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Quantitative variation of LINE-1 sequences in five species and three subspecies of the subgenus *Mus* and in five Robertsonian races of *Mus musculus domesticus*

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Abstract The quantitative variation of a conserved region of the LINE-1 ORF2 sequence was determined in eight species and subspecies of the subgenus *Mus* (*M. m. domesticus, M. m. musculus, M. m. castaneus, M. spicilegus, M. spretus*, *M. cervicolor, M. cookii*, *M. caroli*) and five Robertsonian races of *M. m. domesticus*. No differences in LINE-1 ORF2 content were found between all acrocentric or Robertsonian chromosome races, whereas the quantitative variation of the LINE-1 ORF2 sequences detected among the eight taxa partly matches with the clades into which the subgenus is divided. An accumulation of LINE-1 ORF2 elements likely occurred during the evolution of the subgenus. Within the Asiatic clade, *M. cervicolor*, *cookii*, and *caroli* show a low quantity of LINE-1 sequences, also detected within the Palearctic clade in *M. m. castaneus* and *M. spretus*, representing perhaps the ancestral condition within the subgenus. On the other hand, *M. m. domesticus*, *M. m. musculus* and *M. spicilegus* showed a high content of LINE-1 ORF2 sequences. Comparison between the chromosomal hybridization pattern of *M. m. domesticus*, which possesses the highest content, and *M. spicilegus* did not show any difference in the LINE-1 ORF2

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distribution, suggesting that the quantitative variation of this sequence family did not involve chromosome restructuring or a preferential chromosome accumulation, during the evolution of *M. m. domesticus*.

Keywords LINE-1 · *Mus* · Robertsonian translocation

Abbreviations

3′UTR	3′ untranslated region
5′UTR	5′ untranslated region
ANOVA	analysis of variance
bp	base pair
BSA	bovine serum albumin
DAPI	4′,6-diamidino-2-phenylindole
dCTP	deoxycytidine triphosphate
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleotide
FISH	fluorescence in-situ hybridization
kb	kilobase
LINE-1	long interspersed nuclear element-1
LTR	long terminal repeat
MITE	miniature inverted-repeat transposable element
mtDNA	mitochondrial deoxyribonucleic acid
NIH	National Institutes of Health
ORF1	open reading frame 1
ORF2	open reading frame 2
PBS	phosphate-buffered saline
PCR	polymerase chain reaction
Rb	Robertsonian
RNA	ribonucleic acid
SDS	sodium dodecyl sulfate
SNPs	single-nucleotide polymorphism
SSC	sodium chloride and sodium citrate
TEs	transposable elements

Introduction

The publication of the complete sequence of the mouse genome (Waterston et al. 2002) provides a near-complete census of the transposable, or mobile, elements (TEs) in this species (Furano et al. 2004). TEs make up a major component of the middle repetitive DNA of animals and plants genomes, where each of them is present in copies, their numbers ranging from just a few to thousands *per* genome (Kidwell and Lisch 1997). A characteristic of the mammalian genomes is, in fact, the presence of a high quantity of repetitive sequences, which, as a whole, account for about 70% of the entire genomes. TEs are classified in two different classes, according to their transposition mechanism (Finnegan 1992). Class I elements are retroelements (retrotransposons) that are capable of moving from a target locus using an RNA-mediated transposition mode; this class includes long terminal repeat (LTR) retrotransposons, such as retroviral elements, and non-long terminal repeat (non-LTR) retrotransposons, in which LINE-1 is the dominant clade in both humans (Lander et al. 2001) and mice (Waterston et al. 2002). Class II elements transpose directly from DNA to DNA (transposons *sensu stricto*) and include the well-characterized transposons, such as the *P* element of *Drosophila* (Craig 1990; Castro and Carareto 2004) and the *Tc1* element of *Caenorhabditis elegans* (Eide and Anderson 1988; Vos et al. 1993). Another category of TEs, called miniature inverted-repeat transposable elements (MITEs), has been identified but its transposition mechanism is still unknown (Wessler et al. 1995).

LINE-1 elements represent about 18.78% of the mouse genome (Waterston et al. 2002). A complete active mouse LINE-1 retroposon is approximately 7 kb long and presents a 5′ UTR region with promoter activity and a 3′ UTR region ending in a poly-A-rich tail (Loeb et al. 1986; Kazazian 2000; Deininger and Batzer 2002); a LINE-1 sequence also contains two overlapping open reading frames, which encode for an RNA binding protein (ORF1) and for both a reverse transcriptase and a DNA endonuclease protein (ORF2) (Dombroski et al. 1991; Mathias et al. 1991; Weiner 2002). Thanks to the retrotranscriptase activity, LINE-1 elements are able to proliferate via autonomous duplicative retrotransposition (Furano 2000). Only about 1% of full-length LINE-1 sequences are active. Despite their functional activity, complete LINE-1 elements are extremely rare and this is likely the consequence of an inefficient mechanism of replication; this event generates mostly defective copies that are truncated at their 5′ end. As a consequence, truncated and rearranged LINE-1 elements without autonomous mobile capacity accumulate in the genome (Sassaman et al. 1997; Kazazian 1999). Thus, TEs represent a valuable tool for measuring evolutionary forces acting on the genome (Usdin et al. 1995; Kazazian 1998; Kidwell 2002). TEs have a role in genome function (Spradling 1994; Charlesworth et al. 1994; Feschotte 2008; Böhne et al. 2008) and they have been shown to act as the principal ‘driving force’

