Molecular and Physical Characterization of the Complex Pericentromeric Heterochromatin of the Vole Species *Microtus thomasi*

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Chromosomal races · Heterochromatin · *Microtus* · Pericentromeric region · Pericentromeric repeated sequences · Sex chromosomes

**Abstract**  
A new repeated DNA from *Microtus thomasi*, Mth-Alu2.2, was cloned and characterized and is presented here for the first time. Digestion of genomic DNA from *M. thomasi* with AluI restriction enzyme revealed a 2.2-kb repetitive DNA sequence with a high AT content (69%). This sequence consists of a tandemly repeated nonanucleotide of the consensus sequence CACAATGTA, which constitutes approximately 93–95% of the total unit length. The location of the Mth-Alu2.2 sequence in the karyotype was determined by FISH, demonstrating strong hybridization signals in the pericentromeric regions of all chromosomes and in the heterochromatin blocks of several X chromosome variants. In addition, the distribution of the 4 pericentromeric repeat sequences Msat-160, Mth-Alu900, Mth-Alu2.2, and interstitial telomeric repeats was analyzed by in situ hybridization in *M. thomasi*, in order to shed light on the complex composition of the chromosomal pericentromeric regions in this species. The order and organization of these sequences in the pericentromeric regions are conserved, with slight variations in both the degree of overlapping and the amount of each repeated DNA in the chromosomes. Specifically, Mth-Alu2.2 is localized in the terminal regions of the chromosomes, with Msat-160 occupying the immediately inner region, partially intermixed with Mth-Alu2.2. The sequence Mth-Alu900 is found in internal positions below Msat-160, and the interstitial telomeric repeats are located close to the long-arm euchromatin of the chromosomes.

Pericentromeric and centromeric regions of chromosomes contain diverse arrays of repeated DNA sequences. Despite their relevance in centromeric function, these repeats are highly variable and scarcely conserved even in closely related species, being in most cases species-specific [Plohl et al., 2008]. In fact, pericentromeric repeats, especially satellite DNAs, are extremely diverse in nucleotide sequence, organization, as well as chromosome and genome abundance [Charlesworth et al., 1994]. The biological function of pericentromeric repeats is a key question, with presumed roles proposed in sister chromatid...
cohesion, structure, organization, and segregation of eukaryotic chromosomes [Grewal and Elgin, 2007; Palomeque and Lorite, 2008; Plohl et al., 2008; Carone et al., 2009]. The genomic organization of pericentromeric and centromeric regions in most eukaryotic species remains unclear. The repetitive nature of these regions, consisting of large-sized stretches of satellite or repeat DNA, hampers their complete analysis. In fact, sequencing and assembly of pericentromeric and subtelomeric regions are among the most difficult tasks in genomics [International Human Genome Sequencing Consortium, 2001; Mouse Genome Sequencing Consortium, 2002; Riethman et al., 2004]. Consequently, information concerning the complete organization and functional significance of repeated DNA sequence content of pericentromeric and subtelomeric regions is very scarce. The classical strategy for satellite DNA analysis, which combines genomic DNA restriction, sequence analysis of randomly cloned monomers, and FISH mapping, remains essential for determining the composition and organization of pericentromeric and centromeric sequences in most species.

