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Reticulate Evolution of Parthenospecies of the Lacertidae Rock Lizards: Inheritance of CLsat Tandem Repeats and Anonymous RAPD Markers

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Abstract—The genetic relatedness of several bisexual and of four unisexual "*Lacerta saxicola* complex" lizards was studied, using monomer sequences of the complex-specific CLsat tandem repeats and anonymous RAPD markers. Genomes of parthenospecies were shown to include different satellite monomers. The structure of each such monomer is specific for a certain pair of bisexual species. This fact might be interpreted in favor of co-dominant inheritance of these markers in bisexual species hybridogenesis. This idea is supported by the results obtained with RAPD markers; i.e., unisexual species genomes include only the loci characteristic of certain bisexual species. At the same time, in neither case parthenospecies possess specific, autoapomorphic loci that were not present in this or that bisexual species.

Key words: parthenogenesis, lizard parthenospecies, reticulate evolution, tandem repeats, repeat monomer sequences, RAPD markers

INTRODUCTION

Among the authors of the present paper only V.V.G. was happy to be influenced by the centripetal force generated by the late Roman B. Khesin. In fact, owing to his deep involvement into problems of science, encyclopedic education, bright mind and sincere interest in the high-standard results obtained by his colleagues, Khesin naturally and with silent support of the scientific community became one of the most favorite leaders at the initial period of development of molecular biology in Russia. The Khesin phenomenon was due neither to his high position in the scientific hierarchy or authoritativeness, nor to his ability to impose his personal view of problems and approaches, but to sincere and profound respect for Khesin personally, for the excellent experiments performed in his laboratory, for his just estimates of the results obtained by other scientists, for his clear understanding of future development of science and for his denial of compromise and fearlessness in estimates. Khesin's bright mind and encyclopedicity was not suppressive, did not excite envy. In the scientific flock, he stood apart from self-interested persons. Khesin was an intellectually generous person. This was apparent for everybody who worked with him, attended his lectures, watched him during his actual directorship of "Winter Schools of Molecular Biology," or who read his outstanding, comprehensive books. For his colleagues, Khesin was a tuning fork of scientific estimates.

Turning again and again to his book "Genome Inconstancy" (1984) one cannot but state that its importance does not decrease, but even increases because of its comprehensiveness and educative value. It demonstrates how one must analyze facts, and even now the analytic manner of Khesin serves for the analysis of fresh facts obtained by science. His scientific foresights help us interpret the results of the present-day studies.

The ideology of his book is substantially broader than it follows from the title. The author repeatedly, pithily and wisely draws readers to conclusion that the very fact of lateral transfer of genome fragments from one species to another is of such principal importance that it will inevitably influence our understanding of biodiversity and evolution. Since the time of publication of this book lateral, transfer has been demonstrated not only among microorganisms, plants and protists, but even among higher vertebrates. Transfer of genetic material into a foreign organism with the aid of plasmids, retroviruses, symbionts and parasites, as well as DNA molecules is an established fact which surprised everybody who got familiar with the scientific grounds and its role for biology from Khesin's

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book. This phenomenon could have played a role in speciation and might have been the second mechanism (in addition to the cladistic one) of evolution of living beings. This second mechanism is abundant among plants and, as recently demonstrated, in the animal kingdom: we mean the mechanisms of interspecies and intergeneric hybridization. The latter mechanisms might give rise to new species not via dichotomy, but, in essence, by lateral transfer and exchange of genetic material between two species. This was called "reticulate evolution" and nowadays attracts widespread attention [1]. The role of this mechanism was discussed by one of us in [2].

In this paper, we discuss the results of our study of parthenogenesis, a peculiar form of "hybrid dysgenesis" observed among vertebrates, reptiles in particular. Accumulating evidence testifies that parthenogenesis is a result of interspecies hybridization, but up to now this fact has not been demonstrated experimentally at the genome level. We study one of the most interesting and peculiar objects, lizards of the "*Lacerta saxicola* complex" from the Caucasus. Undoubtedly, direct comparisons with analogous processes observed among *Drosophila* species and described in Khesin's book are impossible, because molecular mechanisms of transformation of a haploid ovum of agamous species into a diploid are still obscure.

Hence, lateral transfer of genes as well as interspecies hybridization could be considered as options of reticulate evolution. This phenomenon is widespread and applies to the origin of not only parthenogenic but new bisexual species as well. For example, numerous hybridogenesis events were demonstrated at the boundaries of species-occupied areas or in the case of sympatry [1]. Species of hybrid origin were described among fishes and amphibians [1–3]. Underestimation of the role of such events might cause false reconstruction of taxa phylogeny, which, as a rule, is based on the assumption of divergence of species. It might also influence development of phylogenetically based systematics. For example, parthenospecies were included into the general system of lacertid lizards before their agamic nature was revealed. From the phylogenetic point of view, it was an erroneous decision. Hence, study of processes taking place during hybridogenesis at different levels and with various genome DNA regions appears to be quite important.

