ISSN 1022-7954, Russian Journal of Genetics, 2009, Vol. 45, No. 2, pp. 203–210. © Pleiades Publishing, Inc., 2009
Original Russian Text © A.V. Omelchenko, V.I. Korchagin, G.A. Sevast'yanova, A.P. Ryskov, O.N. Tokarskaya, 2009, published in Genetika, 2009, Vol. 45, No. 2, pp. 230–238.

ANIMAL GENETICS

Molecular Genetic Characteristic of Dinucleotide Microsatellite Loci in Parthenogenetic Lizards *Darevskia unisexualis*

A. V. Omelchenko[a,b], V. I. Korchagin[a], G. A. Sevast'yanova[b], A. P. Ryskov[a], and O. N. Tokarskaya[a]

[a]*Institute of Gene Biology, Russian Academy of Sciences, Moscow, 119334 Russia;*
e-mail: dna@biogen.msk.su
[b]*Moscow Pedagogical State Univrsity, Moscow, 119882 Russia;*
e-mail: biochem_mpgu@mail.ru

Received April 14, 2008; in final form, July 30, 2008

Abstract—In the present study, the first molecular genetic investigation of dinucleotide $(GT)_n$ microsatellite loci in parthenogenetic lizards *Darevskia unisexualis* was performed. New polymorphic locus, *Du214*, (GenBank Ac. No. EU252542) was identified and characterized in detail. It was demonstrated that allele of this locus differed in the size and structure of microsatellite locus, as well as in point mutations, the combinations of which enabled the isolation of stabile fixed double nucleotide substitutions A–A (alleles 2 and 4) and G–T (alleles 1, 3, 5, and 6). Double nucleotide substitutions described were also identified in the orthlogous loci of the parental species genomes, *D. raddei* (G–T) and *D. valentine* (A–A). Based on the analysis of allele distribution pattern at this locus in all populations of parthenospecies *D. unisexualis*, mathematic model was elaborated and realized. Using this model, frequencies of allelic variants for all populations of the species of interest were calculated and population genetic structure of *D. unisexualis* was characterized. Genetic contribution of each population to the species gene pool was determined. The data obtained demonstrated that microsatellite variation was one of the factors of clonal and genetic diversity of a parthenospecies.

DOI: 10.1134/S1022795409020112

INTRODUCTION

Microsatellite sequences, belonging to tandemly organized moderate repetitive DNA fraction of pro- and eukaryotic genomes, deserve special interest as one of the factors of genome instability. Microsatellites vary in size of the repeat unit that costitute one to six bp, and in the cluster length, constituting 20 to 60 units. High mutation rates of microsatellites, 10^{-2}–10^{-5} [1], result in accumulation of population-specific mutations, which makes it possible to utilize the data of microsatellite loci variation for analysis of the population structure [2, 3]. Among all microsatellite sequences, special attention is focused on dinucleotide microsatellites, which are most evolutionary conserved genetic markers [4]. At present, the most thoroughly studied are microsatellites of humans [5, 6], and those of a number of animals and plants [7]. However, unisexual animal species with clonal type of reproduction still remain scarcely studied relative structural organization of their genomes and genetic variation [8, 9].

Darevskia unisexualis is one of seven parthenogenetically reproducing Caucasian rock lizard species. This species is characterized by a broken range, which is composed of a number of populations of different size, inhabiting northeastern Turkey and Armenia. The populations consist of unisexual genetically identical individuals [10]. Similarly to other members of the genus, *D. unisexualis* has a hybrid origin (the parental species are *D. valentini* and *D. raddei*), is characterized by a diploid chromosome number, high allozyme heterozygosity [11, 12], and low variability of the mitochondrial DNA restriction sites [13, 14]. In our earlier study, using locus-specific PCR analysis, in parthenospecies (*D. unisexualis*, *D. armeniaca*, and *D. dahli*) and bisexual species (*D. raddei*, *D. valentine*, and *D. mixta*) of the genus *Darevskia* intraspecific polymorphism at tetranucleotide loci, containing $(GATA)_n$ microsatellite clusters, was demonstrated [15]. The present study was focused on molecular genetic analysis of dinucleotide microsatellite loci of $(TG)_n$ type in parthenospecies *D. unisexualis* and bisexual species *D. raddei* and *D. valentine*.

