Russian Journal of Genetics, Vol. 40, No. 10, 2004, pp. 1095–1101. Translated from Genetika, Vol. 40, No. 10, 2004, pp. 1336–1342.

GENERAL GENETICS

Study of Allelic Polymorphism of $(GATA)_n$-Containing Loci in Parthenogenetic Lizards *Darevskia unisexualis* (Lacertidae)

V. I. Korchagin[1, 2], I. A. Martirosyan[1], A. V. Omelchenko[2], I. S. Darevsky[3], A. P. Ryskov[1], and O. N. Tokarskaya[1]

[1] *Institute of Gene Biology, Russian Academy of Sciences, Moscow, 119334 Russia; fax: 135-41-05; e-mail: korchagin@newmail.ru*

[2] *Moscow State Pedagogical University, Moscow, 119882 Russia*

[3] *Institute of Zoology, Russian Academy of Sciences, St. Petersburg, 199034 Russia*

Received April 23, 2004

Abstract—The genesis of mini- and microsatellite loci, which is under extensive study in humans and some other bisexual species, has been virtually overlooked in species with clonal mode of reproduction. Earlier, using multilocus DNA fingerprinting, we have examined variability of some mini- and microsatellite DNA markers in parthenogenetic lizards from the genus *Darevskia*. In particular, mutant (GATA)*n*-restriction DNA fragments were found in *Darevskia unisexualis.* In the present study, we examined intraspecific polymorphism of three cloned loci of *D. unisexualis*—*Du323*, *Du215*, and *Du281*—containing $(GATA)_7GAT(GATA)_2$, $GAT(GATA)_9$, and $(GATA)_{10}TA(GATA)$ microsatellite clusters, respectively. Different levels of intrapopulation and interpopulation variability of these loci were found. Locus *Du281* showed the highest polymorphism—(six allelic variants in the sample of 68 DNA specimens). Three alleles were found for locus *Du215*. The *Du323* locus was electrophoretically invariant. The primers chosen for loci *Du323*, *Du215*, and *Du281* were also used for PCR analysis of homologous loci in two presumptive parental bisexual species, *D. valentini* and *D. nairensis.* The PCR products of the corresponding loci of the parental species had approximately the same size (~200 bp) as their counterparts in *D. unisexualis*, but the polymorphism levels of the paternal, maternal, and hybrid species were shown to be somewhat different. These data on the structure of the *D. unisexualis* loci provide a possibility to study genetic diversity in the parthenogenetic species *D. unisexualis* and other related unisexual and bisexual species of this genus, which can provide new information on the origin of parthenogenetic species and on the phylogenetic relationships in the genus *Darevskia*. These data can also be used for resolving problems of marking the lizard genome, which is still poorly studied.

INTRODUCTION

Microsatellites $(GATA)_n$ are widely represented in the eukaryotic genome. The active interest to the $(GATA)_n$ repeats is related to their high variability in all eukaryotic organisms studied, including plants, invertebrates, and vertebrates (mammals, primates, and human among them) [1]. However, the issue of the genesis of $(GATA)_n$ loci, which has been under intense research in bisexual species [2–7], is scarcely studied in species with the clonal mode of reproduction. Parthenogenetic lizard species, whose genotypes are clonally reproduced in generations, is a suitable model for studying variability of hypervariable loci [8, 9].

Earlier, using multilocus DNA fingerprinting we have for the first time ever examined variability of different types of mini- and microsatellite DNA markers in four parthenogenetic species of the genus *Darevskia* (*D. dahli, D. armeniaca, D. unisexualis*, and *D. rostombekovi*) [10–13]. It was shown that, against the background of species-specific DNA fingerprinting patterns, parthenogenetic individuals are genetically heterogeneous with regard to some microsatellite DNA markers. Loci of *D. unisexualis* containing microsatellites $(TCC)_n$, $(TCT)_n$, and $(GATA)_n$ proved to be the most variable. DNA fingerprinting of parthenogenetic families of *D. unisexualis* revealed high frequency of mutations at these loci already in the first-generation progeny [14–16]. Moreover, mutant $(GATA)_n$-fingerprint genotypes in the progeny were also detected in population samples of *D. unisexualis.* This directly indicates the mutational nature of genotypic variability at $(GATA)_n$ loci in *D. unisexualis* populations. At the same time, the molecular nature and mechanisms of appearance of these mutations, as well as the level of allelic polymorphism of microsatellite loci are still unclear. Apparently, these issues can be clarified using genetic engineering studies of the variable loci.

