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A novel sex determination system in a close relative of the house mouse

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Therian mammals have an extremely conserved XX/XY sex determination system. A limited number of mammal species have, however, evolved to escape convention and present aberrant sex chromosome complements. In this study, we identified a new case of atypical sex determination in the African pygmy mouse *Mus minutoides*, a close evolutionary relative of the house mouse. The pygmy mouse is characterized by a very high proportion of XY females (74%, $n = 27$) from geographically widespread Southern and Eastern African populations. Sequencing of the high mobility group domain of the mammalian sex determining gene *Sry*, and karyological analyses using fluorescence *in situ* hybridization and G-banding data, suggest that the sex reversal is most probably not owing to a mutation of *Sry*, but rather to a chromosomal rearrangement on the X chromosome. In effect, two morphologically different X chromosomes were identified, one of which, designated X*, is invariably associated with sex-reversed females. The asterisk designates the still unknown mutation converting X*Y individuals into females. Although relatively still unexplored, such an atypical sex chromosome system offers a unique opportunity to unravel new genetic interactions involved in the initiation of sex determination in mammals.

Keywords: African pygmy mouse; *Mus minutoides*; atypical sex determination system; sex-reversed females; *Sry* gene; X* chromosome

1. INTRODUCTION

Sex poses some of the most fascinating questions in evolutionary biology. Many eukaryotic organisms have separate males and females, but sex is determined by many different strategies, even among closely related species (for a review in fish, see Volff & Schartl 2001; vertebrates, Ezaz *et al.* 2006; Diptera, Saccone *et al.* 2002). By contrast, mammals, or at least therians (i.e. placentals and marsupials) have an extremely conserved sex determination system (for the egg-laying monotremes see Veyrunes *et al.* 2008). Therian sex determination has a unique origin and is syngamic, meaning that sex is fixed by the presence of the sex chromosome complement at fertilization, and not influenced by environmental factors (e.g. temperature, hormones, social structure). In effect, females have two X chromosomes (XX) and males have a single X and a Y chromosome (XY), and variation of this XX/XY dichotomy generally leads to sterility. Mammalian sex is in fact regulated by a single gene on the Y chromosome:

SRY (Sex-determining Region of the Y chromosome). The *SRY* gene is at the top of the gene activity cascade which triggers testis formation and thus male development (Sinclair *et al.* 1990). In the absence of this gene, gonads develop into ovaries. It has generally been assumed that heteromorphic sex chromosomes with a highly degraded and specialized Y chromosome, such as that found in mammals, are a strong barrier to transitions between sex-determining systems. This is owing to formation of lethal genotypes (such as YY) and the potential adverse effects of Y-chromosome genes on the feminization process of neo-XY females or, conversely, the lack of necessary Y-chromosome genes in neo-XX males (Bull 1983; Marin & Baker 1998). A limited number of mammal species do, however, escape this constraint and present atypical sex chromosome systems (see excellent reviews by Fredga 1983, 1988, 1994). So far, these unusual sex-determining systems have been unambiguously identified by molecular or cytogenomic methods in seven genera, all rodents, which group in four categories: (i) XX or XY females and XY males in the lemmings *Myopus schisticolor* and *Dicrostonyx torquatus* (Fredga *et al.* 1976; Fredga 1983, 1988, 1994), as well as

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several species of the South-American field mouse *Akodon* sp. (Hoekstra & Edwards 2000; Bianchi 2002; Ortiz *et al.* 2009); (ii) XO females (only one X) and XY males in the vole *Microtus oregoni* (Ohno *et al.* 1963, 1966; Fredga 1983); (iii) females and males with an identical XO karyotype (loss of the second X chromosome in females and of the Y in males) in the Japanese spiny rats *Tokudaia osimensis* and *Tokudaia tokunoshimensis* (Soullier *et al.* 1998; Sutou *et al.* 2001; Arakawa *et al.* 2002), and the mole vole *Ellobius lutescens* (Matthey 1953; Just *et al.* 1995, 2007), and finally; (iv) XX males and females in two other species of *Ellobius*, *E. tancrei* and *E. talpinus* (Just *et al.* 1995, 2007). Categories (iii) and (iv) are remarkable since sex determination occurs in the absence of a Y chromosome, and even without the *Sry* gene.

