 | 1 F | 18 | 36 | (X.7) | st |
| | Mali | Djoliba, 19 | 1 M | 19 | 36 | (X.7) | st |
| | Mali | Samaya, 8 | 3 M, 3 F | 18–19 | 36 | (X.7) | st, GBG, CBG |
| *M. minutoides* | South Africa | Caledon Reserve, 20 | 2 M | 18 | 35 | (X.1) | st, GBG, CBG |
| | South Africa | Kuruman, 21 | 1 F | 34 | 36 | (X.1)/($X_d$.1) | st, GBG, CBG |
| | South Africa | Stellenbosch, 22 | 1 F | 18 | 36 | (X.1) | st, GBG, CBG |

Geographic origin of sampled localities numbered according to Figure 1.
M = male; F = female; ? = unknown; 2n = diploid number; FN = fundamental number; acro = acrocentric; (X.1) = Rb(X.1) Robertsonian translocation between chromosome X and pair 1; (X.7) = Rb(X.7); $X_d$ = deleted X chromosome; st = standard; GBG = G-banding; CBG = C-banding.

(e.g. specimens from Burkina Faso, Mali and Senegal; Lee & Elder, 1980) or fibroblast cultures established after skin biopsy (e.g. specimens from Chad, Niger and South Africa). The diploid (2n) and fundamental number (FN) for each specimen was established by analysis of standard stained preparations. Identification of chromosomes and/or chromosomal arms was determined by G-banding (GBG; Seabright 1971), and karyotypes constructed following the nomenclature of Jotterand-Bellomo (1986). At least five G-banded metaphases were photographed and karyotyped for each individual using a Zeiss photomicroscope equipped with an image analyzer (Cytovision, Applied Imaging). Heterochromatic regions of the genome were revealed by C-banding (CBG; Sumner 1972) performed on previously G-banded slides in one to four individuals per taxon.

The taxonomic assignation of individuals was performed by comparison of karyotypes with published data (Matthey 1966, Jotterand 1972, Jotterand-Bellomo 1986) and/or known distribution area (Matthey 1966, Jotterand 1972, Musser & Carleton 1993). The skulls and skins of all specimens are deposited at the MNHN, Paris (France), the Institut des Sciences de l'Evolution, Montpellier (France), the Department of Zoology, Stellenbosch (South Africa) and the IRD laboratory of Bamako (Mali).

## Results

The chromosomal analysis carried out on the 65 samples of African pygmy mice revealed considerable variation in diploid number ($18 \leqslant 2n \leqslant 36$), and less so in fundamental number ($35 \leqslant FN \leqslant 38$). Homology of G-banded patterns between specimens was established for all chromosomes, although identification of autosome pairs 16 and 17, when involved in rearrangements, requires confirmation by molecular analyses (e.g. fluorescence *in situ* hybridization). In all, six cytotypes were recognized, four of which are newly described.