in reshaping and remodelling the genome's composition and structure (Akagi et al. 2008). Also, LINE-1 elements exert a direct influence, some beneficial and other detrimental, on genome stability. Retrotransposition can destabilize the genome, shaping genomic landscapes by insertional mutagenesis, deletions and gene rearrangements (occasionally also altering gene expression; Muotri et al. 2007; Akagi et al. 2008). LINE-1 sequences have exerted a significant influence on the composition and architectural organization of the human and mouse genomes. They were shown to be directly involved in karyotypic rearrangements; their sequence similarity allows illegitimate pairing, chromatid breakage and rearrangement (Gray 2000; Boissinot et al. 2006; Song and Boissinot 2007). In primates, illegitimate recombination of LINE-1 sequences induced chromosome inversions (Schwartz et al. 1998; Kehrer-Sawatzki et al. 2002). In *Taterillus*, an increase in LINE-1 correlates with a chromosome-specific localization and to chromosome repatterning in several species of the genus (Dobigny et al. 2004). In four species of the genus *Microtus*, LINE-1 elements preferentially accumulated in the sex chromosomes (Marchal et al. 2006). The preferential accumulation of LINE-1 elements in the X-chromosomes in both *Taterillus* and *Microtus* was suggested to support Lyon's hypothesis (Lyon 2000) of the involvement of these sequences with the X-inactivation process (Dobigny et al. 2004; Marchal et al. 2006). In the mouse, LINE-1 elements are distributed all over the genome giving, when probed *in situ*, a G-banding similar pattern (Boyle et al. 1990).

A recent analysis of over 10 000 intermediate-length genomic variants on several strains and species of the genus *Mus* showed that about 85% of such variants are predominantly constituted by recently originated polymorphic LINE-1 elements (Akagi et al. 2008). LINE-1 active endogenous retrotransposition was suggested to contribute to a profound and rapid diversification of the genomic structures and transcripts distinguishing mouse lineages and driving a major portion of natural genetic variation (Akagi et al. 2008). This analysis also allowed the determination of the genome contribution of ancestral *M. castaneus* and *M. molossinus*, but not that of *M. spretus*, to the genome of some inbred mouse strains. The authors suggested assaying the use of nonpolymorphic LINE-1 sequences to trace the genome contribution of *M. spretus* (Akagi et al. 2008). However, a study of the LINE-1 sequences within the species of the genus *Mus* is lacking.

In this paper, we studied the quantity variation of a conserved region of the LINE-1 ORF2 sequence in eight species and subspecies of the subgenus *Mus* and five Robertsonian (Rb) races of *M. m. domesticus*.

Materials and methods

Animals

A list of the animals used is reported in Table 1.

DNA extraction

DNA from *M. m. domesticus* animals with standard and Rb karyotype and the female *M. spretus* was extracted from either fresh or ethanol-fixed spleens, using GenElute Mammalian Genomic DNA Miniprep Kit (Sigma, St. Louis, MO, USA). DNA from one male of *M. m. domesticus*, the male of *M. m. musculus* and one male of *M. spicilegus* was extracted from either fresh or ethanol fixed spleens using DNeasy Tissue Kit (Qiagen). DNAs of *M. m. molossinus*, *M. m. castaneus*, one male of *M. spicilegus*, *M. caroli*, *M. cervicolor*, *M. macedonicus*, *M. cookii* and the male DNA of *M. spretus* pertained to a DNA collection of one of the authors (S.G.) (see Garagna et al. 1993).

PCR amplification of LINE-1 fragment

Since LINE-1 sequences are very numerous and accumulate as active, truncated or degenerated copies in mammalian genomes, we chose already published PCR primers to produce a fragment of 290 bp that can recognize a conserved region within ORF2 of LINE-1 element (Dobigny et al. 2004). This approach has been used in a previous paper (Dobigny et al. 2004) to obtain a probe for FISH and Southern blot, in order to investigate the amount and distribution of LINE-1 in four species of the genus *Taterillus*. Forward and reverse LINE-1 degenerate primers (LINE-1 R 5′ ATTCTRTTCCATTGGTCTA 3′; LINE-1 F 5′ CCATGCTCATSGATTGG 3′) were designed from a conserved region between mouse, rabbit, rat and the human. The PCR amplification was performed in a final volume of 25 μl in the presence of 200 ng of genomic DNA, 1× PCR buffer containing 2 mM

Table 1 Species, strain, geographic origin, karyotype complement, number and sex of the specimens used in the present study