The rodent species of the subfamily Arvicolinae, and especially from the genus Microtus, are characterized by high karyotypic variation. The sex chromosomes vary in morphology (from acrocentric to metacentric) and size due to variation in the amount of constitutive heterochromatin [Modi, 1987a, b; Burgos et al., 1988a, b; Mitsainas et al., 2009]. The sibling vole species M. thomasi and M. atticus are remarkable models for studies on the chromosomal evolution, since they probably demonstrate the highest rate of karyotypic diversity among Microtus species. In fact, the 2 species show extensive chromosomal polymorphism, resulting in the description of 9 chromosomal races in M. thomasi: ‘thomasi’ (2n = 44, FN = 44), ‘pelponnesiacus’ (2n = 44, FN = 46), ‘Tichio’ (2n = 42, FN = 44), ‘Rb-subalpine’ (2n = 40, FN = 42), ‘subalpine’ (2n = 42, FN = 44), ‘Preveza’ (2n = 40, FN = 42), ‘Kali’ (2n = 40, FN = 42), ‘Aridea’ (2n = 38, FN = 42), and ‘Edessa’ (2n = 38, FN = 40); and 2 chromosomal races in M. atticus: ‘Evia’ (2n = 44, FN = 44) and ‘atticus’ (2n = 44, FN = 46) [Giagia-Athanasopoulou and Stamataopoulos, 1997; Mitsainas et al., 2009; Rovatsos et al., 2011a; Rovatsos, 2012; Rovatsos and Giagia-Athanasopoulou, 2012]. Among them, the chromosomal races ‘thomasi’ and ‘Evia’ are considered the most primitive forms of the species M. thomasi and M. atticus, respectively, with all autosomes and the X chromosomes being acrocentric (2n = 44, FN = 44) [Rovatsos, 2012]. Variation of chromosome numbers in races is probably the consequence of several Robertsonian and tandem fusion events [Giagia and Ondrias, 1973; Giagia, 1985; Giagia-Athanasopoulou et al., 1995; Rovatsos et al., 2011a].

Furthermore, this species shows an extreme polymorphism in size and morphology of its sex chromosomes [Acosta et al., 2009; Mitsainas et al., 2009; Rovatsos et al., 2011a]. At least 9 different X chromosomes have been found, with either acrocentric (X0 to X3) or subtelocentric (Xst0 to Xst2) morphology and variable length of heterochromatin [Acosta et al., 2009; Mitsainas et al., 2009; Rovatsos et al., 2011a, and unpubl. results]. Similarly, 7 Y chromosome variants exist, either acrocentric (Y0 to Y4) or metacentric (Ym and Ym1), all of them almost fully heterochromatic and also highly variable in size [Acosta et al., 2009; Mitsainas et al., 2009; Rovatsos et al., 2011a; Rovatsos and Giagia-Athanasopoulou, 2012, and unpubl. results].

In the species of the Arvicolinae group, 6 repeated DNAs have been reported from the pericentromeric heterochromatin: Msat-160, Msat-21, MS2, STR47, Mth-Alu900, and interstitial telomeric sequences (ITTs) [Modi, 1992, 1993a–c; Ivanov and Modi, 1996; Mayorov et al., 1996; Fernández et al., 2001; Shevchenko et al., 2002; Modi et al., 2003; Acosta et al., 2007, 2009, 2010; Rovatsos et al., 2011b]. Msat-160 is a satellite DNA, with a monomer unit of 160 bp, present in most species of 3 arvicoline genera (Microtus, Chionomys and Arvicola) analyzed to date [Shevchenko et al., 2002; Marchal et al., 2003; Modi et al., 2003; Acosta et al., 2007, 2010]. Msat-21 is a tandemly repeated 21-bp unit localized in 6 species of Microtus [Ivanov and Modi, 1996; Modi et al., 2003]. MS2 is a repeat of 1,194 bp homologous to the mouse B1 element, while STR47 consists of 47-bp-long tandem arrays present in 4 Old World species [Mayorov et al., 1996; Modi et al., 2003]. The sequence Mth-Alu900 of the M. thomasi genome is a non-satellite DNA located in the pericentromeric region of autosomes and in the hetrochromatic block of the subtelocentric Xst1 chromosome [Acosta et al., 2009]. Large blocks of ITTs are also located in the pericentromeric regions of chromosomes in several arvicolid species [Rovatsos et al., 2011b]. The number, localization and degree of amplification of all these sequences vary within the karyotypes, giving rise to a species-specific distribution pattern. Such a specific pattern indicates a complex organization of the pericentromeric regions in arvicolid species, an expected feature in a group of rodents with extensive karyotypic variation and one of the highest rates of evolution among mammals [Maruyama and Imai, 1981; Modi, 1987a, b; Mazurok et al., 2001].
In *M. thomasi*, 3 repeated DNA sequences have been previously characterized from the pericentromeric region: Msat-160 [Acosta et al., 2010], Mth-Alu900 [Acosta et al., 2009] and ITSs [Rovatsos et al., 2011b]. In this paper, we describe a new repeated DNA sequence, Mth-Alu2.2, also contributing to the pericentromeric heterochromatin of this species. Furthermore, we established the relative position of those 4 repeats in *M. thomasi* chromosomes through colocalization analyses. This approach is especially useful to gain a detailed view of the structure and organization of pericentromeric regions in this species, which are fully understood in only few mammals.