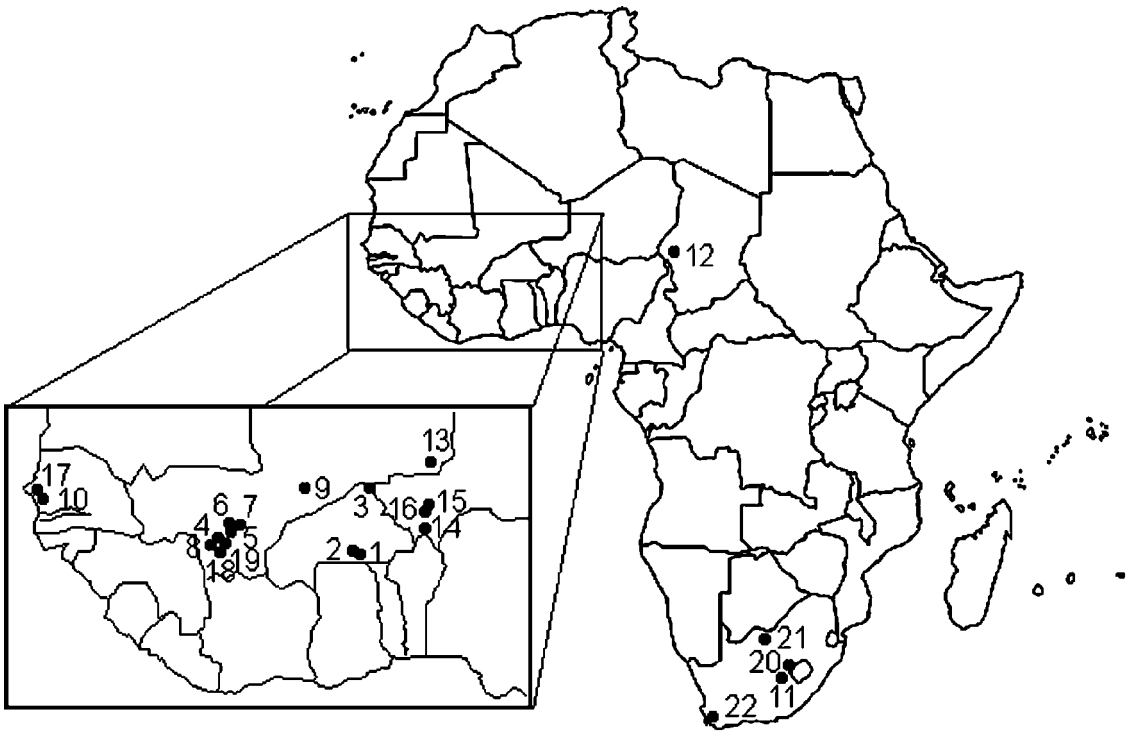


*Figure 1.* Map showing the geographical origin of the samples studied. Locality numbers are indicated in Table 1.

Thirty-four animals from Burkina Faso (7), Mali (24), Senegal (1) and South Africa (2) had a chromosomal formula of $2n = 36$, $FN = 36$, all chromosomes being acrocentric (Figure 2a). The size of the chromosomes varied continuously from large (pairs 1 to 5) to small (pairs 16 and 17). The X chromosome was medium-sized and the Y was approximately two-thirds the size of the X. All chromosomes were free of C-positive heterochromatin even in the centromeric region, except for the Y which was entirely C-band positive (Figure 2b). No differences in G- and C-banding patterns were observed between specimens. The G-banded karyotype was identical to the one published by Jotterand-Bellomo (1986) who assigned the West African cytotype to *Mus* (*Nannomys*) *mattheyi*. The two individuals from Tussen die reviere, South Africa, with the same karyotype, were referred to *Mus indutus*, a species described in South Africa which also possesses 36 acrocentric chromosomes according to Matthey (1966). If confirmed, this would be the first G-banded karyotype published for *M. indutus*.

The four individuals from Thiès in Senegal were characterized by a fundamental number of 38 and a diploid number of 28; the karyotype consisted of nine pairs of acrocentric chromosomes and five pairs of sub-metacentric or metacentric chromosomes. The X and Y chromosomes were medium-sized acrocentrics (Figure 2c). G-banding revealed the presence of four autosomal Robertsonian (Rb) translocations: Rb(2.12), Rb(4.6), Rb(5.7) and Rb(9.10) and a pericentric inversion on chromosome pair 15 that results in an increase of the FN from 36 to 38. The C-banding performed on two animals revealed moderate blocks of centromeric heterochromatin in all of the acrocentric and biarmed chromosomes, including the sex chromosomes (Figure 2d). The pygmy mice from Ménaka, Mali, possessed 32 or 33 chromosomes (two and four individuals respectively) and a $FN = 38$. G-banding (not shown) revealed the presence of three rearrangements which were identical to those in the Senegal specimens: the pericentric inversion of chromosome 15 and the Robertsonian translocations Rb(2.12) and Rb(4.6), the former translocation

