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Molecular Cytogenetic Analysis of Karyotype and Y Chromosome Conservation in Species of the Genus *Talpa* (Insectivora)

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Abstract
The Talpidae family has a highly stable karyotype. Most of the chromosome studies in this mammal group, however, employed classical cytogenetic techniques. Molecular cytogenetic analyses are still scarce and, for example, no repeated DNA sequences have been described to date. In this work, we used sequence analysis, chromosomal mapping of a LINE1 retroelement sequence, as well as chromosome painting with a whole Y chromosome probe of *T. occidentalis* to compare the karyotypes of 3 species of the genus *Talpa (T. occidentalis, T. romana*, and *T. aquitania*). Our results demonstrate that in *Talpa* genomes LINE1 sequences are widely distributed on all chromosomes but are enriched in pericentromeric C-band-positive regions. In addition, these LINE1 accumulate on the Y chromosomes of the 3 *Talpa* species regardless of their euchromatic or heterochromatic condition. Chromosome painting shows that the Y chromosomes in these 3 species are highly conserved. Interestingly, they share sequences with heterochromatic blocks on chromosome pairs 14 and 16 and, to a lesser degree, with the pericentromeric regions of other autosomes. Together, our analyses demonstrate that the repetitive DNA content of chromosomes from *Talpa* species is highly conserved.

The genus *Talpa* (family Talpidae) includes 11 species of fossorial mammals belonging to the order Soricomorpha which is sometimes considered as a suborder of the Eulipotyphla [Hutterer, 2005; Wilson and Reeder, 2005; Nicolas et al., 2015, 2017; Kryštufek et al., 2018].

The species of the Talpidae family – above all moles – are characterized by their highly stable karyotypes [Ye et al., 2006; Biltueva and Vorobieva, 2012]. Most species of the genus *Talpa* have a diploid chromosome number of 2n = 34, the only exceptions being *T. caeca* (2n = 36) and *T. caucasica* (2n = 38). Their autosomal fundamental number is also conserved and varies between 62 and 66 [Fedyk and Ivanitskaya, 1972; Zima, 1983; Jiménez et al., 1984; Kawada et al., 2002; Gornung et al., 2008; Gutiérrez et al., 2019]; their sex chromosomes are also well conserved. Their X chromosome is typically medium-sized and biarmed and, despite appearing submetacentric in

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some cases, is usually metacentric [Gornung et al., 2008]. The Y chromosome in most species is dot-like with no distinguishable morphology and is classified as metacentric, submetacentric, or acrocentric. Recently, in the newly described species *T. aquitania*, the Y chromosome has been shown to be a medium-sized submetacentric biarmed chromosome [Gutiérrez et al., 2019].

As in the rest of the Talpidae family [Yates and Moore, 1990], evolutionary dynamics in *Talpa* is defined by chromosome conservation, although a number of minor differences still account for some karyotypic divergence in this genus [Reumer and Meylan, 1986; Kawada et al., 2002; Gornung et al., 2008; Gutiérrez et al., 2019]. In fact, some studies have revealed interspecific differences due to robertsonian translocations, variation in the morphology of the sex chromosomes, and quantitative changes in heterochromatin. Concerning this latter factor, *T. europaea* shows only pericentromeric heterochromatin [Zima, 1983], while *T. altaica, T. occidentalis*, and *T. aquitania* all also have heterochromatin in some non-centromeric regions [Jiménez et al., 1984; Kawada et al., 2002; Gutiérrez et al., 2019].

Regarding the analysis of repeated DNA sequences, only the distribution and location of the major ribosomal genes and telomeric sequences have ever been determined in the genus *Talpa* [Zurita et al., 1997; Gornung et al., 2008; Gutiérrez et al., 2019]. The localization of the 5S ribosomal genes is less well understood and has only been investigated in *T. romana* and *T. europaea*, where differences in the karyotype distribution have been observed [Gornung et al., 2008]. Telomeric sequences have been found at the chromosome ends but also arranged as interstitial telomeric sequences in some pericentromeric regions in *T. romana*, *T. europaea*, and *T. aquitania* [Gornung et al., 2008; Gutiérrez et al., 2019]. On the basis of the different distributions of interstitial telomeric sequences and 5S rDNA in the chromosomes of *T. romana* and *T. europaea*, it has been suggested that karyotype diversification in *Talpa* may rely on genomic events that are indecipherable using classical cytogenetics [Gornung et al., 2008].

To identify cryptic rearrangements or subtle changes occurring during karyotype evolution in the genus *Talpa*, a molecular analysis of their chromosomal content is required. Hence, in this work we performed (1) a comparative analysis of the karyotype of 3 species of the genus *Talpa* based on sequence analysis and the chromosomal location of a LINE1 retroelement sequence and (2) chromosome painting using the whole Y chromosome of *T. occidentalis* as a probe.