### Material and Methods

#### Genomic DNA Extraction

Genomic DNA from 2 specimens of *M. thomasi* collected in Greece was extracted from ethanol-fixed tissues, following the standard phenol-chloroform procedure. One sample belonged to the chromosomal race ‘thomasi’ with an X0 Y0 sex chromosome constitution, while the other was from ‘peloponnesiacus’ with Xst1 Y1 sex chromosomes.

### Isolation, Cloning, and Sequencing of Repeated DNA

To reveal repeated DNA sequences from *M. thomasi*, we digested genomic DNA from the chromosomal races ‘thomasi’ and ‘peloponnesiacus’ with different restriction endonucleases. We found several strong bands, corresponding to repeated DNA sequences, exclusively in the DNA samples digested with AluI. Two intense bands were observed in gel electrophoresis about 900 bp (previously characterized; see Acosta et al. [2009]) and 2.2 kb in size. The 2.2-kb band of the *M. thomasi* ‘peloponnesiacus’ AluI-digested genome was eluted from the gel and cloned into pGEM-T easy Vector (Promega) as previously described by Sánchez et al. [1996]. Transformed JM109 bacteria were screened using the same digoxigenin-labeled band as a probe, rendering 2 positive clones (Mth-Alu2.2-43 and Mth-Alu2.2-60) that were sequenced.

### Southern Blot

Genomic DNAs were digested with different restriction endonucleases and the resulting fragments were separated in 1% agarose gels and blotted onto nylon membranes (Amersham) following Bullejos et al. [1997]. Membranes were probed overnight at 55°C using the clone Mth-Alu2.2-43 (digoxigenin-labeled by random priming or PCR). Alkaline phosphatase detection was carried out according to the supplier’s recommendations (Roche).
Sequence Analysis

Pairwise and multiple sequence alignments were carried out with the program CLUSTAL W [Thompson et al., 1994]. Sequence homology searches were performed in GenBank using BLASTN with default parameters [Altschul et al., 1997]. Repeated DNA sequences were screened using the program RepeatMasker version 3.1.6 (http://www.repeatmasker.org). Subrepeat units were screened with Tandem Repeats Finder program (http://tandem.bu.edu/trf/trf.html) [Benson, 1999].

Chromosome Preparation and FISH

Chromosomes from several individuals with different sex chromosome constitutions belonging to different chromosomal races from Greece, Albania and Montenegro (fig. 1; table 1) [Mit-sainas et al., 2009; Rovatsos et al., 2011a] were prepared from bone marrow using a modified version of the Hsu and Patton [1969] method. Single indirect FISH for the repeat Mth-Alu2.2 was performed as previously described by Fernández et al. [2001] using the clone Mth-Alu2.2-43 (PCR-labeled with biotin-16-dUTP; Roche) as a probe. Labeling of the probes and double direct FISH was performed as described by Rovatsos et al. [2011b] using as probes Mth-Alu2.2, Mth-Alu900 [Acosta et al., 2009], Msat-160 [Acosta et al., 2010], and telomeric repeat [Rovatsos et al., 2011b], labeled either with Spectrum Green-dUTP or Spectrum Orange-dUTP (Abbott).

