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BIOCHEMISTRY, BIOPHYSICS, AND MOLECULAR BIOLOGY

Genetic Instability of $(GATA)_n$ Microsatellite DNA Repeats and Somatic Mosaicism in the Unisexual Lizards *Darevskia unisexualis*

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$(GATA)_n$ repeats are ubiquitous, evolutionarily conserved nucleotide sequences dispersed over the genomes of eukaryotes. In the snakes *Elaphe radiate* and *Bungarus faciatus*, these repeats are major components of the satellite DNA of the female sex chromosome or W chromosome (females are the heterogametic sex with genotype WZ). In some rodents (*Mus musculus* and related species), they form a specific structural pattern, namely, extremely long clusters (more than 6 kb) in the Y chromosome. Irrespective of the chromosomal location, simple tandemly organized $(GATA)_n$ repeats are unstable and exhibit an unusually high restriction fragment length polymorphism (RFLP) in all eukaryotic organisms studied thus far: plants; invertebrates; and vertebrates, including mammals (besides other species, they were found in humans and other primates) [1].

We used a new model system of organisms with parthenogenetic reproduction, which implies a clonal reproduction of the maternal genotype in successive generations, to study the instability of $(GATA)_n$ microsatellite repeats. Obligately parthenogenetic reptiles, such as parthenogenetic lizards from the genus *Darevskia* (family Lacertidae), whose system of reproduction excludes both recombination with the male genome and activation of ova by spermatozoa (gynogenesis), are unique objects for monitoring genetic variation in highly mutable loci [2].

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D. unisexualis is one of seven parthenogenetic species of Caucasian rock lizards. It is characterized by a disrupted geographic range, which comprises several isolated populations of different sizes living in northeastern Turkey and Armenia, each population consisting of genetically identical animals of the same sex (females) [3]. Like other parthenogenetic species of this genus, *D. unisexualis* has a hybrid origin (its ancestor species were *D. valentini* and *D. nairensis*). It has a diploid chromosome set and is characterized by a high heterozygosity of allozyme loci [4] and an insignificant variation of mitochondrial DNA restriction sites [5]. The results of our earlier studies using multilocus DNA fingerprinting in the parthenogenetic species *D. dahli*, *D. rostombekovi*, and *D. unisexualis* [6–8] demonstrated individual genetic heterogeneity with respect to some mini- and microsatellites, including $(GATA)_n$ DNA markers, against a background of distinct species-specific profiles. The analysis of the variation of natural *D. unisexualis* populations with respect to $(GATA)_n$ microsatellites showed RFLP in the electrophoretic resolution region from 9.4 to 4.3 kb [8]. It was suggested that such polymorphic fragments were new variants of ancestral alleles resulting from mutations. To test this suggestion, we attempted to detect mutant fingerprint phenotypes in the first offspring generation by means of DNA fingerprint analysis of *D. unisexualis* families.

Female *D. unisexualis* preparing for egg-laying were caught in isolated populations from natural habitats near the settlements Kutchak, Lchap, Takyarlu, and Artik, as well as on the coast of Lake Sevan (Cape Noraduz on the Sevan Peninsula and Zagalu on the southeastern Sevan coast), in Armenia in June 2001 and 2002. In the laboratory, each animal was kept in a separate terrarium until individual clutches of eggs were obtained, as described earlier [7]. DNA was isolated from the blood of adult animals and frozen embryos by the standard phenol–chloroform method using protein kinase K. DNA samples were treated with *Bsu*RI, and blot hybridization was performed with the use of two