In this study we report a new atypical sex determination system, this time in the African pygmy mouse *Mus minutoides*. The pygmy mouse is one of the smallest African mammals (adult weight = 4–6 g), but with one of the widest sub-Saharan distributions (Veyrunes *et al.* 2005). It is included in the same genus as the house mouse, *Mus musculus*, but is assigned to a different subgenus, *Nannomys* (Musser & Carleton 2005; Veyrunes *et al.* 2005, 2006). *Mus minutoides* presents an extreme example of karyotypic diversity owing largely to Robertsonian fusion variation, but more unexpectedly to rare chromosomal changes involving the sex chromosomes (Veyrunes *et al.* 2004, 2007). Here, we show that this species possesses a very high proportion of sex-reversed females, that is, individuals that are phenotypically female and fertile, but with a male karyotype. Thus, females are either XX or XY, males being XY. This represents the fourth report of such an unusual sex chromosome system within mammals (see above, category (i)), and given its close phylogenetic association to the emblematic *M. musculus*, may provide valuable insights into the complex initiation of sex determination in therian mammals.

2. MATERIAL AND METHODS

(a) Approach

In the 1960s, several reports described the occurrence of heteromorphic X chromosomes in populations of *Nannomys*, i.e. in *M. minutoides* from Ivory Coast and the Central African Republic and in *M. triton* from Congo (Matthey 1967, 1970; Jotterand 1972). Some females possessed one normal-sized X chromosome, while the other was two-thirds smaller and entirely heterochromatic, being similar in size and staining properties to a typical Y chromosome. The authors considered this chromosome as a partially deleted X, named X_d . Recently, Castiglia *et al.* (2002) and Veyrunes *et al.* (2004), respectively, described in *M. minutoides*, two females (from Mutanda, Zambia) and one female (from Kuruman, South Africa) with heteromorphic sex chromosomes, designated as XX_d in accordance with the previous literature. To further critically investigate the nature of the X_d chromosome, an analysis of the *M. minutoides* sex chromosomes was performed using cytogenetic (G-banding), cytogenomic (fluorescence *in situ* hybridization) and molecular (DNA sequencing) analyses.

Table 1. List of the sampling localities with the number of males and females of each sex chromosome complement.

localities	females			males XY
	X*Y	XX*	XX	
Belfast	0	0	1	0
Caledon NR	11	0	0	10
Kuruman	1	0	0	1
Mutanda	2	0	0	1
Sandveld NR	1	1	0	3
Stellenbosch	3	2	2	3
Sterkfontein NR	2	0	0	1
Willem Pretorius NR	0	0	1	0
total	20	3	4	19

(b) Sampling

A total of 46 wild-caught specimens from eight localities of Southern and Eastern Africa were used in this study: Caledon Nature Reserve (NR), Stellenbosch, Sandveld NR, Sterkfontein NR, Kuruman, Belfast and Willem Pretorius NR from South Africa, and Mutanda from Zambia (table 1). The geographical distribution of the localities is indicated in figure 1. Since several species of African pygmy mice may coexist in sympatry, the assignment of all specimens to *M. minutoides* was established by comparison with published karyotypes and/or cytochrome *b* sequences (Veyrunes *et al.* 2004, 2005). Additionally, we expanded our sample to include specimens of *Mus mattheyi*, *Mus famulus* and *Mus crociduroides* for the analysis of *Sry* (see below).

(c) Karyotype and chromosome identifications

Chromosome preparations of all specimens were made either from bone marrow of yeast-stimulated animals (Lee & Elder 1980) or fibroblast cell-cultures established from skin biopsy following standard procedures. Identification of chromosomes was accomplished by G-banding (Seabright 1971). Several karyotypes of specimens used in this study have previously been published (Castiglia *et al.* 2002; Veyrunes *et al.* 2004, 2007).