In the present work, three $(GATA)_n$-containing loci of *D. unisexualis* are cloned and sequenced, and intraspecific polymorphism of their amplification products is examined in *D. unisexualis* and its bisexual parental species, *D. valentini* and *D. nairensis.*

1022-7954/04/4010-1095 © 2004 MAIK "Nauka/Interperiodica"

Table 1. Molecular-genetic characteristics of $(GATA)_n$-bearing clones of *D. unisexualis*

Clone (locus)	GenBank no.	Insert size, bp	$(GATA)_n$ cluster	Primers (5'–3')*	Annealing temperature, °C	PCR product size, bp
Du323	AY574977	876	$(GATA)_7GAT(GATA)_2$	F: AAGCAGACTGTACAAATCCCTA R: ACTGATCTAAGACAAGGTAAAAT	48	199
Du215	AY574978	453	$GAT(GATA)_9$	F: CAACTAGCAGTAGCTCTCCAGA R: CCAGACAGGCCCCAACTT	50	216–230
Du281	AY442143	556	$(GATA)_{10}TA(GATA)$	F: TTGCTAATCTGAATAACTG R: TCCTGCTGAGAAAGACCA	50	197–212

* F, forward primer; R, reverse primer.

MATERIALS AND METHODS

Females of *D. unisexualis* were captured in June 2001–2002 in isolated localities of central Armenia in the natural habitats near settlements of Kutchak, Lchap, Takyarlu, as well as on the coast of Sevan Lake (Noraduz Cape, southeastern Sevan Lake, village of Zagalu). Nuclear DNA was isolated from lizard blood samples preserved at 4°C in 0.05 M EDTA, pH 8.8, by standard phenol–chloroform extraction with the use of proteinase K. The construction of genomic library of *D. unisexualis* and selection of clones carrying different microsatellite types has been described earlier [17]. Sequencing of DNA clones was carried out using Sanger's method. Based on the sequences obtained, primers were chosen for the polymerase chain reaction using the Primer Select procedure from the LaserGene package (DNASTAR). Amplification was conducted in the volume of 20 μl containing 1 × PCR buffer (Dialat); 200 μM of each dNTP; 2 mM $MgCl_2$; 400 pM of each primer; and 0.125 U *Taq* polymerase. To the reaction mixture, 40–80 ng of DNA was added. Amplification was run on a four-channel thermal cycler TP4-PCR-01 (Tertsik) in the following temperature regime: denaturation for 3 min at 94°C; 30 cycles of amplification (1 min at 94°C, 40 s at 48–50°C, 40 s at 72°C); 5 min at 72°C. After amplification, 5 μl of a mixture of brome phenol blue and xylencyanol was added to the mixture. From 5 to 10 μl of the product was fractionated in 8% native polyacrylamide gel (PAAG) and visualized by staining with ethidium bromide.

Table 2. Population-genetic parameters of *D. unisexualis* loci bearing $(GATA)_n$ microsatellite repeats

Population	Locus *Du323*		Locus *Du215*		Locus *Du281*	
	number of animals	number of alleles	number of animals	number of alleles	number of animals	number of alleles
Kutchak	10	1	10	2	10	3
Lchap	10	1	12	2	11	2
Zagalu	11	1	11	2	11	2
Takyarlu	16	1	17	2	19	1
Noraduz	17	1	15	2	17	3
Total	64	1	65	3	68	6

RESULTS AND DISCUSSION

For studying the molecular nature of $(GATA)_n$ allelic polymorphism at microsatellite loci in parthenogenetic species and bisexual parental species, we have earlier developed a genomic library of *D. unisexualis*. By screening of 4000 recombinant clones, ten clones containing $(GATA)_n$ microsatellites were selected. Sequencing followed by computer-aided analysis of three randomly chosen clones (*Du323*, *Du215*, and *Du281*) confirmed the presence in them long $(GATA)_n$ clusters (nine to fifteen repeated units) and showed that these clusters represent different regions of the lizard genome (Table 1). Taking into