Materials and Methods

Specimens Analyzed and Chromosome Preparations

For this study, we used DNA samples from males of 4 *Talpa* species, namely, *T. occidentalis* (Granada, Spain), *T. europaea* (Pavia, Italy), *T. aquitania* (Torme, Spain), and *T. romana* (Rome, Italy), together with chromosome preparations from 1 *T. occidentalis*, 1 *T. romana*, and 3 *T. aquitania* specimens. DNA samples were extracted following the standard phenol-chloroform procedure. Chromosomes were prepared from bone marrow cells as reported by Burgos et al. [1986]. The chromosomes in this work are numbered according to the karyotypes described for *T. occidentalis* and *T. aquitania* [Jiménez et al., 1984; Gutierrez et al., 2019].

Isolation, Cloning, and Sequencing of Repeated DNA

To identify the repeated DNA sequences from *T. occidentalis, T. europaea, T. aquitania*, and *T. romana,* genomic DNA was digested with *Hin*dIII restriction endonuclease, which produces several bands of repeated DNA sequences (1, 0.8, 0.6, and 0.4 kb) in gel electrophoresis. The 1-kb band of *T. europaea* was eluted from the gel and cloned into pGEM-T easy Vector (Promega), as previously described by Sánchez et al. [1996]. Transformed Z-competent bacteria (Zymo Research) were screened using the same digoxigenin-labeled band as a probe.

Southern Blot

Genomic DNAs were digested with *Hin*dIII restriction endonuclease 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 positive clone TE-1kb-clon 31 (see below) (digoxigenin-labeled by PCR). Alkaline phosphatase detection was carried out according to the supplier's recommendations (Roche).

Sequence Analysis

Plasmids from bacterial clones were Sanger sequenced using universal primers and were analyzed using the Bioedit program (version 7.0.9.0) (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). Pairwise and multiple sequence alignments were carried out with the program CLUSTAL OMEGA [Thompson et al., 1994]. Repeated DNA sequences were screened using the program RepeatMasker version 4.0.6 (http://repeatmasker.org). A GenBank search for sequences was performed with BLASTN [Zhang et al., 2000] and BLASTP [Altschul et al., 1997] (https://blast.ncbi.nlm.nih.gov/).

FISH and Chromosome Painting

Indirect FISH was performed, as previously described by Fernández et al. [2001], using as a probe the clone TE-1kb-clon 31 labeled with biotin-16-dUTP by PCR amplification, with M13 forward and reverse primers.

For chromosome painting, a painting probe was prepared from the whole minute Y chromosome of *T. occidentalis*. For probe preparation, 15 microdissected chromosomes were amplified and labeled by DOP-PCR using Spectrum-Orange dUTP (Abbott). We followed the direct FISH protocol for chromosome painting described by Marchal et al. [2004]. The images were captured using a fluorescence microscope (Olympus BX51) equipped with a CCD camera (Olympus DP70).

Results

Cloning and Chromosomal Localization of the Repeated Sequences

Electrophoresis of the *Hin*dIII-restricted genomic DNAs of *T. occidentalis*, *T. europaea*, *T. aquitania*, and *T. romana* produced several intense bands of approximately 1, 0.8, 0.6, and 0.4 kb (Fig. 1a; only *T. europaea* is illustrated). The 1-kb band of *T. europaea* was eluted, labeled, and used as probe for a Southern blot on the restricted DNAs of the 4 species. The Southern blot results were all very similar for *T. occidentalis*, *T. europaea*, and *T. aquitania* (the results for *T. romana* are not shown as the quality and amount of the DNA was insufficient). In addition to the corresponding 1-kb band, 3 other bands of about 0.8, 1.2, and 1.7 kb in size were clearly visible. These bands could correspond to shorter or larger DNA fragments of the same LINE1 arising as a consequence of sequence modification and variation in restriction pattern, or alternatively correspond to restriction fragments of other similar LINEs. Another additional large intense band was present in *T. europaea*, which was only faintly detectable in the *T. occidentalis* and *T. aquitania* genomes (Fig. 1b).

The 1-kb eluted band from *T. europaea* was cloned in pGEM-T Easy Vector to obtain 10 positive clones that were fully sequenced (TE-1kb-clon 31, 32, 38, 51, 135, 139, 169, 182, 194, 196) (GenBank accession numbers: MN212943–MN212952). The clones contained sequences that varied in length between 1,033 and 1,054 bp. The identity of these sequences was in the range of 88.8–99.3% when compared with the consensus; on the other hand, pairwise sequence comparison produced identities of 86.4–99.5%. The AT content of the cloned sequences was on average 64.4%. The nucleotide variation observed in the alignment was reduced to random base changes and a few small insertions of 1–8 bp in length. The RepeatMasker program identified the analyzed sequences as part of the reverse transcriptase (ORF2) from a LINE1 retrotransposon. A BLAST search of the whole genome shotgun databases of the Soricomorpha identified many sequences with 88.0% identity in contigs from *Scalopus aquaticus*, a species of Soricomorpha phylogenetically related to *Talpa* species.