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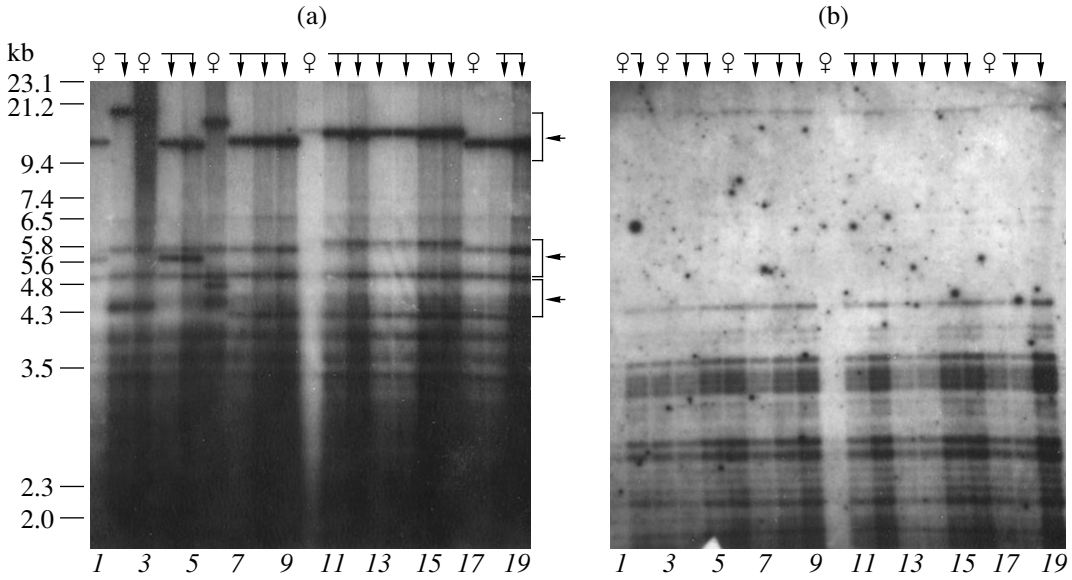

Fig. 1. Multilocus DNA fingerprinting of parthenogenetic families of *D. unisexualis*. Family samples of DNA from the Kutchak (lanes *1* and *2*), Lchap (lanes *3–5*), Artik (lanes *6–9*), and Zagalu (lanes *10–19*) populations. Blot filters were sequentially hybridized with (a) the$(GATA)_4$ oligonucleotide and (b) phage M13 DNA. Arrows indicate the zones of the changes in fingerprint phenotypes in parthenogenetic families. Phage λ DNA digested by *Eco*RI and *Hind*III was the source of marker DNA fragments (their sizes in kilobases are indicated).

probes labeled with ^{32}P: the oligonucleotide $(GATA)_4$ and phage M13 DNA [6–8].

Here, we report the results of the DNA fingerprint analysis of $(GATA)_n$ microsatellite repeats in 25 parthenogenetic families (a total of 84 offspring) from six isolated *D. unisexualis* populations of Armenia. In 21 families, all maternal DNA fragments were inherited by offspring without changes. However, in four families (13 offspring), we found significant changes in $(GATA)_n$ fingerprint phenotypes. The mutant offspring phenotypes differed from maternal ones in the mobilities of one to five DNA fragments in the electrophoretic resolution zones 21–7, 5.8–4.8, 4.3, and less than 3.5 kb. Some mutant fingerprint phenotypes were found not only in families, but also in population samples (Fig. 1a). According to our estimation of the mutational variation of DNA fragments containing $(GATA)_n$ repeats, the frequency of mutant $(GATA)_n$ fingerprint phenotypes in offspring was 15% (13/84). In total, we found 16 mutant fragments in the sample of 84 offspring. For the estimation of mutational variation with the use of multilocus DNA probes, the mutation rate per offspring microsatellite fragment should be introduced. This value is calculated as the number of mutant microsatellite fragments divided by the mean number of microsatellite fragments per individual and divided by the number of offspring (in the given case, $16/(20 \times 84)$) [9]. The mean mutation rate per offspring microsatellite fragment calculated by this method is 0.95%.

The M13 DNA fingerprinting of *D. unisexualis* families mutant and not mutant for $(GATA)_n$ microsatellites demonstrated that the offspring inherited unchanged M13 microsatellite DNAs (Fig. 1b): none of the offspring fingerprint phenotypes differed from the maternal phenotype. Apparently, these differences were related to the difference in the mechanisms of variation of minisatellites and microsatellites.