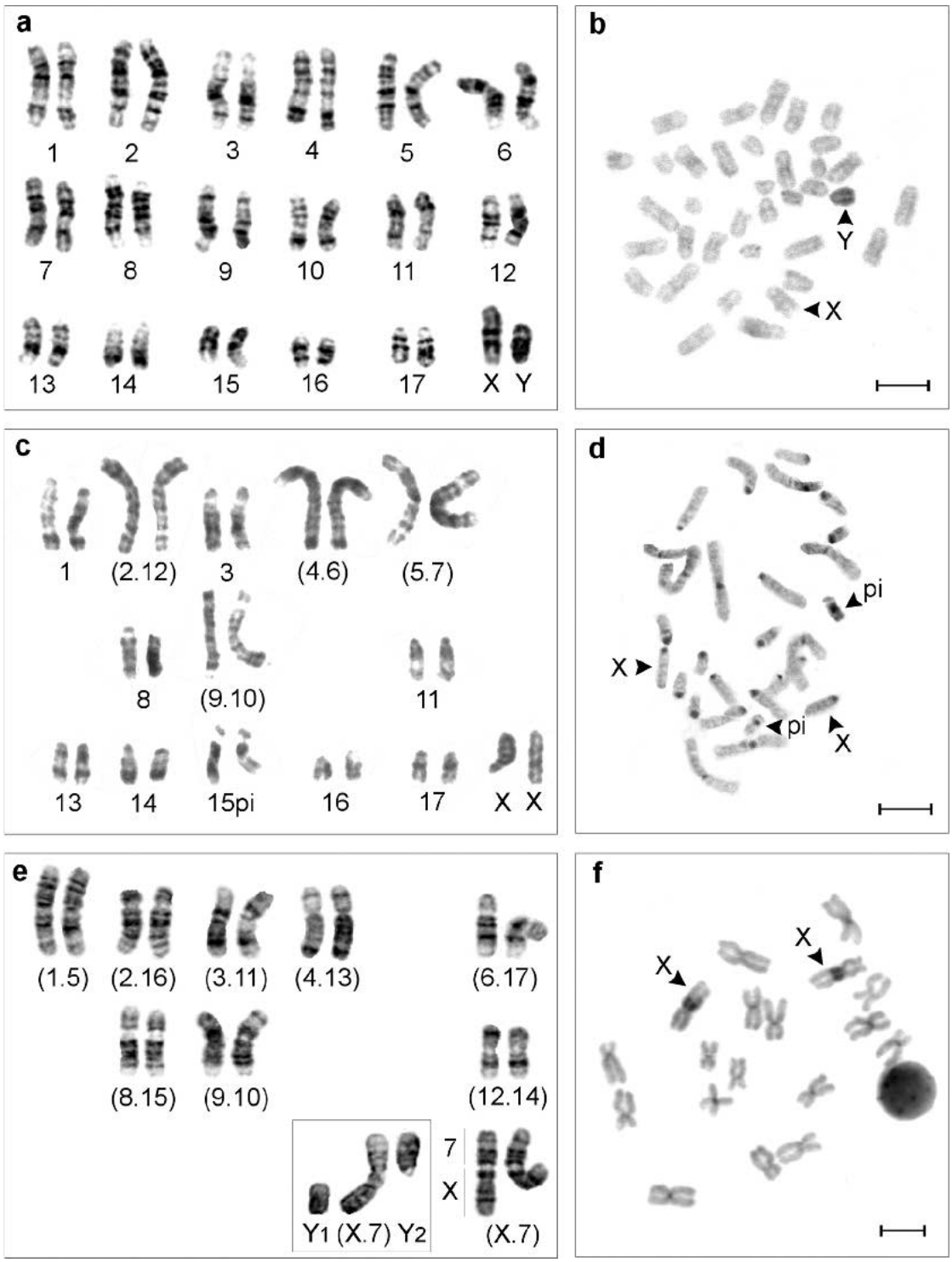


*Figure 2.* (**a**) G-banded karyotype and (**b**) C-banded metaphase of a male *M. mattheyi*. (**c**) G-banded karyotype and (**d**) C-banded metaphase of a female *M. haussa*, pi = pericentric inversion. (**e**) G-banded karyotype (insert: sex chromosomes of a male) and (**f**) C-banded metaphase of a female *M. musculoides*. Arrows indicate sex chromosomes. Scale bars indicate 10 μm.

being heterozygous in the 2n = 33 karyotype. The samples from Niger and Chad showed a diploid number varying from 30 to 34 due to the presence of 6 to 2 biarmed chromosomes respectively. All specimens carried a FN = 38 suggestive of a pericentric inversion which involved a small biarmed chromosome. No G- or C-banding data were available for these mice. All the specimens with a FN = 38 from Senegal, Mali, Niger and Chad were referred to *M. haussa*, a species restricted to the sahelian region and for which Jotterand (1972) described a pericentric inversion involving a small pair of chromosomes and a variation in diploid number between 32 and 34. G-banding patterns represent the first data for this species.

The karyotype of the six animals from Samaya in Mali (plus one male from Djoliba and one female from Diaban, Mali, for which only standard Giemsa stains were available) consisted of 18 and 19 chromosomes in females and males respectively, with a FN = 36 in both sexes (Figure 2e). Except for the Y chromosome which was acrocentric, all chromosomes were biarmed resulting from Robertsonian translocations. Eight of these involved only autosomes, Rb(1.5), Rb(2.16), Rb(3.11), Rb(4.13), Rb(6.17), Rb(8.15), Rb(9.10), Rb(12.14), and one the X chromosome, Rb(X.7). The absence of a fusion involving the Y chromosome led to a $XX/XY_1Y_2$ sex-chromosome system, and thus to the odd diploid number in males (2n = 19). C-banding was performed on a female and showed a large heterochromatic block in the centromeric region of the X chromosome translocated onto chromosome 7, whereas very small heterochromatic blocks were observed in the pericentric regions of most autosomes (Figure 2f). A similar chromosomal configuration (2n = 18–19, FN = 36) was described from Central African Republic and Senegal by Jotterand (1972), who attributed it to the *minutoides/musculoides* species complex. In the absence of a referential G-banded karyotype and considering the distribution area currently assigned to these two species (*minutoides* in South-Eastern Africa, and *musculoides* in Western and Central Africa; Musser & Carleton 1993), we considered this karyotype as representing *M. musculoides*.

The three remaining cytotypes involved specimens from different localities in South Africa. The diploid number ranged from 2n = 18 to 34 due to varying numbers of autosomal Rb translocations; however, they all had in common the same Rb(X.1) translocation but differed in the morphology of the sex chromosomes.