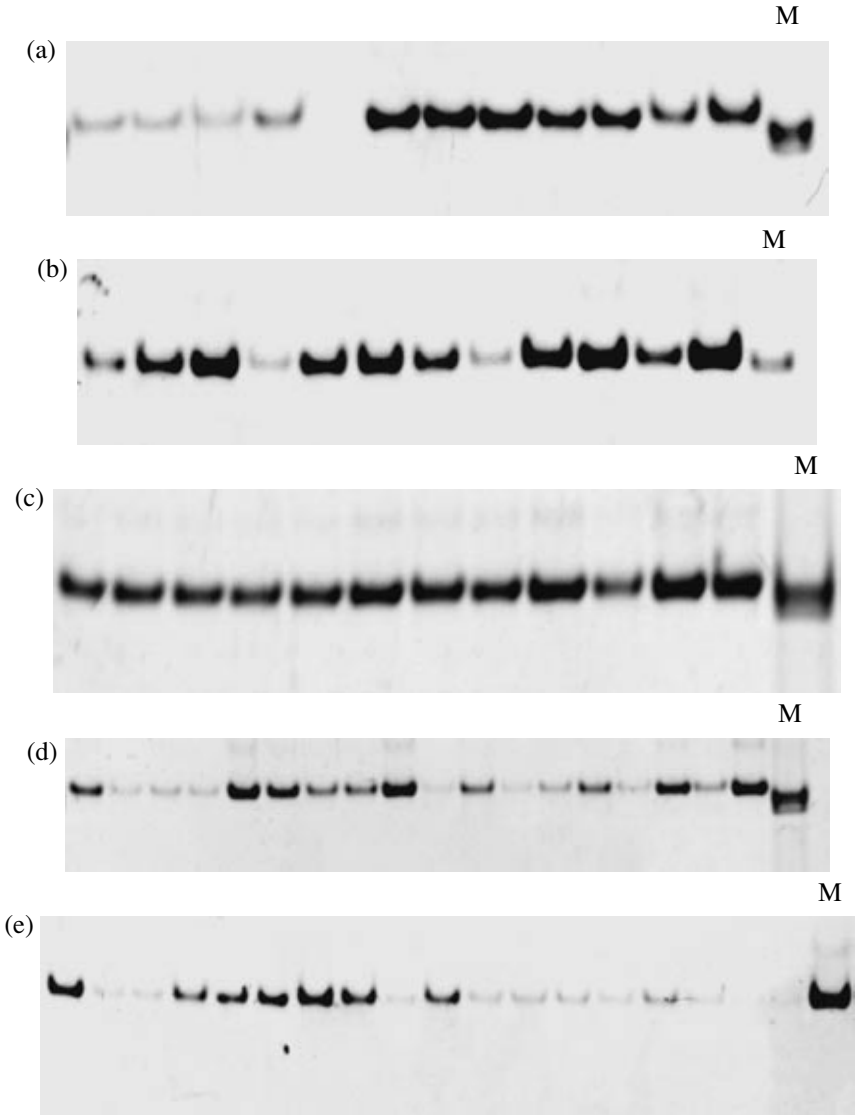

Fig. 1. Electrophoresis of the *Du323* PCR products of the *D. unisexualis* populations. (a) Lchap; (b) Kutchak; (c) Zagalu; (d) Noraduz; (e) Takyarlu. Here and in further figures, marker fragment M is 200 bp (Amersham).

account the screening results and the average size of inserts in the cloned equal to about 700 bp [17], the percentage of $(GATA)_n$ repeats in the *D. unisexualis* genome is ~0.25%, and the possible distance is 280 kb ($4000 \times 700/10 = 280000$) [18, 19]. On the other hand, such approximate computations do not exclude preferential localization of $(GATA)_n$ repeats in definite regions of the lizard genome and even on the definite chromosomes, as was shown for the Y chromosome of mice and humans [1]. Nucleotide sequences of three aforementioned clones were deposited in GenBank, and the primer pairs to obtain PCR products about 200 bp in size were selected on the basis of the data on the primary structure (Table 1).