As the sequence translation gave rise to multiple stop codons in most of the sequenced clones, they were probably from nonfunctional copies (pseudogenes) of LINE1 retroposons. However, clone TE-1kb-clon 139 did not contain premature stop codons in the translated amino acid sequence. A BLASTp search demonstrated 78% identity with the LINE1 reverse transcriptases available in GenBank, including 5 of the conserved domains (3–7) that characterize reverse transcriptase proteins [Xiong and Eickbush, 1990].

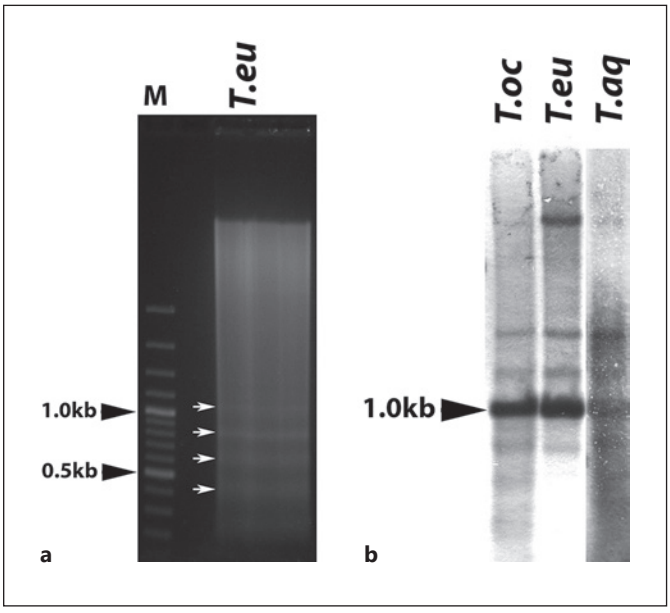

Fig. 1. a Gel electrophoresis of genomic DNA from *Talpa europaea* (*T.eu*) digested with *Hin*dIII. M, 100-bp DNA molecular weight marker. **b** Southern blot of *T. occidentalis* (*T.oc*), *T. europaea* (*T. eu*), and *T. aquitania* (*T.aq*) genomic DNA digested with *Hin*dIII and probed with the eluted 1-kb band labeled with digoxigenin.

To determine the chromosomal location of the cloned LINE1 sequence, we performed FISH on metaphase spreads of *T. occidentalis*, *T. romana*, and *T. aquitania* (Fig. 2). The FISH signals in the karyotype of these 3 species were widely distributed on most autosomes and X chromosomes. Interestingly, LINE1 appeared enriched in the pericentromeric regions of the chromosomes of *T. occidentalis* and *T. romana*, while in *T. aquitania* it was less intense in these regions. LINE1 signals were also detected on the dot-like minute Y chromosomes of *T. occidentalis* and *T. romana*, and on the submetacentric Y chromosome of *T. aquitania* (Fig. 2). In this latter case, the sequences were not homogeneously distributed and seemed to be accumulated on the short arm of the Y chromosome (Fig. 2c).

Painting with the T. occidentalis *Y Chromosome Probe*

As expected, this probe painted the complete Y chromosome of *T. occidentalis*. Notably, it also strongly hybridized on the short arm of autosomal pair 16 and, somewhat less intensely, on the pericentromeric region and on the small arm of submetacentric pair 14. Faint signals

