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Characterization of Retrotransposon Bov-B LINE Reverse Transcriptase Gene Sequences in Parthenogenetic Lizards *Darevskia unisexualis* and Bisexual Species *D. nairensis* and *D. valentini*

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Abstract—Cloning and sequencing of the partial reverse transcriptase gene (750 bp) of the Bov-B LINE retrotransposon have been held in parthenogenetic lizards *Darevskia unisexualis* and its assumed parental bisexual species *D. nairensis* and *D.valentini*. It was shown that the percentage of transcriptionally active copies of this gene, which does not contain a stop codon, is almost the same in the three species and is about 75%. The intragenomic divergence level of these sequences is low and was found to be 2.6% in *D. unisexualis*, 1.9% in *D. nairensis*, and 1.6% in *D. valentini*. The phylogenetic analysis shows the distribution of copies of *D. unisexualis* in each of the two clusters of RT sequences characteristic of *D. nairensis* and *D. valentini*. This result supports the view of the hybrid origin of *D. unisexualis* and does not exclude intraspecific hybridization between *D. nairensis* and *D. valentini*.

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FORMULATION OF THE PROBLEM

Retrotransposons (RTEs) are mobile genetic elements that move in the genome via RNA intermediates. These multicopied sequences are involved in recombination processes that lead to structural rearrangements of the genome, which significantly alters its stability and function. The feature of RTE of the type LINE is the presence of various functional sites in their structure, including the RNA-dependent DNA polymerase gene sequences (reverse transcriptase, RT). RT sequences polymorphism is used to classify mobile elements from different classes and for the phylogenetic analysis of animals of different taxonomic ranks [1, 2]. The retrotransposon Bov-B LINE has a length of about 3.2 kb and belongs to the superfamily that does not contain long terminal repeats (non-LTR RTE). Bov-B LINE was firstly discovered in the family Bovidae, and it was considered specific for the suborder Ruminantia. Later, a similar retrotransposon was found in the snake *Vipera ammodytes* (viper of the order Squamata, family Viperidae). Its appearance in this family was explained by the horizontal transfer of the element between two evolutionary distant classes, which occurred about 40–50 million years ago [3]. Later Bov-B LINE was found in lizards, including the genus *Anolis* (order Squamata, family Iguanidae) [4]. In *A. carolinensis* elements of Bov-B LINE comprise 0.66% of the whole genome, and the number of copies reaches ~30000 [5]. It is believed that this element appeared for the first time in representatives of the order Squamata, and then spread among mammals [3, 6].

It is interesting to study the retrotransposon polymorphisms, Bov-B LINE in particular, in parthenogenetic species of reptiles, which are believed to occur as a result of hybridization between bisexual species [7]. Retrotransposons are important and interesting not only in the study of their role in genomic instability and their own evolutionary dynamics, but they can be used as genetic markers for studying the evolution of the host, such as various species of reptiles. For these purposes, a complex of species of rock lizards of the genus *Darevskia* (order Squamata, family Lacertidae) is a favourable object that was well studied biogeographically and, along with bisexual species, contains a number of unisexual parthenogenetic forms [8]. We previously found short regions of the Bov-B LINE retrotransposon in bisexual and unisexual lizard species of this genus [9].

In this work, we sequenced a 750 bp region of 94 clones of the Bov-B LINE retrotransposon RT gene sequences from three lizard genomes—parthenogenetic species *D. unisexualis* and bisexual species *D. valentini* and *D. nairensis* (*D. raddei nairensis*). It was shown for the first time that the genomes of these three species contain inactive and potentially transcriptionally active copies of the RT gene. The assessment of their intragenomic divergence and the results of phylogenetic analysis support the hypothesis on the hybrid origin of *D. unisexualis* based on the allozyme variations and mtDNA polymorphisms in a group of lizards of the genus *Darevskia* [7].

EXPERIMENTAL

Three DNA samples were used that were isolated from the blood of lizards *D. unisexualis*, *D. nairensis*, and *D. valentini* living on the territory of Armenia.

