[Finding of Bov-B LINE retroelement in parthenogenetic and bisexual lizard species of the genus Darevskia (Lacertidae)].

ARTICLE *in* GENETIKA · AUGUST 2006

Impact Factor: 0.37 · Source: PubMed

5 AUTHORS, INCLUDING:

Vitaly Korchagin
Russian Academy of Sciences
23 PUBLICATIONS **95** CITATIONS

ISSN 1022-7954, Russian Journal of Genetics, 2006, Vol. 42, No. 7, pp. 790–794. © Pleiades Publishing, Inc., 2006.
Original Russian Text © I.A. Martirosyan, V.I. Korchagin, O.N. Tokarskaya, I.S. Darevsky, A. P. Ryskov, 2006, published in Genetika, 2006, Vol. 42, No. 7, pp. 963–967.

ANIMAL GENETICS

Finding of *Bov-B* LINE Retroelement in Parthenogenetic and Bisexual Lizard Species of the Genus *Darevskia* (Lacertidae)

I. A. Martirosyan[a], V. I. Korchagin[a], O. N. Tokarskaya[a], I. S. Darevsky[b], and A. P. Ryskov[a]

[a] *Institute of Gene Biology, Russian Academy of Sciences, Moscow, 119334 Russia; fax: (495) 135-41-05; e-mail: dna@biogen.msk.su*
[b] *Institute of Zoology, Russian Academy of Sciences, St. Petersburg, 119034 Russia; fax: (812) 114-04-44; e-mail: dis@zisp.spb.su*

Received January 30, 2006

Abstract—The *Bov-B* LINE retrotransposon was first discovered in Ruminantia and was long considered to be specific for this order. Later, this mobile element was described in snakes and some lizard species. Analysis of phylogenetic relationships of *Bov-B* LINE elements from different ruminants, snakes, and lizard species led to the suggestion on horizontal transfer of this retrotransposon from Squamata to Ruminantia. In the Squamata group, *Bov-B* LINE element was found in all snakes and some lizard species examined. The element was not detected in the genomes of some species of the genera *Lacerta* and *Podarcis*. In the present study, using PCR amplification and sequencing of PCR products, *Bov-B* LINE element was identified in the genomes of parthenogenetic and bisexual species of the genus *Darevskia* (Lacertidae), as well as in such species as *Lacerta agilis* and *Zootoca vivipara*, where this retrotransposon had not been not detected before.

DOI: 10.1134/S1022795406070143

INTRODUCTION

LINE elements belong to the group of retrotransposons not carrying long inverted repeats and are widely distribution in the genomes of all eukaryotes, where they are represented by multiple 5'-truncated nonfunctional copies and several active copies [1]. The *Bov-B* LINE element is the member of this retrotransposon group. It is characterized by conservative structure [2] and contains short 3' fragments, mostly consisting of variable number of pentamer repeats [3]. Earlier, *Bov-B* LINE was identified in ruminants [4], as well as in all snake and some lizard species from the genera *Lacerta* and *Podarcis* [5]. In reptiles, 5'-truncated *Bov-B* LINE element was first identified in the fourth intron of two *Vipera ammodytes* genes for the PLA_2 toxin. At orthologous positions in other snake PLA_2 genes, the *Bov-B* LINE element is absent [6, 7]. It is suggested that amplification of *Bov-B* LINE might have occurred before the divergence within the order Squamata and played a role in speciation in Squamata and Ruminantia. Analysis of phylogenetic relationships of *Bov-B* LINE elements from different species led to the suggestion on horizontal transfer of retrotransposon from ancient Squamata to the ancestors of Ruminantia [8].

In lizards, the *Bov-B* LINE structure, the copy number, as well as the distribution and phylogenetic relationships remain unclear. In particular, nothing is known on the existence of this element in the genomes of parthenogenetic and bisexual species of the genus *Darevskia*. Sequence analysis (using the GenBank database) pointed to partial homology between the Du 215 (AY574978) clone from the parthenogenetic lizard *Darevskia unisexualis* genomic library [9] and the *Bov-B* LINE containing clone, PM7 (AF013024), from the *Podarcis muralis* lizard. This established the task of the present study, i.e., searching for the *Bov-B* LINE elements in the lizards of the genus *Darevskia*.

In the present study, the *Bov-B* LINE element was identified in the genomes of parthenogenetic and bisexual species of the genus *Darevskia*, as well as in such species as *Lacerta agilis* and *Zootoca vivipara*, in which this retrotransposon of interest had not been detected before.

