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Sex chromosomes, sex determination, and sex-linked sequences in Microtidae

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Abstract. The Arvicolidae is a widely distributed rodent group with several interesting characteristics in their sex chromosomes. Here, we summarize the actual knowledge of some of these characteristics. This mammalian group has species with abnormal sex determination systems. In fact, some species present the same karyotype in both males and females, with total absence of a Y chromosome, and hence of SRY and ZFY genes. Other species present fertile, sex-reversed XY females, generally due to mutations affecting X chromosomes. Furthermore, in *Microtus oregoni* males and females are gonosomic mosaic (the females are XO in the soma and XX in the germ cells, while the males are XY in the soma and OY in the germ cells). Regarding sex chromosomes, some species present enlarged (giant) sex chromosomes because of the presence of large blocks of constitutive heterochromatin, which have been demonstrated to be highly heterogeneous. Furthermore, we also consider the alterations affecting composition and localization of sex-linked genes or repeated sequences. Finally, this rodent group includes species with synaptic and asynaptic sex chromosomes. In fact, several species with asynaptic sex chromosomes have been described. It is interesting to note that within the genus *Microtus* both types of sex chromosomes are present.

The origin of the rodent group Arvicolidae could be in Northern or Central Asia followed by an expansion throughout the Northern hemisphere. They appear in fossil records in early to middle Pliocene in Europe and Asia and in early Pliocene in North America (Carleton and Musser, 1984). The subfamily Arvicolidae includes approximately 125 species widely distributed throughout the Northern hemisphere in a wide range of ecological habitats. Arvicolid rodents are karyotypically one of the most polymorphic groups of mammals studied, with the genus *Microtus* showing the highest rate of karyotypic change (Maruyama and Imai, 1981). Sex chromosomes and sex determination in some species of Arvicolidae present some characteristic features that make it a very interesting group to study sex determination mechanisms and sex chromosome structure, composition, pairing and evolution.

Sex chromosomes

It is assumed that the ancestral karyotype of the group of arvicolid rodents was 2n = 56 chromosomes (27 autosomal pairs plus the sex chromosome pair) (Matthey, 1973). The features of the primitive karyotype are high chromosome number, acrocentric morphology of autosomes and sex chromosomes, presence of large centromeric C-bands in autosomes and X chromosomes and entirely heterochromatic Y chromosomes (Modi, 1987a, b). According to this, the karyotype of several species may be considered as ancestral, e.g. *Microtus nivalis* (Burgos et al., 1988b), *M. richardsoni, M. mexicanus* and *M. agrestis* (Modi, 1987a) (Fig. 1). The morphology of both sex chromosomes can vary in Arvicolidae from acrocentric to metacentric. However, the most interesting feature of the sex chromosome pair is their ability to add large blocks of heterochromatin. In fact, in the genus *Microtus* five species *(M. agrestis, M. cabrerae, M. chrotorrhinus, M. epiroticus* and *M. transcaspicus)* present enlarged sex chromosomes (named as giant sex

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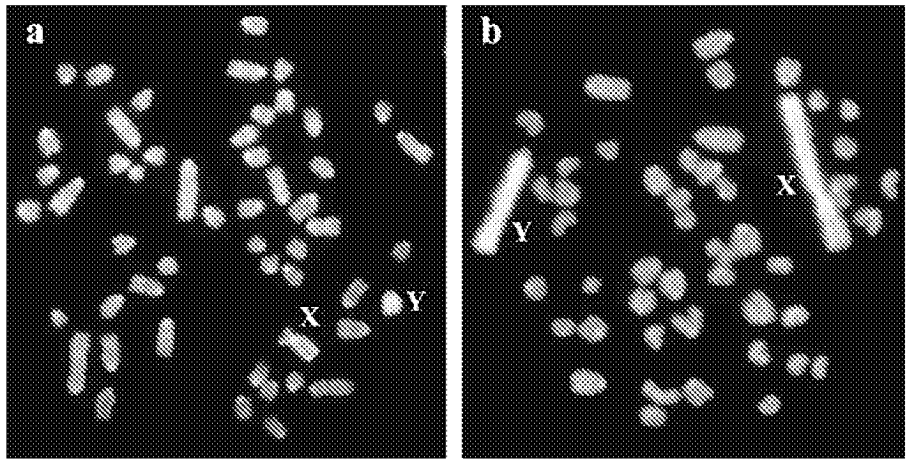