The two males from Caledon Provincial Nature Reserve, South Africa, had 2n = 18 (Figure 3a); all chromosomes were biarmed including both sex chromosomes due to Robertsonian translocations: Rb(2.10), Rb(3.9), Rb(4.7), Rb(5.8), Rb(6.11), Rb(12.17), Rb(13.16), Rb(14.15) and Rb(X/Y.1). The very small size of chromosome 15 in Rb(14.15) suggests that an additional unidentified rearrangement has occurred. Similarly, the chromosomal segment assigned to the Y was extremely reduced in size and was not stained by C-banding (Figure 3b). The X chromosome displayed a sharp C-positive intercalary band, and several of the autosomes possessed weak interstitial heterochromatic bands which were only perceptible after C-banding alone (not shown).

The female from Stellenbosch, South Africa presented a similar chromosomal configuration (2n = 18, FN = 36) (Figure 3c). G-banding of this individual showed that it shared six Rb translocations with the previous specimens: Rb(3.9), Rb(4.7), Rb(5.8), Rb(6.11), Rb(12.17) and Rb(X.1), while the remaining three were unique: Rb(2.13), Rb(10.14) and Rb(15.16). Pair 15 presented the same reduction in size as in the previous cytotype. The X chromosome was heteromorphic, the two forms differing in length and banding pattern. G-banding revealed three main bands on both X chromosomes; however, these bands were of equal intensity on the smallest form, whereas they were spaced wider, with the middle one much darker than the other two, in the larger X. After C-banding, both X chromosomes presented a well-pronounced intercalary band located near the telomere, with an additional less distal band present in the larger form (Figure 3d). Several autosomes possessed weak interstitial heterochromatic bands that were only perceptible after C-banding alone (not shown).

Finally, the karyotype of a female from Kuruman, South Africa, consisted of 2n = 34, FN = 36. It carried a Rb(X.1) translocation, while the remaining autosomes were all acrocentric (Figure 3e). In addition, the two X chromosomes differed in morphology, one being normal-sized, Rb(X.1), while the other was partially deleted,