The hypothesis of the origin of unisexual populations of rock lizards via hybridization between some bisexual species, which might cause ovogenetic distortions, was initially based on morphological studies and allozyme analysis [4, 6]. Further, these preliminary data obtained for a limited number of species and in the studies of a few enzymes found additional support in the results of taxoprint analysis of DNA repeats [7]. Then, we investigated two lizard parthenospecies by using RAPD markers [8]. The results obtained testify that genomes of these species contain only markers which are present in certain bisexual species. Possible "paternal" and "maternal" species were selected among them. However, high genetic similarity revealed by this method among some "clue" species of the complex, presumable "parental" candidates, did not allow coming to definite conclusions.

We continued our search for more specific markers of genetic relatedness of parthenogenic species. In this study we employed monomers of CLsat (Caucasian Lizard satellite) tandem repeats as genome markers discovered during our previous studies of *Lacerta saxicola* (syn. *Darevskia*) genome [9, 10]. Four out of seven known parthenospecies of the complex were investigated.

EXPERIMENTAL

DNA isolation. DNA was isolated from blood of nembutal-treated animals, transferred into 0.05 M EDTA, pH 7.4, and kept frozen at –55°C. Erythrocyte lysate was centrifuged and DNA isolated from nuclear pellet using proteinase K and phenol–chloroform mixture [11]. DNA concentration was determined by comparing the intensity of electrophoretic bands obtained in 1% agarose gels at different dilutions with the intensity of bands of standard DNA (e.g., phage λ) of predetermined concentration, or of marker fragments (Fermentas, Lithuania).

Cloning and sequencing. Genome DNA was hydrolyzed with restriction endonucleases *Hin*dIII, *Cla*I, and *Taq*I (Fermentas, Lithuania). DNA fragments ca. 150 bp long were isolated by 2% agarose gel electrophoresis and transferred onto nitrocellulose filters (NA-45, Schleicher and Schuel). Fragments were inserted into pGEM vectors (Promega) and cloned using *Escherichia coli* XL-1Blue. Positive clones were selected by the blue-white β-galactosidase test. Plasmids were isolated as described in [12]. Clones were sequenced by the Sanger method employing γ-^{32}P-labeled pUC/M13 universal primer by the procedure suggested by Promega.

PCR-RAPD was carried out as in [13, 14] with modifications and primers described in [15]. We are grateful for the primers synthesized by G.E. Pozmogova, Yu.B. Golova, and B.N. Chernov. Amplification protocol: (i) denaturation at 94°C, 5 min; renaturation at 25°C up to 57°C, depending on the structure and length of primer, 1 min; synthesis at 72°C, 2 min, (ii) 39 cycles of 1 min, 1 min, 2 min at the above mentioned temperatures, respectively.

AMPLI-2 (Biocom) and MiMiniCycler™ (MJ Research, GB) were used to carry out reactions. Dye and ethidium bromide were added to the reaction mixture and then it was subjected to electrophoresis in 1.4 or 2% agarose gels in Tris-borate buffer. Gels were UV-photographed onto Micrat-Isopam (Russia) films.

*Hin*dIII or *Eco*RI fragments of phage λ DNA or synthetic markers (Fermentas) were used as markers. Gel photographs were copied to transparent paper, and loci of different bisexual species were marked by different colors. Loci of unisexual species were marked as corresponding loci of bisexuals. A matrix of pairwise similarities for each parthenospecies and for investigated bisexual species was constructed, and the number of similar and specific bands was presented graphically.

RESULTS AND DISCUSSION

Hybridogenesis of Parthenospecies Studied by Comparing Nucleotide Sequences of the CLsat Tandem Repeat Monomer Families

A family of tandem repeats specific for Caucasian rock lizards of the "*L. saxicola* complex" and for two terrestrial species genomes were described in our previous paper [9, 10]. Dot hybridization data show that it is absent from the *Lacerta agilis* group and *Podarcis*, *Eremias*, and *Ophisops* genomes. Monomers of these tandem repeats are 145–146 bp long. The specificity of the repeats might support the idea to isolate this group of Caucasian rock lizards into a separate genus, *Darevskia* [5].

