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# $XY_1Y_2$ chromosome system in *Salinomys delicatus* (Rodentia, Cricetidae)

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**Abstract** *Salinomys delicatus* is considered a rare species due to its restricted and patchy distribution, poor records and low abundances. It is also the phyllotine with the lowest known diploid chromosome number (2n = 18), however its sex chromosome system has never been described. Here, we studied the chromosomes of six females and three males with bands G, C, DAPI/$CMA_3$ and meiosis. In males, the chromosome number was 2n = 19, with one large metacentric X-chromosome and two medium-sized acrocentrics absent in females. The karyotype of females was the same as previously described (2n = 18, FN = 32), with X-chromosomes being metacentric and the largest elements of the complement. In males, the two acrocentrics and the large metacentric form a trivalent in meiotic prophase. This indicates that *S. delicatus* has $XY_1Y_2$ sex chromosomes, which is confirmed by G and DAPI bands. Constitutive heterochromatin (CH) is restricted to small pericentromeric blocks in all chromosomes. The X-chromosome shows the largest block of centromeric CH, which could favor the establishment of this X-autosome translocation. This sex chromosome system is rare in mammals and, compared with other phyllotine rodents, *S. delicatus* seems to have undergone a major chromosome restructuring during its karyotypic evolution.

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## Introduction

The delicate mouse, *Salinomys delicatus*, is considered a rare species due to its restricted and patchy distribution, few records and low population abundances (Lanzone et al. 2005; Rodríguez et al. 2008). It is a small phyllotine rodent mainly associated with desert salt-marsh environments in the temperate Monte desert and Monte–Chaco ecotonal areas of Argentina (Braun and Mares 1995; Ojeda et al. 2001). This rodent has developed important ecophysiological specializations for survival in xeric environments, such as elongated renal papillae as well as one of the highest renal indices and urine concentrations among other world desert rodents (Díaz and Ojeda 1999).

*Salinomys* is a monotypic genus and possesses the lowest diploid number known for the Phyllotini tribe. Its chromosome complement was described in one female from the northernmost locality of its distributional range (Catamarca Province in Argentina) as 2n = 18. All chromosomes in its karyotype were biarmed and the fundamental number was 32; but the sex chromosomes were never identified (Lanzone et al. 2005).

The sex chromosome system in mammals is highly conserved. Males usually have the sex chromosomes XY and females have XX. Among these chromosomes, the gene content of the Xs is virtually identical in several unrelated placental mammals such as mice and humans (Marshall Graves 2006). However, exceptions from this general pattern have been discovered in several orders of mammals for few species, or groups of species, that are phylogenetically closely related (Fredga 1970).

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One of the most studied modifications of this general pattern is the occurrence of multiple sex chromosomes. These sex systems usually result from the translocations between one of the sex chromosomes and one or more autosomes. When one autosome is translocated to the X-chromosome (X-autosome translocation), a $XY_1Y_2$ system results, whereas when the Y-chromosome is involved in the rearrangement (Y-autosome translocation), a $X_1X_2Y$ system arises. Both types of sex chromosomes determination systems can be identified because, in the first case, males have an extra chromosome when compared to the female chromosome complement, with the opposite being observed in the other case (Fredga 1970).

The occurrence of multiple sex chromosomes system is a rare event in mammals. It is associated with the deleterious effect observed in rearrangements involving sex chromosomes that undergo spontaneous mutations and in laboratory conditions (Ashley 2002; Dobigny et al. 2004). However, organisms with multiple sex chromosomes in stable conditions can avoid these deleterious effects, because they transmit the sex chromosomes in a balanced way, and there are no negative effects on fertility.

In this study, we report the presence of a multiple sex chromosome system in *S. delicatus* and analyze the morphology of these sex chromosomes and their meiotic behaviour.