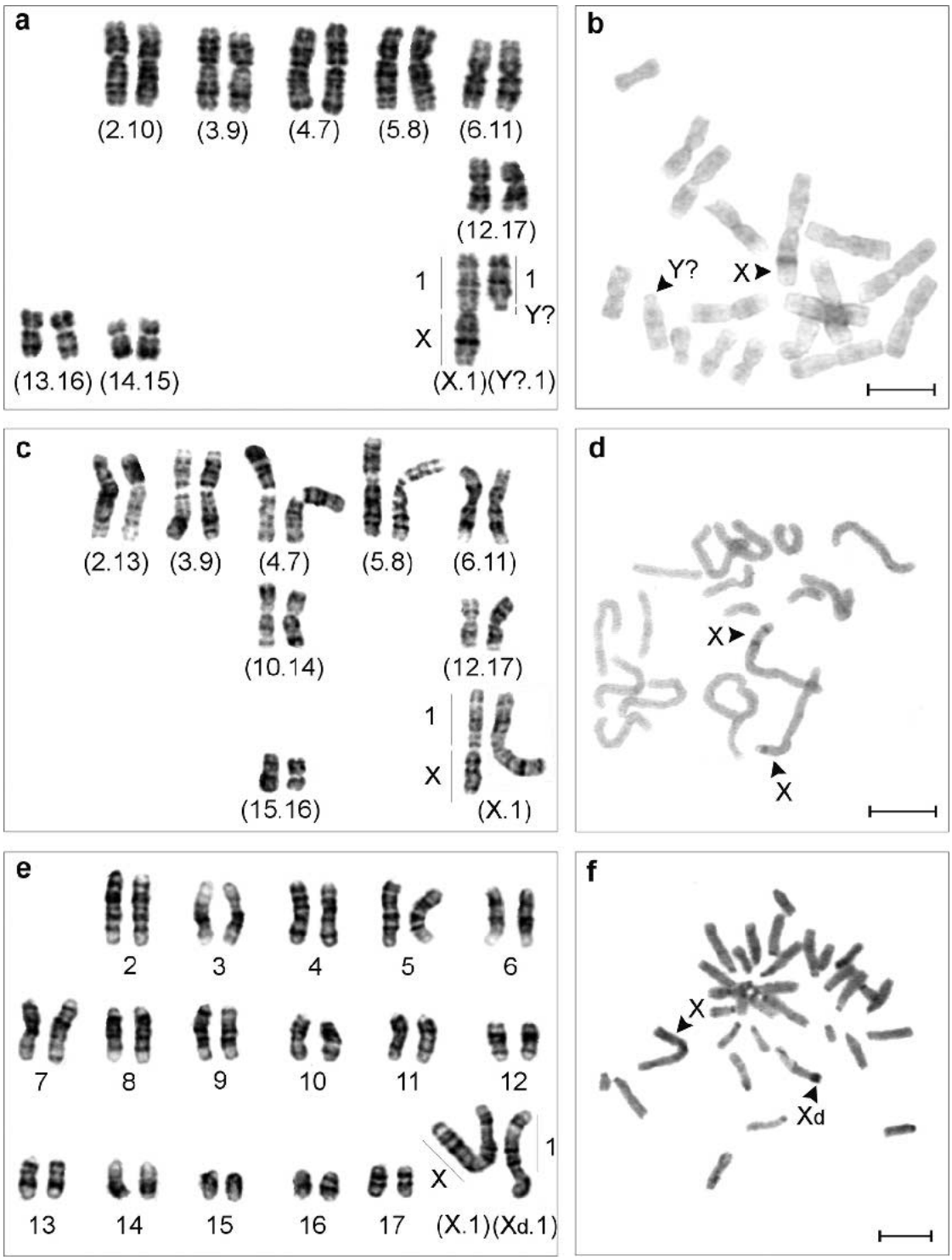


*Figure 3. M. minutoides*: (**a**) G-banded karyotype and (**b**) C-banded metaphase of a male from Caledon. (**c**) G-banded karyotype and (**d**) C-banded metaphase of a female from Stellenbosch. (**e**) G-banded karyotype and (**f**) C-banded metaphase of a female from Kuruman. Arrows indicate sex chromosomes. Scale bars indicate 10 μm.

Rb($X_d$.1). C-banding revealed that the deleted $X_d$ arm was entirely C-positive, whereas the normal-sized X arm was heterochromatin-free. The majority of the autosomal acrocentrics showed diminutive blocks of centromeric heterochromatin (Figure 3f). An identical G-banded karyotype (without the partial deletion of one of the X chromosomes) has been described from Ivory Coast by Jotterand-Bellomo (1986) who assigned this cytotype to the *minutoides/musculoides* complex.

As the last three cytotypes shared the Rb(X.1) translocation which so far has only been described in specimens of the *minutoides/musculoides* complex (Jotterand-Bellomo 1986, Castiglia *et al.* 2002), we have tentatively assigned them to *M. minutoides*, on the basis of the South-Eastern African distribution area provided for this species by Musser & Carleton (1993).

## Discussion

### *Chromosomal diversity*

The chromosomal analysis of *Nannomys* specimens from West and South Africa confirmed the extensive karyotypic diversification detected by Matthey (1966) within the pygmy mice. The G-banding data allowed us to assign the samples to five taxa (*M. mattheyi*, *M. indutus*, *M. haussa*, *M. musculoides* and *M. minutoides;* Table 1) clustered into three major species groups based on their karyotypes and/or type of chromosomal rearrangements. In addition, comparison of the rearrangements differentiating the karyotypes within groups allowed us to infer, in some instances, likely levels of reproductive isolation.

The first group is distinguished by a chromosomal configuration of 2n = 36, FN = 36. This karyotype is shared by *M. mattheyi* and *M. indutus*, and has been reported in the literature for other species such as *M. setulosus*, *M. tenellus*, *M. bufo* and *M. mahomet* (Matthey 1966, Jotterand-Bellomo 1986, Aniskin *et al.* 1998). The G-band karyotypes of the specimens which we assign to this group are identical to those published previously for *M. mattheyi*, *M. bufo* (Jotterand-Bellomo 1986, 1988) and *M. mahomet* (Aniskin *et al.* 1998), whereas differences in C-banding are present between these and *M. setulosus* (Jotterand-Bellomo 1986). This karyotype appears widespread throughout Africa, as it is present in species distributed in West Africa (*M. mattheyi*), Ethiopia (*M. mahomet*), Burundi (*M. bufo*) and Southern Africa (*M. indutus*), and has been regarded as ancestral within the *Nannomys* (Matthey 1966, Jotterand-Bellomo 1984, 1986). However, the phylogenetic relatedness of the 2n = 36 all-acrocentric species remains to be determined by molecular analyses.

The second group comprises specimens with FN = 38 and is assigned to *M. haussa*. All individuals share a pericentric inversion, while the diploid number varies from 2n = 28 to 34, due to varying numbers of autosomal Rb fusions among localities. Such variation in diploid number has also been recorded in specimens from Cameroon (2n = 32–34; Jotterand 1972), and suggests that *M. haussa* may be characterized by a gradual accumulation of Rb fusions throughout its range. This hypothesis is supported by the two Rb fusions shared between the samples from Senegal (2n = 28) and Mali (2n = 33–34), but needs to be confirmed by G-banding for the specimens from the other localities. The existence of Rb chromosomal polymorphism may represent a transitory phase prior to fixation of new Rb fusions, or a hybrid karyotype between chromosomally differentiated populations. Within *Nannomys*, pericentric inversions, although uncommon, have also been reported in *M. oubanguii* (Jotterand-Bellomo 1984).