MATERIALS AND METHODS

The genomic library of *D. unisexualis* used in the study was obtained earlier [16]. Using the method of colony hybridization with oligonucleotide probes, recombinant clones containing microsatellites of $(GT)_n$ type were selected and sequenced. In addition to $(TG)_n$ dinucleotides, these clones contained other types of dinucleotide motifs. Polymorphism of these loci was typed with the help of locus-specific PCR, which was

Table 1. Conditions of PCR amplification of four microsatellite loci from the parthenospecies *D. unisexualis*

Locus	Primer pair	Annealing temperature, °C
Du231 (Ac. No. EU252540)	5'TCAAGAGGCCTCCCGAAAAG 3' (F)	56
	5'TGAGCCAGCTACCGTCATTCA3' (R)	
Du365 (Ac. No. EU252543)	5'GGGGCCCATTGTGTAAATACTGTA 3' (F)	55
	5'GGATTAAGGGGTTTTCTCAGGACA3' (R)	
Du255 (Ac. No. EU252541)	5'TCGCAGAGTGGCAGGAAACAAT 3' (F)	58
	5'TGCATCCAGCTCAACCAAAATACC 3' (R)	
Du214 (Ac. No. EU252542)	5'TCACTTAAGGTTGACGCTGACTCA 3' (F)	50
	5'CTGAACAAGTTGTCCACCTCTGC 3' (R)	

performed using population DNA samples of *D. unisexualis* and bisexual species, *D. raddei* and *D. valentini*. Blood samples were obtained from *D. unisexualis* females collected in five natural populations from Central Armenia (Takyarlu, Kutchak) and the coast of the Lake Sevan (Lchap, Noratus, and Zagalu) ($N = 65$), as well as in four populations of *D. raddei* ($N = 24$) and three populations of *D. valentini* ($N = 18$) from Central and Northern Armenia. The samples were stored in 0.05 M EDTA (pH 8.0) and kept at +4°C. DNA was extracted using standard phenol–chloroform method with proteinase K treatment [17]. Single-locus PCR was carried out using oligonucleotide primers listed in Table 1. Amplification was performed in 20 μl of the reaction mixture (50 ng DNA) using the GenePak^R PCR Core (Isogene, Russia) PCR kit. The reaction was run in the Tertsik four–channel DNA thermal cycler (TPCh–PTsR-01, DNK-Tekhnologia, Russia). The reaction conditions included denaturing for 3 min at 94°C, followed by 30 cycles of amplification (94°C for 1 min; primer annealing for 40 s; 72°C for 40 s) with the 5-min final extension at 72°C. Allelic variants of the genomic loci of *D. unisexualis* were separated by means of denaturing gel electrophoresis in 6% PAAG (40% PAA (acrylamide : bisacrylamide, 19 : 1); 50% urea; and 37% formamide) at 60°C for 4 h. Gels were stained according to the standard protocol of Silver Sequence DNA (Promega). Sequencing of the amplification products was performed according to the method of Sanger with the ABI PRISM BigDye Terminator v. 3.1 reagent kit and subsequent analysis of the reaction products on the automated sequencer DNA ABI PRISM 3100-Avant. Nucleotide sequences were aligned using the MegAlign 4.05 software program.

Table 2. Molecular characteristics of the $(GT)_n$ loci

Locus	Microsatellite cluster	Size of the PCR product, bp
Du231 (Ac. No. EU252540)	$(GT)_{14}$; $(CT)_{10}$	160
Du365 (Ac. No. EU252543)	GTGGA$(GT)_{22}$	254
Du255 (Ac. No. EU252541)	$(GT)_{22}(AT)_{12}$	212
Du214 (Ac. No. EU252542)	$(GT)_{22}$	223

RESULTS

Using PCR amplification, in five population samples of *D. unisexualis* (65 individuals) four dinucleotide loci were examined. The structures of microsatellite clusters form the clones containing $(GT)_n$ microsatellites are presented in Table 2. It was demonstrated that three of the four loci examined, *Du231*, *Du365*, and *Du255*, were electrophoretically monomorphic. One locus (*Du214*, Ac. No. EU252542) was polymorphic and represented by six alleleic variants differing in electrophoretic mobility. Frequencies of the *Du214* alleles are presented in Table 3. In particular, equal numbers of alleles 2 and 3 were found in the populations of Lchap, Zagalu, and Noratus. In population of Takyarlu three alleles were found, 1, 2, and 3. Population of Kutchak was considered as most variable, since its members were the carriers of five allelic variants.

The PCR products of each allelic variant were cloned and sequenced. The sequences of the *Du214* allelic variants are shown in Fig. 1. Alleles of the *Du214* locus demonstrated the differences in the length of microsatellite cluster resulted from the different numbers of (GT) repeats. The range of the differences constituted from 22 to 29 monomers. In addition, from Fig. 1 it followed that in *D. unisexualis* alleles of the *Du214* locus differed in point mutations at the beginning of microsatellite cluster. Combinations of these fixed point mutations formed double nucleotide substitutions, G–T (alleles 1, 3, 5, and 6) and A–A (alleles 2 and 4), which were specific markers for each of the alleles, thought to be inherited from different bisexual parental species. To test this proposal, PCR amplification of the DNA samples from bisexual species *D. raddei* and *D. valentini* was performed (Fig. 2). Figure 2 presents the data on electrophoretic fractioning of the PCR products of the orthologous loci of parental species. From the figure it follows that bisexual species were heterozygous and heterogeneous for the *Du214*

