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SHORT COMMUNICATIONS

Intra- and Interspecific Polymorphism of $(AAT)_n$ in Microsatellite Locus Du47D in Parthenogenetic Species of the Genus *Darevskia*

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Abstract—The molecular structure of the allelic variants of $(AAT)_n$ of the Du47D microsatellite locus was determined in parthenogenetic lizards *Darevskia dahli, D. armeniaca*, and *D. rostombekovi*. Comparative analysis of these alleles showed that they were characterized by perfect structure of microsatellite cluster, and were different in the number of (AAT) monomeric units, as well as in the combinations of species-specific substitutions and deletions in the microsatellite flanking regions. Molecular structure of microsatellite cluster, species-specific single nucleotide polymorphism (SNP), and different representation of alleles Du47 in the samples of parthenogenetic species examined point to the origin of the alleles from different bisexual species, which is consistent with the hybrid nature of unisexual species of the genus *Darevskia*. In addition, these data reflect different combination patterns of interspecific hybridization events with the participation of the same bisexual species upon the formation of hybrid genomes of parthenogenetic species. Possible application of the allelic variants of microsatellite loci of parthenogenetic lizards as the genetic markers for the analysis of the genomes of parthenogenetic species in the light of evolution, ecology, and parthenogenetic type of reproduction in vertebrates is discussed.

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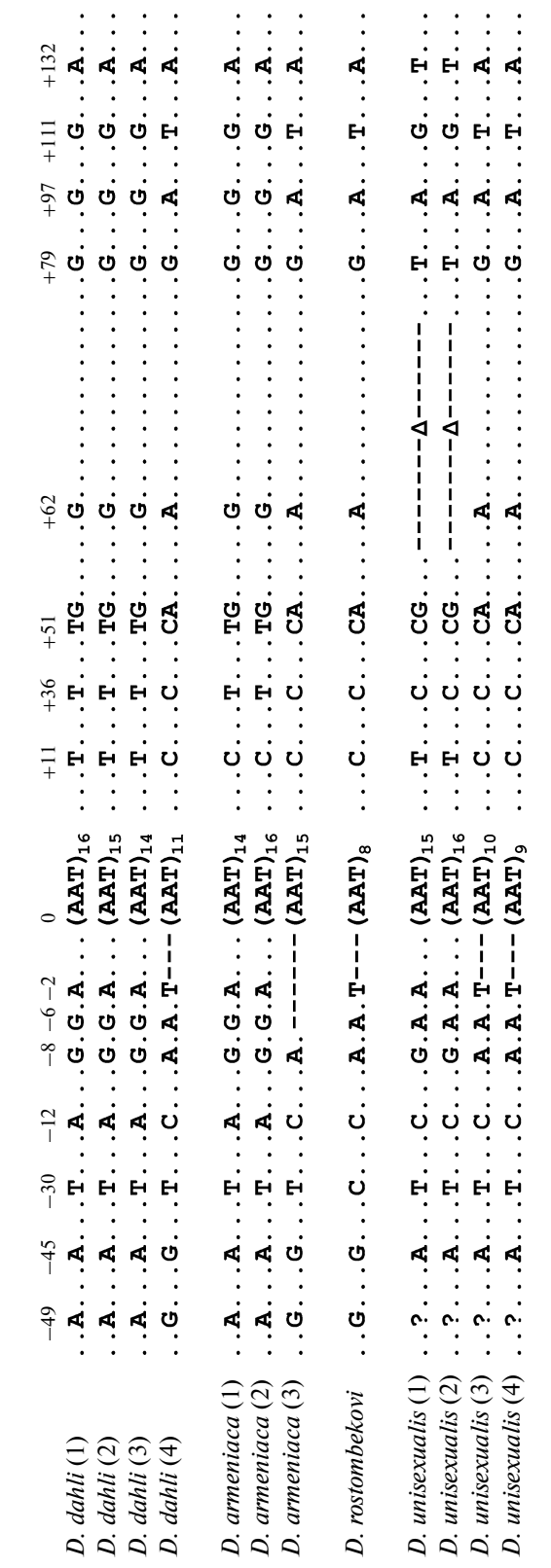
In recent decade, microsatellites have taken the lead among different types of genetic markers, including population studies, evolutionary genomics, and biodiversity. The special place occupied by microsatellites among the genetic markers is primarily determined by their wide representation in the genomes of prokaryotes and eukaryotes, high evolution rates, and usually codominant type of inheritance [1]. Furthermore, in recent years, microsatellite markers are widely used for genetic studies of economically valuable, commercial, and rare animal and plant species [2–5].