The third group, represented by the *minutoides/musculoides* complex, comprises taxa in which at least one of the sex chromosomes is translocated onto an autosome, and 0–16 autosomal Rb fusions (aRb) are present. Two X-autosome translocations are found in this group: Rb(X.7) in *M. musculoides* from Mali, and Rb(X.1) in all *M. minutoides* from South Africa. Moreover, no aRb fusions are shared between specimens from these two countries, suggesting a high level of chromosomal divergence within this morphologically undifferentiated species complex. The Rb(X.1) translocation found in the South African specimens has also been recorded in karyotypes of pygmy mice from Central African Republic (CAR), Ivory Coast (Jotterand-Bellomo 1984, 1986) and recently from Zambia (Castiglia *et al.* 2002). The geographically widespread occurrence of this fusion raises the question of their phylogenetic relationship. Sex-autosome

translocations are highly deleterious chromosomal rearrangements in mammals (King 1993, Ashley 2002), and are thus not expected to be prone to convergence (Rokas & Holland 2000). This would suggest that the Rb(X.1) translocation may result from a unique event, in which case all cytotypes carrying this rearrangement would belong to the same phylogenetic lineage, the female from Kuruman representing an ancestral form since only this fusion is present. Subsequent chromosomal differentiation within this lineage would have occurred through fixation of aRb fusions: one in CAR and Ivory Coast (Jotterand-Bellomo 1984, 1986), five in Zambia (Castiglia *et al.* 2002) and eight in South Africa (specimens from Caledon and Stellenbosch). Identification of the chromosomal arms involved in the aRb fusions showed that none of the latter were shared by mice from these different countries, leading to extensive monobrachial (i.e. single arm) homology (Table 2). The effects of aRb translocation heterozygosity on fertility have been intensively studied in several mammalian species, and have shown that the level of gametogenetic disruption in hybrids depended on the nature and number of chromosomes involved. In particular, the presence of a large number of trivalents and/or of meiotic chain configurations involving more than five chromosomes was found to severely impair gametogenesis leading to complete sterility in some cases (Gropp *et al.* 1982, Redi & Garagna *et al.* 1990, Saïd *et al.* 1993, Hauffe & Searle 1998, Castiglia & Capanna 2000). As this would be the case for hybrids resulting from crosses between either of the $2n = 18$ specimens from South Africa and all the other cytotypes (Kuruman, Zambia, CAR and Ivory Coast), these data strongly suggest that they represent two reproductively isolated groups. However, the extent of meiotic disruption within each of these groups is less straightforward, since meiosis would involve a ring of 6 chromosomes in the former group, and few trivalents (0–5) and/or small chains (4–5 chromosomes) in the latter, configurations that lead to variable levels of subfertility in house mice and shrews (Gropp *et al.* 1982, Redi & Capanna 1988, Garagna *et al.* 1990, Mercer *et al.* 1992, Hauffe & Searle 1998, Banaszek *et al.* 2000, Pialek *et al.* 2001). In summary, among the six different cytotypes that have been described within the Rb(X.1) lineage (this study, Jotterand-Bellomo 1986, Castiglia *et al.* 2002), at least two highly reproductively isolated groups are expected, and may represent cryptic species complexes. Molecular analyses are required to extend these observations and to support the phylogenetic relatedness of all Rb(X.1)-carrying taxa. In addition, extensive geographic sampling, particularly in South Africa, is needed to determine the distributional boundaries between related cytotypes and their taxonomic assignment. In particular, the $2n = 18$ specimens from Caledon, South Africa may represent *M. orangiae* described from the Free State, South Africa (Musser & Carleton 1993, Bronner *et al.* 2003), which until recently was considered as a subspecies of *M. minutoides*. Until further systematics studies are performed, we propose that all samples carrying the Rb(X.1) translocation should be assigned to a '*minutoides* species complex', thereby extending its previous western and northern distribution limit from southern Africa to Ivory Coast.

If the systematics of *Nannomys* species is unsatisfactory owing to the lack of discriminating morphological characters, it is further confounded by the sympatric coexistence of similarly-sized species (Jotterand-Bellomo 1988). In Samaya (Mali), two species were collected in the same locality, but in different sites. Thus, *M. musculoides* ($2n = 18$–$19$) was trapped in rice-cultivated areas, and *M. mattheyi* ($2n = 36$) in neighboring sweet potato fields. No hybrids between the two were found. These results stress the diagnostic value of karyotypic traits in this morphologically homogeneous subgenus.

