Parentage of Caucasian parthenogenetic rock lizard species (Lacerta) as revealed by restriction endonuclease analysis of highly repetitive DNA

Vernata V. Grechkol, Dmitry M. Ryabinin, Larissa V. Fedorova, Alexey N. Fedorov, Alexey P. Ryskov, Ilya S. Darevsky

1 Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Vavilov str., 32, 117984 Moscow, Russia
2 Institute of Gene Biology, Russian Academy of Sciences, Vavilov str., 2/5, 117984 Moscow, Russia
3 Zoological Institute, Russian Academy of Sciences, University nab., 1, 199034 St. Petersburg, Russia

Abstract. The Caucasian parthenogenetic rock lizards of the L. saxicola complex probably originated by hybridisation of some bisexual species. We verified this hypothesis using a new approach based on comparison of repetitive DNA characters, which produce species-specific patterns (named "taxonprint"). The method relies on restriction endonuclease hydrolysis of genomic DNA, with the following 32P-end labeling and polyacrylamide gel electrophoretic separation of the fragments. The parthenogenetic species L. armeniaca and L. dahli possess specific features both of putative maternal L. mixta (in some taxonprints), and of paternal L. valentini (in other ones), whereas L. portschinskii and L. rudis also could be the paternal species. Parthenoclones L. unisexualis and L. uzzelli have the specific DNA features in several taxonprints of L. raddei or L. nairensis (which cannot be discriminated by taxonprints) which are supposed to be maternal species for L. unisexualis and paternal ones for L. uzzelli. The specific features of L. valentini (or L. portschinskii) are observed in L. unisexualis; L. uzzelli has not been investigated in this respect. Parthenogenetic L. rostombekovi also possesses properties of L. raddei or L. nairensis, but we have not be able to find any features of supposed paternal L. portschinskii in this parthenogenetic species.

Introduction

The general characteristics of highly repetitive sequences of DNA, which can be revealed by their amenability to restriction endonuclease digestion, possess species specificity (Cook, 1975; Elizur et al., 1982; Shubina and Mednikov, 1986; Turner et al., 1991; Fedorov et al., 1992; Grechko et al., 1993b; Potapov and Ryskov, 1993; Skurikhina et al., 1993; Bannikova et al., 1995; Chelomina et al., 1995; Grechko et al., 1997). The electrophoretic patterns of the digested DNA fragments are enriched with repeated monomers (Southern, 1975; Donehower and Gillespie, 1979) and have some bands specific for species in general, some for a genus and some for a family.
In previous papers we revised these data using a large pool of taxa and specimens to clarify the level of intrapopulational and intraspecific variability of these characters. Several hedgehogs (Bannikova et al., 1995), hamsters, mice (Potapov and Ryskov, 1993; Ryskov et al., 1994), fish, lizards and insects species, and human populations too (Grechko et al., 1997), were studied in this respect. It was shown that there are no individual (intrapopulational) polymorphisms in electrophoretic restriction patterns in each of the populations studied up to now (about 50 species, more than two hundred of DNA specimens). Meanwhile all the patterns were endonuclease and species specific. For convenience we proposed to name these patterns, which have no individual and sex polymorphisms, “taxonprints” (Fedorov et al., 1992) in contrast (as antonym) to individually specific “fingerprints”.

So far, as all the specimens of a population have identical patterns, it is not necessary to perform statistical treatment of the results obtained, or to use a large number of specimens for each analysis.

These facts permit to study of relationships of species and higher taxa, and also the parentage among the species which are supposed to take part in hybridisation events.

Table 1. *Lacerta* (*L.*) and *Podarcis* (*P.*) species studied.