Results and Discussion

Cloning, Sequencing, and Chromosomal Location of the Mth-Alu2.2 Repeat

AluI digestion of genomic DNA from M. thomasi (chromosomal races ‘thomasi’ and ‘peloponnesiacus’) showed several prominent bands. In fact, a 900-bp band was previously demonstrated to contain a repeat DNA sequence located in the pericentromeric regions of most chromosomes, and in the heterochromatic block of some X chromosome variants of M. thomasi [Acosta et al., 2009]. Here, we analyzed a 2.2-kb band from the chromosomal race ‘peloponnesiacus’ AluI-digested genome. This band was purified from the agarose gel and ligated into the pGEM-T vector obtaining 2 positive clones, named Mth-Alu2.2-43 and Mth-Alu2.2-60, which were completely sequenced (GenBank accession numbers HF570919 and HF570920). Both clones contained the same repeated DNA sequence but included fragments of different size; that is, clone Mth-Alu2.2-43 was 2,147 bp long, while Mth-Alu2.2-60 was only 1,391 bp long. The percentage of identity throughout the shared region was 89.7%.

BLAST searches with the complete sequences of both clones found no relevant homology in GenBank. Also the RepeatMasker software failed to identify any known interspersed repeats. The Tandem Repeats Finder program identified 226 and 144 copies of the consensus sequence CACAATGTA in clones Mth-Alu2.2-43 and -60, respectively (covering 94.8 and 93.2% of the complete sequences). Thus, this new repeated DNA could have arisen from a 9-bp repeat unit through slipped-strand mispairing amplification. In the same way, the sequence Mth-Alu900 was hypothesized to have arisen by amplification of the 3 repeat motifs CAAAT, CAGAT and CAGAC, which represent about 80% of the whole sequence [Acosta et al., 2009]. Also, the satellite-DNA sequence Msat-160 of M. chrotorrhinus was probably derived from the short submotifs GAAA and CTTTCT, which represent 59% of the 160-bp monomer unit [Modi, 1992]. Short repeated submotifs have been described in many satellite DNA se-

Fig. 1. Map showing the sampling localities of M. thomasi and M. atticus specimens (see table 1). 1 = Xirokampi; 2 = Kalavryta; 3 = Stroflyia; 4 = Afidnes; 5 = Chalkida; 6 = Kimassu; 7 = Galaxidi; 8 = Ano Tichio; 9 = Astakos; 10 = Kastrosikia; 11 = Tyrnavos; 12 = Kali; 13 = Aridea; 14 = Edessa; 15 = Kakavia; 16 = Preze; 17 = Donje Selo.
quences from different mammalian species, e.g. the satellite of the kangaroo rat [Hatch et al., 1976; Fry and Salser, 1977], the blue whale [Arnason and Widegren, 1989], the American pronghorn antelope [Denome et al., 1994], the mouse [Zhang and Horz, 1984], and some marsupials [Bulazel et al., 2006].

The Mth-Alu2.2 repeat sequence has a high AT content (69%), a common feature of most repeated DNA sequences in Microtus species. In fact, the other non-satellite pericentromeric Alu I repeated DNA sequence described previously in the M. thomasi genome (Mth-Alu900) has an AT content of 59% [Acosta et al., 2009]. Similarly, the AT content of the Microtus pericentromeric satellite DNAs (Msat-160, Msat-21 and Msat-2750) ranges between 58 and 64% [Modi, 1992, 1993a–c; Ivanov and Modi, 1996; Fernández et al., 2001; Shevchenko et al., 2002]. Furthermore, other repeats without pericentromeric location are also AT-rich as e.g. pMAHAE2, exclusive from the gonsosomal heterochromatin of the giant sex chromosomes from M. agrestis [Kalscheuer et al., 1996], or McaY851, specific for the Y chromosome heterochromatin in most Microtus species [Marchal et al., 2004].