Fig. 1. DAPI-stained male chromosomes from the species *Microtus nivalis* (**a**) and *Microtus agrestis* (**b**). Note that in **a** all the chromosomes are acrocentric with the exception of the X chromosome, which characterizes the primitive condition of this karyotype. In **b** note the enlarged (giant) sex chromosomes of *Microtus agrestis*, which have large heterochromatic blocks.

chromosomes) and seven others show different extents of heterochromatin on their sex chromosomes (Modi, 1987b). These giant X chromosomes do not follow Ohno's rule (1967), that is, they do not comprise 5% of the genome. Thus, the X chromosome of *M. agrestis*, the longest X chromosome found in *Microtus* species, represents approximately 20% of the genome (Nanda et al., 1988) (Fig. 1b).

Conventional cytogenetic analysis (C- and G-banding and fluorochrome staining) has demonstrated that the heterochromatin of giant sex chromosomes of *Microtus* species is highly heterogeneous (Burgos et al., 1988c, 1990; Modi, 1993a). In fact, the existence of six different types of heterochromatin has been demonstrated in the sex chromosomes of *M. cabrerae*, four on the X chromosome and two on the Y chromosome (Burgos et al., 1988c, 1990; Modi 1993a). Also in this species Burgos et al. (1988c) described a length polymorphism due to deletion mutations affecting the heterochromatic block of both X and Y chromosomes. According to this polymorphism these authors described three different X chromosomes (the standard X: X^s, and two deleted X chromosomes: X^{d1} and X^{d2}) and five different Y chromosomes (Y^1, Y^2, Y^3, Y^4, Y^5), with Y^1 the biggest and Y^5 the smallest.

Furthermore, some populations of *M. agrestis* restricted to the southwestern parts of Sweden present the so called Lund Y chromosome, which is characterized by a longer short arm as compared with the standard Y chromosome. The Lund Y chromosome has arisen through a pericentric inversion in the standard Y chromosome (Fredga and Jaarola, 1997).

Sex determination mechanisms

In mammalian species, including rodents, the Y chromosome is male determining. Females are the homogametic sex with the sex chromosome constitution XX while males are heterogametic with XY. Exceptions from this general rule have been described in several species of the Arvicolinae group such as *Dicrostonyx torquatus, Ellobius lutescens, E. tancrey, Microtus cabrerae, M. oregoni* and *Myopus schisticolor* (see Fredga, 1994).

Dicrostonyx torquatus

In *Dicrostonyx torquatus* females have XX, XX* and X*Y karyotypes while males have the normal XY karyotype (Gileva and Chebotar, 1979). This system occurs due to a postulated, still unknown, X-linked mutation (X*) which prevents male development in the presence of the Y chromosome.

Microtus cabrerae

In a population of the vole *Microtus cabrerae* the presence of two XY fertile females has been described (Burgos et al., 1988a). These XY females exhibited variation in the length of the Y chromosome due to variation affecting the heterochromatic block. Breeding in laboratory demonstrated that these XY females could give rise to normal XX females, normal XY males, and in addition to XY females. A Y chromosome mutation was postulated as being responsible for this sex reversal (Burgos et al., 1988a). Unfortunately since 1988 no such XY females have been trapped again, even in the same population where they were described, and due to the absence of genomic DNA, molecular characterization of this sex reversal condition was not possible. A hypothesis is that a mutation occurred in the Y chromosome which caused sex reversal in some individuals of the population and this mutation was definitively lost from the population despite the fertility of XY females.

Microtus oregoni

The species *Microtus oregoni* has one of the most interesting sex determination systems, with both males and females being gonosomal mosaics. In females, the karyotype is XO in somatic cells and XX in germ cells, while in males the somatic karyotype is XY and the germ cell karyotype is OY (Ohno et al., 1963, 1966). Females produce X oocytes and males O and Y sperms that form XO (females) and XY (males) zygotes. Meiotic non-disjunction processes affecting X chromosome in germ cells in both sexes give rise to the XX female germ cells (cells without sex chromosome die) and OY male germ cells (XXY cells die).