The $(GATA)_n$ microsatellite mutations found in these experiments may be somatic and lead to tissue mosaicism. The somatic stability of $(GATA)_n$ repeats was also studied by means of fingerprinting of DNA samples isolated from different tissues and organs (the blood, liver, lungs, oviducts, and intestine) of *D. unisexualis*. As seen from Fig. 2, the fingerprint phenotypes of different tissues were similar in three adult lizards. However, in the fourth animal, the fingerprint phenotype of the lungs differed from those of other organs with respect to several fragments (indicated by arrows). Thus, we found somatic mosaicism in one of four cases. The lungs, blood, and heart are of common (mesodermal) origin; therefore, it is likely that somatic rather than generative mutations were responsible for the variation of $(GATA)_n$ loci in lung tissues.

Our data on clonal variation in the parthenogenetic lizard *D. unisexualis* indicate that the mutation rates of microsatellites in parthenogenetic species are as high as in bisexual species, but they have some specific characteristics discovered in this study. First, new fingerprint phenotypes replaced the old ones in parthenogenetic families; these new phenotypes were more frequent in population samples (Fig. 1a, lanes *1–9*). Second, all offspring in the mutant families studied had the same fingerprint phenotypes, which differed from maternal ones. This indicates a higher probability of these muta-

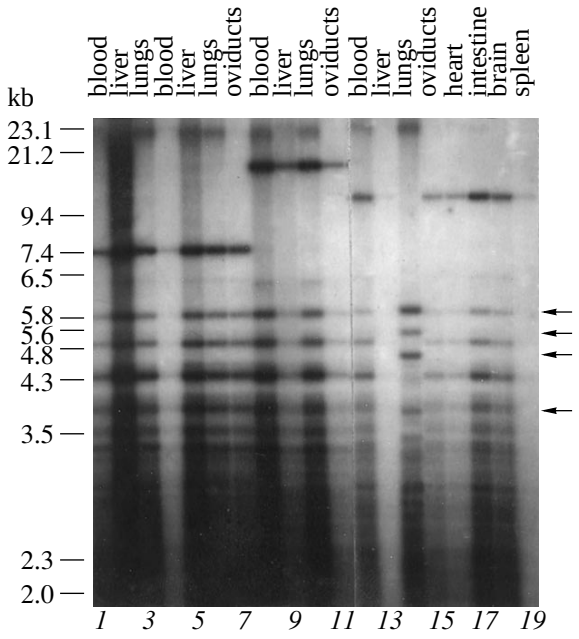

Fig. 2. Multilocus fingerprinting of DNA samples isolated from the tissues and organs of *D. unisexualis.* Blot filters were hybridized with the oligonucleotide probe $(GATA)_4$. The lizards were from the Takyarlu (lanes *1–7*), Kutchak (lanes *8–11*), and Noraduz (lanes *12–19*) populations. Arrows indicate mutant fragments in the fingerprint phenotypes of the tissues in the zone of changes from 21.2 to 3.5 kb. The marker fragments are the same as in Fig. 1.

tions in germ-line (generative) cells, although the aforementioned case of tissue mosaicism was apparently related to somatic mutations. Third, the clonal diversity of fingerprint phenotypes observed in the populations of parthenogenetic species that we detected using nuclear DNA markers resulted from spontaneous mutations of unstable microsatellite loci.

Thus, we demonstrated the existence of unstable $(GATA)_n$-containing microsatellite loci and the somatic mosaicism with respect to these loci in the parthenogenetic lizards *D. unisexualis.*

Note that the genesis and instability of microsatellite loci, which are being intensely studied in humans and bisexual species of animals, remain practically unexplored in species with clonal reproduction. The notions on mutations and microsatellites resulting from DNA polymerase slipping and/or reading-frame shifts during the complementary interaction between one-stranded DNAs [10] have become classical. However, more complex, "noncanonical" mechanisms, including sister chromatid exchange, gene conversion [11], mutations of adjacent regions [12], and DNA conformational polymorphism [13], are also being discussed.

DNA fingerprinting of the populations and pedigrees of some bisexual species of animals demonstrated the existence of multifragment alleles forming a "restriction haplotype" and segregating as a single Mendelian allele [14]. The study of these haplotypes is especially promising in the case of clonal species reproducing maternal haplotypes in successive generations, because it permits more reliable detection and identification of polymorphism and genomic instability in vertebrates.

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