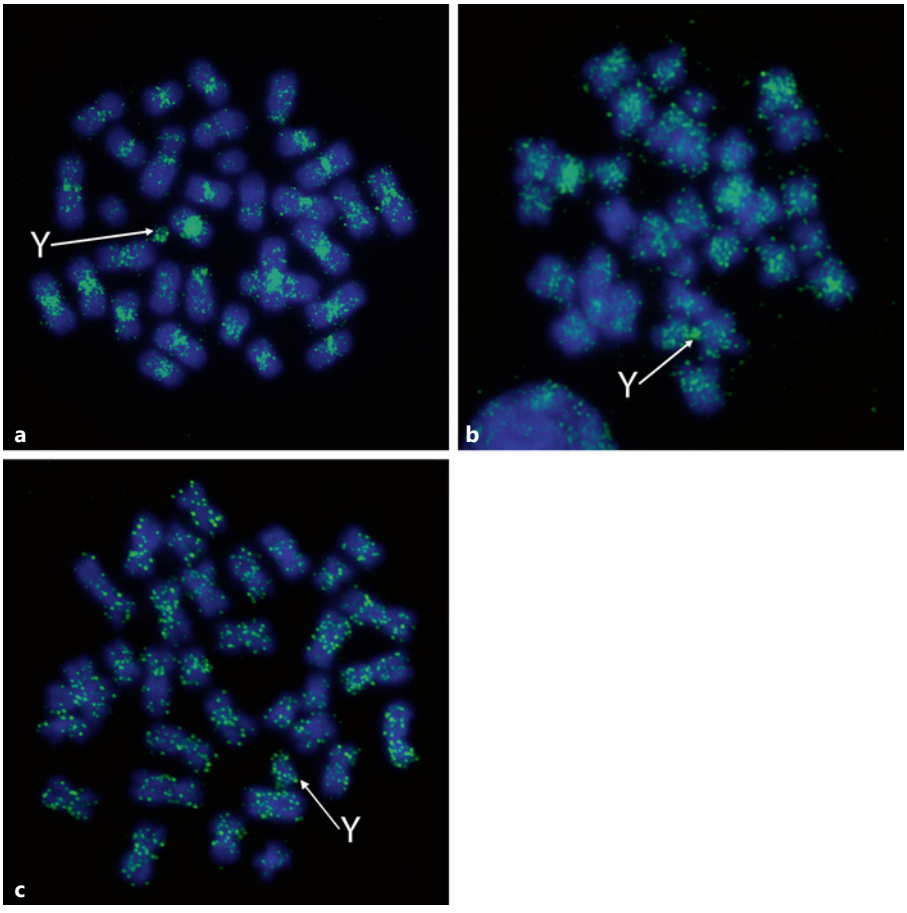

Fig. 2. In situ hybridization with the 1-kb LINE1 probe of *Talpa europaea* on male metaphases of *T. occidentalis* (**a**), *T. romana* (**b**), and *T. aquitania* (**c**).

were also observed in pericentromeric regions of certain other chromosomes (Fig. 3a). Identical results were obtained when this probe was hybridized in the *T. romana* karyotype (chromosome pairs 16 and 14 of *T. occidentalis* correspond to *T. romana* autosomal pairs 9 and 1, according to the chromosome nomenclature used by Gornung et al. [2008]); only the Y chromosome stained less intense than the Y chromosome of *T. occidentalis* (Fig. 3b). In *T. aquitania*, the entire submetacentric Y chromosome, as well as the short arm of submetacentric pair 16, hybridized strongly. The heterochromatin and the short arm of pair 14 of *T. aquitania* were also painted, albeit faintly; no clear signals were observed in the pericentromeric regions of the remaining chromosomes (Fig. 3c).

Discussion

Eukaryotic genomes contain many interspersed repeated sequences derived from mobile genetic elements [Lander et al., 2001; Venter et al., 2001; Waterston et al., 2002; Kirkness et al., 2003; Gibbs et al., 2004]. LINE1 sequences are one of the most important groups of retrotransposons and are abundant in mammal genomes [Hardies et al., 2000; Mears and Hutchison, 2001; Adelson et al., 2009]. It is estimated that one third of the mammal genome originated directly or indirectly through LINE1 retrotransposition [Han and Boeke, 2005]. A good example is the LINE1 content of around 21% revealed by the Human Genome Project [Lander et al., 2001].

Our results demonstrated that LINE1 sequences are widely distributed in the genome of these 3 *Talpa* species and form part of their repetitive DNA content. We cloned and analyzed a 1-kb fragment of the ORF2 from the LINE1 coding for the reverse transcriptase. Most of the sequences obtained in our study correspond to nonfunctional copies of LINE1 retroposons. This is not surprising given that LINE1 retroposition generates mostly defective copies that remain in the genome as mutated or rearranged LINE1 sequences [Furano, 2000; Boissinot and Furano, 2001]. Complete LINE1 sequences with autonomous mobile capacity are in fact very scarce in mam-