Species and strain	Origin	Karyotype	Number and sex	References
M. m. domesticus	CD1 Laboratory strain	2n=40 all acrocentric	2 ♂,1 ♀	–
M. m. domesticus	Varzi and Rieti (Italy)[a] Straas (Germany)[a]	2n=40 all acrocentric	10 ♂	Hauffe and Searle (1998)
M. m. domesticus	Cittaducale[a] (Italy)	2n=22 Rb(1,7), Rb(2,18), Rb(3,8), Rb(4,15), Rb(5,17), Rb(6,13), Rb(9,16), Rb(10,11), Rb(12,14) (CD race)	1 ♂,1 ♀	Capanna et al. (1976)
M. m. domesticus	Milano[a] (Italy)	2n=24 Rb(5,15), Rb (11,13), Rb(9,14), Rb(16,17), Rb(10,12), Rb(2,4), Rb(3,6), Rb(7,8) (Milan I race)	1 ♂,1 ♀	Gropp and Winking (1981)
M. m. domesticus	Milano[a] (Italy)	2n=24 Rb(5,15), Rb (11,13), Rb(9,14), Rb(16,17), Rb(2,8), Rb(10,12), Rb(3,4), Rb(6,7) (Milan II race)	2 ♂	Gropp and Winking (1981)
M. m. domesticus	Lipari[a] (Italy)	2n=26 Rb(1,2), Rb(3,9), Rb(4,13), Rb (5,14), Rb(6,16), Rb(8,12), Rb(10,15) (Lipari race)	3 ♂	Gropp and Winking (1981)
M. m. domesticus	Vulcano[a] (Italy)	2n=26 Rb(1,2), Rb(3,9), Rb(4,13), Rb (5,14), Rb(8,12), Rb(10,16), Rb(15,17) (Vulcano race)	6 ♂	Solano et al. (2007)
M. m. musculus	Czech Republic	2n=40	1 ♂,1 ♀	–
M. m. castaneus	Unknown[b]	2n=40	1 ♂	Garagna et al. (1993)
M. m. molossinus	Unknown[b]	2n=40	1 ♂	Garagna et al. (1993)
M. spicilegus	Dulov Dvor (Slovakia)[a] Attiki (Greece)[a]	2n=40	3 ♂	–
M. macedonicus	Unknown[b]	2n=40	1 ♂,1 ♀	Garagna et al. (1993)
M. spretus	Mouse colony Université Montpellier II (France)	2n=40	1 ♂,1 ♀	–
M. cervicolor	Unknown[b]	2n=40	2 ♀	Garagna et al. (1993)
M. cookii	Unknown[b]	2n=40	1 ♂	Garagna et al. (1993)
M. caroli	Unknown[b]	2n=40	1 ♂	Garagna et al. (1993)

[a] Wild trapped.

[b] DNA collection of the authors.

$MgCl_2$, 200 μM of each dNTP, 500 nM of each primer, 1 IU AmpliTaq polymerase (Applera). Amplification conditions were: 94°C for 2 min, followed by 30 cycles at 94°C for 30 s, 52.5°C for 30 s, 72°C for 30 s, followed by a single step at 72°C for 7 min. The amplicons were run on 2.0% agarose gel and the 290 bp band was then purified from the gel (QIAquick Gel Extraction Kit, Qiagen) for direct sequencing in order to control the specificity of the amplification. The bases of the sequences were all clearly identifiable all along the length of the amplicon; thus we decided that cloning was not necessary.

The 290 bp LINE-1 amplicon, obtained from the genomic DNA of a male CD1 laboratory strain animal, was labelled either by random priming with [α-^{32}P]dCTP (Megaprime Labelling Kit, Amersham, Bucks, UK) and used as a probe for the Southern blotting hybridization or by PCR with digoxigenin and used as a probe for FISH analysis.

Southern blotting analysis

For each sample listed in Table 1, with the exception of *M. m. molossinus* and *M. macedonicus*, 7 μg of high-molecular-weight genomic DNA was digested with 40 U of *Eco*RI (Roche) for 16 h and separated on a 0.7% agarose gel (Biorad). The gel was blotted onto a nylon membrane (Hybond N+, Amersham) and the

DNAs were then hybridized with the [α-^{32}P]dCTP-labelled LINE-1 probe. Hybridization was carried out overnight at 65°C and the final washing was performed in 0.2× SSC, 0.5% SDS. Hybridization signals were detected and revealed using the Cyclone (Storage Phosphore Screen) (Packard) and the hybridization signals were subsequently quantified using the NIH ImageJ software.

Chromosomes preparation and fluorescence in-situ hybridization (FISH)

Chromosome spreads from *M. m. domesticus* and *M. spicilegus* animals were prepared from the bone marrows, according to standard procedure.

For FISH analysis, slides were pretreated with pepsin to a final concentration of 1 mg/ml (pH 2), for 5 min at 37°C. 20 ng of 290 bp LINE-1 digoxigenin labelled probe was hybridized overnight in 50% formamide in 2× SSC and 1× Denhardt's at 37°C in a humid chamber. Washes were performed in 50% formamide in 2XSSC at 42°C. The hybridization signal was revealed with a rhodaminated sheep anti-digoxigenin antibody (10 ng/μl in PBS 1×/BSA 1%, Roche), followed by a rhodamine-conjugated rabbit anti-sheep antibody (50 ng/μl in PBS 1×/BSA 1%, Chemicon International) and by a rhodamine-conjugated goat anti-rabbit antibody (10 ng/μl in PBS 1×/BSA 1%, Calbiochem). Chromosomes were counterstained with DAPI (0.2 μg/ml).