(d) Fluorescence *in situ* hybridization

The commercial (Cambio) Y chromosome-specific painting probe of the house mouse *M. musculus* was hybridized to metaphase spreads of *M. minutoides* females with heteromorphic sex chromosomes from Kuruman (1 specimen) and Caledon NR (2 specimens). Hybridization and detection followed a slightly modified procedure from that described in Robinson *et al.* (2004). The Y chromosome probe (50 ng) was made up to 11 μ l with hybridization buffer (50% deionized formamide, 10% dextran sulfate, $2 \times$ SSC, 0.5 mol l^{-1} phosphate buffer, pH 7.3, and $1 \times$ Denhardt's solution). The slides were pretreated with 10 mM HCl containing 0.01 per cent pepsin at 37°C for 5 min. The probe was denatured at 65°C for 10 min and then preannealed at 37°C for 30 min. Metaphase slides were denatured by incubation in 70 per cent formamide/30 per cent $2 \times$ SSC solution at 65°C for 2 min, quenched in ice-cold 70 per cent ethanol, and dehydrated through a 70, 80, 90 and 100 per cent ethanol series. The pre-annealed paint was applied to slides, and incubated for 24 h at 37°C . Post-hybridization washes involved two successive 5 min incubations at 42°C in

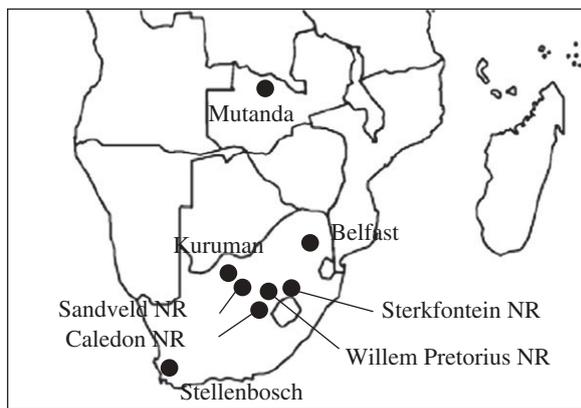


Figure 1. Map of Southern Africa showing the geographical origin of the samples studied.

50 per cent formamide/1 × SSC, 2 × SSC, and one in 4XT (250 μl Tween in 50 ml 4 × SSC). Biotin-labelled probe was visualized using Cy3-avidin (1:500 dilution, Amersham). Slides were mounted in Vectashield mounting medium with DAPI (Vector Laboratories), and images captured using the GENUS software (Applied Imaging).

(e) *Sry* gene sequencing and phylogenetic reconstruction

Total genomic DNA was extracted from tissues preserved in ethanol using a QiaAmp DNA extraction kit (Qiagen). A 472-bp region of the *Sry* gene, beginning 103 bp 5' to the highly conserved 79-amino-acid high mobility group (HMG) box, was amplified from genomic DNA using two oligonucleotide primers (SRY1: 5'-AGATCTTGATTTTT AGTGTTTC-3' and SRY2: 5'-GAGTACAGGTGTGCA GCTCTA-3') as described in Lundrigan & Tucker (1994). Polymerase chain reaction (PCR) parameters were: one step at 94°C for 5 min, followed by 35 cycles (30 s at 94°C, 30 s at 50°C, 1 min at 72°C). The final extension at the end of the profile was at 72°C for 10 min. Double-stranded PCR products were purified from agarose gel using Amicon Ultrafree-DNA columns (Millipore), and both strands were sequenced directly using an automatic sequencer ABI 310 (PE Applied Biosystems) or sent to Cogenics for sequencing. New *Sry* sequences obtained for *M. minutoides* (27), *M. mattheyi*, *M. famulus* and *M. crociduroides* were deposited in the EMBL data bank under the accession numbers FN568 495 to FN568 524. Additional sequences were retrieved from GenBank and added to the alignment: *M. minutoides* (AY057 745), *M. musculus* (MMU03 645), *Mus cervicolor* (L29 548) and *Mus pahari* (L29 543). Sequences were aligned using SEAVIEW (Galtier *et al.* 1996). The maximum likelihood (ML) phylogeny was reconstructed with PHYML (Guindon & Gascuel 2003) under a GTR + G + I model of sequence evolution as specified by the Akaike Information Criterion criteria implemented in MODELTEST v. 3.06 (Posada & Crandall 1998), and 100 ML bootstrap replicates were performed on the resulting topology.