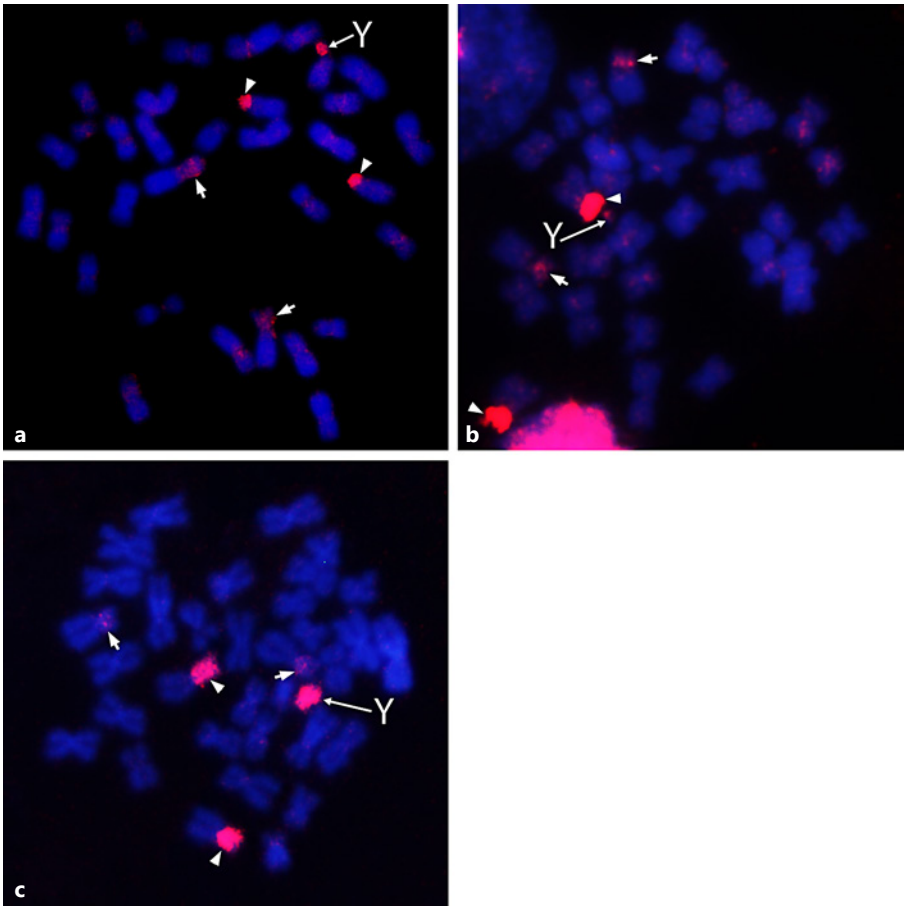

Fig. 3. Chromosome painting with the probe from the whole Y chromosome of *Talpa occidentalis* on male metaphases of *T. occidentalis* (**a**), *T. romana* (**b**), and *T. aquitania* (**c**). The arrows and arrowheads indicate chromosome pairs 14 and 16, respectively.

mal genomes [Sassaman et al., 1997; Kazazian, 1999]. One of the clones analyzed could derive from a functional *Talpa* LINE1 retroelement since it does not contain stop codons, and the resulting polypeptide includes 5 conserved domains of the reverse transcriptase, as described by Xiong and Eickbush [1990]. However, we cannot rule out the possibility that this analyzed sequence could also be part of an inactive LINE1 element if it is truncated or harbors mutations in other regions of the protein sequence.

A key question is why the LINE1 elements in mammal genomes are widely but not randomly spread on chromosomes [Graham and Boissinot, 2006; Kvikstad and Makova, 2010]. Genome projects are currently quantifying the AT-rich genome distribution bias of LINE1, which could have certain functional implications. At the chromosomal level, LINE1 sequences are enriched in heterochromatic regions of autosomes, while in sex chromosomes they are similarly abundant in both euchromatin and heterochromatin [Bailey et al., 2000; Dobigny et al., 2004; Waters et al., 2004; Marchal et al., 2006; Acosta et al., 2008; Kvikstad and Makova, 2010]. Our results provide evidence that in *Talpa* karyotypes, LINE1 possess similar dynamics since they are widely distributed on all chromosomes but accumulate at pericentromeric regions, which have previously been described as C-band-positive heterochromatin [Jiménez et al., 1984; Gornung et al., 2008; Gutiérrez et al., 2019].

Among the factors proposed to explain the enrichment of retrotransposons in mammal sex chromosomes are the lower rates of recombination, AT-composition-biased preferential insertion, local genome landscape features, natural selection, and random genetic drift [reviewed in Kvikstad and Makova, 2010]. LINE1 accumulation in mammal Y chromosomes could be easily tolerated since this chromosome only contains a few genes and is mainly heterochromatic [Marshall Graves, 2000, 2001]. The accumulation of LINE1 in the Y chromosomes of the 3 *Talpa* species described here for the first time is not related to their chromatin content, i.e., if they are mostly euchromatic (C-band negative) or heterochromatic (C-band positive). Indeed, the Y chromosome

is heterochromatic in *T. occidentalis* and *T. aquitania* but euchromatic in *T. romana* [Jiménez et al., 1984; Gornung et al., 2008; Gutiérrez et al., 2019], and all of them are LINE1 enriched. These data show that the repetitive DNA content of the Y chromosome of *Talpa* species is well conserved despite differences in chromosome size and morphology.

Several chromosome painting studies in Soricomorpha (including *Talpa* species) have previously been performed using human [Volleth and Müller, 2006; Yang et al., 2006; Ye et al., 2006; Biltueva and Vorobieva, 2012] and stone marten [Yang et al., 2006] chromosome painting probes and, vice versa, using insectivore chromosomes as probes on mammal or human chromosomes in order to delineate chromosome evolution [Biltueva and Vorobieva, 2012]. Despite the good results for autosomes and X chromosomes, in most of these studies no information has been given for the Y chromosomes. Only Volleth and Müller [2006] indicate that painting with human chromosomes on *T. europaea* yields reproducible results for all probes except for Y chromosomes. In fact, works about painting analyses of the mammalian Y chromosome are scarce [Acosta et al., 2011]. In Soricomorpha, only 1 previous study of chromosome painting in *Sorex* species exists in which both the X and Y chromosomes were analyzed [Biltueva et al., 2011].