MATERIALS AND METHODS

Samples from four parthenogenetic and five bisexual species from the genus *Darevskia* were used in the study. Samples from *Podarcis muralis, P. taurica, Lacerta strigata*, and *Bos taurus* were used as positive controls, while samples from *Lacerta agilis, Zootoca vivipara, Rutilus rutilus, Gallus gallus, Grus leucogeranus, Mus musculus*, and *Homo sapiens* served as

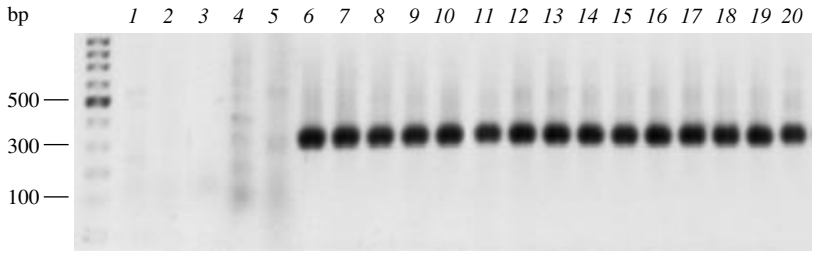

Fig. 1. PCR amplification of the *Bov-B* LINE element conservative 3'-terminal regions in different vertebrates. Amplification products were separated in 1% agarose gel and visualized by staining with ethidium bromide. *1*, *Grus leucogeranus*; *2*, *Gallus gallus; 3, Rutilus rutilus; 4,Mus musculus; 5,Homo sapiens; 6, Podarcis muralis; 7, P. taurica; 8, Lacerta strigara; 9, L. agilis; 10, Zootoca vivipara; 11, Darevskia mixta; 12, D. valentini; 13, D. portchinskii; 14, D. nairensis; 15, D. raddei; 16, D. armeniaca; 17, D. dahli; 18, D. rostombekovi; 19, D. unisexualis; 20, Bos taurus.* The 100 bp Ladder+ (Fermentas, Lithuania) was used as molecular size marker. The sizes of DNA fragments are given in base pairs (bp).

negative controls [5, 8]. Genomic DNA was isolated according to a standard phenol–chloroform method with proteinase K treatment [10]. Amplification of the *Bov-B* LINE conservative 3'-terminal region was performed with 20 ng of genomic DNA and primers used previously for other objects [5]. The reaction was carried out in a final volume of 20 μl, containing 1 × *Taq* polymerase buffer (Dialat, Russia), 2 mM $MgCl_2$, 0.25 mM of each dNTP, 1 μM of each oligonucleotide, and 0.8 units of *Taq* polymerase (Dialat, Russia). The reaction conditions were as follows: 5 min at 95°C, followed by 30 cycles of 95°C for 1 min; 50°C for 1 min; 72°C for 1 min; with the 5-min final extension at 72°C. Amplification products were loaded onto 1% agarose gel. Sequencing of the amplification products was performed according to Sanger's method with the ABI PRISM(r) BigDye™ Terminator v. 3.1 reagent kit and subsequent analysis of the reaction products in the automated sequencer DNA ABI PRISM 3100-Avant. The *Bov-B* LINE fragment sequences from different samples were aligned using the MegAlign 4.05. software program. Genetic distances between the nucleotide sequences were determined using different methods implemented in the MEGA 3. software program.

Genetic distances between the *Bov-B* LINE fragments from different lizard species calculated using two-parametric Kimura's model implemented in the MEGA 3 software package

	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*
1													
2	0.0290												
3	0.0216	0.0216											
4	0.0904	0.0904	0.0825										
5	0.0440	0.0440	0.0366	0.0439									
6	0.0440	0.0440	0.0366	0.0439	0.0000								
7	0.0440	0.0440	0.0366	0.0439	0.0000	0.0000							
8	0.0364	0.0364	0.0290	0.0514	0.0071	0.0071	0.0071						
9	0.0364	0.0364	0.0290	0.0514	0.0071	0.0071	0.0071	0.0000					
10	0.0669	0.0669	0.0592	0.0668	0.0216	0.0216	0.0216	0.0289	0.0289				
11	0.0440	0.0440	0.0366	0.0439	0.0000	0.0000	0.0000	0.0071	0.0071	0.0216			
12	0.0364	0.0364	0.0290	0.0514	0.0071	0.0071	0.0071	0.0144	0.0144	0.0289	0.0071		
13	0.0364	0.0364	0.0290	0.0514	0.0071	0.0071	0.0071	0.0144	0.0144	0.0289	0.0071	0.0000	

Note: *1*, *Podarcis muralis*; *2*, *P. taurica*; *3*, *Zootoca vivipara*; *4*, *L. agilis*; *5*, *Darevskia mixta*; *6*, *D. valentini*; *7*, *D. portchinskii*; *8*, *D. nairensis*; *9*, *D. raddei*; *10*, *D. armeniaca*; *11*, *D. dahli*; *12*, *D. rostombekovi*; *13*, *D. unisexualis*.