3. RESULTS AND DISCUSSION

(a) Phenotypic versus karyotypic sex

Phenotypic sex of the 46 specimens analysed herein (table 1) was identified by examination of gonad morphology, yielding 27 females and 19 males. All specimens were then karyotyped by G-banding. Our

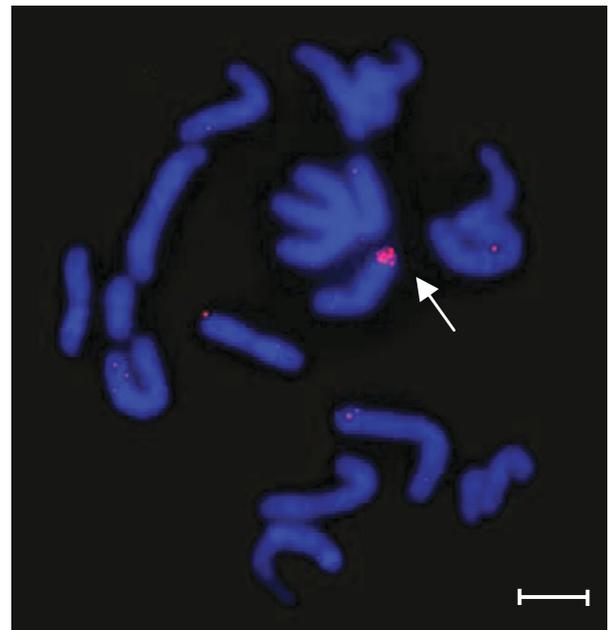


Figure 2. Fluorescence *in situ* hybridization using a house mouse Y chromosome painting probe on a *Mus minutoides* XX_d female from Caledon NR. The strong signal on the X_d arm (arrow) indicates that this female is in fact XY. Scale bar, 10 μm.

analysis included published data from Mutanda ($2n = 24-25$; Castiglia *et al.* 2002), Caledon NR ($2n = 18$; Veyrunes *et al.* 2004, 2007), Stellenbosch ($2n = 18$; Veyrunes *et al.* 2004) and Kuruman ($2n = 34$; Veyrunes *et al.* 2004), that we expanded with additional samples from Caledon NR, Stellenbosch and new localities where G-banded karyotypes were determined for the first time: Belfast and Sandveld NR ($2n = 34$, similar to the karyotype of Kuruman), Sterkfontein NR ($2n = 18$, similar to the one of Caledon NR), and finally, Willem Pretorius NR ($2n = 26$). The difference among karyotypes results from variation in the number and combination of Robertsonian fusions (i.e. fusion by the centromere of two non-homologous chromosomes), but all specimens have two fusions in common, those involving the sex chromosomes and pair 1, (X.1) and (Y.1), which represent a diagnostic signature for this species (Veyrunes *et al.* 2004, 2005).

The karyotypic analyses identified the presence of a small, atypical heterochromatic X chromosome in 20 of the 27 females, named X_d according to Matthey (1967) and subsequently followed in recent studies (Castiglia *et al.* 2002; Veyrunes *et al.* 2004). The X_d chromosome varied from medium-sized in the Mutanda and Kuruman localities to minute in the Caledon NR and Stellenbosch localities, but was invariant within populations. The observation that the X_d arm always matched the size of the Y arm in a given population (see Veyrunes *et al.* 2004), prompted our efforts to critically re-examine the derivation of this chromosome. Fluorescence *in situ* hybridization of the mouse Y chromosome painting probe on metaphase spreads resulted in a strong signal covering the entire X_d arm (figure 2), indicating unambiguously that X_d is in fact a Y chromosome and had been incorrectly assigned to an X chromosome in previous studies. The females carrying this derivative