The results of Y chromosome painting in *Microtus* species and *Sorex* species demonstrated that a small region is conserved, corresponding to the euchromatic part or the pseudoautosomal region (PAR) of the X and Y chromosomes, but not the rest of the Y chromosomes, even in closely related species [Acosta et al., 2011; Biltueva et al., 2011].

By contrast, our painting results using the Y chromosome of *T. occidentalis* as a probe show that the Y chromosome content of the 3 studied *Talpa* species is well preserved. If the dot-like Y chromosomes of *T. occidentalis* and *T. europaea* are considered to be similar to their ancestral Y chromosomes, the enlargement of the Y chromosome occurring in *T. aquitania* involved the amplification of sequences already present in that minute chromosome. Interestingly, the Y chromosomes of the 3 *Talpa* species contain sequences that are very similar to the sequences that form the heterochromatin of autosomal pairs 14 and 16. Two evolutionary contexts could explain this homogeneous pattern of heterochromatin in the Y chromosome and autosomal pairs 14 and 16. Both contexts are similar and probably only differ in the original source of the repeated heterochromatic sequences – either autosomal or Y chromosomal – before amplification and transference to their counterparts. Regardless of the particular evolutionary dynamics that took place, it is highly probable that these repeated sequences were already emplaced in the heterochromatin arranged in the Y chromosome and in the heterochromatin of chromosomes 14 and 16 in the karyotype of the ancestral species of the genus *Talpa*.

It is significant that the scenario described in *Talpa* species for Y chromosome evolution – that is, the conservation of the sequence content – differs significantly from that of other mammals. Many studies have shown that, even between closely related species, the DNA content of the Y chromosome is poorly conserved not only in the heterochromatin but also in the euchromatin regions [Sitnikova et al., 2007; Kirsch et al., 2008; Gifalli-Iughetti and Koiffmann, 2009; Acosta et al., 2011]. Such rapid and independent evolution probably reflects the degenerative process that has driven the evolution of this chromosome [Wilson and Makova, 2009].

In conclusion, we demonstrate here that the repetitive DNA content of the genome of *Talpa* species is enriched in LINE1 sequences, which are distributed in the karyotypes following a conserved pattern. Furthermore, the Y chromosomes of these species are composed of similarly conserved repeated sequences. Finally, the origin of the heterochromatic blocks arranged in some autosomal pairs of these species, in particular pair 16, was parallel to the evolution of the repetitive DNA content of the Y chromosome.

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Statement of Ethics

All capture and sacrifice protocols were approved by the Junta de Andalucía Ethics Committee for Animal Experimentation (code 22/05/2018/094)

Disclosure Statement

The authors have no conflicts of interest to declare.

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Author Contributions

J.G., G.A.M., and M.A. cloned the L1 sequences, performed chromosome preparations, FISH and painting. J.A.M. and A.S. carried out the chromosome microdissection and painting probes. R.C. prepared *Talpa romana* chromosomes. J.G., J.A.M., and A.S. analyzed the results and drafted the first version of the manuscript. All authors revised, approved, and edited the manuscript.

References

Acosta MJ, Marchal JA, Fernández-Espartero CH, Bullejos M, Sánchez A: Retroelements (LINEs and SINEs) in vole genomes: differential distribution in the constitutive heterochromatin. Chromosome Res 16:949–959 (2008).

Acosta MJ, Romero-Fernández I, Sánchez A, Marchal JA: Comparative analysis by chromosome painting of the sex chromosomes in arvicolid rodents. Cytogenet Genome Res 132:47–54 (2011).

Adelson DL, Raison JM, Edgar RC: Characterization and distribution of retrotransposons and simple sequence repeats in the bovine genome. Proc Natl Acad Sci USA 106:12855–12860 (2009).

Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, et al: Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25:3389–3402 (1997).

Bailey JA, Carrel L, Chakravarti A, Eichler EE: Molecular evidence for a relationship between LINE-1 elements and X chromosome inactivation: the Lyon repeat hypothesis. Proc Natl Acad Sci USA 97:6634–6639 (2000).

Biltueva L, Vorobieva N: Chromosome evolution in Eulipotyphla. Cytogenet Genome Res 137:154–164 (2012).

Biltueva L, Vorobieva N, Perelman P, Trifonov V, Volobouev V, et al: Karyotype evolution of Eulipotyphla (Insectivora): the genome homology of seven *Sorex* species revealed by comparative chromosome painting and banding data. Cytogenet Genome Res 135:51–64 (2011).