Parthenogenetic vertebrate species, including parthenogenetic Caucasian rock lizards of the genus *Darevskia*, occupy a special place in biodiversity studies [6, 7]. Based on the allozyme and mitochondrial DNA analyses, it was demonstrated that seven diploid ($2n = 38$) parthenogenetic lizard species of the genus *Darevskia* (*D. dahli, D. americana, D. unisexualis, D. rostombekovi, D. uzzelli, D. sapphirina,* and *D. bendimahiensis*) arose as a result of reticulate evolution, i.e., via hybridization between four ancestral bisexual species (*D. raddei, D. mixta, D. valentine,* and *D. portschinskii*). Furthermore, hybridization in different combinations of the same parental forms resulted in the appearance of different parthenogenetic hybrid species [8]. Unisex species living under the same conditions as parental species compete with them in the zone of sympatry, which can result in the isolation of bisexual species from one another and lead to the impossibility of the appearance of new clones through their hybridization [9, 10]. Populations of parthenogenetic species are represented by clones, i.e., groups of identical individuals that originate from a common ancestor. Clonal reproduction is characterized by the absence of combinatorial variations; all changes are random and probably reflect mutation processes, which take place in the genomes of parthenogenetic individuals. Thus, these lizard species represent a unique model for genetic investigations [11–13]. In the previous study, we examined the Du47D polymorphic locus (GenBank Ac. no. FJ8044739) in parthenogenetic species *D. unisexualis* and in its bisexual parental species *D. raddei* and *D. valentine* [14].

In the present study, a system of PCR amplification with primers D47F1 (ACCTCCTAATGATGTTTGACG) and D47R3 (TAAGCAGCTATCCTATTTT) was used. In our previous studies, this system was used to identify the homeologous locus alleles in parthenogenetic species *D. armeniaca, D. dahli*, and *D. rostombekovi*. Lizard DNA was isolated using the standard proteinase K phenol–chloroform procedure. The samples of *D. armeniaca* ($N = 16$), *D. dahli* ($N = 17$), and *D. rostomnekovi* ($N = 10$) were examined. Amplification products were separated by electrophoresis in 8% native polyacrylamide gel (PAAG) and visualized by staining with ethidium bromide. Amplificates that differ in electrophoretic mobility were



Variable regions of Du47D alleles in unisexual species of the genus *Darevskia*: *D. armeniaca, D. rostombekovi*, and *D. unisexualis*. Conservative nucleotide regions are designated by dots; single nucleotide deletions are designated by dashes; (−Δ−) indicates the region of 14 nucleotide deletion. Numbering is given relative to the starting point of $(AAT)_n$ cluster. 1–4. numbers of allelic variants.

sequenced using the ABI PRISM 3100 Avant automated sequencer (Applied Biosystems, United States). Nucleotide sequences were aligned using the ClustalW algorithm implemented in the MEGA 4.0 software program.

Schematic representation of the Du47D allelic variants of parthenogenetic species *D. armeniaca*, *D. dahli*, and *D. rostombekovi*, as well as of previously described alleles of *D. unisexualis* [14], is given in the figure.