The results of PCR analysis of population samples of *D. unisexualis* DNA at loci *Du323, Du215*, and *Du281* are presented in Figs. 1–3. Apparently, the loci examined exhibit different levels of polymorphism for allele size. Locus *Du323* proved to be electrophoretically invariant and homozygous (Fig. 1, Table 2). In all populations studied, the PCR product of this locus produced one band of ~200 bp. Locus *Du215* had three alleles of different size (Fig. 2, Table 2). A comparison of the data for five populations examined showed that in four of them (Kutchak, Lchap, Zagalu, and Noraduz), two alleles of this locus occurred. The third allele was found in the Takyarlu population. However, the monomorphism of these loci for allele size do not exclude polymorphism of their nucleotide sequences, point mutations, nucleotide substitutions, small deletions, and insertions among them, including those in the regions adjacent to microsatellite repeats [20–22].

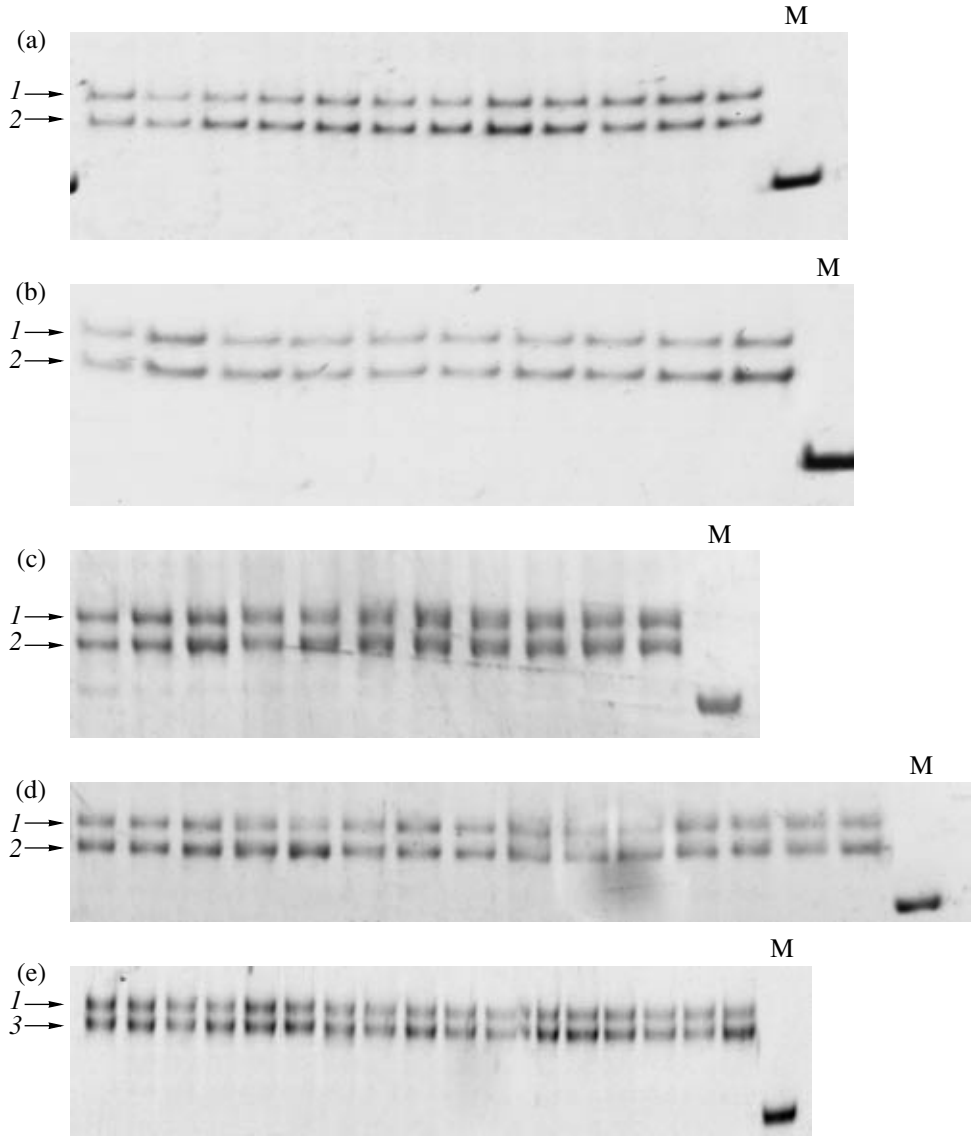

Fig. 2. Electrophoresis of the *Du215* PCR products of the *D. unisexualis* populations. (a) Lchap; (b) Kutchak; (c) Zagalu; (d) Noraduz; (e) Takyarlu. Alleles are indicated by arrows.

Locus *Du281* proved to exhibit the highest polymorphism. The distribution of alleles of this locus in the populations is shown in Fig. 3. A comparison of the data across five populations examined shows that *D. unisexualis* has at least six allelic variants of *Du281* (Table 2).