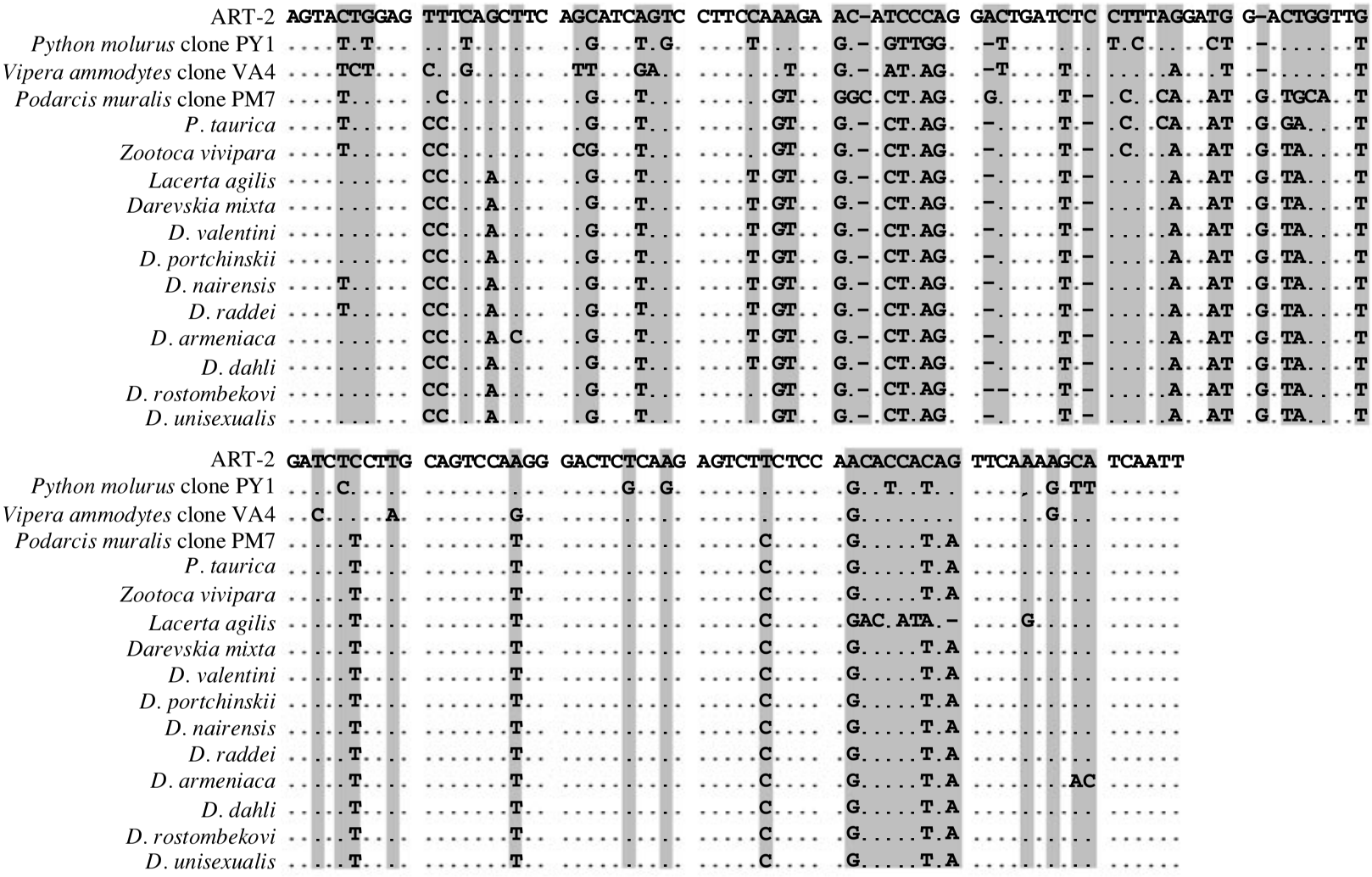

Fig. 2. Sequence comparison of the *Bov-B* LINE element 3'-terminal regions in different lizards and snakes. The sequences taken for the comparison were represented by: ART-2 (X82879) consensus; *Python molurus* clone PY1 (AF048710); *Vipera ammodytes* clone VA4 (AF012992); *Podarcis muralis* clone PM7 (AF013024); *P. taurica* (AY847650); *Zootoca vivipara* (AY847652); *Lacerta agilis* (AY847651); *Darevskia mixta* (AY847657); *D. valentini* (AY847658); *D. portchinskii* (AY847659); *D. nairensis* (AY847660); *D. raddei* (AY847661); *D. armeniaca* (AY847653); *D. dahli* (AY847654); *D. rostombekovi* (AY847655); *D. unisexualis* (AY847656). Sequences of the clones PM7 from *Podarcis muralis*, PY1 from *Pyton molurus*, and VA4 from *Vipera ammodytes* were taken from the GenBank. Variable sites are in gray color.

RESULTS AND DISCUSSION

The data on PCR amplification of different DNA samples are demonstrated in Fig. 1. The 300-bp amplification products were produced in all lizard species studied, including *L. agilis* and *Z. vivipara* used as negative controls. Sequence comparisons of the amplification products with the GenBank database revealed their homology to the analogous *Bov-B* LINE fragment of ruminates. For further comparative analysis, not the whole 300-bp sequence, but its 143-bp fragment, containing no doubtful N nucleotides, was used (Fig. 2). In most of the cases, the differences in this region were represented by single nucleotide substitutions. The level of differences was calculated using two-parametric method of Kimura as implemented in the MEGA 3 software package (table). The highest difference (0.0904) was revealed between *L. agilis* and the two *Podarcis* species. The difference between the *Podarcis* species and *Z. vivipara* was small (0.0216). The difference of the *Darevskia* species from the *Podarcis* species, and from *Z. vivpara* and *L. agilis* constituted on average 0.0432, 0.0323, and 0.0531, respectively.