Sequences of CLsat monomers of more than 20 species of the *Lacerta* genus were determined. These data will be published elsewhere. Here we shall only mention that there exist at least three subfamilies of CLsat monomers (I–III). The representation of subfamilies varies in the analyzed genomes. CLsatI subfamily is characteristic of *L. saxicola* and closely related species (*L. valentini*, *L. portschinskii*, *L. rudis*, *L. raddei*, and *L. alpina)* and comprises the bulk of the satellite sequences (50–80%). CLsatII subfamily quantitatively dominates in *L. mixta* group of species (*L. caucasica*, *L. daghestanica*, *L. driada*, and *L. clarcorum*), but CLsatI sequences are present in a few copies. The content of CLsatIII subfamily is the highest in *L. lindholmi* and *L. parvula*, and in other species it might constitute 1/10 to 1/3 of the total satellite content. Monomer subfamilies are 70–80% similar by the number of nucleotide substitutions, and the homology of clones within each subfamily is 90–98%.

We compared the content of CLsat subfamilies in genomes of unisexual and bisexual species as well as the sequences of CLsatI and CLsatII monomers.

***Lacerta armeniaca* parthenospecies.** Two types of monomers, arm1 and arm2, were found in this parthenospecies (Fig. 1), differing in the number of substitutions relative the consensus sequence (20%, i.e., 30 mutations per 146 bp). In Fig. 1 both monomers are in the center, and sequences of bisexual species most similar to them are located higher or lower depending on the level of difference. It is evident that structures of arm1 and val monomers, on the one hand, and of arm2 and mix, on the other are nearly identical. Differences in monomer structure of the species of the "upper" group (port, rad, and rud) versus arm1 are also negligible (one substitution at different positions), and those of other species of the "lower" group (dri3 and cla1) versus arm2 are small (up to three substitutions in the same positions). Differences from other bisexual species are substantially more pronounced. Hence, the *L. armeniaca* genome appears to be composite and to contain two types of CLsat, the first being characteristic of the *saxicola* group of lizards (CLsatI) and the second of the *mixta* group (CLsatII).

In further experiments we analyzed another subfamily of repeats, CLsatIII, discovered later. Southern hybridization experiments demonstrated that the content of this monomer in *L. armeniaca* is rather high and comparable to that in *L. valentini* and *L. portschinskii*, but it is practically absent from *L. mixta*.

***Lacerta dahli* parthenospecies.** Basing on the analysis presented in Fig. 1 and performed for three other parthenospecies, a matrix of monomer sequence comparisons was constructed employing the data on the total number of substitutions in consensus sequences of the compared pairs of species (Fig. 2). Bisexual species were grouped together depending on the presence of the first and second subfamilies of the repeats in their genomes. Two monomers, dah1 and dah2, were found in *L. dahli* parthenospecies, differing by some 20%, as in *L. armeniaca*. The dah1 monomer sequence appeared to be nearly identical to the port monomer and closely resembles that of val, rud, and rad monomers, differing by three, four, and two substitutions, respectively. The dah2 monomer is nearly identical to the mix monomer (one substitution) and is highly similar to dri3 and cla1 monomers (two substitutions in each). It is worth noting that pairs of monomers of the two investigated parthenospecies are similar. Arm1–dah1 and arm2–dah2 differ by two and three substitutions, respectively. Summing up, each of the two parthenospecies genomes includes two subfamilies of CLsat, one of which is similar to the CLsatI subfamily and another one to the CLsatII subfamily of bisexual species of the corresponding group. The content of CLsatIII in *L. dahli* is slightly lower that in the parental *L. portschinskii*.

Noteworthily, differences of both parthenospecies in the character analyzed from the four bisexual species of the *saxicola* group (*valentini*, *portschinskii*, *raddei*, and *rudis*) are not pronounced enough to unequivocally choose one of them as a parental species. One might point out only a certain tendency, and it is in accord with the concepts of morphologists who hypothesize that *L. valentini* might have been one of the parental species of *L. armeniaca* and, correspondingly, *L. portschinskii* of *L. dahli*; *L. mixta* might have