Results

Sequence analysis of a 290 bp LINE-1 ORF2 region

A target region of the ORF2 of the LINE-1 element was amplified from specimens of *M. m. musculus*, *M. m. castaneus*, *M. m. molossinus*, *M. spicilegus*, *M. macedonicus*, *M. spretus*, *M. cervicolor*, *M. cookii*, *M. caroli* and of *M. m. domesticus* with all acrocentric or with Rb metacentric chromosomes. The results of the amplification procedure showed an intense prevalent band of 290 bp, which was purified from the gel and subsequently directly sequenced. The 290 bp sequence derived from *M. m. musculus* and *M. m. domesticus* with 2n=40 or Rb karyotypes showed no differences when compared to that present in GenBank (ref. no. M13002), suggesting a complete conservation of the sequence itself. In contrast, some nucleotide substitutions, deletions or insertions, and single-nucleotide polymorphisms (SNPs) were found in individuals of the remaining species analysed (Fig. 1). The sequences differing most, although to a limited extent, are those obtained from *M. cookii*, *M. caroli* and *M. cervicolor*. In particular, in *M. cookii* a deletion at position 69, two SNPs at positions 143 (C or T) and 224 (G or T) and 7 other nucleotide substitutions were found. In *M. caroli*, three deletions were observed at positions 53, 57, and 69 respectively; two SNPs at positions 119 (T or G) and 224 (C or G) and five other nucleotide substitutions were also found. The SNP at position 224 (G/C or T) was also found in *M. macedonicus* and in *M. spretus*. In *M. cervicolor*, an insertion of a nucleotide was detected at position 93 of the amplified LINE-1 fragment, never found in the other *Mus* taxa, and in addition one deletion, two SNPs and four substitutions were also observed; *M. m. molossinus* displayed few differences, in fact only a deletion and a single nucleotide substitution were detected (at position 57 and at position 119, respectively); the *M. m. castaneus* fragment differed only by a single substitution at position 295. In addition, very few differences, in the order of 1–3 nucleotides, were detected when comparing the two individuals of *M. spicilegus*, *M. macedonicus* and *M. spretus*, as reported in Fig. 1.

The comparison between the sequence of *M. m. domesticus* and the sequences of the other *Mus* taxa showed differences ranging from none (*M. m. musculus*) to 3% (*M. cookii*). This low variability in the sequences of the PCR products allowed us to use the amplicon obtained from *M. m. domesticus* 2n=40 as a probe for FISH on *M. spicilegus* chromosomes and Southern blotting on the DNAs of all the subspecies and species used in our research. The very low level of mismatch between the probe and the target DNA sequences guarantees the appropriate signal detection with the hybridization conditions used.

Quantitative Southern blot analysis of LINE-1 sequences

Quantification of LINE-1 elements in house mice with differentiated karyotypes

The LINE-1 probe displayed a continuous smear, probably due to the presence of degenerated or