In view of the fact that parthenogenetic species *D. unisexualis*, like other parthenogenetic *Darevskia* species, has a hybrid origin [8], it is of interest to study these loci in the putative parental species (*D. valentini* and *D. nairensis*). In Fig. 4, the PCR products of the loci of the maternal (*D. nairensis*) and paternal (*D. valentini*) species, homologous to *Du215*, *Du323*, and *Du281* are presented. Note that the PCR products of the homologous loci have approximately the same electrophoretic mobilities in the parental species as in *D. unisexualis* (i.e., are confined to the electrophoretic zone of 190 to 230 bp), but they exhibit different levels of polymorphism. For instance, the electrophoretically invariant locus *Du323* is variable in *D. valentini* (eight PCR variants) (Fig. 4a), but it is not amplified in *D. nairensis*. Apparently the absence of PCR amplification of *Du323* in *D. nairensis* is explained by the divergence of the adjacent to the repeat regions and sites of primer annealing, which does not exclude amplification of this locus by other pairs of primers. Locus *Du215* shows a completely different pattern of PCR polymorphism of the parental species (Fig. 4b). In the sample of the parental species *D. valentini* this locus is amplified producing two PCR products, whereas in the sample of nine *D. nairensis* individuals, this locus is highly polymorphic (11 PCR vari-