According to the data obtained, all *D. armeniaca* individuals were heterozygous for the Du47D locus. The locus was represented by three allelic variants that differ in the microsatellite structure (number of $(AAT)_n$ repeats), as well as in nucleotide substitutions in the flanking regions. A comparative sequence analysis of the Du47D alleles in *D. armeniaca* showed that two alleles of this locus belonged to one type, which suggests that they originated from common ancestral species. At the same time, with respect to its molecular structure, the third allele belonged to another type and probably originated from another ancestral species. Moreover, the third allele was found in all examined *D. armeniaca* individuals and was the major allele. The distribution of the first two alleles was as follows. The first allele was found in 11 individuals, and the second allele was found in four out of the 16 individuals examined. Similarly, it was demonstrated that all *D. dahli* individuals examined were the Du47D heterozygotes. The locus was represented by four alleles that differ in the number of (AAT) repeat units in the microsatellite cluster and in nucleotide substitutions in the flanking regions. In *D. dahlia*, three Du47D alleles were of one type, and the fourth allele belonged to another type. It is suggested that these two allele types originated from different parental species. The representation of the alleles in *D. dahli* sample was different. The fourth allele was found in all individuals examined and was one of the major alleles. The third allele was detected in 14 out of 17 individuals examined and was also considered to be major allele. Alleles 1 and 2 were revealed in one or two individuals, and were minor alleles. In the populations of parthenogenetic species *D. rostombekovi*, the Du47D PCR products were found to be electrophoretically monomorphic and represented by a single allelic variant. Sequencing of PCR products from the sample of *D. rostombekovi* showed the presence of a single allelic variant of 292 bp in size. The microsatellite cluster of the this allele contained eight (AAT) repeats. The structure of this allele was consistent with that of the third allele of *D. dahli*, except for the species-specific substitution of T for C. The presence of a single Du47D allele in parthenogenetic species *D. rostombekovi* is thought to be associated with the absence of the amplification of the second allele in the system of PCR analysis used. Thus, the present study was the first to acquire the information on the molecular nature of the structural variations of Du47D alleles and their prevalence in parthenogenetic species

D. armeniaca, D. dahli, and *D. rostombekovi*. Specific features of the structural variations of the allele, including microsatellite clusters and species-specific SNPs in flanking regions, suggested that, in the hybrid genomes, they originated from different bisexual species. Furthermore, alleles with SNP combinations that are similar but differ in the number of microsatellite monomeric units identified in different parthenogenetic species reflected different combination patterns of interspecific hybridization events with the participation of the same bisexual species upon the formation of hybrid genomes of parthenogenetic species.

At present, the data on parthenogenetic species genomics and interspecific variation of microsatellite loci in unisexual vertebrate is rather scarce. For example, the polymorphism of the dinucleotide and trinucleotide loci was examined in the parthenogenetic mourning gecko *Lepidodactylus lugubris* [15]. Gardner et al. [16] reported the isolation and sequencing of six polymorphic loci containing $(AAC)_n$ and $(AAAG)_n$ microsatellites from the parthenogenetic form of the Australian lizard *Menetia greyii*. These loci were further used for the population analysis of bisexual species of the same genus. Eight highly polymorphic dinucleotide loci were described in the Australian gecko *Heteronotia binoeli*, including parthenogenetic triploid clones formed as a result of hybridization between two diploid races [17]. In most cases, parthenogenetic species have a hybrid origin [18]. Due to the specific features of meiosis, specifically the formation of bivalents from genetically identical sister chromosomes, in parthenogenetic species, a high level of fixed heterozygosity is observed, along with the low level of genomic variability [19]. For these reasons, microsatellite markers are effectively used to identify possible parents of unisexual species. However, hybrid speciation is not the only way that parthenogenetic populations form. For instance, an analysis of 14 microsatellite loci in two parthenogenetic lizard species of the genus *Lepidophyma* (*L. reticulatum* and *L. flavimaculatum*) revealed an extremely low heterozygosity level of these loci in unisexual lizards *L. reticulatum* (14.7–19.3%) along with homozygosity for these loci in *L. flavimaculatum* individuals. These data serve as the first reasonably good evidence of the nonhybrid origin of parthenogenesis in natural populations of vertebrates [20]. The authors of the study suggest the spontaneous origin of both parthenogenetic species from the populations of bisexual ancestral species. Microsatellite markers are effectively used to study obligatory, as well as facultative, parthenogenesis in vertebrates [21–23]. For instance, the in female Komodo dragon *Varanus komodensis* held in captivity under conditions of long-term isolation from males, a switch to asexual reproduction was observed. An analysis of microsatellite markers in female and parthenogenetic offspring of Komodo dragons in the London Zoo revealed homozygosity for all loci and the identity of their genotypes [22]. Furthermore, all parthenogenetic offspring were represented by males. This reproductive plasticity makes it possible for these species to survive under the conditions of a considerable population decline and/or inability to sexually reproduce. In addition, it becomes possible for new populations to form by a single female founder. Similar switch between the reproduction types was described in the Burmese python *Python molurus bivittatus* from the Amsterdam Zoo [21]. An analysis of seven microsatellite loci and 692 AFLP markers in the female python and its parthenogenetic offspring (females) revealed an extremely low level of polymorphism. However, it should be noted that facultative parthenogenesis described in all cases as only one type of reproduction under difficult survival conditions, and did not result in the formation of a new clonal species.

