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Variation of Mini- and Microsatellite DNA Repeats in Parthenogenetic Lizard *Darevskia armeniaca* as Revealed by DNA Fingerprinting Analysis

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Abstract—Population and family samples of two morphological forms (mutant and normal with respect to dorsal color) of parthenogenetic lizard species *Darevskia armeniaca* were examined by means of DNA fingerprinting using M13 mini- and $(GACA)_n$ and $(TCC)_n$ microsatellite DNA markers. The morphological forms examined were characterized by clonally inherited, species-specific patterns of the DNA markers, which were different from the species-specific DNA fingerprints of the other parthenogenetic species of the genus *Darevskia* (*D. dahli, D. unisexualis,* and *D. rostombekovi*). The mean index of similarity (*S*) obtained for a sample of 36 individuals from three isolated populations using three types of DNA markers was 0.966. This was similar to those observed in *D. dahli* (0.962) ($P > 0.05$), but higher than that in *D. unisexualis* (0.950) ($P < 0.05$) and *D. rostombekovi* (0.875) ($P < 0.01$). Inheritance of M13 minisatellite and $(TCC)_n$ microsatellite DNA markers in the F1 offspring of parthenogenetic lizards was examined. It was shown that variability and clonal diversity of the fingerprint phenotypes observed in the populations and families of *D. armeniaca* could be at least partly explained by RFLP mutations in microsatellite repeats.

INTRODUCTION

Hypervariable mini- and microsatellites represent the most common and universal system of molecular markers used in population and evolutionary studies, genome marking, assessment of paternity, and medical genetic investigations [1–8]. Mini- and microsatellite DNA repeats belong to most unstable sequences of eukaryotic genomes displaying maximal mutation rates observed for the genetic loci [2, 9–12]. Despite intense studies performed during the last decade, the nature and mechanisms of instability and genesis of mini- and microsatellite DNA markers remain unclear [13–16].

Obligatory-parthenogenetic reptiles, whose reproductive system implies clonal reproduction of maternal genotype in a series of generations, represent a unique animal model for monitoring of genetic variation in loci exhibiting high mutation rate [17–19]. Earlier studies on variation of multilocus DNA markers in parthogenetic species of Caucasian rock lizards of the genus *Darevskia* showed a high level of intraspecific similarity (e.g., mean similarity indices in *D. dahli* and *D. unisexualis* were 0.962 and 0.950, respectively) along with DNA fingerprint phenotypic differences attaining several DNA fragments. At the same time, at the background of species-specific DNA fingerprint profiles of parthenogenetic individuals, typical genetic heterogeneity and instability of some microsatellite DNA fragments were observed [18–21].

Our aim was examining variability of mini- and microsatellite DNA repeats in parthenogenetic Armenian lizard, *D. armeniaca*, widely distributed across central Armenia and southern Georgia [17]. Like other unisexual species of the genus *Darevskia, D. armeniaca* has hybrid descent (parental species are *D. valentini* and *D. mixta*), and is characterized by diploid chromosome number, high level of fixed heterozygosity of allozyme loci [22], and extremely low restriction site polymorphism of miotochondrial DNA [23]. Analysis of *D. armeniaca* allozyme spectra revealed the variation attaining 3 of 35 loci manifested as the occurrence of two major and two minor allozyme variants in the population [24]. In addition to allozyme clones, *D. armeniaca* was shown to have two morphological forms with typical variation of dorsal color [25]. Incubations of eggs obtained from the mutant females showed that mutant color was clonally inherited.

DNA fingerprinting analysis of population and family samples of lizards with mutant and normal color revealed no differences between the morphological forms. Based on the data obtained, intraspecific variability of micro- and minisatellite DNA markers (S = 0.966) in *D. armeniaca* was estimated. It was shown

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Table 1. Variability of mini- and microsatellite DNA repeats in *D. armeniaca*

Combination of DNA probe and restriction enzyme	Populations	Number of individuals, *N*	Mean number of fragments per individual *n* (*SE*)	Mean index of similarity *S* (*SE*)	95% confidence interval for *S* values
M13–*Bsu*RI	Stepanavan, Medved'-Gora	36	39.75 (0.124)	0.974 (0.001)	0.972–0.976
$(TCC)_{50}$–*Bsu*RI	Pushkinskii Pereval, Stepanavan	36	37.86 (0.1)	0.967 (0.001)	0.965–0.969
$(GACA)_4$–*Mva*I	Pushkinskii Pereval, Takyarlu	36	16.08 (0.047)	0.944 (0.001)	0.942–0.946
Combined data from three matrices		36	93.69 (0.213)	0.966 (0.001)	0.965–0.967

that variability and diversity of fingerprint phenotypes observed in the populations of the parthenogenetic species at least partly resulted from RFLP mutations in microsatellite DNA repeats.