Within the genus *Darevskia* variation of the *Bov-B* LINE 3'-terminal region between the species was low, varying from 0 to 0.0289. The highest values were revealed in pairwise comparisons of parthenogenetic species *D. armeniaca* with parthonogenetic species *D. unisexualis* and *D. rostombekovi*, as well as in its comparisons with bisexual species *D. nairensis* and *D. raddei* (note that based on some molecular genetic data, *D. nairensis* and *D. raddei* are assigned to in one complex, *D. raddei* [11, 12]). According to the data obtained, bisexual species *D. nariensis* and *D. raddei*, as well as *D. portchinski* and *D. valentini*, form two groups, which reflect their evolutionary relationships [13–15]. In addition, it was demonstrated that parthenogenetic species *D. unisexualis* and *D. rostombekovi* appeared as a result of hybridization between evolutionary close bisexual species (*D. nariensis, D. raddei,* and *D. portchinskii, D. valentini*), were characterized by identical genetic distances.

Thus, in this study the existence of retrotransposons in unisexual vertebrates was demonstrated for the first time. The contribution of retrotransposons (in this case, of the *Bov-B* LINE) to the variation and/or instability of the hybrid genomes (the genomes of all parthenogenetic *Darevskia* species belong to this type) remains unclear. It can be suggested that the formation of a hybrid genome can switch on transcription of previously inactive retroelement, resulting in the increased instability of some chromosomes, or genomic regions of parthenogenetic species. Earlier it was demonstrated, that genomes of parthenogenetic species of the genus *Darevskia*, especially of *D. unisexualis*, possess hyperinstable regions that contain microsatellite sequences [16, 17]. The reasons for this instability are still unknown. The role of microsatellites, retrotransposons, and of their combination in this process is suggested. Hopefully, further investigation of the *Bov-B* LINE structure and distribution among bisexual and unisexual lizards of the genus *Darevskia* will shed the new light on the mechanisms of genomic instability and extend the knowledge on the origin and evolution of parthenogenetic species.

ACKNOWLEDGMENTS

This work was supported by the Russian Foundation for Basic Research (grant no. 05-04-48923), the Presidential Program "Leading Scientific Schools of Russia" (grant no. NSh-6156.2006.4), Program of Support for Young Scientists (grant no. MK-2240.2005.4), and the Program of the Presidium of the Russian Academy of Sciences "Dynamics of Plant, Animal, and Human Gene Pools" (grant no. 142/200404-112).