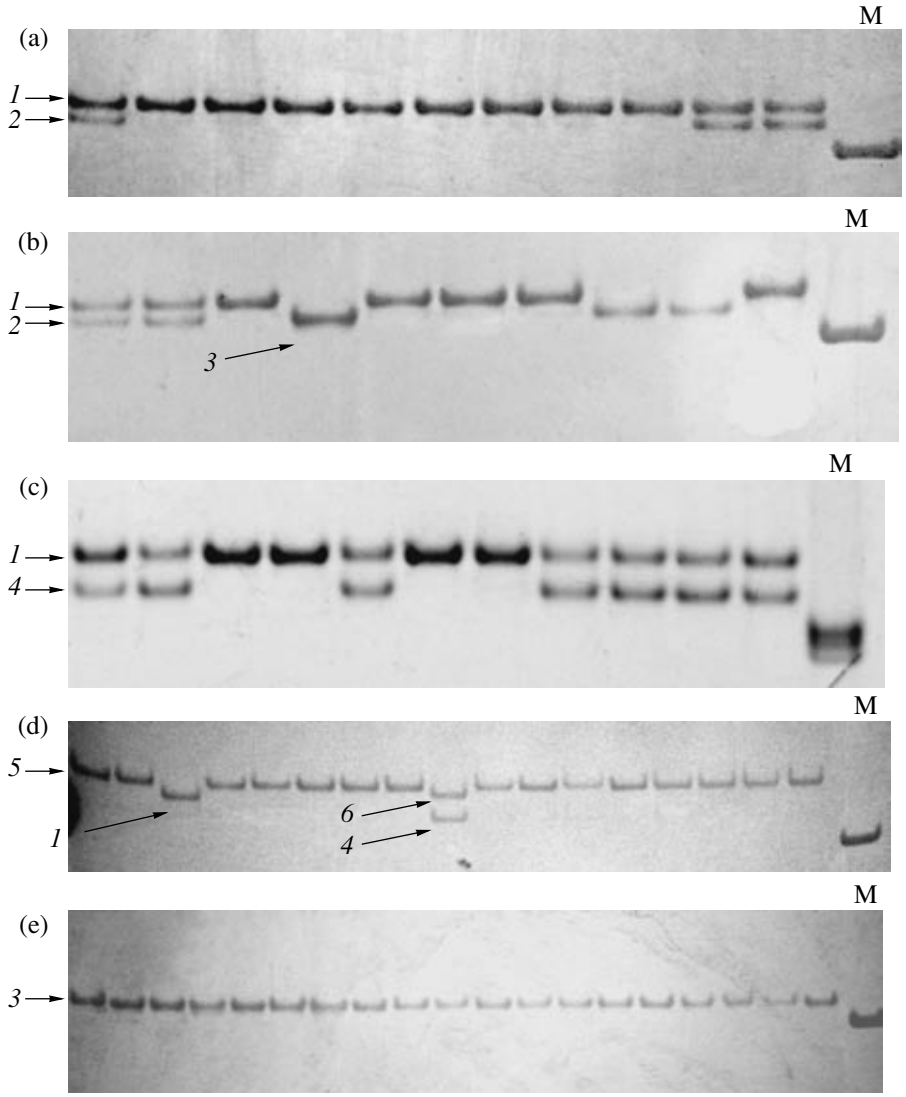

Fig. 3. Electrophoresis of the *Du281* PCR products of the *D. unisexualis* populations. (a) Lchap; (b) Kutchak; (c) Zagalu; (d) Noraduz; (e) Takyarlu. Alleles are indicated by arrows.

ants). The most polymorphic locus *Du281* has two PCR variants in the sample of six *D. valentini* individuals and two variants in the *D. nairensis* samples of five individuals (Fig. 4c). Apparently, the number of the amplified variants may be greatly underestimated because of the small sample size of the parental species. These results show that the cloned loci *Du323*, *Du215*, and *Du281* have highly conserved homologs in the parental species, which is in concordance with the hybrid origin of the parthenogenetic species *D. unisexualis.*

Thus, in the present work, intraspecific variability at three cloned microsatellite loci has been studied for the first time in parthenogenetic species *D. unisexualis* and its two bisexual parental species *D. valentini* and *D. nairensis*. A comparison of the nucleotide sequences of $(GATA)_n$ clusters in three clones studied (Table 1) indicates that the electrophoretic allelic variants are probably related to microsatellite mutations caused by DNA polymerase slippage and (or) frame shift errors in complementary interaction of single-stranded DNAs [23]. In this connection, it is of interest to determine the primary sequence of the PCR products of the cloned loci in the populations of both the parthenogenetic species and the bisexual parental species. Thus, our results may shed light on the origin of parthenogenetic species and on the phylogenetic relationships in the group of lizards from the genus *Darevskia.* The further development of this work is related to molecular genetic characterization of the *D. unisexualis* clones bearing different types of variable microsatellites in view of their future use not only for resolving the above problems but also for