To investigate the organization of this repeated sequence, we compared the restriction pattern of genomic DNA from M. thomasi using several endonucleases: Alu I, which revealed the cloned band on genomic DNA, and endonucleases that cut once (DraI and FspI) or 3 times (PciI) inside the sequence of the largest clone (Mth-Alu2.2-43). Restrictions were probed in a Southern blot using the same clone as probe (fig. 2). In the Alu I digestion, a major prominent band of ∼2.2 kb was present. PciI yielded 3 bands corresponding to ∼0.6-, 0.8- and 1.7-kb fragments (the latter one being the most prominent). In DraI and FspI restriction, most of the repeated sequences appeared at the top of the digested DNA fragments (about 19 kb), and only in DraI-digested samples an additional band of 2.2 kb was detected. Hence, in any case, the result was a ladder pattern, which indicated that this repeated DNA sequence is not organized in tandem arrays.

To determine the chromosomal location of Mth-Alu2.2, we performed FISH on metaphase spreads of 18 males and 2 females from different chromosomal races of M. thomasi/M. atticus with 2n = 38–44 (one individual had 2n = 45 (XXY)) and different sex chromosome constitutions, as described by Rovatsos et al. [2008] (fig. 3; table 1). C-banding of all the individuals was previously performed to investigate the sex-chromosome constitutions, following Mitsainas et al. [2009]. The FISH results indicated that this repeated DNA sequence was located mainly in the pericentromeric heterochromatin of the autosomes (fig. 3a–i). However, differences existed in the number of chromosomes carrying this repeat and the signal intensity between the different chromosomal races, and even between individuals of different populations from the same chromosomal race (fig. 3a–c). In fact, the individuals from populations in Albania (fig. 3b) and Montenegro (fig. 3c) showed a reduction in the amount of this repeat in relation to individuals from the same chromosomal race but from different Greek populations (fig. 3a). These results indicate that, in addition to changes in sex chromosome constitution, the chromosomal races also differ in the amount and composition of autosomal heterochromatin, probably due to deletion, amplification or substitution of repeated DNA sequences.

In relation to the sex chromosomes, we analyzed the distribution of this repeat in 5 acrocentric (X_0, X_2, X_3, X_4, X_5) and 1 subtelocentric (X_s1) X chromosomes, and in 5 acrocentric (Y_0, Y_1, Y_2, Y_3, Y_4) and 2 metacentric (Y_m, Y_m1) Y chromosomes (see figs. 3 and 4; table 1) [Mitsainas...
et al., 2009; Rovatsos et al., 2011a]. This sequence is present in the pericentromeric regions of most acrocentric X chromosomes, and 2 of them (X4 and X3) have 1 and 2 additional faint interstitial bands, respectively, in the heterochromatic block. In the subtelocentric Xst chromosome this repeated sequence is present in the heterochromatic block of the long arm. Notably, our results demonstrate that at least 2 variants of the X2 and X3 chromosomes exist, either containing or lacking this repeat in the pericentromeric region (fig. 4).

On the acrocentric Y chromosomes this repeat is found mainly in pericentromeric regions with variable intensity, being very faint in Y2 and Y4. Also, there are at least 2 variants of the Y0 chromosome, each or either of them containing or lacking this repeat (fig. 4). In addition, some of the largest chromosomes (Y2, Y3 and Y4)
show a signal in the telomere of the long arm. In the metacentric Y chromosome, which proved to have 3 variants of size polymorphism, faint signals are seen in some cases in the telomere of one arm from the largest variants (Y m1 ) (fig. 4). However, no clear signals were detected in the short variant (Y m ). Therefore, the X and Y chromosome polymorphism described based on C-banding seems to be much more complex when the repeated DNA composition is analyzed.