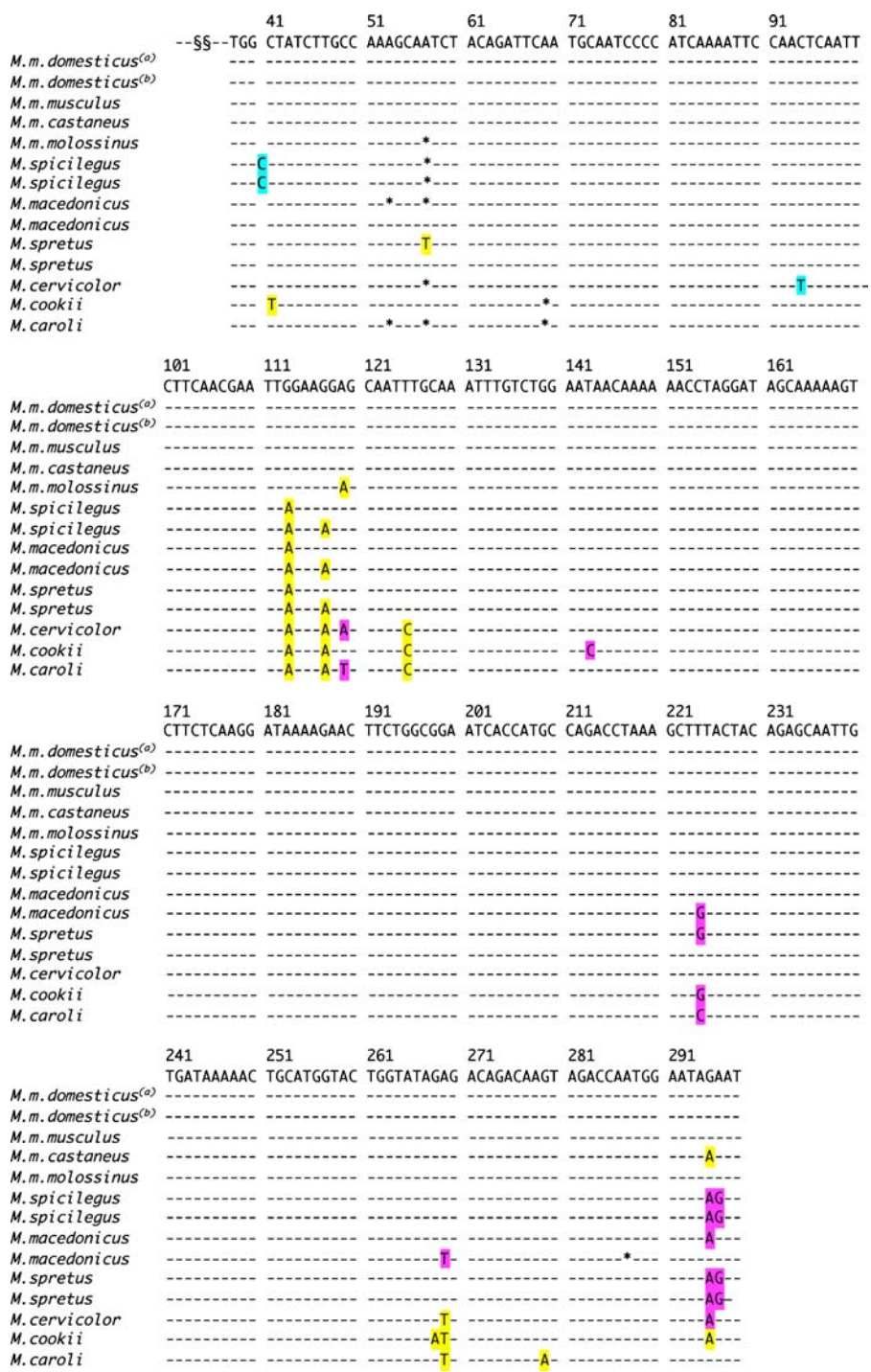

Fig. 1 Sequence of the 290 bp LINE-1 ORF2 fragments obtained from individuals of the ten species and subspecies of the subgenus *Mus*. [(a)]2n= 40 sample; [(b)]Rb sample; — identical nucleotide; N, nucleotide substitution; N SNP; N insertion; * deletion

truncated LINE-1 sequences, and a strong signal at 1.3 kb (Fig. 2a) expected after *Eco*RI restriction enzyme digestion, which is known to delimit a 1.3 kb fragment within the LINE-1 ORF2, in both *M. m. domesticus* all acrocentric and Rb chromosome complement mice (listed in Table 1). For the quantitative estimation of the LINE-1 sequences, we first evaluated the potential differences in the DNA that was loaded onto the gel for each sample. For this purpose, for each lane, we measured the intensity of

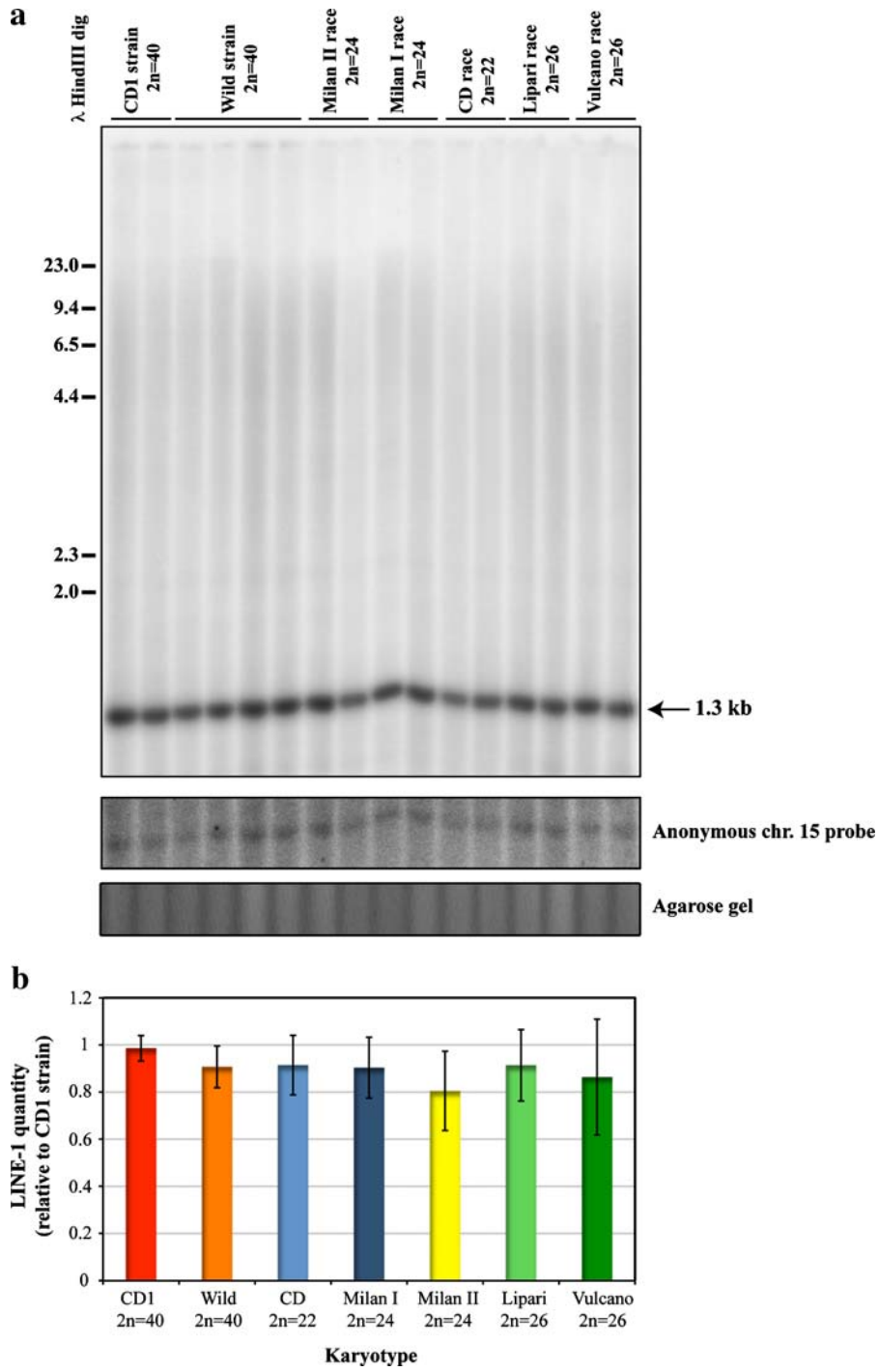

Fig. 2 **(a)** A representative Southern blotting analysis of LINE-1 elements of *M. m. domesticus* specimens with all acrocentric and differentiated karyotypes. Each lane corresponds to a single individual. **(b)** Histogram representation of LINE-1 quantification. No quantitative differences were found among the different races. Bar represents the mean standard deviation