## Materials and methods

We studied nine specimens of *Salinomys delicatus* (six females and three males) from two localities from Mendoza Province in the central part of Argentina: Laguna del Rosario (32°9′17.62″S; 68°14′25.73″W, 532 masl) and Estancia El Tapón (33°06.2′S; 67°12.3′W; 463 masl). Chromosomal preparations of specimens were obtained using the standard hypotonic technique for bone-marrow (Ford and Hamerton 1956, with modifications) and stained with Giemsa. We also performed G banding (Seabright 1971), C banding (Sumner 1972), DAPI/$CMA_3$ staining (Schweizer 1980, with modifications) and studies of male meiosis (Evans et al. 1964).

## Results

In *S. delicatus* males, the chromosome number was 2n = 19, with two large submetacentric autosomal pairs and six small biarmed pairs, a single large metacentric and two medium acrocentrics of different sizes (Fig. 1a). In females, the karyotype was identical to that previously described (2n = 18/FN = 32; Lanzone et al. 2005), with Xs chromosomes being large metacentrics (Fig. 1b). In fact, in *S. delicates*, X-chromosomes are the largest elements of the complement.

Our results show that *S. delicatus* has a multiple sex chromosome system $XY_1Y_2$, which is confirmed by G and DAPI banding (Fig. 1c, d). According to these bandings, the $Y_1$ showed a homogeneous staining pattern after treatment with trypsin (Fig. 1c) and is the most brilliant element of the complement when stained with DAPI (Fig. 1d), thus corresponding to the Y chromosome. The $Y_2$, however, showed three bands (resistant to trypsin and DAPI positive) that are similar to a single arm of the X chromosome, thus corresponding to the unfused homologous partner of the autosome translocated to the X (Fig. 1c, d).

Constitutive heterochromatin (CH) was restricted almost exclusively to small pericentromeric blocks (Fig. 1b, g). The X is the one with the largest block of CH in this region, which is DAPI negative/$CMA_3$ positive (Fig. 1e, f), with $Y_1$ being almost completely heterochromatic (Fig. 1g).

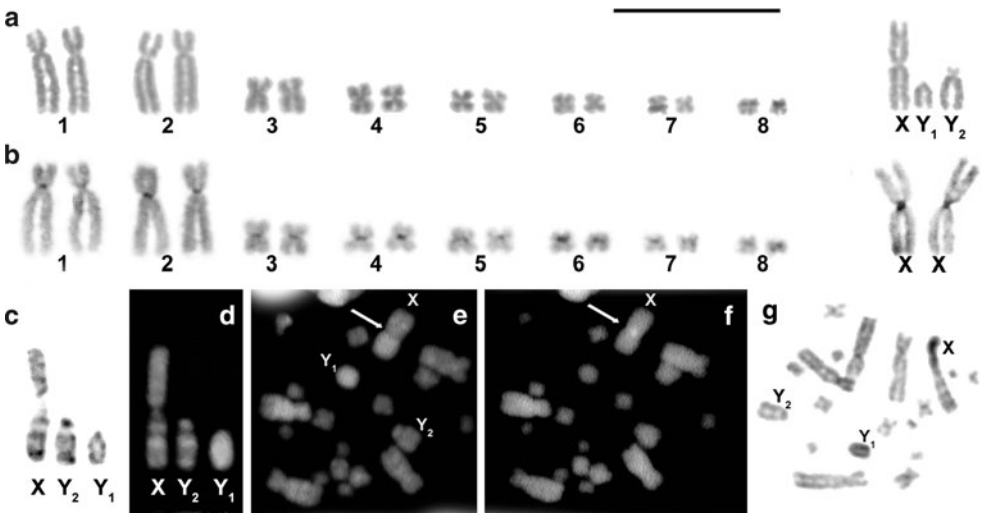


**Fig. 1** Mitosis in *S. delicatus*. **a** Giemsa-stained karyotype of a male. **b** C-bands of female karyotype. **c** G-bands of male sex chromosomes. **d** Detail of DAPI banding pattern in male sex chromosomes. **e**, **f** Sequential DAPI/$CMA_3$ staining respectively, of male mitotic chromosomes. *Arrows* indicate the DAPI-negative and $CMA_3$-positive blocks in the X-chromosome. **g** C-bands in male. *Bar* corresponds to 10 μm