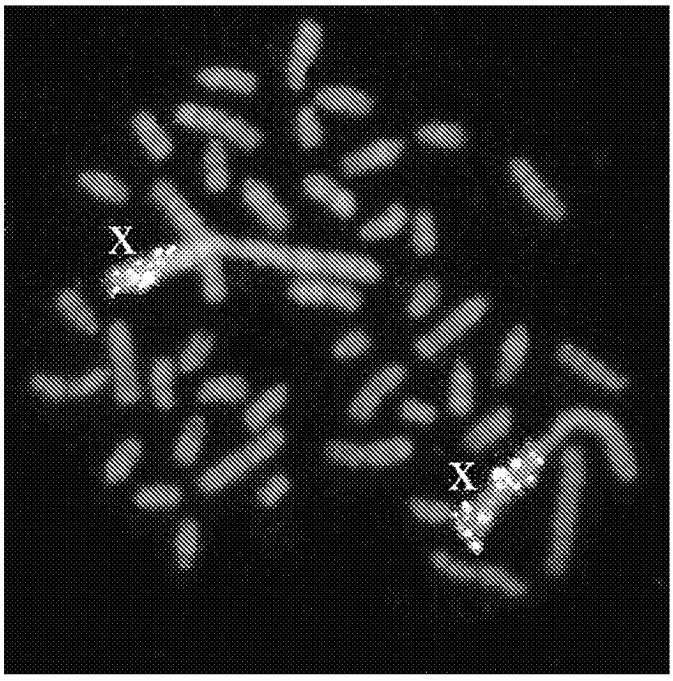

Fig. 2. Female chromosomes from the species *Microtus cabrerae* hybridized with a probe for the SRY gene. Note the presence of several signals in the heterochromatic block in both X chromosomes.

Genus Ellobius

Two species of the genus *Ellobius (E. lutescens* and *E. tancrei)* show another interesting sex determination system. In both species males and females have the same karyotype: 2n = 17,XO in *E. lutescens* (Matthey, 1953) and 2n = 34–54,XX in *E. tancrei* (Fredga and Lyapunova, 1991). By cytogenetic techniques a Y chromosome or Y chromosome fragment was never observed in these two species, although its existence was always assumed. However, using molecular studies it was demonstrated that the Y chromosome was completely lost in the genome of these two *Ellobius* species (Just et al., 1995; Vogel et al., 1998). On the contrary, in the species *E. fuscolapillus* normal sex determination occurs, with XX females and XY males. A very interesting issue arising in these species is therefore how sex determination occurs in the absence of the Y chromosome.

Myopus schisticolor

Myopus schisticolor females, like *Dicrostonyx torquatus,* have three karyotypes (XX, XX*, X*Y) while males have only one (XY). A chromosomal deletion, affecting the short arm of the normal X chromosome (Xp21-23), gives rise to the X* chromosome, which prevents male differentiation in X*Y individuals (Liu et al., 1998). Hence, in this species each type of female (XX, XX*, X*Y) produces males and females at different ratios. XX females produce females and males at 1:1 ratio, XX* female offspring show 3:1 female to male ratio, and X*Y females produce only females because of a double non-disjunction that impedes the formation of Y-carrying oocytes in foetal ovaries (see Fredga, 1994).

Y-linked genes

SRY gene

In mammals, male sex determination is switched on by the SRY gene (sex determining region of the Y chromosome), which initiates the development of a testis from the bipotential, undifferentiated gonadal primordium (Gubbay et al., 1990; Sinclair et al., 1990). SRY is a single-copy, male-specific gene, located on the Y chromosome in most eutherian (placental) and metatherian (marsupial) mammals.

Exceptions to this general rule have been found in several rodent species, with the most interesting ones described in Microtidae species. Just et al. (1995) demonstrated that *Ellobius lutescens* and *E. tancrei* lack the SRY gene and confirm previous results indicating that in these species sex determination occurs in the absence of a Y chromosome. Normal sex determination in absence of the SRY gene also occurs in the spiny rat *Tokudaia osimensis* (Soullier et al., 1998), a species where males and females show the same karyotype (2n = 25,XO) (Honda et al., 1977).