MATERIALS AND METHODS

Parthenogenetic females of *D. armeniaca* were collected in June and July 1999 through 2001 in the natural habitats of central and northeastern Armenia, in the populations of Medved'-Gora (isolated population, 16 individuals), Pushkinskii Pereval (20 individuals), as well as in the vicinity of the mt. Stepanavan (20 individuals) and the settlement of Takyarlu (20 individuals, including 10 females with mutant color and 10 females with normal color). The females ready for egg-laying were maintained in the laboratory in separate terrariums until obtaining individual clutches. Laid eggs were incubated for 30 days and then dissected. Embryos were rinsed in physiological saline and quickly frozen in liquid nitrogen. Blood samples obtained from adult females were preserved in 0.05 M EDTA, pH 8.0, as described earlier [18, 19]. DNA was extracted according to Mathew [26] with the use of proteinase K. Frozen embryos were homogenized into powder in liquid nitrogen, and DNA was extracted using the standard phenol–chloroform procedure and incubation with Proteinase K [26]. DNA samples (10 μg) were digested with the *Bsu*RI and *Mva*I restriction endonucleases (Fermentas) according to the recommendations of the manufacturer. Multilocus DNA fingerprinting was carried out using M13, $(GACA)_4$, and $(TCC)_{50}$ probes labeled with [^{32}P] in standard conditions as described earlier [18–20]. The data of fingerprint analysis were represented as object–character binary matrices and treated according to the earlier described method [18] using BIOSYSTEM 1.0 software package [27]. DNA fingerprint phenotypes were determined using hierarchical cluster analysis [28] as the set of individuals displaying 100% similarity in all three DNA marker types used.

RESULTS

The data on DNA fingerprinting analysis and quantitative estimates of mini- and microsatellite DNA markers variation in *D. armeniaca* are presented in Fig. 1 and Table 1. Similarly to other parthenogenetic lizards of the genus *Darevskia* [18, 20, 21, 29], *D. armeriaca* displayed some microsatellite DNA fragment variability (indicated by arrows in Fig. 1) at the background of high intaspecific similarity of fingerprint phenotypes of the individuals from different populations (see Materials and Methods).

Quantitative estimates of the fingerprint data showed that $(GACA)_n$ and $(TCC)_n$ microsatellite DNA markers exhibited higher variability than the M13 minisatellite marker. Mean indices of similarity (*S*) obtained using $(GACA)_n$ and $(TCC)_n$ loci were 0.944 (0.001) and 0.967 (0.001), respectively, while the value of this parameter estimated using M13 minisatellites was 0.974 (0.001) (Table 1, $P < 0.05$). The mean index of similarity obtained from three data matrices was 0.966 (0.001). Therefore, intraspesific variability of *D. armeniaca* (with respect to the DNA marker types used) was similar to that determined earlier for *D. dahli* ($P > 0.05$) [18], and lower than that in *D. unisexualis* ($P < 0.05$) [20] and *D. rostombekovi* ($P < 0.01$) [29].

M13 DNA fingerprint analysis of *D. armeniaca* individuals with mutant and normal dorsal color revealed differences in one marker DNA fragment (Fig. 2, indicated by an arrow). This fragment was not detected in all mutant individuals from the sample examined and also in some normal individuals from the same population. Within one of two M13 fingerprint phenotypes, two morphological forms, mutant and normal, were observed. Thus, differentiation of the individuals with respect to this type of minisatellite DNA repeats did not correlate with the changes of dorsal color. In all parthenogenetic offspring of the mutant females examined clonal inheritance of one out of two (alternative) M13 fingerprint phenotypes was observed (Fig. 2b).