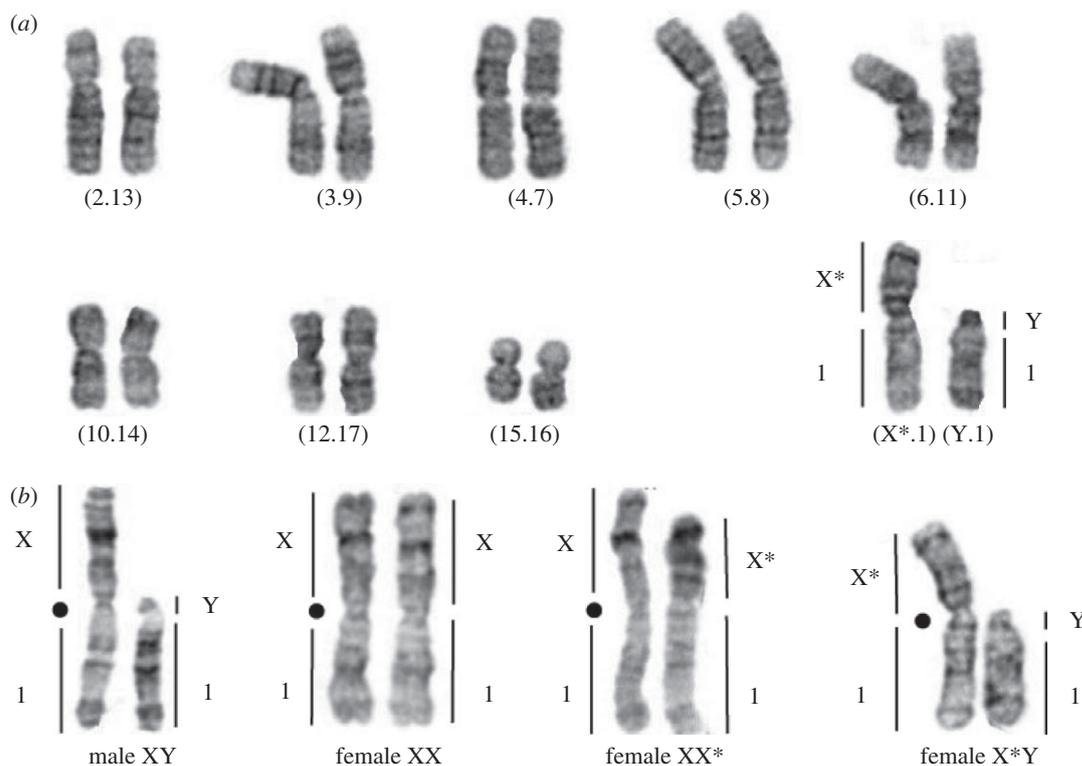


Figure 3. (a) G-banded karyotype of an X*Y female from Stellenbosch, the numbers in parentheses designate the chromosome pairs involved in the Robertsonian fusions. (b) The different sex chromosome complements (X, X* and Y chromosomes fused to autosome pair 1); black dots indicate centromere position.

chromosome are therefore XY females (figure 3a). Remarkably, all the females ($n = 11$) from the Caledon NR population were diagnosed as being sex-reversed (table 1).

(b) *Sry* sequence in *M. minutoides*

An obvious explanation for the pattern of sex-reversal outlined above would involve the male-promoting gene, *Sry*. In effect, since the Y chromosome is present in the females, we hypothesized that the *Sry* gene was either deleted in these specimens or, if present, was not expressed. Given that the window for *Sry* expression in the mouse is extremely narrow (a few hours during embryogenesis; Hacker *et al.* 1995; Bullejos & Koopman 2001), we chose an indirect approach to test the integrity/functionality of the *Sry* gene in sex-reversed females. Our decision to target 472 bp of the *Sry* gene (whole HMG domain and part of the flanking regions) in 27 individuals (18 XY females and nine males), was based on the observation that most *SRY* mutations leading to sex-reversal in humans have fallen within the HMG box (O'Neill & O'Neill 1999). Surprisingly, the sequencing results revealed that the gene was present in XY females (but not in XX females), and that there was no evidence of deletion or nucleotide substitution that would result in frameshift or stop codon mutations. In fact, the sequences were identical between males and XY females of a given population (with the exception of Mutanda; figure 4). The *Sry* sequences were clustered into four haplotypes corresponding to the different cytotypes: one shared by all specimens analysed with 18 chromosomes, i.e. Stellenbosch, Sterkfontein NR and Caledon NR (14 females; six males); one shared by all specimens with 34