On the other hand, multiple monomorphic or polymorphic Y-linked copies of *Sry* have been described in nine species of Microtidae belonging to the genera *Arvicola, Microtus* and *Pitymys* (Bullejos et al., 1999). Hence, *Sry* could have been amplified in the Y chromosome of the ancestral species of these genera. The occurrence of multiple copies of the SRY gene in the genome is not an exclusive feature of Microtidae, but is quite common in rodents: as in *Akodon* species (Bianchi et al., 1993), Old World rodents (Nagamine, 1994), and African murine rodent species (Lundrigan and Tucker, 1997).

The vole species *Microtus cabrerae* also has multiple polymorphic copies of *Sry*, but strikingly, they are present in both males and females (Bullejos et al., 1997). FISH analysis with an *Sry* probe of this species demonstrated that multiple copies of the SRY gene are located on the euchromatic region of the Y chromosome, and hence outside constitutive heterochromatin and repetitive sequences. *Sry* copies are also located on the heterochromatic region of the X chromosome, interspersed within constitutive heterochromatin and satellite DNA. X-linked *Sry* copies are organized in multiple clusters of several copies arranged in tandem, distributed on the heterochromatic region of this chromosome (Fernández et al., 2002) (Fig. 2).

Cytogenetic studies have shown that the Y chromosome is male determining in *M. cabrerae* (Burgos et al., 1988a, b). Therefore, at least one functional copy of *Sry* located on the Y chromosome should act as the testis-determining gene in this species. In the case that several functional copies exist, they would not interfere with normal male development, as occurred in African murine rodents with multiple functional polymorphic copies of *Sry* (Lundrigan and Tucker, 1997). Logically, X-linked *Sry* sequences must be non functional. In fact, HMG box of several *Sry* copies from this species showed that most of them have base substitutions and deletions that give rise to stop codons (Bullejos et al., 1997). The most feasible explanation is that these *Sry* copies were inactivated by heterochromatinization after their insertion into the constitutive heterochromatin, since if they were active they would have originated sterile XX males and, thus the X chromosome with *Sry* would have been lost (Fernández et al., 2002).

ZFY gene

The zinc finger Y gene (ZFY), considered as the testis determining gene before SRY was identified, is located on the Y chromosome of placental mammals (Page et al., 1987; Sinclair et al., 1988). A highly homologous gene to ZFY has been located on the X chromosome, zinc finger X (ZFX) (Page et al., 1987). In human, ZFY is a single copy gene (Page et al., 1987) while in mouse there are two copies: *Zfy*1 and *Zfy*2 (Mardon and Page, 1989; Nagamine et al., 1990).

Southern blot analysis demonstrated that *Zfy*, like *Sry*, is lost in *E. lutescens* and *E. tancrei*. These results support the assumption that the entire Y chromosome has been lost from the genome of these two *Ellobius* species. However, in the spiny rat *Tokudaia osimensis Zfy* seems to be present in both males and females, despite the absence of a Y chromosome, suggesting a possible translocation of *Zfy* from the Y chromosome to the X chromosome or any autosome (Sutou et al., 2001). Since this possibility is only inferred by Southern blot analysis, more detailed studies are necessary to discern between the presence or absence of the ZFY gene in this species.

In *Myopus schisticolor* several copies (at least 15 copies) of *Zfy* are present on the Y chromosome. Northern blot analysis indicated that these copies are not transcribed in somatic or gonadal tissues (Lau et al., 1992). However, ZFY genes are transcribed in the gonads of other mammalian species analyzed (Zambrowicz et al., 1994).

X linked genes

In *Myopus schisticolor* the *Zfx* gene was located on both X chromosomes (X and X*) in the region p12→p11, close to the deletion breakpoint that originated the mutated X chromosome (X*). Although restriction fragment length polymorphism was detected between X and X* *Zfx* genes, both produced similar transcripts (Lau et al., 1992).

The deleted region on the mutated X* chromosome (Xp21→23) of *Myopus schisticolor* has also been characterized. Microdissection of this region, amplification by DOP-PCR and FISH onto mouse chromosomes yielded an interesting result. The probe hybridized to the centromeric regions of mouse chromosomes with the same pattern as the gamma satellite DNA (Liu et al., 1998). Screening of mouse embryonic gonadal cDNA libraries with this probe identified several clones that contained sequences homologous to gamma satellite DNA, confirming the presence of these sequences in the deleted region (Liu et al., 2001). Furthermore, a mouse clone containing the *Cct7* gene (chaperonin containing t-complex polypeptide 1, η subunit) was isolated. This gene mapped on the X chromosome of *Myopus schisticolor* close to the deleted region of the X* chromosome (Liu et al., 2001).