Observed variability of mini- and microsatellite DNA repeats in the population of *D. armeniaca* implies the performance of the family analysis with the purpose of detection in the offspring of the "new" mutant DNA

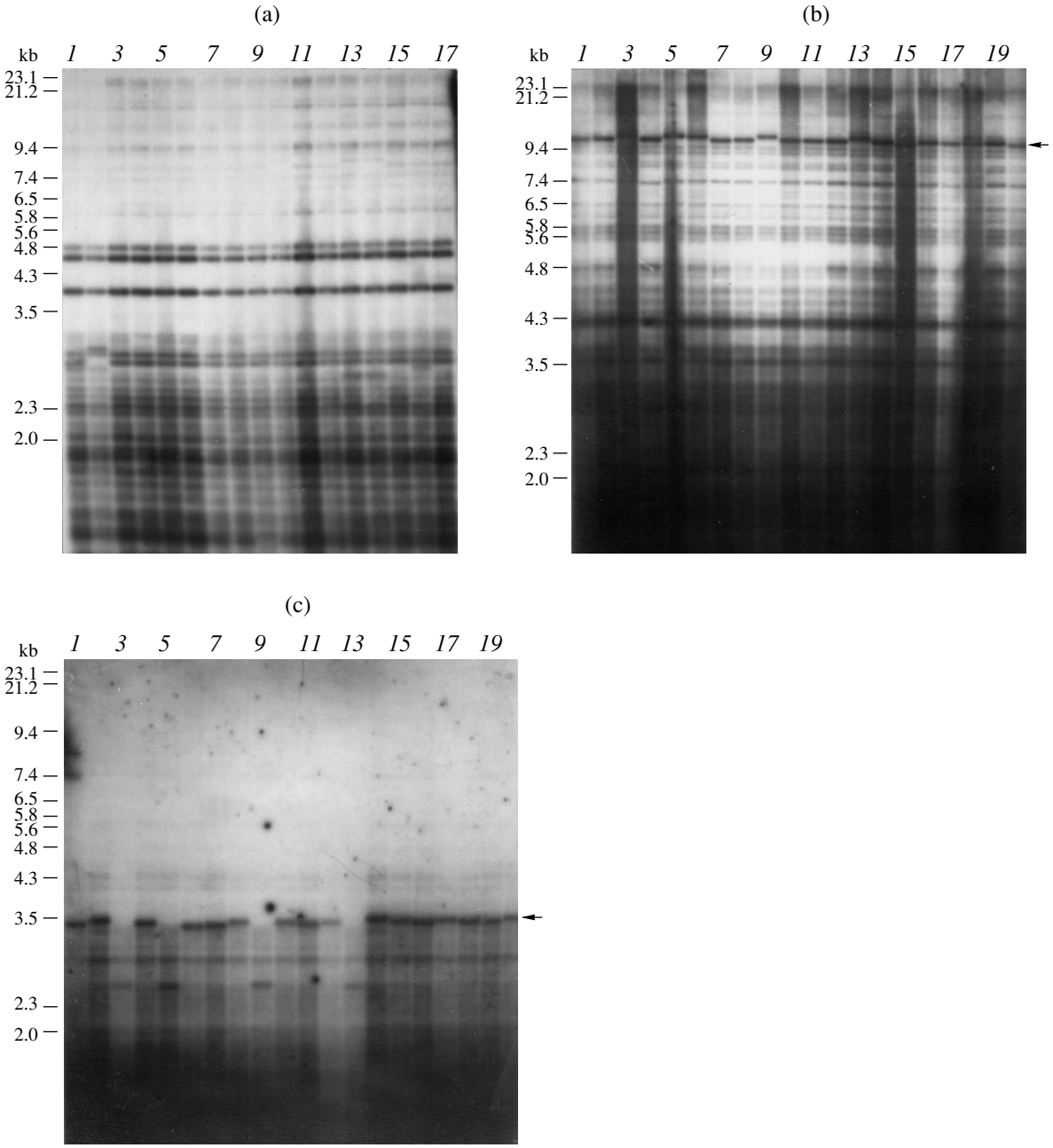

Fig. 1. Multilocus DNA fingerprinting analysis of *D. armeniaca*. DNA samples digested either with the *Bsu*RI, or *Mva*I restriction endonuclease were hybridized with the probes as follows: (a) M13, individuals from the population of the Tezh mountain (lanes *1* and *2*) and Stepanavan (lanes *3* to *17*); (b) lanes *1* to *13*, individuals from Medved'-Gora population, lanes *14* to *20*, individuals from Pushkinskii Pereval population; (c) $(GACA)_4$, individuals from populations Medved'-Gora (lanes *1* to *13*) and Pushkinskii Pereval (lanes *14* to *20*). Variable microsatellite fragments are indicated by arrows. The λ phage DNA digested with the *Eco*RI and *Hind*III restriction endonuclases was used as molecular weight marker.