Table 3. Quantitative estimate of the *Du214* variability in parthenospecies *D. unisexualis*

Population	Allelic variant						Total number of individuals
	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
Noratus	0	17	17	0	0	0	17
Takyarlu	12	17	5	0	0	0	17
Over all populations	12	63	50	2	2	1	65

locus. Sequences of variable regions of this locus in parental species are demonstrated in Fig. 3. It can be seen that allelic variants were different not only in the lengths of microsatellite $(GT)_n$ cluster, but also in specific single nucleotide substitutions, forming the desired fixed stabile double nucleotide substitutions. Furthermore, substitution G–T was stably present within the sequences of this locus in *D. raddei*, while substitution A–A was detected in the sequences of allelic variants of this locus in *D. valentini*.

To treat the data demonstrated in Table 3, mathematical model was elaborated based on the application of index analysis [18, 19]. The input data of this model were the elements of the matrix, where each of the elements quantitatively characterized the presence of an allelic variant in a population. This model was designed for qualitative and quantitative evaluation of different allele frequencies in the species gene pool, as well as for identification of the population genetic structure of the species examined based on qualitative estimate of the genetic contribution of each population in the species gene pool.

The mathematic model was based on the following assumptions (limitations) characterizing the physical (biologic) nature of the application object of the model:

(1) the contribution to the species gene pool depend on the genetic diversity and the sizes of the populations forming the given species;

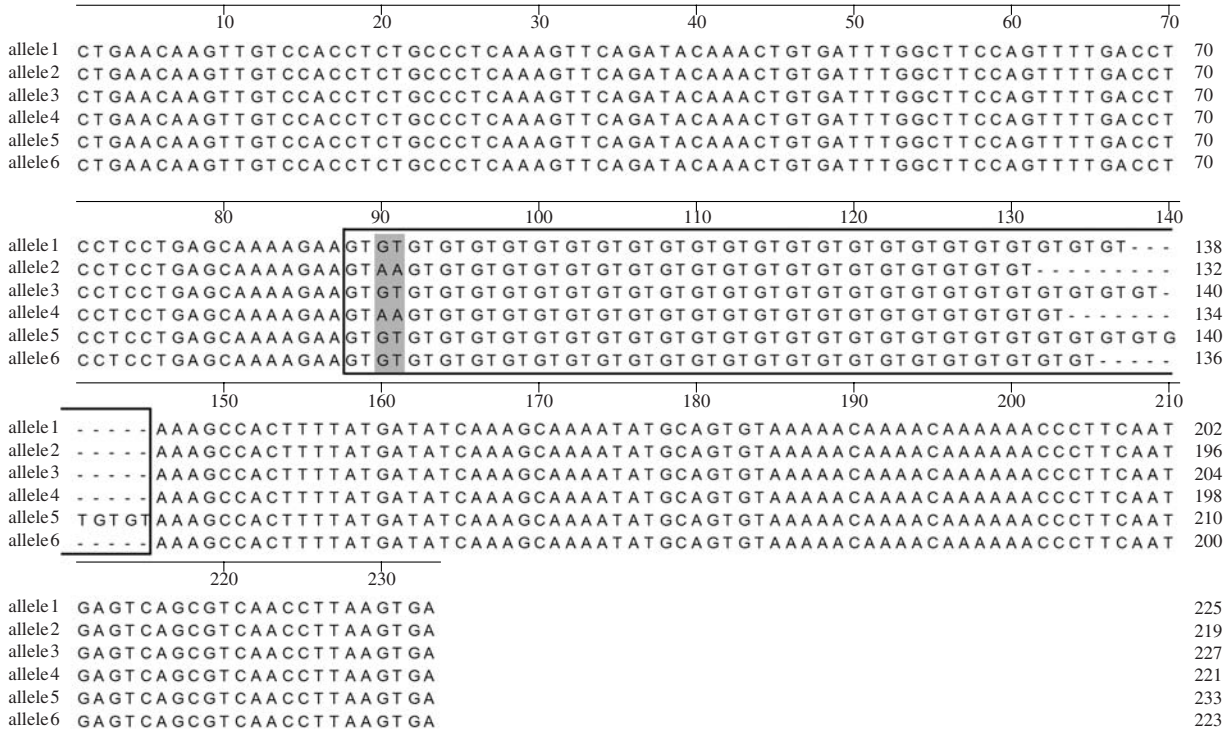

Fig. 1. Sequences of the *Du214* alleles in parthenospecies *D. unisexualis*. The $(GT)_n$ clusters are isolated by a frame. Fixed nucleotide variations are designated by the gray box.