Five genes, α-galactosidase *(Gla)*, glucose-6-phosphate dehydrogenase *(G6pdx)*, hypoxanthine phosphoribosyltransferase *(Hprt)*, phosphoglycerate kinase 1 *(Pgk1)* and *Xist* (X inactive-specific transcript gene), have been mapped on the X chromosomes of *M. rossiaemeridionalis, M. tanscaspicus, M. kirgisorum, M. arvalis* and *M. agrestis*. However, in the X chromosomes of these species the gene order is different between each species and different to the gene orders observed in human, *Mustela vison*, and in the marsupial *Planigale maculata* (Nesterova et al., 1994, 1998a, b). These results confirm the proposed conservation of gene content of the mammalian X chromosome although this conservation does not affect gene order, which has changed mainly by rearrangements between large blocks, but not by small gene rearrangements (Nesterova et al., 1998a).

Mammalian X chromosome inactivation is regulated by the XIST gene (X inactive-specific transcript gene) (Brockdorff, 2002), which encodes for large untranslated RNA that initiates the inactivation of the X chromosome from which it is transcribed (Clemson et al., 1996). The *Xist* gene has been analyzed in four *Microtus* species *(M. arvalis, M. rossiaemeridionalis, M. transcaspicus, M. kirgisorum)* showing a similar structure to this gene in mouse. The gene is conserved in the four species, although differences between species affecting the length of the exon and intron sequences exist. The average sequence identity is 92.8%, with the differences mainly due to deletions, insertions and nucleotide substitutions. All these genes present eight exons of different sizes. Some of them are large, such as exons 1 and 7, and the rest are small, varying between the 83 bp of exon 2 and the 393 bp of exon 8 (Nesterova et al., 2001). In addition to species specific repeats in the upstream regions present in all species, the *M. arvalis Xist* gene has SINE elements inserted in exons 1 and 7 and in introns 1 and 7, also present in *M. rossiaemeridionalis* in intron 7. Northern blot analysis of the *Xist* gene demonstrated the existence of two RNA transcripts which implied the existence of alternative splicing sites.

Random inactivation of the X chromosome has been described in mouse (Clerc and Avner, 2003). However, in interspecific crosses of several vole species a phenomenon of nonrandom X chromosome inactivation occurs. Thus, in crosses between *M. arvalis* and *M. rossiaemeridionalis, M. transcaspicus* or *M. kirgisorum* the X chromosomes of the latter species are normally inactivated (Zakian et al., 1987, 1991). This nonrandom inactivation of X chromosomes in interspecific crosses cannot be explained by the differences described on the *Xist* gene between these species (Nesterova et al., 2001).

Furthermore, a nucleolar organizer region (NOR) containing rRNA genes has been located on the short arm of the acrocentric X chromosome in the species *M. kirgisorum* (Nesterova et al., 1998b).

Repetitive DNA sequences in sex chromosomes

Several repetitive DNA sequences located in the sex chromosomes have been described in different species of the genus *Microtus*. These sequences showed interspecific variation. In fact, they could be completely absent in the sex chromosomes of some species or present in several regions of the sex chromosomes of others, located in pericentromeric regions, in heterochromatic blocks or in both pericentromeric regions and heterochromatic blocks (Table 1).

Table 1. Distribution of different repeat DNA sequences in the genome of several species of the genus *Microtus*