Boissinot S, Furano AV: Adaptive evolution in LINE-1 retrotransposons. Mol Biol Evol 18:2186–2194 (2001).

Bullejos M, Sánchez A, Burgos M, Hera C, Jiménez R, Díaz de la Guardia R: Multiple, polymorphic copies of SRY in both males and females of the vole *Microtus cabrerae*. Cytogenet Cell Genet 79:167–171 (1997).

Burgos M, Jiménez R, Díaz de la Guardia R: A rapid, simple and reliable combined method for G-banding mammalian and human chromosomes. Stain Technol 61:257–260 (1986).

Dobigny G, Ozouf-Costaz C, Waters PD, Bonillo C, Coutanceau JP, Volobouev V: LINE-1 amplification accompanies explosive genome repatterning in rodents. Chromosome Res 12:787–793 (2004).

Fedyk S, Ivanitskaya EY: Chromosomes of Siberiain shrews. Acta Theriol 27:475–492 (1972).

Fernández R, Barragán MJ, Bullejos M, Marchal JA, Martínez S, et al: Molecular and cytogenetic characterization of highly repeated DNA sequences in the vole *Microtus cabrerae*. Heredity 87:637–646 (2001).

Furano AV: The biological properties and evolutionary dynamics of mammalian LINE-1 retrotransposons. Prog Nucleic Acid Res Mol Biol 64:255–294 (2000).

Gibbs RA, Weinstock GM, Metzker ML, Muzny DM, Sodergren EJ, et al: Genome sequence of the brown Norway rat yields insights into mammalian evolution. Nature 428:493–521 (2004).

Gifalli-Iughetti C, Koiffmann CP: The Y chromosome of the Atelidae family (Platyrrhini): study by chromosome microdissection. Cytogenet Genome Res 125:46–53 (2009).

Gornung E, Volleth M, Capanna E, Castiglia R: Comparative cytogenetics of moles (Eulipotyphla, Talpidae): chromosomal differences in *Talpa romana* and *Talpa europaea*. Cytogenet Genome Res 121:249–254 (2008).

Graham T, Boissinot S: The genomic distribution of L1 elements: the role of insertion bias and natural selection. J Biomed Biotechnol 2006:75327 (2006).

Gutiérrez J, Aleix-Mata G, Lamelas L, Arroyo M, Marchal JA, Sánchez A: Karyotype analysis of the new *Talpa* species *Talpa aquitania* (Talpidae; Insectivora) from northern Spain. Cytogenet Genome Res 159:26–31 (2019).

Han JS, Boeke JD: LINE-1 retrotransposons: modulators of quantity and quality of mammalian gene expression? Bioessays 27:775–784 (2005).

Hardies SC, Wang L, Zhou L, Zhao Y, Casavant NC, Huang S: LINE-1 (L1) lineages in the mouse. Mol Biol Evol 17:616–628 (2000).

Hutterer R: Order Soricomorpha, in Wilson DE, Reeder DM (eds): Mammal Species of the World. A Taxonomic and Geographic Reference, 3rd ed, pp 220–311 (Johns Hopkins University Press, Baltimore 2005).

Jiménez R, Burgos M, Díaz de la Guardia R: Karyotype and chromosome banding in the mole (*Talpa occidentalis*) from the south-east of the Iberian Peninsula. Implications on its taxonomic position. Caryologia 37:253–258 (1984).

Kawada S, Harada M, Grafodatsky AS, Oda S: Cytogenetic study of the Siberian mole, *Talpa altaica* (Insectivora: Talpidae) and karyological relationships within the genus *Talpa*. Mammalia 66:53–62 (2002).

Kazazian HH Jr: An estimated frequency of endogenous insertional mutations in humans. Nat Genet 22:130 (1999).

Kirkness EF, Bafna V, Halpern AL, Levy S, Remington K, et al: The dog genome: survey sequencing and comparative analysis. Science 301:1898–1903 (2003).

Kirsch S, Munch C, Jiang Z, Cheng Z, Chen L, et al: Evolutionary dynamics of segmental duplications from human Y-chromosomal euchromatin/heterochromatin transition regions. Genome Res 18:1030–1042 (2008).

Kryštufek B, Nedyalkov N, Astrin J, Hutterer R: News from the Balkan refugium: Thrace has an endemic mole species (Mammalia: Talpidae). Bonn Zool Bull 67:41–57 (2018).

Kvikstad EM, Makova KD: The (r)evolution of SINE versus LINE distributions in primate genomes: sex chromosomes are important. Genome Res 20:600–613 (2010).

Lander ES, Linton LM, Birren B, Nusbaum C, Zody MC, et al: Initial sequencing and analysis of the human genome. Nature 409:860–921 (2001).

Marchal JA, Acosta MJ, Nietzel H, Sperling K, Bullejos M, et al: X chromosome painting in *Microtus*: origin and evolution of the giant sex chromosomes. Chromosome Res 12:767–776 (2004).