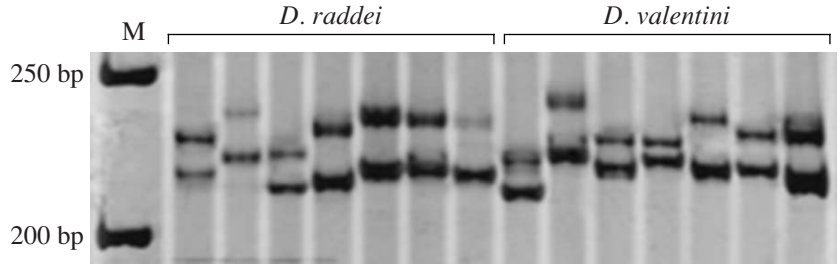

Fig. 2. Electrophresis in native 8% PAAG of the sample of PCR products corresponding to the loci of *D. raddei* and *D. valentini* investigated. The 50-bp Ladder (Fermentas, Lithuania) with the step of 50 bp was used as molecular size marker.

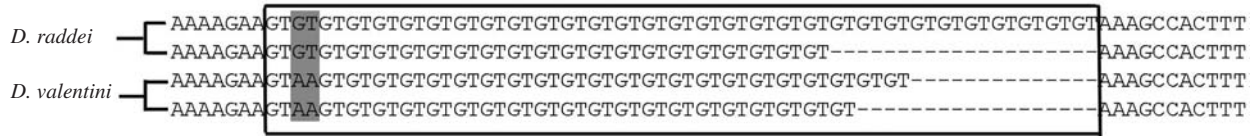

Fig. 3. Sequences of microsatellite clusters in orthologous loci of parental species. The $(GT)_n$ clusters are isolated by a frame. Nucleotide variations are designated by the gray box.

(2) the measures of the population genetic diversity are the number and the frequencies of the allelic variants of each of the loci examined in the genomes of the individuals from this population;

(3) mutations in the locus occur independently from the mutations in the other loci;

(4) mutations in the allelic variants of the locus occur independently from the mutations in the other allelic variants of the same locus; and

(5) the allele frequency ratios and population sizes are thought to correspond to natural values.

The mathematical apparatus suggested operated with the following formulas:

$$f_{ij} = \frac{m_{ij}b_{ij}}{\sum_{i=1}^{k} m_{ij}b_{ij}}; \quad F_i = \frac{\sum_{j=1}^{l} m_{ij}b_{ij}}{\sum_{i=1}^{k}\sum_{j=1}^{l} m_{ij}b_{ij}};$$

$$F_{ij} = \frac{m_{ij}b_j}{\sum_{i=1}^{k}\sum_{j=1}^{l} m_{ij}b_{ij}}; \quad s_{ij}^{i} = \frac{m_{ij}b_{ij}f_{ij}}{\sum_{j=1}^{l} m_{ij}b_{ij}f_{ij}};$$

$$S_j = \sum_{i=1}^{k} s_{ij}^{i}w_i; \quad S_j^F = \left(\sum_{i=1}^{k} F_{ij}\right),$$

where:

m_{ij}, the number of individuals in population j, containing allele i;

f_{ij}, the frequency of alleleic variant i in population j;

F_i, the frequency of allelic variant i in the species;

F_{ij}, the frequency of allelic variant i from the population j in the DNA of the whole species;

s_{ij}^{i}, the proportion of allele i from the population j in the DNA of the whole species with respect to allele i (genetic contribution of the carriers of allele ii from population j in the species gene pool with respect to allele i);

S_j, the summarized weighted share of all alleles from population j in the DNA of the whole species (genetic contribution of population j to the species gene pool);

S_j^F, the sum of the frequencies of all alleles from population j in the DNA of the whole species;

k, the number of allelic variants;

l, the number of the populations in the species;

w_i, the weighting coefficient for each allelic variant i, numerically equal to the relative frequency of allelic variant i in the form of: $w_i = F_i$;

b_j, the normalization (relative to the size) factor for the population j, determined by the following expression:

$$b_j = \frac{n^{\max}}{n_j},$$

where $n^{\max}$ is the number of individuals in of the population of the largest size;

$n^{\min}$, the number of individuals in the smallest population; n_j, the number of individuals in population j.

$$i, j, n, k, l \in Z > 0; \quad i = 1, 2, 3, \ldots, k;$$
$$j = 1, 2, 3, \ldots, l; \quad l = \text{const}.$$