chromosomes, i.e. Kuruman and Sandveld NR (two females; two males); one for the two females from Mutanda ($2n = 24-25$); and one specific to the male of Mutanda ($2n = 25$). Within *M. minutoides*, the bootstrap values supporting clades are extremely low since only one or two nucleotide substitutions characterize each haplotype (figure 4). Although Nagamine (1994) reported the presence of several Y-specific *Sry* copies in *Mus*, including *M. minutoides*, no ambiguities were recorded in the PCR products or sequences in our study, suggesting that either the gene copies were identical over the sequenced region, or the PCR primers were specific for only the functional copy (see also Lundrigan & Tucker 1994; Lundrigan *et al.* 2002). These results suggest that the *Sry* gene, or at least the HMG box is intact (mutations outside the analysed region, such as promoter sequences, have not been considered), and is thus potentially functional in both sexes. In light of these results, therefore, the sex reversal mechanism in *M. minutoides* does not appear to involve an *Sry* mutation.

(c) *Sex-reversal mutation in M. minutoides*

In addition to the presence of a Y chromosome (and *Sry* gene) in some of the females, the G-banding data detected two X chromosome morphs that differ in length and banding pattern: one that is similar to other pygmy mouse species (Veyrunes *et al.* 2004), designated as X herein and which is always associated with XY males, and a second, always associated with XY females that we termed X* (figure 3b). The difference in banding pattern and size between the two morphs suggests that a complex rearrangement has occurred possibly involving a deletion and an inversion. When the phenotypic sex

was compared to the genotypic sex established from karyotypes, three types of females were identified: XX, XX* and X*Y; whereas all males were XY (figure 3b). Of the 27 females examined in our study, four were XX, three were XX* and 20 were X*Y females from geographically widespread populations (table 1). There was one exception to this: both the male and sex-reversed female from Kuruman possessed the same X chromosome morph, which was slightly longer, and differed in banding pattern from the X and X* chromosomes of the other specimens (data not shown). This inconsistency could be attributed to an independent event in this lineage, or to a further rearrangement on the X chromosome. We also identified a pregnant X*Y female (from Stellenbosch) carrying five embryos, all but one of which were karyotyped, and sexed according to their sex chromosome complement. This pregnancy comprised two X*Y females, one XX* female, and one XY male, suggesting that X*Y females are fertile in marked contrast to pathological sex-reversal in humans, where the affected individuals are invariably sterile (e.g. McElreavey & Fellous 1999).

These data allowed us to infer the presence of a mutation on the X* chromosome that prevents masculinisation of X*Y specimens. Although, it might appear counterintuitive that the mutation causing male-to-female sex reversal is on the X chromosome and not the Y, the X is known to have accumulated a disproportionate number of genes controlling sex and reproductive traits, and even genes expressed in spermatogonia (Saifi & Chandra 1999; Wang *et al.* 2001; Graves *et al.* 2002). For example, over-expression of the *DAX1* gene on the X chromosome appears to over-ride *SRY*, and is associated with male-to-female sex reversal in humans (Bardoni *et al.* 1994). It is noteworthy that similar, atypical sex determination systems have been described in three other rodent genera (see §1). The genetic bases of these modifications are all unknown, but are associated with an X-linked mutation in *Myopus* (deletion of Xp²¹⁻²³; Liu *et al.* 1998), and possibly so too in *Dicrostonyx* and *Akodon* (Fredga 1988; Ortiz *et al.* 2009). Although these mutations have appeared independently in the four different lineages (even the two lemmings are not closely related; Buzan *et al.* 2008), they are all X-linked, and may therefore involve the same gene(s). Clearly, further molecular/genomic analyses are required in *M. minutoides* to identify the mutational event changing the normal X chromosome into the derivative X*. Once the rearranged synteny block is identified, it should be possible to pinpoint sex-reversal candidate genes by comparison to the mouse genome assembly.