Species	Chromosome	Repeat sequences						
		MSAT-160	MSAT-2750	MSAT-21	pMAHAE2	MS2	MS3	MS4
M. chrotorrhinus	X chromosome	Heterochromatin Pericentromeric	Heterochromatin	Heterochromatin	Not tested	Not tested	Not tested	Not tested
	Y chromosome	Heterochromatin	Heterochromatin	Heterochromatin				
	Autosomes	All centromeres	Not present	Not present				
M. agrestis	X chromosome	Pericentromeric	Not present	Not tested	Heterochromatin Pericentromeric	Not tested	Not tested	Not tested
	Y chromosome	Not present	Not present		Heterochromatin			
	Autosomes	Some centromeres			Not present			
M. cabrerae	X chromosome	Heterochromatin Pericentromeric	Not present		Present but not localized	Not tested	Not tested	Not tested
	Y chromosome	Interstitial bands in heterochromatin	Not present	Not tested				
	Autosomes	All centromeres	Not present					
M. rossiaemeridionalis	X chromosome	Not present				Heterochromatin	Heterochromatin	Heterochromatin
(*M. subarvalis*)	Y chromosome	Not present	Not tested	Not tested	Not tested	Heterochromatin	Heterochromatin Two bands	Heterochromatin
	Autosomes	Some centromeres				Not present	Not present	Not present
M. arvalis	X chromosome	Not present	Not present			Not present	Not present	Heterochromatin One band
M. arvalis arvalis					Present but not localized			
M. arvalis obscurus	Y chromosome	Not present	Not present	Not tested		Not present	Not present	Heterochromatin
	Autosomes	Some centromeres	Not present			Only one pair	Pair no. 1	Not present
M. transcaspicus	X chromosome	Not present				Heterochromatin	Heterochromatin	Heterochromatin One band
	Y chromosome	Not present	Not tested	Not tested	Not tested	Heterochromatin	Heterochromatin	Heterochromatin
	Autosomes	Some centromeres				Not present	Not present	Not present
M. ochrogaster minor	X chromosome	Pericentromeric	Not present					
	Y chromosome	Not present	Not present	Not tested	Not tested	Not tested	Not tested	Not tested
	Autosomes	Some centromeres	Not present					
M. ochrogaster ochrogaster	X chromosome	Pericentromeric	Not present					
	Y chromosome	Not present	Not present	Not tested	Not tested	Not tested	Not tested	Not tested
	Autosomes	All centromeres Some interstitial bands	Not present					
M. kirgisorum	X chromosome	Pericentromeric				Pericentromeric	Pericentromeric	Pericentromeric
	Y chromosome	Not present	Not tested	Not tested	Not tested	Heterochromatin	Heterochromatin	Heterochromatin
	Autosomes	Some centromeres				Only one pair	Pair no. 13	Not present
M. miurus	X chromosome	Pericentromeric	Not present					
	Y chromosome	Pericentromeric	Not present	Not tested	Not tested	Not tested	Not tested	Not tested
	Autosomes	All centromeres	Not present					
M. guentheri	X chromosome	Not present	Not present					
	Y chromosome	Pericentromeric	Not present	Not tested	Present but not localized	Not tested	Not tested	Not tested
	Autosomes	All centromeres Some interstitial bands	Not present					

MSAT-Repeats (MSAT-160, 2570 and 21)

The satellite DNA sequence, MSAT-160, with a monomer length of 160 bp, has been described at molecular and cytogenetic levels in *M. chrotorrhinus, M. agrestis,* (Modi, 1992, 1993a, b) *M. cabrerae* (Fernandez et al., 2001), *M. arvalis, M. rossiaemeridionalis, M. transcaspicus* and *M. kirgisorum* (Shevchenko et al., 2002). For these species, in situ hybridization showed patterns of species specific chromosome distribution. This repetitive family is mainly located in a variable number of autosomal centromeric regions and in sex chromosome constitutive heterochromatin (Table 1; Fig. 3). Related to X chromosomes, it could be completely absent, present only in the centromeric regions or present in both centromeric and heterochromatic regions. The centromeric regions of the Y chromosomes do not present this satellite, with the exception of *M. guentheri* and *M. miurus* (Modi, 1993a).

The distribution of MSAT-160 in species with giant sex chromosomes is highly variable (Table 1). In *M. chrotorrhinus* this family of repetitive DNA hybridizes to the centromere of the X chromosome, but not to the Y centromere, and to the entire heterochromatic regions of both sex chromosomes (Modi, 1993a). In *M. agrestis*, with the exception of the X chromosome centromere, no other hybridization signal is observed on the euchromatic or heterochromatic regions of the sex chromosomes (Modi, 1993a). In *M. cabrerae*, MSAT-160 hybridized intensely with the X chromosome centromere, but no sig-