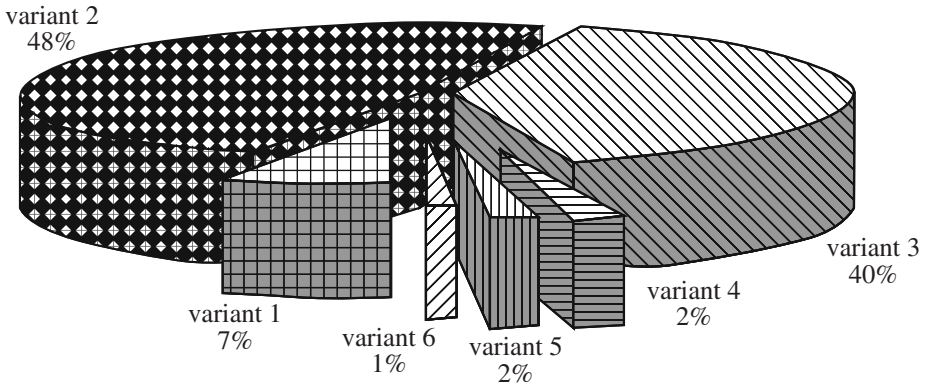

Fig. 4. Frequencies of the *Du214* alleles in the parthenospecies *D. unisexualis*. Sectors of the diagram corresponding to quantitative contribution of each of the allelic variants are designated by different shading.

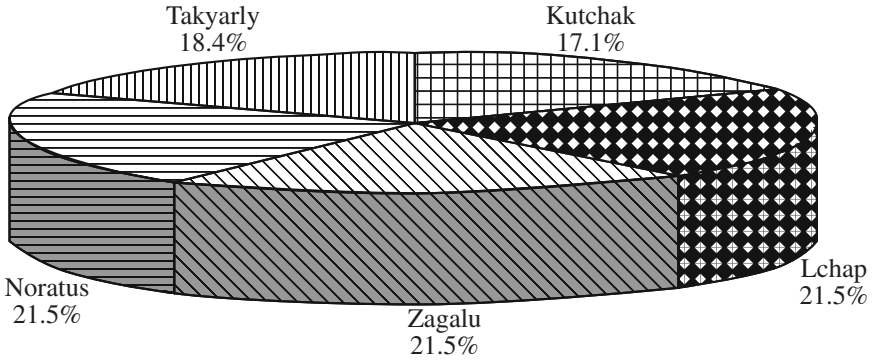

Fig. 5. Genetic population structure of the parthenospecies *D. unisexualis* based on the analysis of the *Du214* marker allele frequencies. Sectors of the diagram corresponding to quantitative contribution of each of the allelic variants are designated by different shading.

Taking into consideration the expression for normalization factor b_j, some formulas of this mathematic model take the following form:

$$f_{ij} = \frac{m_{ij}}{\sum_{i=1}^{k} m_{ij}}; \quad F_i = \frac{\sum_{j=1}^{l} \frac{m_{ij}}{n_j}}{\sum_{i=1}^{k}\sum_{j=1}^{l} \frac{m_{ij}}{n_j}};$$

$$F_{ij} = \frac{m_{ij}}{n_j \sum_{i=1}^{k}\sum_{j=1}^{l} \frac{m_{ij}}{n_j}}; \quad s_{ij}^{i} = \frac{m_{ij}^2}{n_j \sum_{i=1}^{k} m_{ij} \sum_{j=1}^{l} \frac{m_{ij}^2}{n_j \sum_{i=1}^{k} m_{ij}}}.$$

It should be noted that the condition for applying the mathematic model is the absence of twofold numerical superiority of one population over another:

$$\frac{n^{\max}}{n^{\min}} < 2.$$

Application of this mathematic model made it possible to determine the frequencies of all allelic variants in the gene pool of parthenospecies *D. unisexualis* (Fig. 4). The species genetic population structure demonstrating total genetic contribution of each population and considering contribution of a population over each allelic variant is shown in Fig. 5. From the diagram it can be seen that the largest contribution to the species gene pool is made by the populations of Lchap, Zagalu, and Noratus, each of which forms by 21.5% of the gene pool over the locus of interest. These populations are followed by the populations from Takyarlu and Kutchak (18.4 and 7.1%, respectively). In addition, evaluation of the frequencies of different *Du214* alleles in each of the populations of *D. unisexualis* showed

that for a single population, the sum of different allele frequencies in the gene pool of *D. unisexualis* was constant and equal to:

$$S^F_{\text{Kutchak}} = S^F_{\text{Lchap}} = S^F_{\text{Zagalu}} = S^F_{\text{Noratus}} = S^F_{\text{Takyarly}} = 0.2.$$

DISCUSSION

Investigations of individual microsatellite loci showed that the changes occurring in these loci were greatly variable depending on the species, repeat types, alleles, age, and gender [20]. Parthenogenetic reptiles, the populations of which consist of genetically identical individuals originating from a single ancestor, represent a unique natural model for investigation of the molecular mechanisms of variation and evolution of hypervariable DNA repeats, including microsatellites [21].