It has been suggested that the further investigation of the polymorphism and variations at microsatellite loci of the parthenogenetic species of the genus *Darevskia* will contribute considerably to the understanding of fundamental issues, such as the molecular nature of variations, the origin and evolution of the genomes of unisexual and bisexual species of vertebrates, and issues of animal ecology and biodiversity.

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REFERENCES

1. Liu, Z.J. and Cordes, J.F., Review: DNA Marker Technologies and Their Applications in Aquaculture Genetics, *Aquaculture*, 2004, vol. 238, pp. 243–250.
2. Korchagin, V.I., Badaeva, T.N., Tokarskaya, O.N., et al., Molecular Characterization of Allelic Variants of $(GATA)_n$ Microsatellite Loci in Parthenogenetic Lizards *Darevskia unisexualis* (Lacertidae), *Gene*, 2007, vol. 392, no. 1–2, pp. 126–133.
3. Wang, S.L. and Niu, D.H., Isolation and Characterization of 10 Polymorphic Microsatellites in *Metrix meretrix*, *Cons. Genet. Res.*, 2009, vol. 1, pp. 111–113.
4. Mokhtar, M.A.A., Baharum, S.N., Noor, N.M., and Kumar, V., Isolation and Identification of Microsatel-

lite Repeat Motifs from the *Epinephelus fuscoguttatus* Genome, *Afr. J. Biotechnol.*, 2011, vol. 10, no. 43, pp. 8553–8557.