six different regions of the DNA smear stained with propidium iodide before blotting (Fig. 2a). Then, for each sample, the mean value obtained was normalized against the mean value determined for the CD1 specimen. Moreover, for the same purpose, we analysed the hybridization signal intensity of a single band obtained using an anonymous sequence located on chromosome 15 after blotting (Rebuzzini et al. 2007) (Fig. 2a). After the normalization procedure, the ratios between the mean intensity values of the

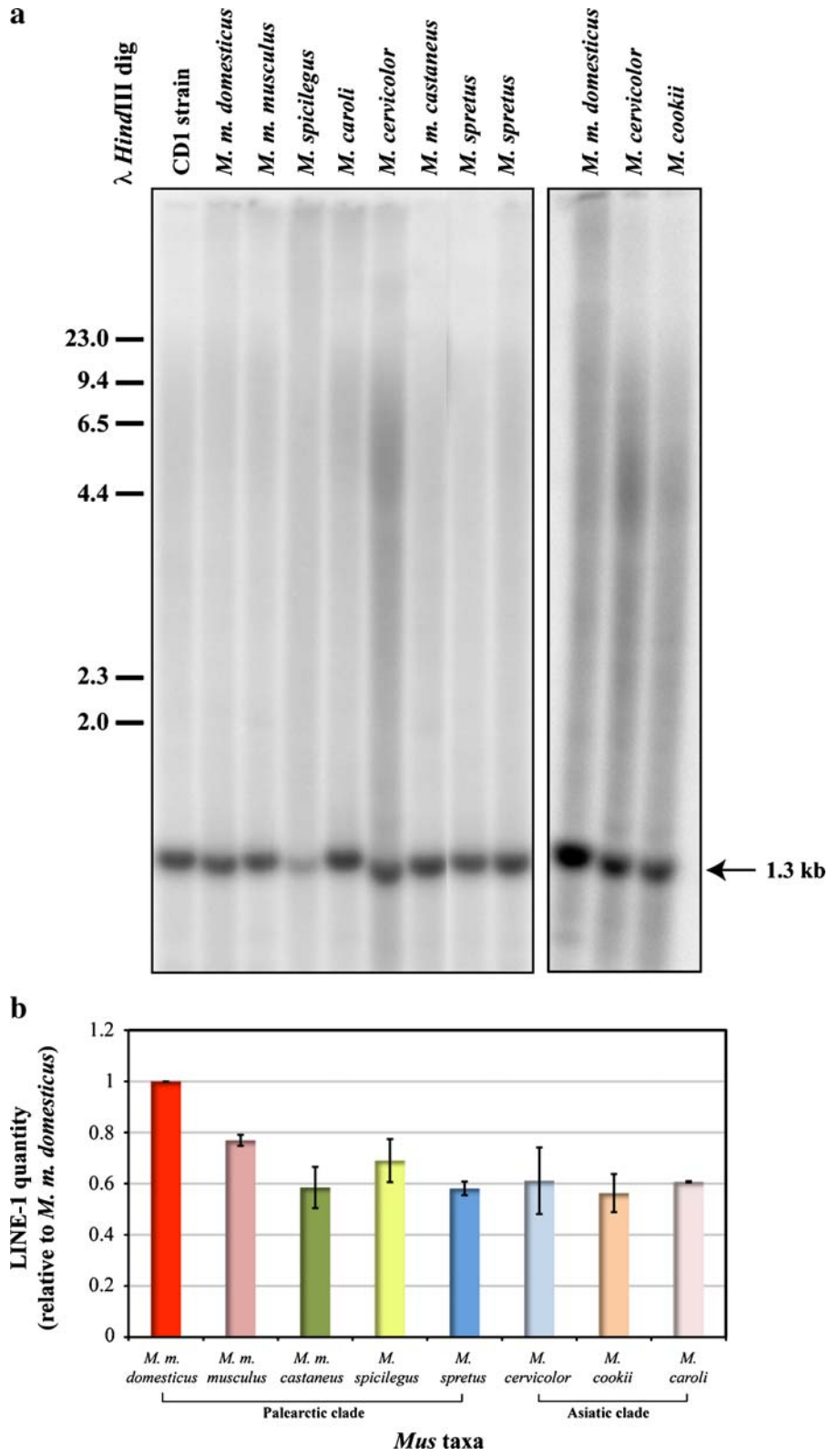

Fig. 3 **(a)** A representative Southern blotting analysis of LINE-1 elements of individuals from eight species and subspecies of the subgenus *Mus*. **(b)** Histogram representation of LINE-1 quantification. For the description of the significant differences, see text. Bar represents the mean standard deviation

hybridization signals of each lane of the CD1 strain and the mean intensity values obtained for each species were calculated (Fig. 2b). The statistical analysis (ANOVA test) showed no variation in the LINE-1 content among the individuals, irrespective of karyotype complement (Fig. 2b).