To explain the microsatellite loci variation, a number of models were put forward [22–25]. Most of these models confine to the so-called stepwise mutation model (SMM), according to which changes in the lengths of microsatellite clusters occur sequentially due to either gain or contraction of one repeat unit. The most common mutational mechanism affecting microsatellites is replication slippage. Tandem repeats provide base mismatch, and as a consequence, formation of the loops. In most of the cases these mutations are successfully corrected by the repair mechanisms. However, in isolated cases, microsatellite DNA length change happens. The differences in the lengths of microsatellite clusters observed in *Du214* allelic variants constituted from one to seven repeat units, which was consistent with the stepwise (SMM) model.

Comparative analysis of five $(GT)_n$ loci has revealed a correlation between the locus structure and the level of its polymorphism. It is known that microsatellite repeats with complex structures, i.e., containing different types of microsatellites, or imperfect monomeric units in the cluster, more often appear to be monomorphic. At the same time, the loci containing extended perfect microsatellite repeats demonstrated rather high level of polymorphism. In other words, imperfect repeats are more stable, compared to perfect repeats. Furthermore, extended microsatellite clusters demonstrated the tendency towards the contraction, while short repeats were prone to cluster extension [26]. Based on this finding, it can be suggested that deletions in the *Du214* locus can be a preferable process compared to insertions.

In addition, the data on polarity of mutations in microsatellite DNA [27], as well as on the increased frequency of single nucleotide substitutions in the microsatellite DNA flanking regions [28], were reported. Furthermore, the mutation frequency in these regions can be higher than that in microsatellite cluster, as it was demonstrated for dinucleotide microsatellites of invertebrates [29].

In our earlier studies, the structure of allelic variants was described for tetranucleotide microsatellite loci *Du215*, *Du323*, and *Du281* [15] in *D. unisexualis*, and in the populations of other parthenogenetic species, *D. armeniaca* for *Du215* [30]; and *D. dahli*, for *Du323* [31]. The differences between the alleles of these loci observed were expressed as the microsatellite cluster length variations by one or more repeat units (which was consistent with the single-step classical model), as well as in single point mutations (haplotypes) in the microsatellite-adjacent regions. For the *Du215* and *Du281*, as well as for *Du323* it was demonstrated that haplotypes originated from bisexual ancestral forms [15]. In this study, stabile fixed double nucleotide substitutions, G–T (alleles 1, 3, 5, and 6), and A–A (alleles 2 and 4) were detected. These mutations in dinucleotide locus *Du214* originated from the maternal species, *D. raddei*, and from the paternal species, *D. valentini*. However, contrary to tetranucleotide loci, point mutations in *Du214* were located in the microsatellite cluster itself.

To evaluate allelic diversity, a set of equations, comprising a single mathematic model, was suggested. Application of the given mathematic model produced the data characterizing the contribution of each population over each allelic variant to the species gene pool. Note that in the course of these procedures the *Du214* allele frequency distribution patterns in each population were taken into account, and total genetic contribution of each population to the species gene pool was calculated. In addition to quantitative estimate of the population contribution to the species gene pool, these data provide the conclusion that the sum of different allelic variant frequencies in a certain population of fixed heterozygous parthenospecies is constant and equal between the populations of this parthenospecies, irrespectively of the ratio between the allele frequencies at a given locus in certain population, as well as in the species as a whole:

$$S^F_1 = S^F_2 = \ldots = S^F_j = \text{const} | \forall j \in Z > 0, \quad j = 1, 2, 3, \ldots, l.$$

Let us prove this statement in general form for any fixed-heterozygous parthenospecies using the mathematic model suggested. It should be noted that in this case fixed heterozygosity means that all the species members can be haploid, diploid, or tetraploid, etc., for the locus of interest, i.e.:

$$c = \text{const} | \, c \in Z > 0,$$

where c is the individual ploidy.

From the condition of fixed heterozygosity it follows that

$$\sum_{i=1}^{k} m_{ij} = cn_j.$$

From here

$$\frac{1}{n_j}\sum_{i=1}^{k} m_{ij} = c; \quad \sum_{i=1}^{k}\frac{m_{ij}}{n_j} = c = \text{const}.$$

Based on commutativity of addition

$$\sum_{i=1}^{k}\sum_{j=1}^{l}\frac{m_{ij}}{n_j} = \sum_{j=1}^{l}\sum_{i=1}^{k}\frac{m_{ij}}{n_j}.$$

Using the expression $\sum_{i=1}^{k}\frac{m_{ij}}{n_j} = c = \text{const}$, we obtain

$$\sum_{j=1}^{l}\sum_{i=1}^{k}\frac{m_{ij}}{n_j} = \sum_{j=1}^{l} c = cl.$$

From here it follows that expression $S_j^F = \sum_{i=1}^{k}\frac{m_{ij}}{n_j\sum_{i=1}^{k}\sum_{j=1}^{l}\frac{m_{ij}}{n_j}}$ takes the form of

$$S_j^F = \sum_{i=1}^{k}\frac{m_{ij}}{n_j cl} = \frac{1}{cl}\sum_{i=1}^{k}\frac{m_{ij}}{n_j}.$$

Using the expression $\sum_{i=1}^{k}\frac{m_{ij}}{n_j} = c = \text{const}$, we obtain

$$S_j^F = \frac{1}{cl}\sum_{i=1}^{k}\frac{m_{ij}}{n_j} = \frac{1}{cl}c = \frac{1}{l}.$$

Since $l = \text{const} \longrightarrow \frac{1}{l} = \text{const}$, then

$$S_j^F = \text{const} \mid \forall j \in Z > 0, j = 1, 2, 3, \ldots, l.$$

The statement is proved.