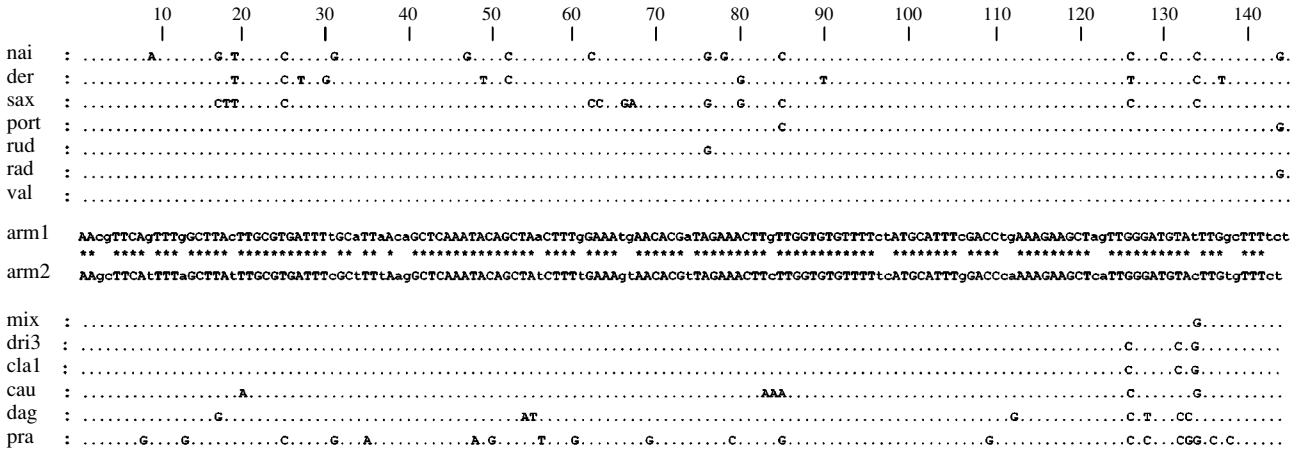

Fig. 1. Sequences of monomers of CLsat tandem repeats of genomes of bisexual "*L. saxicola* complex" species and of two options of this monomer (arm1 and arm2) found in *L. armeniaca* parthenospecies. Substitutions in arm1 and arm2 are designated by gaps and small letters. Broken line in sequences of bisexual species indicates its identity to the monomer of a unisexual one. Substitutions are indicated by corresponding letters. Sequences of monomers of bisexual species above the arm1 sequence belong to CLsatI subfamily, and those below it to CLsatII subfamily. Monomers of bisexual species are arranged with increasing number of substitutions beginning from arm1 and arm2. Abbreviations: nai, *L. nairensis*; der, *L. derjungini*; sax, *L. saxicola*; port, *L. portschinskii*; rud, *L. rudis*; rad, *L. raddei*; val, *L. valentini*; mix, *L. mixta*; dri, *L. driada*; cla, *L. clarcorum*; cau, *L. caucasica*; dag, *L. daghestanica*; pra, *L. praticola*; arm, *L. armeniaca*.

been the other parental species of both parthenospecies.

Pronounced similarity of arm1 not only with val sequences (no substitutions), but also with rud, rad (one substitution), and port (two substitutions) might indicate that either a certain predecessor participated in parthenogenesis of all these species, or that parthenospecies have a polyclonal origin: at different stages of the process three similar species might have participated in it. The latter hypothesis seems to be less grounded, because fingerprints of these species might be interpreted in favor of their monoclonal origin [16, 17]. The higher similarity of dal1 and port (one substitution) in comparison with the same bisexual species (val, one substitution; rud, four substitutions; rad, two substitutions) may support the hypothesis that hybridogenesis occurred after divergence of all the four bisexual species and one of them. Negligible differences in arm1–dah1 (three substitutions) and arm2–dah2 (two substations) seem to support the second hypothesis, although direct evidence is lacking.

Data demonstrating relatedness of arm2 and dah2 monomers with CLsatII monomers may be treated in the same manner. *L. mixta* seems to be a better candidate parental species (one substitution against two or three substitutions in *L. dahli* and *L. clarcorum* monomers). This testifies to very close relatedness if not identity of these three bisexual species, or their recent divergence.

***Lacerta rostombekovi* parthenospecies.** Data obtained for two other parthenospecies are equivocal (see below). Figure 2 demonstrates similarity of one of them (*L. rostombekovi*) with the same group of bisexual, CLsatI-carrying species. The same result was obtained with two other parthenospecies, but differences are slightly more pronounced (three or four substitutions). This may indicate earlier divergence of *L. rostombekovi* from the common ancestor of these four species. All the CLsatI monomers of other bisexual species under study are less similar with monomers of this parthenospecies (10 to 17 substitutions) and could scarcely be parental. Noteworthily, genomes of this and another parthenospecies, *L. unisexualis*, do not contain CLsatII monomers, and monomers rost and uni typical of such genomes by this character have no similarity with any bisexual species of this group. The differences are well pronounced, varying from 29 to 45 substitutions in different species pairs. Dot hybridization data show that more than half of the satellite in this species is CLsatIII. Hence, subfamilies I and III are found in this parthenospecies, the first of which is characteristic of the *saxicola* group (*valentini*, *portschinskii*, *raddei*, *rudis*, *saxicola*, *derjugini*), while the second could be found in *valentini* and *portschinskii*, as well as of *lindholmi* and *parvula*. However, as CLsatIII sequences have not yet been determined, one cannot choose one of the species mentioned above, although the hybrid origin of *L. rostombekovi* from two bisexual species (one from the group with predominant CLsatI, and the other from the CLsatIII group) is highly probable. We cannot but mention that participation of *L. parvula*, *L. lindholmi*, and *L. driada* in this process is less probable, according to morphological and zoogeographic comparative data.

