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

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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.

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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 PLA2 toxin. At orthologous positions in other snake PLA₂ 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 rela-

tionships 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 $1 \times Taq$ polymerase buffer (Dialat, Russia), 2 mM MgCl₂, 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) BigDyeTM 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.

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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	

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

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.

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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.

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