fragments differing by their mobility from the maternal ones (mutant fingerprint phenotypes). Analysis of eight parthenogenetic families of *D. armeniaca* (with the total number of offspring constituting 26 animals) showed that M13 minisatellite fragments were inherited by the F1 progeny without alterations (Fig. 3a). However, analysis of the same samples using $(TCC)_{50}$ microsatellite DNA probe showed a change in the mobility of a DNA marker fragment in the zone between 3.5 and 2.5 kb in one family. Furthermore, the female and one of its offspring were different from the two other offspring (Fig. 3b). Both fingerprint phenotypes of the $(TCC)_n$ loci observed in the family sample were also detected in the population sample (Fig. 3c).

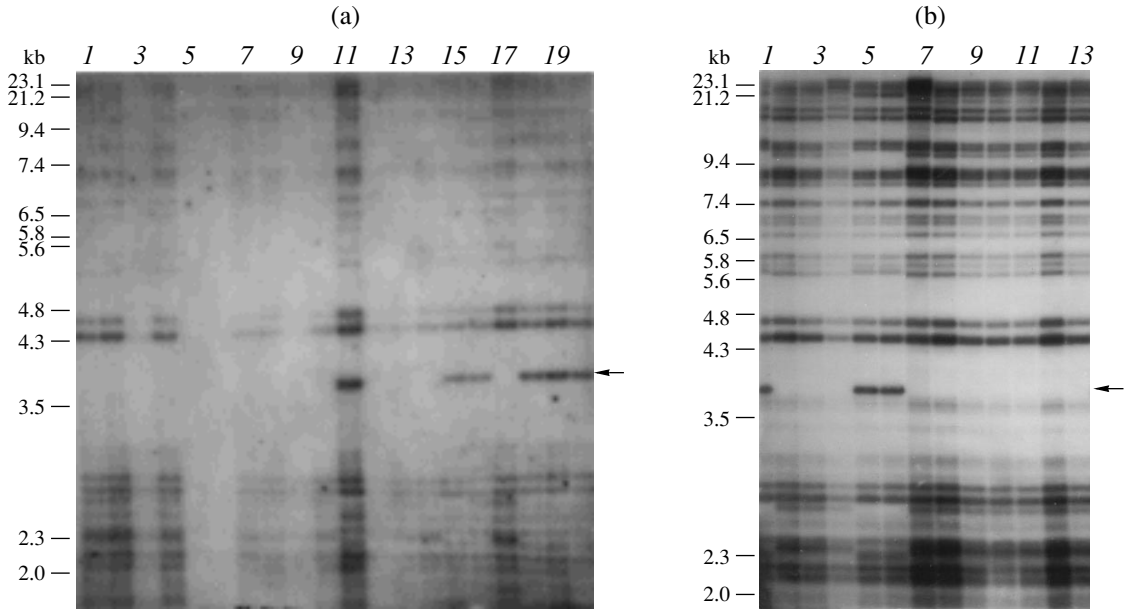

Fig. 2. M13 DNA fingerprint analysis of mutant and normal *D. armeniaca* individuals. (a) Population samples of mutant (lanes *1* to *10*) and normal (lanes *11* to *20*) lizards; (b) family samples of mutant females (lanes *4* and *7*) and their progeny (lanes *2*, *3*, *8–13*) and normal female (lane *1*) and its progeny (lanes *5* and *6*). The position of a fragment absent from the individuals with mutant color is indicated by an arrow. Markers (kb) as in Fig. 1.

DISCUSSION

Using parthenogenetic lizards as a model organism, we have shown that DNA fingerprinting is the most effective tool for monitoring total genome changes, the nature and the mechanisms of which, however, still remain obscure. To explain the observed microsatellite allele frequency distribution patterns in the populations of bisexual animals, as well as in human populations, a model was suggested, according to which mutations in microsatellites occur via subsequent addition or loss (elimination) of repetitive units (stepwise mutation model) [30, 31].