Quantification of LINE-1 elements in mice from different species and subspecies

Using the above approach, we performed a quantitative analysis of LINE-1 elements of eight samples, belonging to five different species (*M. spicilegus*, *M. spretus*, *M. cookii*, *M. caroli* and *M. cervicolor*) and three subspecies (*M. m. domesticus*, *M. m. musculus* and *M. m. castaneus*; the DNAs of *M. m. molossinus* and *M. macedonicus* were of insufficient quantity to allow us to perform Southern analysis). Hybridization signals of different intensity were observed among the different *Mus* taxa, with *M. m. domesticus* displaying the highest content of LINE-1 sequences (Fig. 3b). Within the Palearctic clade, *M. m. castaneus* and *M. spretus* genomes carry about 60% of the quantity of LINE-1 elements in comparison with that of *M. m. domesticus*. The same 60% was found when comparing the species of the Asiatic clade with *M. m. domesticus*. The statistical analysis (ANOVA and Fisher test) showed significant difference between *M. m. domesticus* and all the other subspecies and species (Fig. 4). Moreover, significant differences were also found when comparing *M. m. musculus* versus *M. m. castaneus*, *M. spretus*, *M. cookii*, *M. cervicolor* or *M. caroli* (Fig. 4).

FISH analysis of LINE-1 elements in mouse with acrocentric and Rb karyotypes

We also determined the distribution pattern of LINE-1 elements by FISH analysis of metaphase spreads obtained from bone marrow of mice from *M. m. domesticus* (Milan II Rb race) and *M. spicilegus*. The hybridization pattern is similar in all samples analysed (Fig. 5), with LINE-1 elements distributed along the entire chromosomes, with the exception of the pericentromeric heterochromatic regions, where no hybridization signals were detected (Fig. 5a, c).

Discussion

In this study, we analysed the quantitative variation of the LINE-1 ORF2 fragment in eight species and subspecies and in five Rb races of *M. m. domesticus*, in order to trace its evolution in the subgenus *Mus*. We also compared the LINE-1 ORF2 chromosome distribution in *M. m. domesticus* (Milan II chromosome race) and *M. spicilegus*.

When comparing specimens with all acrocentric or Rb chromosome karyotypes within the subspecies *M. m. domesticus*, no quantitative differences in the

Species	*M. m. domesticus*	*M. m. musculus*	*M. m. castaneus*	*M. spicilegus*	*M. spretus*	*M. cervicolor*	*M. cookii*	*M. caroli*
M. m. domesticus	-							
M. m. musculus	yes	-						
M. m. castaneus	yes	yes	-					
M. spicilegus	yes	no	no	-				
M. spretus	yes	yes	no	no	-			
M. cervicolor	yes	yes	no	no	no	-		
M. cookii	yes	yes	no	no	no	no	-	
M. caroli	yes	yes	no	no	no	no	no	-

Fig. 4 Comparison between LINE-1 quantitative differences among different species and subspecies of the subgenus *Mus*. Pairwise multiple comparison (Fisher test, $p<0.001$)