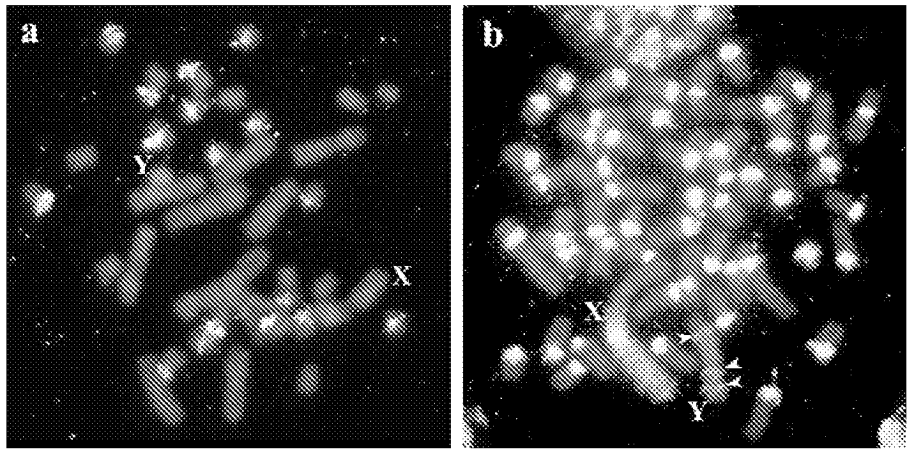

Fig. 3. MSAT-160 fluorescence in situ hybridization on male chromosomes from the species *Microtus arvalis* (**a**) and *Microtus cabrerae* (**b**). Note that in *M. arvalis* this satellite is located only in pericentomeric heterochromatin in some autosomal pairs, while in *M. cabrerae* it is located in all pericentromeric regions, with the exception of the Y chromosome. Furthermore, in *M. cabrerae* a faint signal can be seen along the X heterochromatin and three interstitial bands on the Y heterochromatin (arrowheads).

nal is detected on the Y centromere. Also, a faintly positive signal can be observed on the entire heterochromatic region of the X chromosome, while on the heterochromatic region of the Y chromosome only three faint interstitial bands are present (Fig. 3b). In *M. rossiaemeridionalis* MSAT-160 is completely absent in both sex chromosomes (Shevchenko et al., 2002).

MSAT-2570 satellite DNA has a length of 2570 bp and is located on the heterochromatic region of both sex chromosomes of *M. chrotorrhinus*, representing approximately 3–4% of the genome of this species (80,000 copies) (Modi, 1993c). MSAT-21 is also located on the heterochromatin of both sex chromosomes in *M. chrotorrhinus* (Ivanov and Modi, 1996).

pMAHAE2

This middle repetitive element (not organized in tandem) was characterized in the *M. agrestis* genome. It was estimated that the diploid genome of this species has approximately 10,000 to 20,000 copies. This element is located on the non centromeric heterochromatin of the entire long arm of the X chromosome and in most of the Y heterochromatin, with the exception of the telomeric ends (Kalscheuer et al., 1996; Singh et al., 2000). This element is also present in four other *Microtus* species analyzed, which present from 100 to 500 copies per diploid genome, although their chromosomal localization remains unknown at the moment (Kalscheuer et al., 1996).

MS2, MS3 and MS4

These three repeat sequences were obtained from the genome of *M. subarvalis (M. rossiaemeridionalis)*. They are also present in other *Microtus* species, although they showed variation between species. These repeat elements are very complex, containing several B1 elements and B2-like SINE repeats within them. All of them are located in both sex chromosomes of *M. rossiaemeridionalis, M. transcaspicus, M. kirgisorum* and *M. arvalis* with species specific distribution, but MS2 and MS3 are absent in *M. arvalis* sex chromosomes. Strikingly in some species, in addition to sex chromosome location, some interstitial bands of these repeats are also located on some autosomal pairs (Table 1) (Mayorov et al., 1996; Elisaphenko et al., 1998).

The high number of different repetitive DNA sequences cloned from the sex chromosomes of species of the genus *Microtus* and the species specific distribution that most of them show suggest that sex chromosome heterochromatin in this genus is highly complex in composition and organization. It seems that sequences in the gonosomal heterochromatin have been randomly chosen for amplification in the sex chromosomes in each species. Furthermore, the amplification degree and the localization of the amplified sequences are different between species. Several authors have proposed that sex chromosomes of *Microtus* species could have a natural predisposition to accumulate heterochromatin on sex chromosomes, although each species amplified different sequences (Burgos et al., 1990; Modi, 1993c; Singh et al., 2000). Since the genus *Microtus* is thought to have radiated about 1.6 million years ago (Chaline and Graf, 1988), this implies that *Microtus* genomes are susceptible to fast amplification of different repetitive sequences.