The reverse transcriptase gene of Bov-B LINE was amplified using 20 ng of genomic DNA and degenerate primers ME1_liz (CACAGTARTCCAAGYYTAYRCMCCAAC) and ME2_liz (CWGCATATCTGAGGTTRTTGATATTTCT), which we designed based on the known sequence of the Bov-B LINE of the horned viper (*V. ammodytes*) and is based on conserved regions in the apurine/apirimidine endonuclease and RT domains ([11], GeneBank, accession number AF332691). GenePak PCR Core reagent kit was used (Litekh, Russia) in a volume of 20 μL under the following conditions: initial denaturation (at 95°C for 5 min); PCR product amplification for 35 cycles, consisting of denaturation at 95°C for 1 min, primer annealing at 55°C for 1 min, and extension of DNA at 72°C for 1 min; and final elongation at 72°C for 5 min. The amplification products were fractionated by electrophoresis using a 1% agarose gel, eluted from a gel, purified using a Wizard SV Gel kit and PCR Clean-up System (Promega, United States), and ligated with the vector pGEM using a pGEM-T-easy kit (Promega); sequencing was carried out according to the manufacturer's protocol. The nucleotide sequence of the clones insertion was determined by the Sanger method using the ABI PRISM® BigDye™ Terminator v. 3.1 reagent kit (Applied Biosystems, United States) and the analysis of the reaction products using an automated DNA sequencer ABI PRISM 3100-Avant (Applied Biosystems).

A total of 94 sequences were obtained, including 38 of *D. unisexualis*, 27 of *D. nairensis*, and 29 of *D. valentini*. The multiple sequence alignment was performed using the algorithm CLUSTALW and edited manually. The program MEGA 6 was used (http://www.megasoftware.net) in the search for the stop codons; pairwise sequence divergence (the minimum-maximum range, the *p*-distance); and the construction of phylogenetic trees based on the maximum likelihood method of the selected model, which has the lowest value of AIC and BIC criteria. The RT Bov-B LINE sequences of the horned viper (*V. ammodytes*, accession number AF332691) and the green anole lizard (*A. carolinensis*, accession numbers FJ158981 and FJ158982) formed the outgroup. The blastn, blastx, and tblastx algorithms (http://www.ncbi.nlm.nih.gov) were used to identify the homology of the nucleotide sequence.

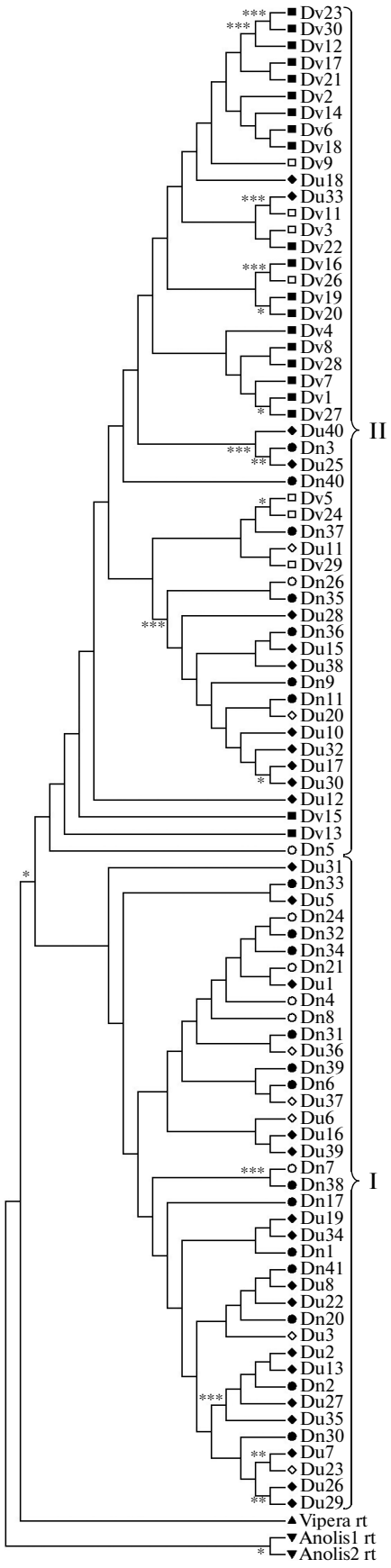
RESULTS AND DISCUSSION

As a result of the PCR amplification of DNA, sequences with lengths of approximately 1.8 kb that consist of an apurinic/apirimidinic endonuclease and RT gene regions were revealed. The studied 750-bp fragment located closer to the 3'-end of the Bov-B LINE sequence that constitutes about two-thirds of the reverse transcriptase gene length was used for phylogenetic analysis. The length of the sequences in *D. unisexualis* varies from 712 to 752 bp, from 749 to 753 bp in *D. nairensis*, and from 719 to 750 bp in *D. valentini*. The differences are the result of indels of different lengths. Most of them are single-nucleotide substitutions, but the clone Dv10 has a deletion of 30 bp at the 5'-end, and clones Du4 and Du9 carry two deletions (36 and 12 bp) in the middle part of the reverse transcriptase gene. Some sequences in each of the three species contain premature stop codons; their number varies among different species and within species, with maximum variations in the range of 1–10, as can be seen in the unisexual *D. unisexualis*. However, the ratio between the numbers of the potentially active copies that do not contain stop codons (20, 21 and 29 copies, respectively) (see table) and inactive copies is almost the same in the three species, i.e., about 3 : 1 in each of them.