Subfamily of CLsat		PARTHENOSPECIES						BISEXUAL-CLsat I								BISEXUAL-CLsat II						
PARTHENOSPECIES		arm1	arm2	dah1	dah2	rost	uni	val	por	rud	rad	nai	sax	alp	der	mix	dri3	cla1	cau	dag	pra	
	arm1	0	30	3	28	3	15	0	2	1	1	15	13	13	11	27	30	29	31	35	40	
	arm2		0	28	2	29	38	28	26	29	30	38	34	32	36	1	3	3	6	8	20	
	dah1			0	31	4	13	3	1	4	2	14	14	11	15	30	31	30	32	36	43	A
	dah2				0	38	38	24	28	29	28	36	35	32	36	1	2	2	6	10	16	
	rost					0	13	3	3	4	3	13	12	10	13	29	32	31	34	29	43	
	uni						0	14	10	13	13	2	12	10	17	37	37	37	38	41	45	
BISEXUAL-CLsat I	val							0	2	1	1	15	13	12	12	27	29	29	31	33	41	
	port								0	2	1	14	12	13	12	29	31	31	32	36	42	
	rud									0	2	14	12	12	13	27	30	29	38	38	42	
	rad										0	14	14	17	12	27	30	35	32	34	42	B
	nair											0	14	15	17	36	37	37	32	40	45	
	sax												0	13	15	33	34	33	34	37	43	
	alp													0	13	34	35	34	35	35	43	
BISEXUAL-CLsat II	der														0	35	35	36	38	39	46	
	mix															0	1	1	5	9	15	
	dri3																0	0	4	7	14	
	cla1																	0	5	7	15	C
	cau																		0	12	18	
	dag																			0	20	
	prat																				0	

Fig. 2. Matrices of pairwise comparisons by the number of differences between CLsat monomer sequences. Shaded are areas of the most similar sequences with each of monomers of unisexual species and with bisexual species under study. A, region of comparisons of genomes of bisexual and parthenospecies; B, region of comparisons of bisexual species genomes including CLsatI with genomes of the same group and with bisexual species genomes including CLsatII sequences; C, region of comparison of bisexual species including CLsatII sequences with bisexual species of the same group.

***Lacerta unisexualis* parthenospecies.** Analysis of CLsatI and II sequences of the last parthenospecies under study and the results obtained by dot hybridization with CLsatIII (preliminary data) show that only CLsatI is certainly present in the genome of this parthenospecies, whereas the content of CLsatII and CLsatIII is very low. At the present stage, we are unable to demonstrate the hybrid origin of this parthenospecies using the above-mentioned markers. In subspecies *raddei nairensis* rad and uni monomer sequences appeared to be the most similar (Fig. 2). However, as the hybrid origin of Caucasian lacertids by hybridogenesis is most probable, one might suppose that either only bisexual species containing mostly CLsatI subfamily sequences participated in hybridogenesis, or there exists some other, still unidentified monomer family, which does not hybridize with primers used in sequencing all the three repeat families revealed by *Hin*dIII and *Taq*I treatment. The homology of such a subfamily with all others might be less than 75%. Alternatively, it does not contain the restriction sites mentioned above.

The problem remains unresolved. Note that the *L. unisexualis* parthenospecies genome has some unique characters of a vague nature. For example, fingerprint analysis revealed very low heterogeneity of *L. armeniaca* and *L. dahli* patterns, but two out of the six probes demonstrated pronounced polymorphism of its genome. To shed light on this problem, other populations of *L. unisexualis* are being studied in our laboratory, as well as other families of tandem repeats.

Some additional evidence on the role of various bisexual species in parthenogenesis of this and other parthenospecies could be obtained by the RAPD method. The results of such analysis are presented in the next section.

Hybrid Origin of Parthenospecies Demonstrated by RAPD Analysis

As found earlier, RAPD markers do not reveal intrapopulation, individual variability of parthenospecies genomes [8, 15]. Analysis of *armeniaca*, *dahli*, and *rostombekovi* parthenospecies by fingerprinting with several probes also demonstrated absolute identity of all the investigated specimens from the same or different populations of any of these parthenospecies [16, 17]. It might be concluded that the parthenospe-