### *Sex chromosome diversity*

In addition to the occurrence of sex-autosome translocations, African pygmy mice show further modifications of sex chromosome morphology. A case in point is the partially deleted and entirely C-positive $X_d$ chromosome arm of the female *M. minutoides* from Kuruman. The occurrence of such chromosomes is not unusual among the *Nannomys*, as similar reports have been described in *M. minutoides/musculoides* from Zambia (Castiglia *et al.* 2002), Ivory Coast (Matthey 1966), and CAR (Jotterand 1972), and in *M. triton* from Congo (Matthey 1967). In all cases, the $X_d$ chromosome is only observed in females

*Table 2.* List of known Rb translocations in *Nannomys*.

| Species | Country (locality) | Chromosome number | | | | | | | | | | | | | | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | X | Y | 1 | 2 | 3 | 4 | 5 | 6 | 8 | 9 | 10 | 12 | 13 | 14 | 15 | |
| *M. haussa* | Senegal (Thiès) | | | | 2.12 | | 4.6 | 5.7 | | | 9.10 | | | | | | this study |
| | Mali (Ménaka) | | | | 2.12* | | 4.6 | | | | | | | | | | this study |
| *M. musculoides* | Mali (Samaya) | X.7 | | 1.5 | 2.16 | 3.11 | 4.13 | | 6.17 | 8.15 | 9.10 | | 12.14 | | | | this study |
| *M. minutoides* | SA (Caledon) | X.1 | | | 2.10 | 3.9 | 4.7 | 5.8 | 6.11 | | | | 12.17 | 13.16 | 14.15 | | this study |
| | SA (Stellenbosch) | X.1 | ? | | 2.13 | 3.9 | 4.7 | 5.8 | 6.11 | | | 10.14 | 12.17 | | | 15.16 | this study |
| | SA (Kuruman) | X.1 | ? | | | | | | | | | | | | | | this study |
| | Zambia | X.1 | Y.1 | | 2.7 | 3.12 | 4.5 | | 6.8 | | 9.16* | | | | | | Castiglia *et al.* 2002 |
| | Ivory Coast | X.1 | Y.1 | | 2.17* | | | | | | | | | | | | Jotterand-Bellomo 1986 |
| | CAR | X.1 | Y.1 | | | 3.7* | | | | | | | | | | | Jotterand-Bellomo 1986 |
| *M. oubanguii* | CAR | X.15 | Y.15 | | | | 4.10 | | | 8.13 | | | | | | | Jotterand-Bellomo 1986 |
| *M. triton* | Burundi | X.12 | | | 2.15* | | | | | | | | | | | | Jotterand-Bellomo 1988 |

? = unknown event; * = polymorphic Rb translocations.

and is always in a heterozygous form, the proportion of $XX_d$ individuals varying from 20% to 60% of the female population. The absence of $X_dX_d$ and $X_dY$ individuals suggests that the latter combination is likely lethal. The evolutionary mechanisms leading to and maintaining this apparently selectively disadvantageous X chromosome form remain unknown. Other rodent species such as *Akodon* show a comparable system (females are XX or XY*), and Bianchi (2002) has shown that XY* females have a larger ovulation rate or a longer reproductive lifespan than XX females, thereby compensating for the loss of YY* embryos. Furthermore, it is of note that partially deleted X chromosomes have so far only been noted in *Nannomys* taxa carrying X-autosome translocations.

Variation in length of the Y chromosome between species is a common feature in mammals, but seems particularly pronounced in African pygmy mice. The Y chromosome, when acrocentric, is slightly larger than the smallest autosome pair (e.g. *M. mattheyi*: this study and Jotterand-Bellomo 1986; *M. setulosus*: Jotterand-Bellomo 1986; $Y_1$ of *M. musculoides*: this study), but may decrease dramatically, becoming the smallest arm of the karyotype (e.g. *M. minutoides*/*musculoides* and *M. oubanguii*: Jotterand-Bellomo 1986; *M. mahomet*: Aniskin *et al.* 1998). An even larger decrease is observed in the diminutive Y of the *M. minutoides* males from Caledon (South Africa), although assigning these sequences to a functional Y chromosome requires molecular confirmation using Y-specific probes, as they showed no $C^+$ staining, contrary to the other species. Such a molecular identification is essential as total deletion of the Y chromosome has been observed in *M. triton* (Jotterand-Bellomo 1988), resulting in a weird sex determination mechanism known for only three other species of mammals in which males and females are XO: two spiny rats species of the genus *Tokudaia* (Sutou *et al.* 2001, Arakawa *et al.* 2002), and the mole vole *Ellobius lutescens* (Just *et al.* 1995, 2002).