(d) Evolution of sex determination in *M. minutoides*

The question remains of the selective pressures favouring the establishment of the atypical sexual system in pygmy mice given the reproductive cost associated with X*Y females (loss of YY embryos, i.e. 25% lethality). Several evolutionary mechanisms for compensating for the loss of the YY embryos have been identified in the literature. In effect, the X*Y females may bypass this reproductive disadvantage by meiotic drive (i.e. preferential transmission of the X* in gametes). This is particularly evident with the meiotic double non-disjunction that

occurs in *M. schisticolor* in which only X*-carrying ova are formed, producing only XX* and X*Y females, thus avoiding embryo loss (Fredga 1983). Additionally X*Y females may compensate for foetal loss through higher reproductive efficiency, as is the case in *Akodon azarae* where X*Y females have both a longer reproductive lifespan and higher rates of preimplantation embryonic development compared to XX females (Espinosa & Vitullo 2001). Although constrained by sample size, the pregnant X*Y female in our analysis provided a preliminary clue to its ability to establish: (i) no litter size reduction was evident, since five embryos were formed, marginally higher than the documented mean litter size of four (Willan & Meester 1978); and (ii) the presence of a XY male offspring does not support a *Myopus*-like double non-disjunction mechanism. An additional feature of the X*Y female sex system is an anticipated biased sex-ratio since the XX* and X*Y females are theoretically expected to produce 75 and 66 per cent female offspring, respectively. Skewed sex-ratios such as these may be under selection and contribute to evolutionary changes in sex determination systems (e.g. Werren & Beukeboom 1998; Uller *et al.* 2007; Vuilleumier *et al.* 2007). For example, in the two gregarious and polygamous lemming species *M. schisticolor* and *D. torquatus*, cycles of high densities followed by population crashes (boom and bust scenario) have been recorded, suggesting that the production of an excess of females may represent an adaptive strategy for rapid recovery from low densities. Coincidentally, surveys of *M. minutoides* populations have shown similarly seasonal population cycles (Monadjem 1999; Fichet-Calvet *et al.* 2009).

(e) How pervasive is this sex determining system within African pygmy mice?

By extension, our results suggest that all females previously described with heteromorphic X chromosomes in *M. minutoides* from Ivory Coast, the Central African Republic and even in a population of *M. triton* from Congo may in fact be X*Y females (Matthey 1967, 1970; Jotterand 1972). If confirmed, *M. minutoides* X*Y females would not be restricted to Southern and Eastern Africa, but would be widespread throughout sub-Saharan Africa, and would extend to related species. Additionally, the apparently unique situation in mice collected from the Caledon NR population deserves closer scrutiny. All females analysed from this population ($n = 11$) were identified as X*Y (table 1). If this unexpected result is confirmed by additional sampling, it would mean that the XX and X*X females are no longer produced or viable (and that half of the embryos produced are lethal). A sex chromosome system such as this, where all females are X*Y and males XY, has never been described, and would lead to a unique mechanism in which the sex determining switch would no longer be determined by the Y chromosome, but by the presence of a modified X chromosome (either X or X*). Interestingly, Jotterand-Bellomo (1988) reported a further sex chromosome system in a population of *M. triton* from Burundi, where both males and females are XO, mimicking the situation in *E. lutescens* and *T. osimensis* (see §1). Consequently, there are at least two sex determining systems in the African pygmy mice: the standard XX/