These FISH results indicate that the pericentromeric Mth-Alu2.2 repeat is a minor component of the heterochromatin of both sex chromosomes, except in the X st1 variant, where it is enriched. Consequently, it is not the major sequence responsible for the variation in heterochromatin size that raised the current polymorphism of the sex chromosomes.

**Colocalization of Repeat Sequences in Pericentromeric Regions**

Diverse collections of repeated DNA sequences with varying patterns of chromosome and/or species distributions have been characterized in Arvicolinae species [reviewed in Marchal et al., 2003; Modi et al., 2003]. Many of them are located in pericentromeric C-positive bands, as Msat160, Msat21, MS2, STR47, Mth-Alu900, and ITSs [Marchal et al., 2003; Modi et al., 2003; Acosta et al., 2009], which indicates a complex organization of those regions. However, no colocalization studies to establish the relative position along the chromosome have been conducted until now.

*M. thomasi/M. atticus* chromosomes are remarkable examples of complexity at pericentromeric regions which are composed of 4 different repeated sequences: Msat-160 [Acosta et al., 2010], Mth-Alu900 [Acosta et al., 2009], Mth-Alu2.2 (present work), and ITSs [Rovatsos et al., 2011b]. In a previous work, we demonstrated that Msat-160 and ITSs colocalize in the pericentromeric regions (in the order telomere→Msat-160-ITSs→qter) in *M. thomasi* (R14) (table 1), but not contiguously, as in most chromosomes a region of unknown composition separates the 2 sequences [Rovatsos et al., 2011b].

To characterize the organization of the pericentromeric regions in detail, we performed double FISH, combining the 4 above-mentioned DNA sequence repeats, either red or green labeled, on the individual R14 of the ‘thomasi’ race, which has the primitive karyotype with all acrocentric autosomes and sex chromosomes. The results demonstrated that all the sequences were variably distributed among the pericentromeric regions of the chromosomes. Thus, the amount of each repeat is quite different, and none of them is heavily amplified or distributed over all chromosomes (fig. 5).

Despite the lack of a unique molecular organization common to all chromosomes, we noticed that the relative position of the 4 sequences along the pericentromeric regions of *M. thomasi* is conserved (figs. 5, 6). Msat-160 and Mth-Alu900 are located contiguously in the order telomere→Msat-160-Mth-Alu900→qter, partially overlapping in some chromosomes, as evidenced by the yellow signals (fig. 5a–c). The Mth-Alu2.2 sequence presents a dot hybridization pattern throughout the pericentromeric regions which is superposed (but not completely) with Msat-160 signals (fig. 5d–f). Meanwhile, Mth-Alu2.2 and ITSs are located in the order telomere→Mth-Alu2.2-ITSs→qter, either contiguously arranged in some chromosomes or split in others by a region probably composed of Msat-160 and Mth-Alu900 (fig. 5g–i). Mth-Alu2.2 and Mth-Alu900 are located in the order telomere→Mth-Alu2.2-Mth-Alu900→qter, and appear to be contiguous in most chromosomes (fig. 5j–l). Only in a few chromosomes, a region without a signal is found between them, most likely containing Msat-160 sequences. Finally, Mth-Alu-900 and ITS repeats are located in the order telomere→Mth-Alu900-ITSs→qter (fig. 5m–o).
Fig. 5. Double FISH with different combinations of the 4 probes Msat-160, Mth-Alu900, Mth-Alu2.2, and ITSs labeled in red or green in chromosomes of the individual R14 of *M. thomasi* (chromosomal race ‘thomasi’). Left column: probes labeled in red; middle column: probes labeled in green; right column: the 2 probes merged. The pictures of the right column include an enlarged chromosome hybridized with the corresponding 2 probes.
In conclusion, the pericentromeric regions of *M. thomasi* acrocentric chromosomes are organized following this pattern: the sequence described in this work, Mth-Alu2.2, is in the terminal region, the Msat-160 satellite occupies the immediately inner region, partially intermixed with Mth-Alu2.2. More internally, the sequence Mth-Alu900 is located; and finally, ITSs are more distal, close to the euchromatin (fig. 6). This main organization presents slight variations between chromosomes in both the degree of overlapping and the amount of each particular repeat.