Marchal JA, Acosta MJ, Bullejos M, Puerma E, Díaz de la Guardia R, Sánchez A: Distribution of L1-retroposons on the giant sex chromosomes of *Microtus cabrerae* (Arvicolidae, Rodentia): functional and evolutionary implications. Chromosome Res 14:177–186 (2006).

Marshall Graves JA: Human Y chromosome, sex determination, and spermatogenesis – a feminist view. Biol Reprod 63:667–676 (2000).

Marshall Graves JA: From brain determination to testis determination: evolution of the mammalian sex-determining gene. Reprod Fertil Dev 13:665–672 (2001).
Mears ML, Hutchison CA 3rd: The evolution of modern lineages of mouse L1 elements. J Mol Evol 52:51–62 (2001).
Nicolas V, Martínez-Vargas J, Hugot JP: Preliminary note: *Talpa aquitania* nov. sp. (Talpidae, Soricomorpha) a new mole species from southwest France and north Spain. Bull Acad Vet Fr 168:329–334 (2015).
Nicolas V, Martínez-Vargas J, Hugot JP: *Talpa aquitania* sp. nov. (Talpidae, Soricomorpha), a new mole species from SW France and N Spain. Mammalia 81:641–642 (2017).
Reumer JWF, Meylan A: New developments in vertebrate cytotaxonomy IX. Chromosome numbers in the order Insectivora (Mammalia). Genetica 70:119–151 (1986).
Sánchez A, Bullejos M, Burgos M, Jiménez R, Díaz de la Guardia R: An alternative to blunt-end ligation for cloning DNA fragments with incompatible ends. Trends Genet 12:44 (1996).
Sassaman DM, Dombroski BA, Moran JV, Kimberland ML, Naas TP, et al: Many human L1 elements are capable of retrotransposition. Nat Genet 16:37–43 (1997).
Sitnikova NA, Romanenko SA, O'Brien PC, Perelman PL, Fu B, et al: Chromosomal evolution of Arvicolinae (Cricetidae, Rodentia). I. The genome homology of tundra vole, field vole, mouse and golden hamster revealed by comparative chromosome painting. Chromosome Res 15:447–456 (2007).
Thompson JD, Higgins DG, Gibson TJ: CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680 (1994).
Venter JC, Adams MD, Myers EW, Li PW, Mural RJ, et al: The sequence of the human genome. Science 291:1304–1351 (2001).
Volleth M, Müller S: Zoo-FISH in the European mole (*Talpa europaea*) detects all ancestral Boreo-Eutherian human homologous chromosome associations. Cytogenet Genome Res 115:154–157 (2006).
Waters PD, Dobigny G, Pardini AT, Robinson TJ: LINE-1 distribution in Afrotheria and Xenarthra: implications for understanding the evolution of LINE-1 in eutherian genomes. Chromosoma 113:137–144 (2004).
Waterston RH, Lindblad-Toh K, Birney E, Rogers J, Abril JF, et al: Initial sequencing and comparative analysis of the mouse genome. Nature 420:520–562 (2002).
Wilson DE, Reeder DM: Mammal Species of the World: A Taxonomic and Geographic Reference, 3rd ed (Johns Hopkins University Press, Baltimore 2005).
Wilson MA, Makova KD: Genomic analyses of sex chromosome evolution. Annu Rev Genomics Hum Genet 10:333–354 (2009).
Xiong Y, Eickbush TH: Origin and evolution of retroelements based upon their reverse transcriptase sequences. EMBO J 9:3353–3362 (1990).
Yang F, Graphodatsky AS, Li T, Fu B, Dobigny G, et al: Comparative genome maps of the pangolin, hedgehog, sloth, anteater and human revealed by cross-species chromosome painting: further insight into the ancestral karyotype and genome evolution of eutherian mammals. Chromosome Res 14:283–296 (2006).
Yates TL, Moore DW: Speciation and evolution in the family Talpidae (Mammalia: Insectivora). Prog Clin Biol Res 335:1–22 (1990).
Ye J, Biltueva L, Huang L, Nie W, Wang J, et al: Cross-species chromosome painting unveils cytogenetic signatures for the Eulipotyphla and evidence for the polyphyly of Insectivora. Chromosome Res 14:151–159 (2006).
Zhang Z, Schwartz S, Wagner L, Miller W: A greedy algorithm for aligning DNA sequences. J Comput Biol 7:203–214 (2000).
Zima J: The karyotype of *Talpa europaea kratochvili* (Talpidae, Insectivora). Folia Zoologica 32:131–136 (1983).
Zurita F, Sánchez A, Burgos M, Jiménez R, Díaz de la Guardia R: Interchromosomal, intercellular and interindividual variability of NORs studied with silver staining and in situ hybridization. Heredity 78:229–234 (1997).