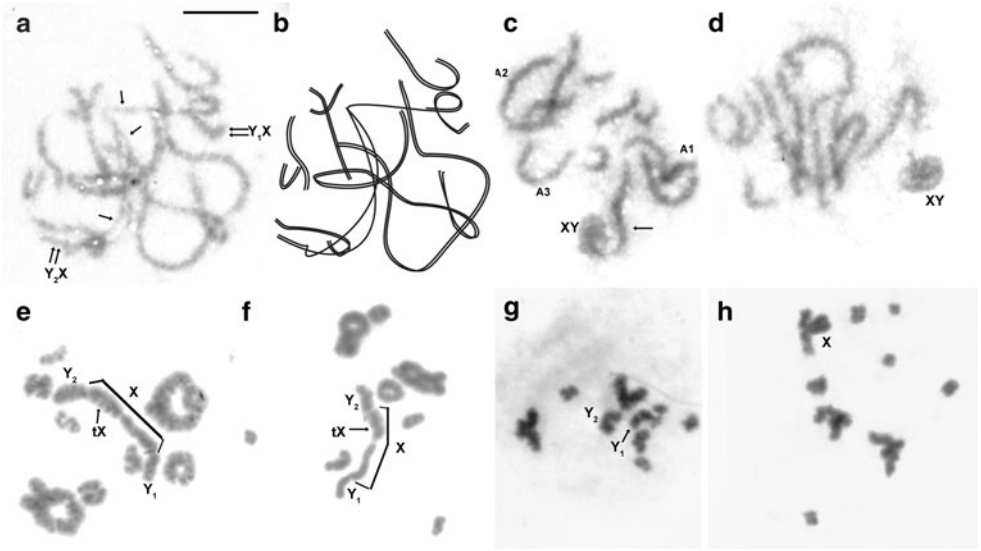


**Fig. 2** Male meiosis. **a**–**d** Pachytene cells. **a** Early pachytene showing the sex trivalent paired regions at the ends (*double arrows*) and a large unpaired internal region (*single arrows*) **b** Schematic representation of the pachytene cell shown in (**a**). **c** XY body continuous with an autosomal region (*arrow*). **d** Advanced pachytene in which the entire sex trivalent is included in the sex body. **e**, **f** Diakinesis cells. Note the difference in condensation between the original arm of the X-chromosome and tX arm (tX = translocated arm of the X-chromosome). **g**, **h** Metaphase II cells with 10 (**g**) and 9 (**h**) chromosomes. *Bar* corresponds to 10 μm

In male meiosis, cells in pachytene (N = 114) were classified in early (N = 12), middle (N = 68) and late (N = 34) substage, in accordance to the degree of bivalents condensation and the sex chromosome differentiation (Lanzone et al. 2002). Early pachytene was the least frequent substage and can be recognized by the lack of differentiation of sex chromosomes and low condensation of the bivalents (Fig. 2a). In this substage, few cells (N = 2/12) showed an initial delay in pairing of the sex trivalent (Fig. 2a, b). The middle pachytene was the most frequent substage (N = 68/114), beginning with the progressive differentiation of the sex body and the shortening of the bivalents. In middle pachytene, the sex chromosomes form the sex body characteristic of mammals, which continues with a region of pairing with autosomal behaviour (Fig. 2c). In few cells (N = 3/68), a small unpaired region can be observed in the union between the sex body and the region with autosomal behaviour. Finally, in late pachytene, the sex body includes all of the translocated autosomal region inside (Fig. 2d).