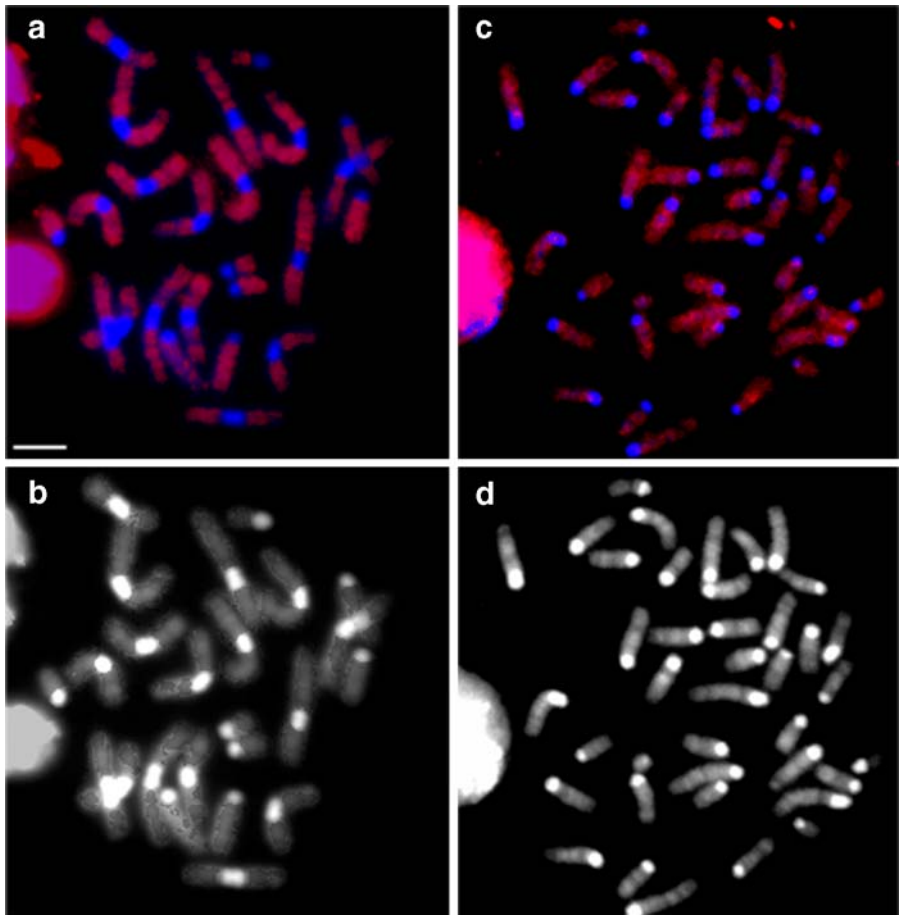

Fig. 5 Merging of the LINE-1 hybridization signals (red) and DAPI counterstaining (blue) in *M. m. domesticus* 2n=24 Milan II **(a)** and in *M. spicilegus* **(c)** metaphase chromosomes. In **(b)** and **(d)**, chromosomes are counterstained with DAPI. Bar represents 10 μm

LINE-1 sequences were detected. Comparison between the published hybridization pattern of *M. m. domesticus* 2n=40 (Boyle et al. 1990) and 2n=24 mitotic spreads showed that LINE-1 elements were distributed along the entire chromosomes in both races, with the exception of the pericentromeric heterochromatic regions, where no signal was detected. These data suggest that the very recent karyotypic differentiation in *M. m. domesticus* did not involve any detectable quantitative or distributional differences of LINE-1 elements along the chromosomes.

The quantitative variation of the LINE-1 ORF2 fragment partly matches with the clades into which the subgenus is divided. In fact, the three species, *M. cervicolor*, *cookii* and *caroli*, which belong to the Asiatic clade (Lundrigan et al. 2002; Chevret and Dobigny 2005), show a low quantity of LINE-1 sequences, although similar to the quantity of *M. m. castaneus* and *M. spretus*. The three Asiatic species must have separated from each other almost simultaneously (She et al. 1990) and there exists, therefore, disagreement with regard to the order of their evolution (Guénet and Bonhomme 2003; Tucker et al. 2005). It is nevertheless almost certain that *M. cervicolor* and *M. caroli* cannot be sister taxa, whereas the remaining two combinations are possible (Lundrigan et al. 2002; Guénet and Bonhomme 2003; Chevret and Dobigny 2005; Tucker et al. 2005). Yet *M. caroli* might be the oldest of the three (Tucker et al. 2005). In any case, the quantity of LINE-1 ORF2 found in the species of the Asiatic clade might reflect the ancestral condition. Besides, the similar quantity of ORF2 LINE-1 sequences detected in the three subspecies of the Asiatic clade is in agreement with their more basal positioning with respect to the Palearctic species in the proposed phylogenetic trees of the subgenus *Mus* (Lundigran et al. 2002, Chevret and Dobigny 2005; Tucker et al. 2005).

As observed in other mammalian species (Bailey et al. 2000; Dobigny et al. 2004; Waters et al. 2004;

Marchal et al. 2006), an accumulation of LINE-1 elements occurred during the evolution of the subgenus *Mus*. Within the Palearctic clade, *M. m. musculus* and *M. m. domesticus* show the highest content of LINE-1 sequences. *M. m. domesticus* possesses a significantly higher quantity of LINE-1 when compared to both *M. m. castaneus* and *M. m. musculus*, the latter displaying an intermediate quantity between that of the other two subspecies. Interestingly, the divergence of *M. m. domesticus* from the other *Mus* subspecies, and in particular the recent evolutionary events (dated about 7×10^5 years ago) that led to the differentiation of the two *M. m. domesticus* and *M. m. musculus* subspecies (Yonekawa et al. 1982; Ferris et al. 1983), seem to have been accompanied by the amplification of repetitive sequences. Amplification and homogenization of block repeats of satellite DNAs has already been reported for *M. m. domesticus* in comparison with *M. m. musculus* and with other taxa of the subgenus (Redi et al. 1990; Garagna et al. 1993). It has been suggested that the large quantity of satellite DNA organized in tandem repeats all over the chromosomes in *M. m. domesticus* genome could be related to the extensive karyotype restructuring based on Rb translocations (Redi et al. 1990; Garagna et al. 1993), which is restricted to this subspecies within the subgenus *Mus*.

Several studies support that within the Eurasian clade, *M. musculus* and *M. spicilegus* are sister species, while *M. spretus* is considered basal in respect to them (Prager et al. 1996; Chevret and Dobigny 2005), although the conflicting tree topology of mtDNA and nuclear sequence data does not allow for a definitive decision on their phylogeny (Tucker et al. 2005). In this evolutionary context, it might be plausible that the high content of LINE-1 in *M. m. musculus*, *M. m. domesticus* and *M. spicilegus* represents an accumulation, whereas the low quantity of LINE-1 in *M. m. castaneus* and *M. spretus* may represent the ancestral condition, as shown for the Asiatic clade.

Despite the differences in quantity of LINE-1 sequences, their chromosome distribution detected after FISH hybridization was similar between *M. m. domesticus* and *M. spicilegus*. Interestingly, as in other rodent species, these quantitative variations did not involve chromosome restructuring or a preferential chromosome accumulation during the evolution of *M. m. domesticus*.

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