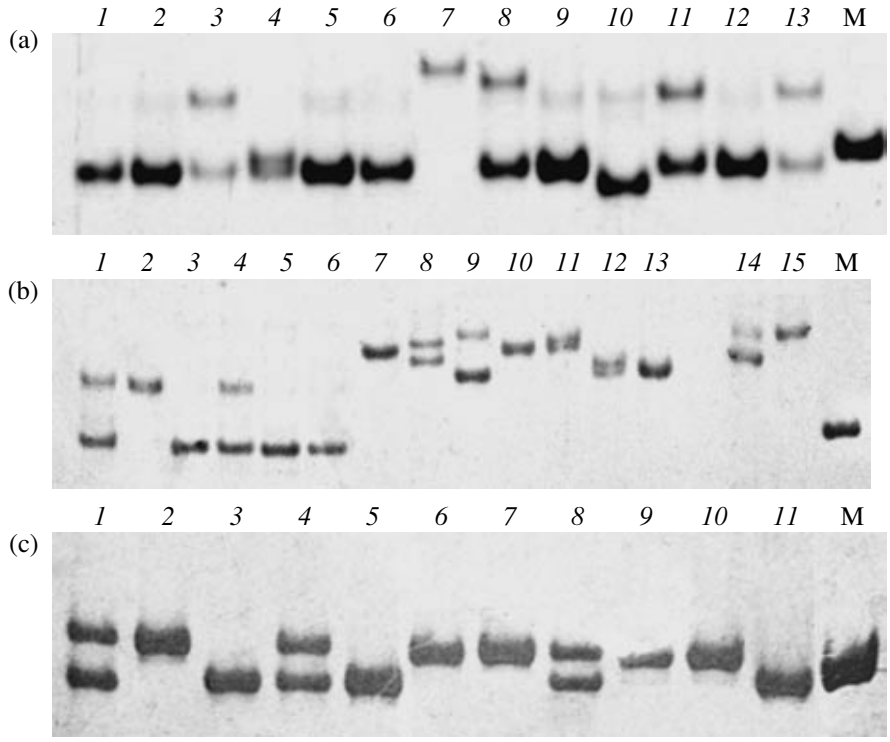

Fig. 4. PCR amplification of *Du323* (a), *Du215* (b), and *Du281* (c) in the parental species. (a) Lanes *1–13*, *D. valentini*. (b) Lanes *1–6*, *D. valentini*; *7–15*, *D. nairensis*. (c) Lanes *1–5*, *D. nairensis*; *6–11*, *D. valentini*.

complete marking the lizard genome, which is still poorly studied.

ACKNOWLEDGMENTS

This study was supported by the Russian Foundation for Basic Research (grant nos. 02-04-48516, 04-04-49436, 02-04-49548), Ministry of Industry and Science (grant no. 43.073.11.2501), and the programs "Leading Scientific Schools of Russia" (grant no. NSh-1995.2003.4), "Integration" (grant nos. GK Ch0064/885, Ya0033/1990), and "Dynamics of Gene Pools" (contract no. 10002-251/P-24/154-142/200404-112).

REFERENCES

1. Epplen, J.T., On Simple Repeated GATA/GACA Sequences in Animal Genomes: A Critical Reappraisal, *Heredity*, 1988, vol. 79, no. 6, pp. 409–417.
2. Jeffreys, A.J., Alien, M.J., Armour, J.A., *et al.*, Mutation Processes at Human Minisatellites, *Electrophoresis* (Weinheim, Fed. Repub. Ger.), 1995, vol. 16, pp. 1577–1585.
3. Ryskov, A.P., DNA Fingerprinting in Population Genetic Studies of Biodiversity, *Mol. Biol.* (Moscow), 1999, vol. 33, no. 6, pp. 880–892.
4. Bois, P., Willianson, J., Brown, J., *et al.*, A Novel Unstable Mouse VNTR Family Expanded from SINE B1 Elements, *Genomics*, 1998, vol. 49, pp. 122–128.
5. Ellegren, H., Microsatellite Mutations in the Germline: Implication for Evolutionary Inference, *Trends Genet.*, 2000, vol. 16, no. 12, pp. 551–558.
6. Xu, X., Peng, M., and Fang, Z., The Direction of Microsatellite Mutations Is Dependent upon Allele Length, *Nat. Genet.*, 2000, vol. 24, pp. 396–399.
7. Li, Y.-C., Korol, A.B., Fahima, T., *et al.*, Microsatellites: Distribution, Putative Functions and Mutational Mechanisms: A Review, *Mol. Ecol.*, 2002, vol. 11, pp. 2453–2465.
8. Darevsky, I.S., Kupriyanova, L.A., and Uzzell, T., Parthenogenesis in Reptiles, *Biology of the Reptilia*, New York: Wiley, 1985, vol. 15, pp. 412–526.
9. Tokarskaya, O.N., Martirosyan, I.A., Badaeva, T.N., *et al.*, Instability of $(GATA)_n$ Microsatellite Loci in the Parthenogenetic Caucasian Rock Lizard *Darevskia unisexualis* (Lacertidae), *Mol. Genet. Genomics*, 2004, vol. 270, pp. 509–513.
10. Kan, N.G., Petrosyan, V.G., Martirosyan, I.A., *et al.*, DNA Fingerprinting in Parthenogenetic Lizart Species *Lacerta dahli*: The Effect of Genomic Polymorphism of Mini- and Microsatellite Loci, *Mol. Biol.* (Moscow), 1998, vol. 32, no. 5, pp. 805–812.
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. Genet. Genomics*, 2001, vol. 265, pp. 812–819.
12. Martirosyan, I.A., Ryskov, A.P., Petrosyan, V.G., *et al.*, Variation of Mini- and Microsatellite DNA Markers in Population of Parthenogenetic Rock Lizard *Darevskia*