It should be noted that this statement is not true when the condition of fixed heterozygosity for any of the loci is not followed.

Constant sums of different allele frequencies in the populations of parthenospecies allow the suggestion on the existence of the genetic population mechanism which, in terms of the allele frequencies, brings the gene pool of a parthenospecies to equilibrium. A departure from this equilibrium state occurs if the condition of fixed heterozygosity of the individuals comprising the parthenospecies breaks down as a result of either mutations, or interspecific and intraspecific hybridization, which is rather often in the lizards of the genus *Darevskia* [32].

Thus, the data obtained demonstrated that microsatellite variation was one of the factors of clonal and genetic diversity of a parthenospecies. The mathematic model, designed based on quantitative estimates of the ratio between the *DU214* polymorphic allele frequencies, provided determination of the population genetic contribution to the species gene pool. This model can serve as a tool for interpretation of molecular genetic data in population genetic and evolutionary investigations.

ACKNOWLEDGMENTS

We thank V.G. Petrosyan for many helpful comments.

This work was supported by the Russian Foundation for Basic Research (grant no. 08-04-0668), the Program of the President of the Russian Federation (grant no. MK-4748.2007.4), a Subprogram of the Presidium of the Russian Academy of Sciences “Dynamics of Gene Pools”, and the Program “Leading Scientific Schools of Russia.”

REFERENCES

1. Ellegren, H., Microsatellites: Simple Sequences with Complex Evolution, *Nat. Rev. Genet.*, 2004, vol. 5, pp. 435–445.
2. Rosenberg, N.A., Pritchard, J.K., and Weber, J.L., Genetic Structure of Human Populations, *Science,* 2002, vol. 298, pp. 2381–2385.
3. Jorde, L.B., Rogers, A.R., and Bamshad, M., Microsatellite Diversity and the Demographic History of Modern Humans, *Proc. Natl. Acad. Sci. USA*, 1997, vol. 94, pp. 3100–3103.
4. Neff, B.D. and Gross, M.R., Microsatellite Evolution in Vertebrates: Inference from AC Dinucleotide Repeats, *Evolution*, 2001, vol. 55, no. 9, pp. 1717–1733.
5. Zhivotovsky, L.A., Microsatellite Variation in Human Populations and Methods of Its Study, *Vestn. VOGiS*, 2006, vol. 10, no. 1, pp. 74–96.
6. Fan, H. and Chu, J.-Y., A Brief Review of Short Tandem Repeat Mutation, *Geno. Prot. Bioinfo.*, 2007, vol. 5, no. 1, pp. 7–14.
7. Sharma, P.C., Grover, A., and Kahl, G., Mining Microsatellites in Eukaryotic Genomes, *Trends Biotechnol.*, 2007, vol. 25, no. 11, pp. 490–498.
8. Ryskov, A.P., Multilocus DNA Fingerprinting in Genetic Population Studies of Biodiversity, *Mol. Biol.*, (Moscow), 1999, vol. 33, no. 6, pp. 997–1011.
9. Tokarskaya, O.N., Kan, N.G., and Petrosyan, V.G., 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.
10. Darevsky, I.S., Kupriyanova, L.A., and Uzzel, T., Parthenogenesis in Reptieles, *Biol. Reptilia*, 1985, vol. 15, pp. 412–526.
11. Fu, J., MacCulloch, R.D., and Murphy, R.W., The Parthenogenetic Rock Lizard *Lacerta unisexualis*: An Example of Limited Genetic Polymorphism, *J. Mol. Evol.*, 1998, vol. 46, pp. 127–130.
12. Fu, J., MacCulloch, R.D., and Murphy, R.W., Allozyme Variation Patterns and Multiple Hybridization Origins: Clonal Variation among Four Sibling Parthenogenetic

Caucasian Rock Lizards, *Genetics,* 2000, vol. 108, pp. 107–112.