The DNA polymerase slippage in replication is considered to be the key factor changing allele length. This model received indirect and direct experimental support [32, 33]. However, numerous observations showed that microsatellite behavior was far from being always concordant with the consensus variant of the stepwise model. A modified variant of this model, the two phase mutation model [34], suggests that in some microsatellite loci mutations can occur either subsequently, step-by-step, or discontinuously, leading to tremendous one-time enhancement of the repeat length (expansion) [7, 8]. A large body of data has been accumulated pointing to even more complex processes taking place in microsatellites, including asymmetric distribution of the mutations along the cluster [35], high frequency of insertions and deletions [35, 36], and also numerous mutations, predominantly nucleotide substitutions, in the flanking DNA sequences [4, 37]. For example, in crab *Limutus polyphemus*, a total of 34 570-bp allelic DNA fragments containing the $(CA)_n$ repeat were found. Though the length of the $(CA)_n$ repeat varied from five to eleven monomeric units, the main allele differences were caused by polymorphism of the flanking regions containing nucleotide substitutions in 22 sites [37]. The mutation rate of microsalellite can be lower than the substitution rate in the flanking DNA regions (cited from [36]).

In some *Drosophila* species (*D. melanogaster, D. simulans,* and *D. sechelia*) the differences in the flanking regions are more profound, compared to microsatellite repeats [4]. In limiting cases the differences between allelic variants lied only in that in the flanking DNA regions [37].

One of human $(TATC)_n$ containing hypervariable locus, *DXS981* (mutation rate per gamete is 1.5%), is characterized by insertion–deletion polymorphism in the DNA regions immediately adjacent to the repeat sequence. Linked inheritance of two alternative insertion–deletion haplotypes means that new mutant alleles in this locus arise either as a result of polymerase slippage or are caused by unequal sister chromatid exchange, completely excluding, however, unequal exchange (recombination) between the alleles. The authors of the cited article also suggested that some of the mutations observed at this locus were somatic [11].

Mutations leading to the changes of fingerprint phenotypes in parthenogenetic lizards can be of complex, yet unknown, nature. Possibly, they are caused by processes in mini- and microsatellites, analogous to that observed in bisexual species [11, 30–37]. Since mini- and microsatellite repeats belong to unstable regions of eukaryotic genomes, these processes result in substan-