*Pattern of autosome and sex chromosome evolution*

The present study supports previous observations that suggest Rb translocations involving both autosomes and sex chromosomes as the most common rearrangement in the karyotypic differentiation of the African pygmy mice. Twenty-four Rb fusions were identified in the present investigation, 23 of which are newly described, raising to 38 the number of known Rb translocations within African pygmy mice (Table 2). All chromosomes were involved in a minimum of two and up to seven (e.g. chromosome 2) distinct Rb fusions. These rearrangements were present in several species, but only one was identical in two of them: Rb(9.10). These results highlight a particularly proneness for autosomal Rb fusions in *Nannomys*, similar to that described in another species of a closely related subgenus of *Mus*, i.e. the well studied *Mus musculus domesticus*. Several authors (Redi *et al.* 1990, Garagna *et al.* 2001) have proposed that the recurrence of aRb fusions in a genome is related to large amounts and high degree of homology of pericentromeric satellite DNA sequences. However, C-banding analyses revealed contrasting results within *Nannomys*. No autosomal pericentromeric C-positive heterochromatin was observed in *M. mattheyi*, *M. indutus* and *M. minutoides* from Caledon and Stellenbosch (this study), and from *M. minutoides*/*musculoides* from CAR and Zambia (Jotterand-Bellomo 1984, Castiglia *et al.* 2002). Additionally, very small heterochromatic blocks in the pericentric regions of most autosomes in *M. haussa*, *M. musculoides*, *M. minutoides* from Kuruman (this study) and *M. oubanguii* (Jotterand-Bellomo 1984) were observed, whereas large amounts were present in *M. setulosus* (Jotterand-Bellomo 1984) and *M. mahomet* (Aniskin *et al.* 1998). Thus, the data for *Nannomys* provide only partial support for these Rb-enhancing molecular requirements, unless these sequences were present initially and subsequently lost.

A prominent feature among the Rb fusions identified in the pygmy mice studied was the presence of two sex-autosome translocations, one of which is newly described (Rb(X.7) in *M. musculoides*). Thus, a total of no less than four different X-autosome Rb fusions are now known in the subgenus (Table 2), making *Nannomys* the lineage of mammals having the greatest diversity of sex-autosome translocations known so far. Such rearrangements are known to have a highly deleterious effect on gene expression and gametogenesis in mammals, due to the differential inactivation and

replication requirements of the X and autosome genomes (King 1993, Ashley 2002, Dobigny *et al.* submitted). The existence of several X-autosome fusions in *Nannomys* suggests that specific genomic traits allowing a higher rate of appearance and/or fixation of this rearrangement may be present within this subgenus. Several authors (Viegas-Péquignot *et al.* 1982, Ratomponirina *et al.* 1986, Jaafar *et al.* 1993, Dobigny *et al.* submitted) have proposed that the presence of a large heterochromatic block between the original X and the translocated autosome may represent such a feature. This would serve as a boundary preventing X-inactivation from spreading to the autosomal segment, and would also allow an independent regulation of replication timing on both the sexual and autosomal arms (reviewed in Dobigny *et al.* submitted). The pathological consequences of such a rearrangement will thus be reduced, increasing the viability and fertility of the individuals carrying it. Support for this hypothesis is found in most of the mammal species known to possess sex-autosome translocations, in which a large amount of centromeric heterochromatin located between the autosomal and sexual segments has been observed (Viegas-Péquignot *et al.* 1982, Tucker 1986, Ratomponirina *et al.* 1986, Jaafar *et al.* 1993, Yang *et al.* 1997, Metcalfe *et al.* 1998, Rodrigues Noronha *et al.* 2001, Dobigny *et al.* 2002, submitted). The C-banding analyses performed in *Nannomys* showed that, whereas no centromeric heterochromatin was present on the acrocentric X chromosomes of *M. mattheyi* and *indutus*, and only a small amount in *M. haussa*, a large pericentromeric block was evident on the X chromosome arm of Rb(X.7) in *M. musculoides*. However, this was not the case for the *M. minutoides* taxa carrying Rb(X.1), since no $C^+$ staining was observed on the "normal" X arm of the sample from Kuruman, and only interstitial ones in those from Caledon and Stellenbosch. Similar observations were made in the Rb(X.1)-carrying specimens from CAR and Zambia (Jotterand-Bellomo 1984, Castiglia *et al.* 2002). So, while results for *M. musculoides* follow the expected pattern for X-autosome translocations, those for *M. minutoides* clearly do not. However, Castiglia *et al.* (2002) reported the presence of centromeric telomeric sequences on both the Rb(X.1) and Rb(Y.1) chromosomes of the samples from Zambia. In addition, in the European shrew *Sorex araneus*, a heterochromatic C-negative but late-replicating block was observed between the sexual and autosomal segments (in Dobigny *et al.* submitted). Therefore, isolation of the sex and autosome genomic compartments in *M. minutoides* may involve other types of repetitive sequences, such as telomeres and rDNA clusters (Parish *et al.* 2002), or genomic characteristics specific to chromosome 1 such as a low density of LINE-1 elements, as they appear to act as booster elements of the X-inactivation process (Bailey *et al.* 2000, Parish *et al.* 2002). This study has revealed a high diversity of rearrangements involving the sex chromosomes in the African pygmy mice, which may be unique among mammals, and undoubtedly makes this subgenus a choice model for investigating the evolution of sex determination mechanisms.

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