Genetic transposable elements on sex chromosomes

Several L1 and non-L1 retroposons have been described in species of the genus *Microtus*, most of them showing species specific localization and distribution. In *M. agrestis*, L1 (pMAECO14) and non-L1 (pMA11/3) retroposons have been described (Neitzel et al., 1998; 2002). As expected for these mobile genetic elements, they are interspersed in all autosomes and are especially abundant in the whole heterochromatin regions of the X and Y chromosomes. Furthermore, both elements are actively transcribed from the constitutive heterochromatin (Neitzel et al., 1998, 2002).

In the genome of *M. subarvalis* a LINE-1 element (MS1) has been characterized. It is distributed along the entire genome with a preferential localization on both sex chromosomes (Kholodilov et al., 1993). This element was also present in four other *Microtus* species analyzed. A very similar element (MK1) cloned from the *M. kirgisorum* genome is preferentially located on the sex chromosomes of the species of the *M. arvalis* group (Mayorov et al., 1999).

Synaptic and asynaptic sex chromosomes

Synaptic associations between sex chromosomes in pachynema and chiasma formation have been considered standard and obligatory events in most mammals (Solari, 1989). However, this is not the case in arvicolid species, which can be divided into two groups according to the synaptic behaviour of their sex chromosomes. The "synaptic group" includes species with pairing and synapsis between sex chromosomes at pachynema. On the other hand, the "asynaptic group" includes those species with sex chromosomes that do not pair or synapse at pachynema (see Megías-Nogales et al., 2003).

Several hypotheses have been proposed to explain the mechanisms involved in the appearance of the asynaptic condition in mammalian sex chromosomes. One of them stated that large heterochromatic regions on sex chromosomes could interfere with synapsis (Solari and Ashley, 1977). However, asynapsis also occurs in species without heterochromatic regions on sex chromosomes (Ashley et al., 1989; Carnero et al., 1991; Borodin et al., 1995; Megías-Nogales et al., 2003). Also, the loss or malfunction of the pairing regions in sex chromosomes has been proposed as the cause of asynapsis (Carnero et al., 1991; Jiménez et al., 1991). Nevertheless, no molecular studies have been performed in asynaptic sex chromosomes to prove or refute this hypothesis.

Synapsis of sex chromosomes is a prerequisite for crossing over, chiasmata formation and correct sex chromosome segregation. Hence, several hypotheses have been proposed to explain the correct disjunction of sex chromosomes in asynaptic species. These hypotheses include: (a) attachment of sex chromosome ends to the nuclear envelope (Ashley et al., 1989; Carnero et al., 1991; Jiménez et al., 1991); (b) existence of fibrillar structures that attach the sex chromosomes (Carnero et al., 1991; Jiménez et al., 1991) and (c) the sex vesicle could help to keep the X and Y chromosomes together (Jiménez et al., 1991).

A very interesting issue is the phylogenetic origin of asynaptic sex chromosomes in Arvicolidae. Two possibilities could be considered to explain the evolution of the asynaptic condition in Arvicolidae: (a) synaptic condition was the primitive trait and it was lost in some groups; (b) synaptic sex chromosomes were replaced by asynaptic chromosomes in the ancestral species of Arvicolidae, becoming the primitive trait. Afterwards some groups restored the synaptic condition.

The fact that most of the genera analyzed in this family present species with synaptic sex chromosomes supports the hypothesis of the synaptic condition as the ancestral situation in Arvicolidae (Megias-Nogales et al., 2003).

The genus *Microtus* is very striking because it includes both synaptic and asynaptic species. Thus, we could suppose that the asynaptic condition originated independently in each *Microtus* species. Nevertheless, this possibility is highly improbable from an evolutionary point of view. The alternative to this hypothesis is the existence of a common origin for the species with asynaptic sex chromosomes. Megías-Nogales et al. (2003) have suggested that asynaptic sex chromosomes originated only once in the ancestral species of the lineage that gave rise to the *M. arvalis/agrestis* group of species, while in the rest of *Microtus* species the ancestral synaptic condition remained.

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