5. McGlaughlin, M.E., Riley, L., Wallace, L.E., and Helenurm, K., Isolation of Microsatellite Loci from Endangered Members of *Lotus* (Fabaceae) Subgenus *Syrmatium*, *Conserv. Genet. Resour.*, 2011, vol. 3, pp. 117–121.
6. Darevsky, I.S., Evolution and Ecology of Parthenogenetic Reproduction in Reptiles, in *Sovremennye problemy teorii evolyutsii* (Modern Problems of Evolution Theory), Moscow: Nauka, 1993, pp. 89–109.
7. Ryskov, A.P., Genetically Unstable Microsatellite-Containing Loci and Genome Diversity in Clonally Reproduced Unisexual Vertebrates, *Int. Rev. Cell Mol. Biol.*, 2008, vol. 270, pp. 319–349.
8. Murphy, R.W., Fu, J., MacCulloch, R.D., et al., A Fine Line between Sex and Unisexuality: The Phylogenetic Constraints on Parthenogenesis in Lacertid Lizards, *Zool. J. Linn. Soc.*, 2000, vol. 130, pp. 527–549.
9. Tarkhnishvili, D., Gavashelishvili, A., Avaliani, F., et al., Unisexual Rock Lizard Might Be Outcompeting Its Bisexual Progenitors in the Caucasus, *Biol. J. Linn. Soc.*, 2010, vol. 101, pp. 447–460.
10. Danielyan, F., Arakelyan, M., and Stepanyan, I., Hybrids of *Darevskia valentini, D. armeniaca* and *D. unisexualis* from a Sympatric Population in Armenia, *Amphibia–Reptilia*, 2008, vol. 29, pp. 487–504.
11. Tokarskaya, O.N., Kan, N.G., Petrosyan, V.G., et al., Genetic Variation in Parthenogenetic Caucasian Rock Lizards of the Genus *Lacerta* (*L. dahli, L. armeniaca, L. unisexualis*) Analyzed by DNA Fingerprinting, *Mol. Gen. Genet.*, vol. 256, pp. 812–819.
12. Malysheva, D.N., Vergun, A.A., Tokarskaya, O.N., et al., Nucleotide Sequences of the Microsatellite Locus *Du215*(*arm*) Allelic Variants in Parthenospecies *Darevskia armeniaca* (Lacertidae), *Russ. J. Genet.*, 2007, vol. 43, no. 2, pp. 116–120.
13. Badaeva, T.N., Malysheva, D.N., Korchagin, V.I., and Ryskov, A.P., Genetic Variation and de novo Mutations in the Parthenogenetic Caucasian Rock Lizard *Darevskia unisexualis*, *PLoS One*, 2008, vol. 3, no. 7, e2730, http://www.plosone.org.
14. Korchagin, V.I. and Tokarskaya, O.N., Molecular Structure of the Allelic Variants of $(AAT)_n$ Microsatellite Locus *Du47D* in the Parthenogenetic Species *Darevskia unisexualis* and Bisexual Parental Species *D. valentine* and *D. raddei*, *Russ. J. Genet.*, 2010, vol. 46, no. 5, pp. 630–632.
15. Wilmhoff, C.D., Csepeggi, C.E., and Petren, K., Characterization of Dinucleotide Microsatellite Markers in Parthenogenetic Mourning Gecko (*Lepidodactylus lugubris*), *Mol. Ecol.*, 2003, vol. 3, pp. 400–402.
16. Gardner, M.G., Otewell, K., and Adams, M., Isolation of Microsatellites in Parthenogenetic Lizard *Menetia greyii* (Scintidae) and Their Utility in Sexual Species of the *Menetia greyii* Complex, *Mol. Ecol. Notes*, 2004, vol. 4, no. 2, pp. 219–221.
17. Strasburg, J.L., Eight Highly Polymorphic Microsatellite Loci for the Australian Gecko *Heteronotia binoei*, *Mol. Ecol. Notes*, 2004, vol. 4, pp. 456–458.
18. Dawley, R.M., An Introduction to Unisexual Vertebrates, *Evolution and Ecology of Unisexual Vertebrates*, Dawley, R.M., Bogart, J., and Bull, P., Eds., Albany, in *Bull. N.Y. State Mus.*, 1989, vol. 466, pp. 1–8.
19. Lutes, A.A., Neaves, W.B., Baumann, D.P., et al., Sister Chromosome Pairing Maintains Heterozygosity in Parthenogentic Lizards, *Nature*, 2010, vol. 464, pp. 283–286.
20. Sinclair, E.A., Pramuk, J.B., Bezy, R.L., et al., DNA Evidence for Nonhybrid Origins of Parthenogenesis in Natural Populations of Vertebrates, *Evolution*, 2009, vol. 64, no. 5, pp. 1346–1357.
21. Groot, T.V.M., Bruins, E., and Breeuwer, J.A.J., Molecular Genetic Evidence for Parthenogenesis in the Burmese Python, *Python molurus bivittatus*, *Heredity*, 2003, vol. 90, no. 2, pp. 130–135.
22. Watts, P.C., Buley, K.R., Sanderson, S., et al., Parthenogenesis in Comodo Dragons, *Nature*, 2006, vol. 444, pp. 1021–1022.
23. Germano, D.J. and Smith, P.T., Molecular Evidence for Parthenogenesis in the Sierra Garter Snake, *Thamnophis couchii* (Colubridae), *South. Nat.*, 2010, vol. 55, no. 2, pp. 130–135.