At diakinesis, the sex chromosomes form a trivalent ($XY_1Y_2$), with $Y_1$ and $Y_2$ connected by a distal chiasma to opposite ends of X. This trivalent has a differential condensation, with one part having autosomal behaviour and another one with a typical mammal sex chromosomes condensation, evidenced by a more homogeneous staining (Fig. 2e, f). In metaphase II, cells with n = 10 ($Y_1 + Y_2$) and with n = 9 (X) were observed in the same frequency (N = 20), indicating a normal segregation of the sex trivalent during anaphase I (Fig. 2g, h).

## Discussion

In mammals, sex-autosome fusions are considered one of the most deleterious chromosomal rearrangements, and as such, are considered rare events (Ashley 2002). However, the occurrence of multiple sex chromosome systems due to rearrangements among sex chromosomes and autosomes has been described in natural populations of some species of several orders such as: Insectivora, Chiroptera, Artiodactyla and Rodentia (Fredga 1970; Ashley 2002). Furthermore, multiple sex chromosome systems often occur in more than one genus within a family, as seen in bats and gazelles (Ashley 2002). In rodents, X-autosome translocations have been described for species of *Gerbillus*, *Tatterillus* and *Mus* (*Nannomys*) genus (Fredga 1970; Dobigny et al. 2004; Veyrunes et al. 2004, 2007). X-autosome translocations have also been identified in other rodents such as mole-rats (Deuve et al. 2006), and also in lemmings, i.e. *Dicrostonyx torquatus* (Fredga 1988).

The first known sigmodontine rodent with multiple sex chromosomes was *Deltamys kempi*, with $X_1X_2Y$ due to a Y-autosome translocation (Sbalqueiro et al. 1984). Therefore, *S. delicatus* is the second sigmodontine with multiple neo-sex chromosomes, but the first with $XY_1Y_2$ due to an X-autosome translocation. Both species belong to a monotypic genus, have restricted geographic distributions and present low abundances (Castro et al. 1991; Braun and Mares 1995; D'Elía et al. 2003; Lanzone et al. 2005). Also, it is highly feasible that these population attributes may

have contributed to the establishment of very unusual chromosome rearrangements in these rodents.

Some authors have proposed that the deleterious effects of these rearrangements may be avoided by the addition of CH blocks or repetitive sequences that isolate the autosomal and ancestral sexual components of the X-chromosome (Dobigny et al. 2004). The centromeric CH block in the X-chromosome of *S. delicatus* supports this hypothesis and thus could have allowed the establishment of this rearrangement.

The homology of $Y_2$ with a single arm of the X and the autosomal behaviour of the translocated region on pachytene and diakinesis suggest a recent origin for this rearrangement. However, the observation of a delay in pairing (although at a low frequency) and the presence of only distal chiasma suggest restriction to genetic exchange at least in the translocated region, which comprises the pericentromeric and interstitial region of neo-sex chromosomes. This restriction in recombination can occur due to two causes: (a) a lack of homology for principles of genetic degeneration in the neo-Y chromosomes (Marshall Graves 2006), which can only be detected with high resolution chromosome banding, or (b) suppression of the recombination due to mechanical interference during meiosis, or the distortion of the original starting points of pairing and homologue recognition during early meiosis (Davisson and Akeson 1993).

According to molecular data, the sister-genus of *Salinomys* is *Andalgalomys* (Steppan et al. 2007). *A. pearsoni* is the phyllotine with the highest known diploid number ($2n = 78$; Olds et al. 1987) eventhough there are other species of the same genus with $2n = 60$ (*A. olrogi* and *A. roigi*; Mares and Braun 1996). This suggests that this phylogenetic clade of *Andalgalomys* and *Salinomys* went through a major karyotypic restructuring during their evolution. Also, the presence of sex-autosome translocations often define lineages, as observed in bats and gazelles (Ashley 2002). In *Andalgalomys,* males and females have the same diploid number without the presence of multiple sex chromosomes; but these chromosome characterizations were based on few specimens, without chromosome banding or meiotic studies (Olds et al. 1987; Mares and Braun 1996). Because of this, the presence of other multiple sex chromosome systems in *Andalgalomys* should not be ruled out.

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