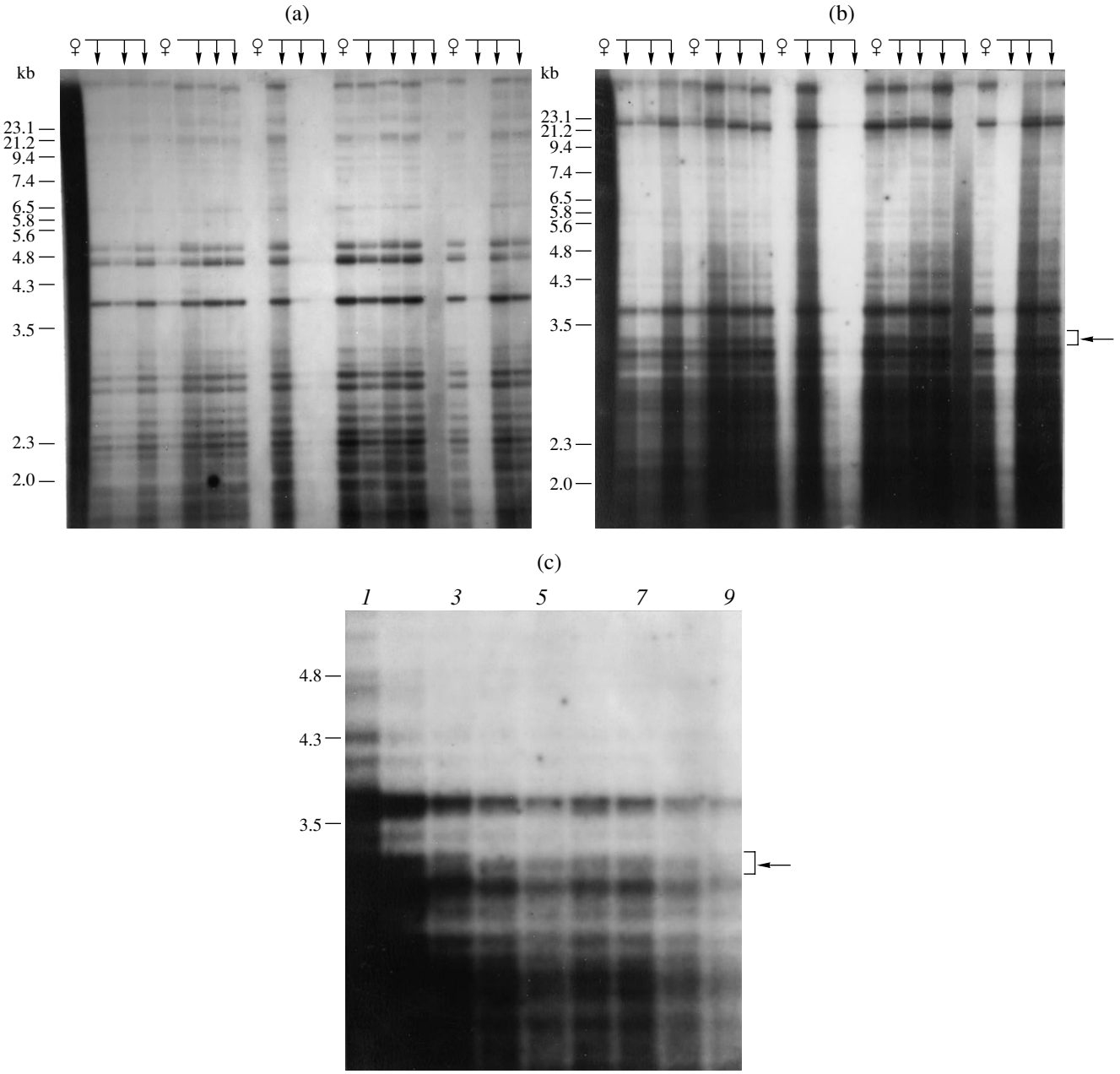

Fig. 3. DNA fingerprint analysis of the family and population samples of *D. armeniaca*. DNA samples obtained from parthenogenetic females and their progeny were digested with the *Bsu*RI restriction endonuclease and hybridized to the M13 (a) and $(TCC)_{50}$ (b and c) DNA probes. Variable fragments are indicated by the arrows; (c) magnified image of the $(TCC)_n$ zone, a population DNA fingerprint sample. Markers (kb) as in Fig. 1.

tially higher clonal diversity and differentiation of partenogenetic lizards regarding mini- and microsatellite loci, when compared to allozyme loci (Table 2) and (or) especially to the loci controlling the skin pattern and color.

It was shown that variability levels of partenogenetic species with respect to the skin color and pattern, allozyme loci, histocompatibility, and finally, nuclear DNA markers cannot be compared [8, 19, 38–40]. If clonal diversity is estimated in respect to each system of morphological and genetic characters, then partenogenetic lizards of the genus *Darevskia* should be considered as a multiclonal species system. Some clonal species, however, are characterized by stronger correlation between variability in respect of different types of markers [41–43].

From the viewpoint of evolution history, parthenogenetic species of Caucasian rock lizards are considered young (8000 to 10 000 years) [25] and being in the process of establishment and development of clonal

Table 2. Comparative analysis of allozyme and fingerprint markers variation in parthenogenetic lizards of the genus *Darevskia*

Species	Number of allozyme loci examined	Number of individuals, *N*	Number of variable loci	Number of clones	Number of fingerprint markers	Number of individuals tested, *N*	Total number of fingerprint phenotypes*
Darevskia dahli	35	161	2	5	1791	25	13
D. unisexualis	36	57	1	2	1777	40	16
D. rostombekovi	35	65	0	1	1802	21	16
D. armeniaca	35	117	3	4	3373	36	23

Notes: The data on allozyme analysis are form [22–24, 38–40], and data on fingerprint analysis are from [18–21, 29].

* Includes the individuals distinguished for at least one marker DNA fragment [19, 29].

diversity. It is still unknown whether they include already developed clones (and/or populations), which would be in fact different from each other in different parameters (morphological, biochemical, immunological, and cytogenetic characters) or whether mutation processes at different gene loci form a variegated, chaotic picture of genetic diversity and false multiclonality, pointing rather to the early stages of differentiation and clone formation in these species.

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