13. Moritz, C., Donnelan, S., Adams, M., and Baverstock, P.R., The Origin and Evolution of Parthenogenesis in *Heteronotia binoei* (Gekkonidae): Extensive Genotypic Diversity among Parthenogens, *Evolution*, 1989, vol. 43, pp. 994–1003.
14. Fu, J., Murphy, R.W., and Darevsky, I.S., Divergence of the Cytochrome b Gene in the *Lacerta raddei* Complex and Its Parthenogenetic Daughter Species: Evidence for Recent Multiple Origins, *Copeia*, 2000, vol. 2, pp. 432–440.
15. Korchagin, V.I., Badaeva, T.N., and Tokarskaya, O.N., Molecular Characterization of Allelic Variants of $(GATA)_n$ Microsatellite Loci in Parthenogenetic Lizards *Darevskia unisexualis* (Lacertidae), *Gene*, 2007, vol. 392, pp. 126–133.
16. Korchagin, V.I., Churikov, N.A., Tokarskaya, O.N., et al., Molecular Cloning and Characteristic of Allelic Variants of $(GATA)_n$ Microsatellite Locus *Du281* in Parthenogenetic Lizard *Darevskia unisexualis* Genome, *Dokl. Akad. Nauk*, 2004, vol. 394, no. 5, pp. 693–695.
17. Mathew, C.G.P, The Isolation of High Molecular Weight DNA, *Methods in Molecular Biology*, Walker, J.M., Ed., New York: Humana, 1984, vol. 2, pp. 31–34.
18. Eliseeva, I.I. and Yuzbashev, M.M., *Obshchaya teoriya statistiki* (General Theory of Statistics), Moscow: Finansy i Statistika, 2008.
19. Baklanov, G.I., *Nekotorye voprosy indeksnogo metoda* (Some Issues of the Index Method), Moscow: Statistika, 1972.
20. Ellegren, H., Microsatellite Mutations in the Germline: Implication for Evolutionary Inference, *Trends Genet.*, 2000, vol. 16, no. 12, pp. 551–558.
21. Tokarskaya, O.N., Darevsky, I.S., and Martirosyan, I.A., Genetic Instability of $(GATA)_n$ Microsatellite DNA Repeats and Somatic Mosaicism among Unisexual Lizards *Darevskia unisexualis*, *Dokl. Akad. Nauk*, 2003, vol. 388, no. 6, pp. 825–828.
22. Schlutterer, C. and Tautz, D., Slippage Syntesis of Simple Sequences DNA, *Nucleic Acids Res.*, 1992, vol. 20, pp. 211–215.
23. Weber, J.L. and Wong, C., Mutation of Human Short Tandem Repeats, *Hum. Mol. Genet.*, 1993, vol. 2, pp. 1123–1128.
24. Di Rienzo, A., Peterson, A.C., and Garza, J.C., Mutational Processes of Simple-Sequence Repeat Loci in Human Populations, *Proc. Natl. Acad. Sci. USA*, 1994, vol. 91, pp. 3166–3170.
25. Kruglyak, S., Durrett, R.T., Schug, M.D., and Aquadro, C.F., Equilibrium Distributions of Microsatellite Repeat Length Resulting from a Balance between Slippage Events and Point Mutations, *Proc. Natl. Acad. Sci. USA*, 1998, vol. 95, pp. 10774–10778.
26. Xu, X., Peng, M., Fang, Z., and Xu, X.P., The Direction of Microsatellite Mutations Is Dependent upon Allele Length, *Nat. Genet.*, 2000, vol. 24, pp. 396–399.
27. Sturzeneker, R., Haddad, L.A., and Bevilacqua, A.U., Polarity of Mutations in Tumor-Associated Microsatellite Instability, *Hum. Genet.*, 1998, vol. 102, pp. 231–235.
28. Colson, I. and Goldstein, D.B., Evidence for Complex Mutations at Microsatellite Loci in *Drosophila, Genetics*, 1999, vol. 152, pp. 617–627.
29. Orti, G., Pearse, D.E., and Avise, J., Phylogenetic Assessment of Length Variation at a Microsatellite Locus, *Proc. Natl. Acad. Sci. USA*, 1997, vol. 94, pp. 10745–10749.
30. Malysheva, D.N., Vergun, A.A., Tokarskaya, O.N., et al., Nucleotide Sequences of the Microsatellite Locus *Du215 (arm)* Allelic Variants in Parthenogenetic Species *Darevskia armeniaca* (Lacertidae), *Russ. J. Genet.,* 2007, vol. 43, no. 2, pp. 170–175.
31. Vergun, A.A., Markelova, E.S., and Martirosyan, I.A., Molecular Genetic Characteristic of the *Du323* Locus Containing Different Types of Microsatellite in Parthenogenetic Lizards *Darevskia dahli* (Lacertidae), *Dokl. Akad. Nauk*, 2007, vol. 416, no. 5, pp. 690–692.
32. Darevsky, I.S., Evolution and Ecology of Parthenogenetic Reproduction in Reptiles, in *Sovremennye problemy teorii evolyutsii* (Current Problems of the Theory of Evolution), Moscow: Nauka, 1993, pp. 89–109.