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Polymorphism of Microsatellite Dinucleotide Loci in Parthenogenetic Lizards *Darevskia unisexualis*

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Genomic instability and variability of microsatellite loci is poorly studied in organisms with clonal inheritance. This was the first work to perform a molecular-genetic study of individual loci containing $(GT)_n$ microsatellites in clone-breeding (parthenogenetic) lizards *Darevskia unisexualis.* We revealed and characterized in detail the polymorphic loci and showed that alleles of this locus differ in size and structure of the microsatellite locus.

Microsatellite sequences, which are classified with tandemly arranged moderately repeated DNA sequences of eukaryotic genomes, are of great interest as a genome instability factor. They vary in cluster length and repeat unit size and are characterized by high mutation rates (10^{-2} to 10^{-5}) depending on the microsatellite type [1], which leads to accumulation of population-specific mutations and makes it possible to use information on the microsatellite locus variability for analyzing the structure of populations [2]. Microsatellites of humans and some animals and plants have been studied most extensively [3, 4]. However, unisexual species of animals with clonal breeding remain poorly studied in terms of the structural organization of their genomes and genetic variability [5]. Among the great variety of microsatellite sequences, dinucleotide microsatellites are of special importance, because they are not only the most abundant microsatellites in the eukaryotic genome but also the most evolutionarily conserved genetic markers [6]. In view of this, we performed a molecular-genetic study of dinucleotide microsatellites in a unique natural model, obligate parthenogenetic species of Caucasian rock lizards *Darevskia unisexualis*, a hybrid derived from the ancestral species *D. raddei* and *D. valentini* [7].

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In this study, we used the genomic library of *D. unisexualis*, which has been created earlier [8]. Hybridization with colonies using oligonucleotide probes allowed us to select and sequence the recombinant clones containing $(GT)_n$-type microsatellites *Du231* (GenBank Ac. no. EU252540), *Du365* (Ac. no. EU252543), *Du255* (Ac. no. EU252541), *Du183* (Ac. no. EU252539), and *Du214* (Ac. no. EU252542). These loci contain not only dinucleotide but also mono- and trinucleotide microsatellite motifs. The polymorphism of these loci was analyzed by locus-specific PCR using the *D. unisexualis* population sample DNA.

Blood of female *D. unisexualis* from five Armenian natural populations ($n = 65$) was conserved in 0.05 M EDTA (pH 8.0) and stored as 4°C. DNA samples were isolated by the standard phenol–chloroform method using proteinase K [9]. Oligonucleotides listed in Table 1 were used as primers for monolocus PCR. DNA (50 ng) was amplified in 20 μl of the reaction mixture using the GenePak® PCR Core reagent kit (Izogen, Russia) in a TP4-PCR-01 Tercyc four-channel DNA thermocycler (DNA-Technology, Russia) under the following conditions: denaturation at 94°C for 3 min; amplification at 94°C for 1 min, primer annealing for 40 s, and at 72°C for 40 s (30 cycles); and final elongation at 72°C for 5 min. The nucleotide sequence of amplification products was determined according to Sanger using the ABI PRISM®BigDye™ Terminator v.3.1 reagent kit, with subsequent analysis of reaction products in an ABI PRISM 3100-Avant automatic DNA sequences. The sequences were aligned using the MegAlign 4.05 software.

Five dinucleotide loci were analyzed by PCR amplification using the sample from five populations of *D. unisexualis*. We found that only one of these loci, *Du214* (Ac. no. EU252542) was polymorphic, whereas others were monomorphic. Figure 1 shows the patterns of electrophoretic fractionation of PCR products obtained by amplification of DNA samples from different populations of *D. unisexualis*. It can be seen that lizards *D. unisexualis* are heterozygous for the *Du214* locus and that the latter is represented in this partheno-

Table 1. Conditions of PCR amplification of five microsatellite loci of the parthenogenetic species *D. unisexualis*

Locus name	Primer pair	Annealing temperature, °C
Du231 (Ac. no. EU252540)	5'-TCAAGAGGCCTCCCGAAAAG-3' (F) 5'-TGAGCCAGCTACCGTCATTCA-3' (R)	56
Du365 (Ac. no. EU252543)	5'-GGGGCCCATTGTGTAAATACTGTA-3' (F) 5'-GGATTAAGGGGTTTTCTCAGGACA-3' (R)	55
Du255 (Ac. no. EU252541)	5'-TCGCAGAGTGGCAGGAAACAAT-3' (F) 5'-TGCATCCAGCTCAACCAAAATACC-3' (R)	58
Du183 (Ac. no. EU252539)	5'-ACAGACAAACACTCATAGAACACAT-3' (F) 5'-AATCAACAAGCCAAGAACAAAC-3' (R)	51
Du214 (Ac. no. EU252542)	5'-TCACTTAAGGTTGACGCTGACTCA-3' (F) 5'-CTGAACAAGTTGTCCACCTCTGC-3' (R)	50