rostombekovi, Genetika (Moscow), 2002, vol. 38, no. 6, pp. 828–835.

13. Martirosyan, I.A., Kan, N.G., Petrosyan, V.G., *et al.*, Variation of Mini- and Microsatellite DNA Repeats in Parthenogenetic Lizard *Darevskia armeniaca* as Revealed by DNA Fingerprinting Analysis, *Genetika* (Moscow), 2003, vol. 39, no. 2, pp. 215–222.
14. Ryskov, A.P., Martirosyan, I.A., Badaeva, T.N., *et al.*, Hyperunstable $(TCT/TCC)_n$ Microsatellite Loci in Parthenogenetic Lizards *Darevskia unisexualis* (Lacertidae), *Genetika* (Moscow), 2003, vol. 39, no. 9, pp. 1172–1179.
15. Tokarskaya, O.N., Kan, N.G., Petrosyan, V.G., *et al.*, Variation of GATA Microsatellite DNA in Populations of Parthenogenetic Lizard Species *Lacerta unisexualis* Darevsky, *Genetika* (Moscow), 2000, vol. 36, no. 5, pp. 693–698.
16. Tokarskaya, O.N., Darevskii, I.S., Martirosyan, I.A., *et al.*, Genetic Instability of $(GATA)_n$ Microsatellite Repeats and Somatic Mosaicism in Unisexual Lizard *Darevskia unisexualis, Dokl. Akad. Nauk*, 2003, vol. 388, no. 6, pp. 825–828.
17. Korchagin, V.I., Churikov, N.A., Tokarskaya, O.N., *et al.*, Molecular Cloning and Characterization of Allelic Variants of the $(GATA)_n$ Microsatellite Locus *Du281* from the Genome of Parthenogeneitc Lizard *Darevskia unisexualis, Dokl. Akad. Nauk*, 2004, vol. 394, no. 5, pp. 693–695.
18. Estoup, A., Solignac, M., *et al.*, Characterization of $(GT)_n$ and $(CT)_n$ Microsatellites in Two Insect Species: *Apis mellifera* and *Bombus terrestris, Nucleic Acids Res.*, 1993, vol. 21, no. 6, pp. 1427–1431.
19. Gullberg, A., Tegelström, H., and Olsson, M., Microsatellites in Sand Lizard (*Lacerta agilis*): Description, Variation, Inheritance, and Applicability, *Biochem. Genet.*, 1997, vol. 35, nos. 7–8, pp. 281–295.
20. Orti, G., Pearse, D.E., and Avise, J.C., Phylogenetic Assessment of Length Variation at a Microsatellite Locus, *Proc. Natl. Acad. Sci. USA*, 1997, vol. 94, pp. 10 745–10 749.
21. Colson, I. and Goldstein, D.B., Evidence for Complex Mutation at Microsatellite Loci in *Drosophila, Genetics*, 1999, vol. 152, pp. 617–627.
22. Mahtani, M.M. and Willard, H.F., A Polymorphic X-Linked Tetranucleotide Repeat Locus Displaying a High Rate of New Mutation: Implications for Mechanisms of Mutation at Short Tandem Repeat Loci, *Hum. Mol. Genet.*, 1993, vol. 2, no. 4, pp. 431–437.
23. Schlötterer, C. and Tautz, D., Slippage Synthesis of Simple Sequence DNA, *Nucleic Acids Res.*, 1992, vol. 20, no. 2, pp. 211–215.