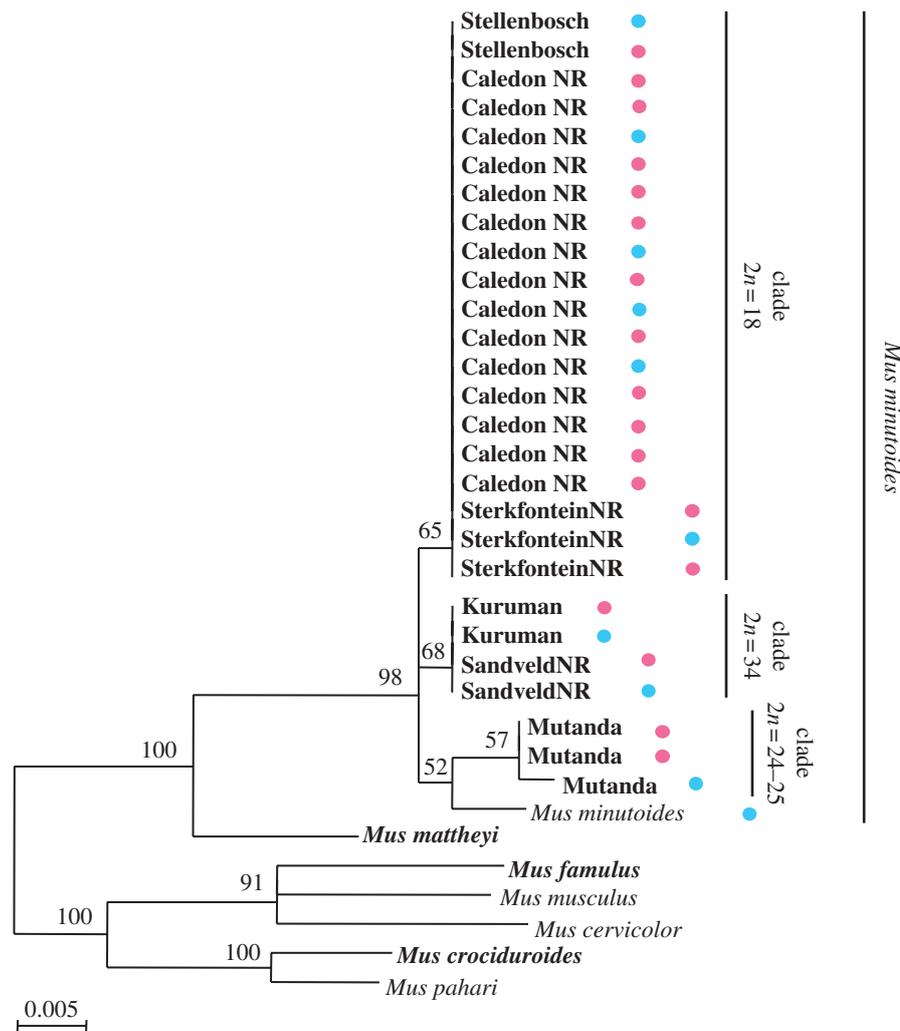


Figure 4. Maximum likelihood phylogeny using the 472 bp sequences of the *Sry* gene. The samples in bold are those sequenced in this study. Bootstrap values supporting each clade are indicated on nodes. Within *Mus minutoides*, specimens are characterized by their locality of capture. Blue and pink dots represent males and X*Y females, respectively. $2n$ = diploid number.

XY and the one described in the present study, and maybe two more if the extraordinary systems of *M. minutoides* from Caledon NR and *M. triton*, respectively X*Y/XY and XO/XO, are confirmed. Whereas modification of sex determination is extremely rare in mammals, and is usually associated with a significant decrease in fitness, our study confirms that the African pygmy mice have an unprecedented predisposition to accumulate chromosomal rearrangements involving sex chromosomes. This makes them an excellent model for investigating the evolution of mammalian sex determination and the evolutionary modification of sex chromosomes (Veyrunes *et al.* 2007).

4. CONCLUSIONS

Notwithstanding the accumulation of karyotypic data and the considerable advances that have been made in genomic/cytogenetic methods, the present study reports, to our knowledge, the first instance of a new atypical sex determination system recorded in a therian mammal in almost 30 years. *Mus minutoides* is one of a few species that escapes the constraints of the conventional mammalian XX/XY system. It shows a widespread occurrence and high prevalence (74%, $n = 27$) of fertile sex-reversed

females. Atypical sex determination systems in mammals are poorly studied. The genes involved in the mutations, the mechanisms of expression and regulation of the genes, and/or organization of the genome are completely unknown, owing in a large part to a lack of knowledge on the complexity of the standard sex determination pathway in mammals itself. For example, still remarkably little is known about the mode of action and identity of the downstream genes involved in sex determination (see reviews by Brennan & Capel 2004; Sekido & Lovell-Badge 2009). However, in return, the study of the atypical sex determination systems discussed herein, and evidenced by the pygmy mice, may provide valuable clues to better understanding standard mammalian sex determination. In effect, most of the major advances in the field (including the identification of *SRY*) have come from the analysis of variant sex determination systems and pathological sex reversal cases in humans and mice (McElreavey & Fellous 1999; Vaiman & Pailhoux 2000; Camerino *et al.* 2006). As a result, the finding of an unusual sex determining system in a close phylogenetic relative of the house mouse, an index species for biomedical research, raises the possibility that comparative genomic approaches may provide novel insights into sex determination in mammals in general, and the

identification of candidate genes involved in the pathological reversal of sex in humans in particular.

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