In order to confirm that the RT genomic sequences of the three lizard species of the *Darevskia* genus belong to the Bov-B LINE family, homologous seg-

Intragenomic variability of Bov-B LINE reverse transcriptase gene copies in three lizard species of the genus *Darevskia*

Species	Number of sequenced copies	Length (bp)	Minimum–maximum distances (mean value), %	Inactive copies	Active copies
				copy number (%)	copy number (%)
D. nairensis	27	749–753	0.1–4.7 (1.9)	7 (25.9)	20 (74.1)
D. valentini	29	719–750	0.0–4.0 (1.6)	8 (27.6)	21 (72.4)
D. unisexualis	38	712–752	0.0–12.1 (2.6)	9 (23.7)	29 (76.3)



Phylogenetic tree constructed using the maximum likelihood method from the polymorphism of 90 regions, each with a length of about 750 bp, of the Bov-B LINE reverse transcriptase gene sequences of the parthenogenetic species *D. unisexualis* and bisexual species *D. nairensis* and *D. valentini* (Model T92 + G, BIC = 10868.90, AIC = 9169.25, −Ln = 4398.12). Inverted triangles are the lizard *Anolis carolinensis*, triangle is the snake *Vipera ammodytes*. Du (rhombus) is *D. unisexualis*; Dn (circle) is *D. nairensis*; Dv (square) is *D. valentini*. Black icons are active copies, white are inactive. Asterisks indicate bootstrap value: * (95–100%); ** (75–94%); *** (50–74%). I is the cluster comprising only sequences of *D. unisexualis* and *D. nairensis*, II is the cluster comprising mainly sequences of *D. valentini* and *D. unisexualis*.

ments were searched for in the database of nucleic acids using the three BLAST algorithms. Maximum similarity of the studied sequences of the three lizard species ($I = 83–86\%$, scores > 700, $E = 0$) was found during a comparison with the 748-bp fragment of Bov-B LINE viper retrotransposon (*V. ammodytes*, AF332691). Deduced hypothetical amino acid sequences of the protein translated from the 750-bp region belong to the domain of RNA-dependent DNA polymerases (pfam00078, E = 1.3e–09) that make up the retrotransposon of this snake ($I = 90–92\%$), E = ~2e–155).

The table shows the intragenomic variation of the sequenced copies in a pairwise comparison of the sequences and the number of transcriptionally active and inactive copies in the three presented species. The minimum and maximum divergence of all RT copies of parental species ranges from 0 to 4.7%, with an average value of less than 2%. In parthenogenetic species, two copies are more divergent, i.e., divergence reaches 12% with an average of 2.6%. The genomes of the three species do not differ in the ratio of transcriptionally active and inactive RT Bov-B LINE gene copies (approximately 3 : 1). This supports the view that the parthenogenetic species appeared relatively recently, estimated about 10000 years ago [10, 11].

The figure shows a tree that reflects the genetic differences between the studied sequences. Four of them, i.e., from different species with long deletions (Dv10, Du4 and Du9) and with an unusually high variability (Du14), were excluded from the analysis. All RT copies form a main cluster, which indicates that new RT copies probably belong to a single line within a Bov-B LINE retrotransposon subfamily of reptiles. Several groups (or subclusters) can be distinguished within this cluster regardless of the supposed functional activity of RT. There is some tendency of the two clusters of bisexual species to separate and for parthenogenetic species sequences to be distributed within each of these clusters. Cluster I includes 21 sequence of the parthenogenetic species and 17 sequences of one of the bisexual species, i.e., *D. nairensis*; cluster II consists of 14 sequences of the parthenogenetic species and 27 of the second, *D. valentini*. This distribution of sequences supports the hybrid origin of *D. unisexualis* as a result of interspecific hybridization of *D. nairensis*

and *D. valentine* [7]. However, eight sequences of the first bisexual species *D. nairensis* also fall into the second cluster II. This may be due to the initially higher genetic heterogeneity of the parental species, which can have additional diverged RT copies resulting from the reticulate evolution of rock lizards of the genus *Darevskia*. In interspecific hybridization, in addition to *D. nairensis* and *D. valentini*, representatives of *D. raddei* could have participated in interspecific hybridization, modern populations of which are genetically close to *D. nairensis*, while those of *D. portschinskii* are close to *D. valentini* [11, 12]. Apparently, definite conclusions of the parental species for *D. unisexualis* (as well as for other parthenogenetic species) can only be made based on a comparison of greater number of orthologous copies of Bov-B LINE of unisexual and bisexual species of *Darevskia*.

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