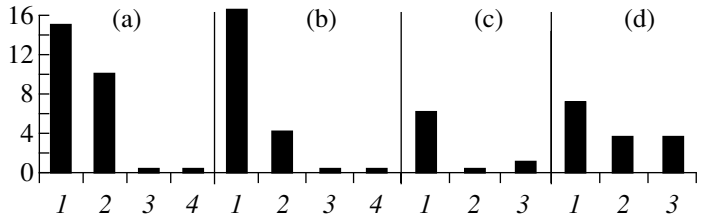

Fig. 3. Number of autoapomorphic characters revealed in genomes of bisexual species under study shared with genomes of parthenospecies. (a) in *L. armeniaca*: (*1*) *L. mixta*; (*2*) *L. valentini*; (*3*) *L. portschinskii*; (*4*) *L. saxicola*; (b) in *L. dahli*: (*1*) *L. mixta*; (*2*) *L. valentini*; (*3*) *L. portschinskii*; (*4*) *L. saxicola*; (c) in *L. rostombekovi*: (*1*) *L. portschinskii*; (*2*) *L. valentini*; (*3*) *L. raddei*; (d) in *L. unisexualis*: (*1*) *L. valentini*; (*2*) *L. portschinskii*; (*3*) *L. raddei*.

cies are monoclonal and hence individual animals might be taken for analysis. Our investigations were favored by the low level of intraspecies polymorphism of lizard bisexual species in RAPD markers, allowing us to study constant, species-specific apomorphic bands [15]. Only statistically valid marker sets of 200–300 bands obtained with different primers were employed in these experiments.

Comparative RAPD analysis of various bisexual species of "*L. saxicola* complex" and patterns obtained in parthenospecies analysis allowed us to exclude species with the most pronounced differences in total, species-specific loci, and to select the most similar ones characteristic of bisexual parental species and of their unisexual progeny for further analysis. This group of bisexual species included *L. mixta*, *L. valentini*, *L. portschinskii*, *L. raddei raddei*, and *L. raddei nairensis*, i.e., the same set of species as indicated in the preceding section. High similarity with parthenospecies was also revealed by the taxoprint method [7]. For control experiments with RAPD markers, closely related species or populations were taken.

Number of RAPD markers (6 primers) in individual species and in various pairs of species (for *L. armeniaca* parthenospecies)

Species of *Lacerta* genus	Total number of characters	Number of synapomorphic RAPD bands of *L. armeniaca* with bisexuals mentioned
saxicola	53	**23**
mixta	72	**25**
armeniaca (*p*)	59	**0**
valentini	68	**10**
portschinskii	53	**14**
armeniaca/*saxicola*	20	0
armeniaca/*mixta*	41	15
armeniaca/*valentini*	35	10
armeniaca/*portschinskii*	20	0
armeniaca/*saxicola* + *mixta*	19	8
armeniaca/*saxicola* + *valentini*	14	2
armeniaca/*saxicola* + *portschinskii*	10	0
armeniaca/*mixta* + *valentini*	17	11
armeniaca/*mixta* + *portschinskii*	12	0
armeniaca/*valentini* + *portschinskii*	20	7
armeniaca/*saxicola* + *mixta* + *valentini* + *portschinskii*	305	7

Note: Species-specific, autoapomorphic bands/characters are given in bold.

As expected, patterns of presumable parental and the most closely related bisexual species as well as parthenospecies have many common bands (i.e., synapomorphies) suggesting their common origin. Some of such bands could be observed in unisexual species as well. The number of specific, apomorphic bands (characters) is low for each of species. Hence, the aim of our work was to reveal and register the characters that are presumably common only for each of bisexual and a definite unisexual species.

***L. armeniaca* parthenospecies.** The total number of bands in all patterns, the number of apomorphic bands for each species, the number of common bands for each pair and combination of species were registered, as well as the number of bands specific only for a parthenospecies and any of bisexual species. The table gives the data obtained in the study of *L. armeniaca*. Out of 305 bands registered, 50–70 bands pertain to an individual species. Patterns of all the species except the unisexual one have apomorphic bands, but in the pattern of parthenospecies only *L. mixta* bands (15 out of 25) and *L. valentini* bands (10 out of 10) could be found. As to 14 *portschinskii*-specific and 23 *saxicola*-specific bands, none of them was revealed in the parthenospecies. Usually, not all the autoapomorphic bands of bisexual species are present in parthenospecies patterns. In addition, the *L. armeniaca* pattern does not have individual apomorphic bands, and they are few (1–2) in other parthenospecies patterns. One might speculate that gross differences in bisexual species patterns are due to long independent evolution

since the hybridization event and to meiotic recombination. It seems plausible that such events did not take place in the evolutionary history of parthenospecies, or their evolutionary age is too short. Otherwise, the remarkable conservatism of some genome apomorphies of contemporary bisexual species would not have been observed. There are no data on genome structure and inheritance of molecular traits in F1 bisexual hybrids between the same bisexual parental species, although such data might help interpret the results of this study. The number of apomorphic bands in pairs of *L. armeniaca* with bisexual species is shown in Fig. 3a. It is evident that in RAPD markers *L. mixta* and *L. valentini* are the most similar to the parthenospecies

Unfortunately, other species of the *mixta* group, i.e., *driada*, *clarcorum* and *caucasica*, were not at our disposal, except the *daghestanica* species, which differs dramatically from parthenospecies, much more than all the species studied. Examination of these species, though, might not have substantially added to the results of RAPD analysis, which are suggestive of hybridogenesis between ancestral species of the *mixta* and *saxicola* groups. The data obtained with *L. armeniaca* are in accord with those described in the preceding section.