Hence our results reveal the complexity of pericentromeric regions in *M. thomasi* chromosomes, which are composed of different repeated sequences arranged following a conserved pattern in all chromosomes. Similar results were demonstrated for the minor and major satellite DNA in mouse pericentromeric regions, where the minor satellite is located in the inner part flanked by 2 domains containing the major satellite [Kuznetsova et al., 2006]. Also in human and other primates the satellite DNA is divided in 2 families differing in the molecular organization and disposition along the pericentromeric heterochromatin [Rudd and Willard, 2004; Rudd et al., 2006].

Also several specific satellite and non-satellite DNA sequences are located in the centromeric regions of Cervidae chromosomes [Cheng et al., 2009]. As observed in *M. thomasi*, in Indian muntjac, colocalization analysis of satellites I and II showed overlapping of the 2 sequences in most pericentromeric regions, the satellite II hybridization signals being distal to those of satellite I [Li et al., 2000]. The characterization of a large segment of centromeric DNA from a BAC clone of Indian muntjac demonstrated a complex organization with 2 types of satellite DNA included (I and IV) interspersed with SINEs, LINEs, and LTR sequences as well as other unassigned DNA elements [Cheng et al., 2009]. One of the most complex examples are the centromeric and pericentromeric regions of pea chromosomes, with 13 repeated DNA sequences and 1 family of retrotransposons which are unevenly distributed among pea chromosomes [Neumann et al., 2012]. In Microtus species, however, retrotransposons (LINEs and SINEs) are especially scarce in pericentromeric regions of chromosomes, as demonstrated by FISH analysis [Marchal et al., 2006; Acosta et al., 2008, and unpubl. results].

Pericentromeric regions tend to evolve fast, giving rise to rapid variations in the DNA sequences which could result in the appearance of new families of repeat sequences in a very short time [Bachmann and Sperlich, 1993]. The expansion of each particular repeat along the pericentromeric heterochromatin is a random process that results in huge variations of their copy number within chromosomes. This scenario might explain the rapid replacement of an abundant satellite sequence by a new one, due to the fast amplification of pre-existing sequences of reduced copy number (‘the library hypothesis’) [Ugarković and Plohl, 2002; Plohl et al., 2008]. This model assumes the existence of a collection of repeated sequences in the pericentromeric regions which would be differentially amplified in each chromosome during the karyotypic evolution. A recent bioinformatics analysis of pericentromeric tandem DNA sequences in hundreds of plant and animal species demonstrated little sequence conservation beyond ~50 million years of divergence. In addition, the analysis revealed that these sequences share similar modes of evolution and thus gives support to the ‘library hypothesis’ [Melters et al., 2013]. A representative example of such a theory is *M. thomasi*, with 4 pericentromeric sequences presenting variable patterns of amplification within chromosomes and also within individuals.
Finally, the repeated DNAs are considered as hotspots for several structural chromosome rearrangements, playing an important role in evolution of mammalian karyotypes. Concretely, it has been demonstrated that the orientation, disposition and composition of satellite repeats in the pericentromeric regions could facilitate mechanisms of interchromosomal exchange between repeats promoting Robertsonian translocations [Garagna et al., 2001]. Hence, we postulated a possible relation between the complex pericentromeric regions in *M. thomasi* and the high number of chromosomal races, with variation in autosomal chromosome number and morphology, mainly due to autosomal Robertsonian translocations. In the same way, the pericentromeric repeated DNA sequences described in other arvicolid species could be related to the high karyotypic variation present in this rodent group, one of the highest described in mammals [Rovatsos et al., 2011a].

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Microtus thomasi Complex
Pericentromeric Heterochromatin