Table 2. Quantitative estimation of the *Du214* locus variability in the parthenogenetic species *D. unisexualis*

Population	Allelic variant						Total number of animals
	1	2	3	4	5	6	
Kutchak	0	8	7	2	2	1	10
Lchap	0	10	10	0	0	0	10
Zagalu	0	11	11	0	0	0	11
Naraduz	0	17	17	0	0	0	17
Tokyarlu	12	17	5	0	0	0	17
All populations	12	63	50	2	2	1	65
Allele frequency	0.092	0.485	0.385	0.015	0.015	0.008	

genetic species by six variants differing in the electrophoretic mobility. Data on locus frequencies are summarized in Table 2. In particular, alleles *2* and *3* were represented in the same proportion in populations Lchap, Zagalu, and Naraduz. Population Takyarlu contained three alleles (variants *1, 2*, and *3*). Population Kutchak proved to be most variable: lizards of this population carried five allelic variants. The PCR products of each allelic variant were cloned and sequenced. Figure 2 shows the nucleotide sequences of allelic variants of the *Du214* locus. All alleles of the *Du214* locus differed in the length of the microsatellite cluster, which contained different number (21–29) of $(GT)_n$ repeats. In addition, as can be seen from the data shown in Fig. 2, *Du214* alleles also differed by point mutations at the beginning of the microsatellite cluster. Combinations of such fixed point mutations yield haplotypes G–T (alleles *1, 3, 5*, and *6*) and A–A (alleles *2* and *4*), which are specific markers of each allele, presumably inherited from different bisexual parental species. Apparently, to confirm this assumption, it is necessary to detect these alleles and haplotypes in the parental species *D. raddei* and *D. valentini*.

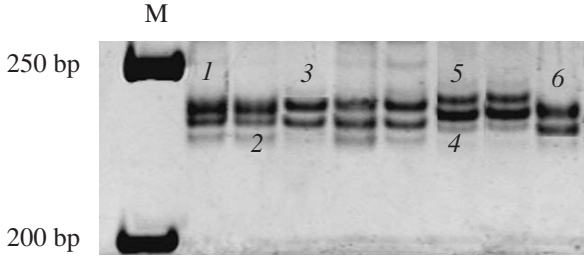

Fig. 1. PAGE in 8% native polyacrylamide gel of PCR products corresponding to allelic variants of the *Du214* locus of *D. unisexualis*. The 50 bp Ladder (Fermentas, Lithuania) with a step of 50 bp was used as a molecular-weight marker. Numerals indicate the allelic variants.

A comparative analysis of five $(GT)_n$-containing loci revealed correlation between the locus structure and the level of its polymorphism. The microsatellite loci with a complex structure, containing different types of microsatellites or imperfect monomeric units in the cluster (*Du231*, *Du365*, *Du255*, and *Du183*), are monomorphic, whereas the loci that contain extended perfect microsatellite clusters (*Du214*) show a high degree of polymorphism.

Thus, this was the first study to clone and sequence five loci containing $(GT)_n$ microsatellites, one of which proved to be polymorphic. The molecular structure of

Fig. 2. Nucleotide sequences of microsatellite clusters of the *Du214* locus of the parthenogenetic species *D. unisexualis.* $(GT)_n$ clusters are shown with the rectangular frame. The nucleotide polymorphisms are shown with gray rectangle.

alleles of this locus was determined. We showed that the differences between the alleles were determined by variations in the size of microsatellite clusters and point mutations in the microsatellite DNA. Earlier, similar allelic variations were demonstrated for the tetranucleotide microsatellite loci *Du215, Du323*, and *Du281* in *D. unisexualis* [10] and in populations of other parthenogenetic species (the *Du215* locus in *D. armeniaca* [11] and the *Du323* locus in *D. dahli* [12]). However, the authors of the cited papers discovered point mutations that formed haplotypes in the regions adjoining the microsatellite cluster. In contract to the tetranucleotide loci of *D. unisexualis*, the *Du214* locus contains point mutations immediately in the microsatellite cluster. The results of this study demonstrate that microsatellite variability is a factor of clonal and genetic diversity of parthenogenetic species.

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