***L. dahli* parthenospecies.** Using the same markers, we have demonstrated [8] that this species might be a hybrid of *L. mixta* and *L. portschinskii*. Comparison of patterns by methods employed in the experiments with *L. armeniaca* supports this inference (table). All three bands specific for *L. portschinskii* patterns were revealed in the parthenospecies pattern. However, none of the 23 *valentinin*-specific bands could be observed in it. Likewise, 16 out of 26 *mixta*-specific bands were found in the *dahli* genome. As follows from Fig. 3b, the most probable parental species of this parthenospecies are *L. mixta* and *L. portschinskii*.

***L. rostombekovi* parthenospecies.** Like the *L. unisexualis* parthenospecies described below, this species might be a hybrid of *L. raddei* and *L. portschinskii* or *L. valentini*. These species are difficult to analyze because of the great number (ca. 50) of *L. raddei* populations inhabiting the Transcaucasian region. Usually this species is treated as the "*L. raddei* complex," reflecting imperfect systematics. During the initial stage of this project, we examined populations from the sympatry region or from the regions close to the area of these two parthenospecies, northwest of the Sevan lake. However, such a method in itself has no advantage, because *L. mixta* nowadays does not have sympatric regions with any of the hybridogenesis participants and with the parthenospecies themselves. Hence, for control experiments we collected animals from populations southwest of the Sevan lake, in the vicinity of Khosrov, Gegard, and Egegnadzor. It has been demonstrated that taxoprints of genomes of two of these populations are nearly identical, whereas that of the third one has minor differences [9]. RAPD analysis gave the same result: populations differed slightly within the limits inherent to interpopulation polymorphism, and again the most southern Egegnadzor population exhibited the highest deviation [8].

Patterns of none of the examined populations have individual apomorphic bands present exclusively in parthenospecies (Fig. 3c). All these populations have five common bands, which are present also in *L. rostombekovi*, as well as many other bands characteristic of various pairs or triples of *raddei* populations, which are present in parthenospecies too. The impact of the Egegnadzor population in such loci is about half that of other populations.

As to the second parental species, *L. portschinskii* appears to be favored as it has five common loci unique for it and for the parthenospecies, whereas the impact of *valentini* is limited to a number of bands shared by these bisexual species [8].

It might be concluded that *L. rostombekovi* and two other parthenogenic species described earlier originated by hybridization, on the one hand, of the predecessors of the *L. raddei* complex and, on the other hand, presumably of *L. portschinskii* but not *L. valentini*.

***L. unisexualis* parthenospecies.** It was hypothesized that this species originated from *L. raddei nairensis* and from *L. valentini*. Analysis of the number of autoapomorphies registered in various populations of *L. raddei* and in *L. valentini* and *L. portschinskii* give grounds to conclusion (Fig. 3d) that the genome of this parthenospecies shares only unit autoapomorphic bands with Gegard, Khosrov, and Lchashen populations, the area inhabited by *nairensis*. The total number of such bands for different combinations is three. No advantage of the Lchashen *nairensis* population is evident, although the structure of the satellite monomer demonstrates it (Fig. 2). Among two other bisexual candidates, *L. valentini* is more probable (six bands).

We can do no more than mention that comparison of several *raddei* populations with the specific "bench mark," i.e., with the parthenospecies allows one to draw some useful systematic conclusions. The problem of a population status within a species complex remains obscure and cannot be solved basing solely on morphological characters. Molecular markers are indicative of pronounced similarity of the Lchashen population having the subspecies *nairensis* status and of the Gosh population which does not possess such a status. At the same time, Egegnadzor and Gosh populations differ more that Lchashen and Gosh populations and, logically, the Egegnadzor population has

more rights to a subspecies status than the *nairensis* one.

Coincidence of data obtained with different markers in the study of one and the same object testifies to the validity of the derived phylogeny and systematic conclusions. This is of utmost importance when discrepancies reflecting minor differences inside taxa of the lower rank are small and congruency of the data obtained with different markers becomes crucial for the validity of the conclusions [19]. Unfortunately, such examples of complex analysis are rare in the literature, our paper being one more attempt. The main conclusion one might draw is that molecular and zoogeographic approaches give congruent data, and the arising problems and contradictions find rational explanation.

Summing up, the results presented in this section support the conclusion of the study on tandem monomers of two parthenospecies, *L. armeniaca* and *L. dahli*: the most probable ancestor of the first one is *L. valentini*, and of the second one, *L. portschinskii*. The preference of the second parental species, *L. mixta*, will remain obscure until its relatedness and the systematic position of *L. driada* and *L. clarcorum* become known.