REFERENCES

1. Hutchison, III C.A., Yardies, S.C., Loeb, D.D., et al., *Mobile DNA*, Berg, D.E. and Howe, M.M., Eds., American Society of Microbiology, Washington DC, 1989, pp. 593–617.
2. Malik, H.S. and Eckbush, T.H., The RTE Class of Non-LTR Retrotransposons is Widely Distributed in Animals and is the Origin of Many SINEs, *Mol. Biol. Evol.*, 1998, no. 15, pp. 1123–1134.
3. Kordiš, D. and Gubenšek, F., Horizontal Transfer of Non-LTR Retrotransposons in Vertebrates, *Genetica*, 1999, no. 107, pp. 121–128.
4. Jobse, C., Buntjer, J.B., Haagsma, N., et al., Evolution and Recombination of Bovine DNA Repeats, *J. Mol. Evol.*, 1995, no. 41, pp. 277–283.
5. Kordiš, D. and Gubenšek, F., Unusual Horizontal Transfer of a Long Interspersed Nuclear Element between Distant Vertebrate Classes, *Proc. Natl. Acad. Sci. USA,* 1998, no. 95, pp. 10704–10709.
6. Kordiš, D. and Gubenšek, F., The Bov-B LINEs Found in *Vipera ammodytes* Toxic PLA_2 Genes are Widespread in Snake Genomes, *Toxicon*, 1998, no. 36, pp. 1585–1590.
7. Kordiš, D. and Gubenšek, F., Bov-B Long Interspersed Repeated DNA (LINE) Sequences are Present in *Vipera ammodytes* Phospholipase A_2 Genes and in Genomes of Viperidae Snakes, *Eur. J. Biochem.*, 1997, no. 246, pp. 772–779.
8. Kordiš, D. and Gubenšek, F., Molecular Evolution of Bov-B LINEs in Vertebrates, *Gene*, 1999, no. 238, pp. 171–178.
9. Korchagin V. I., Martirosyan I.A., Omelchenko A.V., et al., Study of Allelic Polymorphism of $(GATA)_n$-Containing Loci in Parthenogenetic Lizards *Darevskia unisexualis* (Lasertidae), *Rus. J. Genet.*, 2004, vol. 40, no. 10, pp. 1095–1101.
10. Mathew, C.G.P., *Methods in Molecular Biology*, Walker, J.M., Ed., New York: Humana, 1984, vol. 2, pp. 31–34.
11. Grechko, V.V., Fedorova, L.V., Fedorov, A.N., et al., Restriction Endonuclease Analysis of Highly Repetitive

DNA as a Phylogenetic Tool, *J. Mol. Evol.*, 1997, no. 45, pp. 332–336.

12. Ryabinina, N.L., Grechko, V.V., Semenova, S.K., and Darevsky, I.S., On the Hybridogenus Origin of the Parthenogenetic Species *Lacerta dahli* and *Lacerta rostombekovi* Revealed by RAPD Technique, *Rus. J. Herpetol.*, 1999, vol. 6, no. 1, pp. 55–60.
13. Fu, J., Murphy, R.W., and Darevsky, I.S., Towards the Phylogeny of Caucasian Rock Lizards: Implication from Mitochondrial DNA Gene Sequences (Reptilia: Lacertidae), *Zool. J. Linn. Soc.*, 1997, no. 121, pp. 463–477.
14. Fu, J., MacCulloch R.D., Murphy R.W., and Darevsky I.S., Clonal Variation in the Caucasian Rock Lizard *Lacerta armeniaca* and its Origin, *Amphibia–Reptilia*, 2000, no. 21, pp. 83–88.
15. Moritz, C., Uzzel O., Spolsky C., et al., The Maternal Ancestry and Approximate Age of Parthenogenetic Species of Caucasian Rock Lizards (Lacerta: Lacertidae), *Genetica,* 1992, no. 87, pp. 53–62.
16. Tokarskaya, O.N., Martirosyan, I.A., Badaeva, T.N., et al., $(GATA)_n$ Microsatellite Instability in the Parthenogenetic Caucasian Rock Lizard *Darevskia unisexualis* (Lacertidae), *Mol. Genet. Genom.*, 2004, no. 270, pp. 509–513.
17. Ryskov, A.P., Martirosyan, I.A., Badaeva, T.N., et al., Hyperunstable $(TCT/TCC)_n$ Microsatellite Loci in Parthenogenetic Lizards *Darevskia unisexualis* (Lasertidae), *Rus. J. Genet.,* 2003, vol. 39, no. 9, pp. 983–992.