The presumable role of *L. raddei nairensis* in the *L. unisexualis* origin is a moot point, because RAPD data (this paper) and taxoprint analysis [9] revealed no specific properties of this subspecies. However, tandem repeat comparisons demonstrate higher similarity of this subspecies to *unisexualis* rather than to the Gosh *raddei* population (Fig. 2). Differences in satellite structure of the *nairensis* and Gosh populations are statistically valid, but data on other *raddei* populations have not been obtained as yet. RAPD markers and taxoprint data suggest that *nairensis* does not substantially differ from other populations.

Note that analysis of mitochondrial genes and 35 enzyme loci also indicates that *nairensis* is conspecific to *raddei* [20].

In conclusion, although the hybrid origin of Caucasian lizard parthenospecies was predicted by morphological analysis and was supported by the allozyme data [4, 6], the problem needs further investigation. It was postulated [21] that the hybrid phenotype might be composed of dominant and dominant-recessive characters, and one is unable to predict which of parental characters would be manifested in it. It is also difficult or even impossible to differentiate convergent or monophyletic similarity. For example, in squamation *L. mixta* resembles *L. parvula* and *L. derjungii* to such an extent that its hybrid origin from these two species was hypothesized [22]. However, allozyme analysis rejects such a possibility [6]. Testing of the hybrid origin of *Etheostoma* fishes gave the same result. Morphologically it was similar to two other species, but application of molecular markers ruined this idea. On the other hand, allozyme analysis itself sometimes gives erroneous results. For example, artificial hybrids *Bufo bufo* × *B. viridis* and *B. regularis* × *B. mauritanicus* demonstrated lactate dehydrogenase spectra characteristic of *B. viridis* and *B. regularis*, respectively (cited in [21]), i.e., absence of one of the loci of the allozyme does not exclude hybridogenesis.

Obviously, the problem of reticulate evolution in general and of hybridogenesis in particular cannot be explored if genetic material is not studied, as it is done in the present paper. Data demonstrating the presence of satellite monomers characteristic of presumable parental species support the RAPD data, which are less informative if treated independently.

Summing up, it might be concluded that broad studies of bisexual species and their populations nowadays do not shed enough light on the origin of parthenospecies. Moreover, this problem can hardly be definitely solved even in future. It is likely that none of the modern bisexual species is identical to the one having participated in the hybridization event. The problem seems to be of a scholastic nature, and one should give up the idea to solve it, the more so that the systematic status of some genetically and morphologically closely related bisexual species still remains obscure. The *mixta–driada–clarcorum* triad and numerous *L. raddei* populations might be an example.

However, at the present state of our knowledge, with all the markers employed in such studies, one may conclude that parthenospecies genomes possess characters of at least two bisexual, presumably parental species, and that it does not have dominant, species-specific, autoapomorphic characters. Recent hybrid origin of parthenospecies suggests itself because loci common for bisexual and unisexual species are quite similar. The presumable revised genealogy of parthenospecies might be as follows:

L. armeniaca = "*L. mixta*" × "*L. valentini*"

L. dahli = "*L. mixta*" × "*L. portschinskii*"

L. rostombekovi = "*L. portschinskii*" × "*L. raddei*" complex

L. unisexualis = "*L. valentini*" × "*L. raddei*" complex

Inverted commas are taxonomically irrelevant and indicate only that participation of the contemporary species in the ancient hybridization event remains uncertain.

In addition, our data demonstrating that dominant, apomorphic characters are practically absent from parthenospecies under study do not allow the latter to be formally considered as cladistic and phylogenetic species. Here we cannot but mention the imperfection of cladism claiming that systematics should be based only on divergence and the apomorphic characters of

taxa. If such ideology is accepted, numerous plant, bacterial, and, as it has become obvious recently, animal hybridogenic populations appear to be out of system. Hybridogenic origin of species also does not correspond to the cladistic postulate of the inevitable disappearance of the progenitor species as a result of its divergence into two (or more) clades, because hybridizing species still persist in nature. As to parthenospecies, their characteristics of reproductively isolated individuals united by similar morphology and genetic material transferred through generations and occupying a certain ecological niche are in accord with the species definition [23] even more tightly than the bisexual species parameters. Hence, the possibility of horizontal transfer of hereditary characters should be taken into account in the attempts to construct systematics of large taxa [22, 24]. A palliative decision might be to use "parthenospecies," "clonospecies," and a more general "hybridospecies" terms. American authors employ such terms for unisexual species of Teiidae. The hybrid origin of a species may be specially indicated in phylogenetic trees, reflecting its reticulate evolution origin.

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