<table>
<thead>
<tr>
<th>Species</th>
<th>Localities</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. agilis agilis</em></td>
<td>Germany, München</td>
</tr>
<tr>
<td><em>L. agilis boemica</em></td>
<td>Daghestan, Makhachkala</td>
</tr>
<tr>
<td><em>L. agilis chersonensis</em></td>
<td>Russia, Kaliningrad</td>
</tr>
<tr>
<td><em>L. armeniaca</em> (parth)</td>
<td>Armenia, Dilijan</td>
</tr>
<tr>
<td><em>L. caucasicas daghestanica</em></td>
<td>Daghestan, Makhachkala</td>
</tr>
<tr>
<td><em>L. dahl</em> (parth)</td>
<td>Armenia, Dilijan</td>
</tr>
<tr>
<td><em>L. d. derjugini</em></td>
<td>Georgia, Akhaldaba</td>
</tr>
<tr>
<td><em>L. m. mixta</em></td>
<td>Georgia, Akhaldaba</td>
</tr>
<tr>
<td><em>L. n. nairensis</em></td>
<td>Armenia, Sevan</td>
</tr>
<tr>
<td><em>L. p. portschinkii</em></td>
<td>Armenia Gosh</td>
</tr>
<tr>
<td><em>L. praticola pontica</em></td>
<td>Russia, Sochi</td>
</tr>
<tr>
<td><em>L. r. raddei</em> (E)</td>
<td>Armenia, Eggegnsador</td>
</tr>
<tr>
<td><em>L. r. raddei</em> (G)</td>
<td>Armenia, Gosh</td>
</tr>
<tr>
<td><em>L. r. raddei</em> (Kl)</td>
<td>Armenia, Khosrov</td>
</tr>
<tr>
<td><em>L. rostombechovi</em> (parth)</td>
<td>Armenia, Dilijan</td>
</tr>
<tr>
<td><em>L. rudis chechenica</em></td>
<td>Daghestan, Tijara</td>
</tr>
<tr>
<td><em>L. rudis obscura</em></td>
<td>Georgia, Akhaldaba</td>
</tr>
<tr>
<td><em>L. saxicola darevskii</em></td>
<td>Russia, Sochi</td>
</tr>
<tr>
<td><em>L. saxicola lindholmi</em></td>
<td>Crimea, Yalta</td>
</tr>
<tr>
<td><em>L. unisexualis</em> (parth)</td>
<td>Armenia, Takjarlu</td>
</tr>
<tr>
<td><em>L. uzzelli</em> (parth)</td>
<td>Turkey, Cars</td>
</tr>
<tr>
<td><em>L. v. thalassina</em></td>
<td>Armenia, Geham ridge</td>
</tr>
<tr>
<td><em>L. v. viridis</em></td>
<td>Ukraine, Dnepropetrovsk</td>
</tr>
<tr>
<td><em>L. v. vivipara</em></td>
<td>Russia, St. Petersburg</td>
</tr>
<tr>
<td><em>P. m. murlis</em></td>
<td>Spain, Almazzzo</td>
</tr>
<tr>
<td><em>P. i. taurica</em></td>
<td>Crimea, Yalta</td>
</tr>
</tbody>
</table>
Table 2. Putative parental species and parthenogenetic species of hybrid origin. The maternal ancestors were suggested by Moritz et al. (1991).

<table>
<thead>
<tr>
<th>Parthenogenetic species (p)</th>
<th>Bisexual species</th>
</tr>
</thead>
<tbody>
<tr>
<td>♀♀</td>
<td>♀♀ L. mixta × L. valentini</td>
</tr>
<tr>
<td>L. armeniaca</td>
<td></td>
</tr>
<tr>
<td>L. dahli</td>
<td>L. mixta × L. portschinskii</td>
</tr>
<tr>
<td>L. unisexalis</td>
<td>L. nairensis × L. valentini</td>
</tr>
<tr>
<td>L. uzzelli</td>
<td>L. valentini × L. nairensis</td>
</tr>
<tr>
<td>L. rostombelevi</td>
<td>L. raddei × L. portschinskii</td>
</tr>
</tbody>
</table>

This approach could be especially useful in analysis of possible hybridogeneous origin of parthenogenetic lizard species.

The Caucasian rock lizards (Lacerta) group is united on the basis of some ecological and morphological characters (Darevsky, 1993; Arnold, 1989); it includes at least nine bisexual and five parthenogenetic species (Darevsky, 1993) (table 1). Arnold (1989) placed all the species in the conventional “archaeolacertas” group together with some European Archaeolacerta species. Two forest species (L. praticola and L. derjugini) are usually also included in this group. According to morphological and zoogeographic data the parthenogenetic lizard species seem to have hybridogeneous origins, the parental species being some of the bisexual species of the same group (Darevsky, 1993). Mitochondrial DNA analysis (Moritz et al., 1992) has established several bisexual species as maternal ones for some hybridogeneous clones. The determination of paternal species has not yet been clarified (table 2).

We have studied the relations among parthenogenetic and bisexual species by the taxonprint method which allows to identify parental species in hybrids. We wanted to determine if the taxonprint data correlate with mtDNA data of Moritz et al. (1992), and if there are some other species which have not been studied earlier and could be parental species in hybridogenesis.

Materials and methods

Isolation and restriction endonuclease hydrolysis of genomic DNA

DNA was isolated from blood nuclei by routine methods including proteinase K treatment, sodium dodecyl sulfate and phenol-chloroform extraction, followed by precipitation with isopropanol or ethanol (Sambrook et al., 1989). About 1 μg of the DNA was hydrolyzed with appropriate restriction endonuclease. The reaction was running overnight in total volume of 20 μl under conditions recommended by the supplier (Biopol, Russia; Fermentas, Lithuania; Boehringer Mannheim, Germany).
DNA fragment labeling and electrophoresis

The "sticky" 3'-ends of the restriction fragments were labeled with \([\alpha-\text{32P}]dNTP\) (Physico-energetical Institute, Russia) and the Klenow fragment of \textit{Escherichia coli} DNA polymerase I. Aliquots of the hydrolyzate were added to 20 \(\mu\)l of the buffer solution for end-labeling (50 mM NaCl, 10 mM Tris-HCl buffer, pH 7.5, 10 mM MgCl, 1 mM dithiothreit), which contained the Klenow fragment and the label (about 25 \(\mu\)Ci). After 20 min incubation at room temperature the reaction was stopped with EDTA (final concentration 10 mM). 2-3 \(\mu\)l of the mixture were taken for electrophoresis in 10% non-denaturing polyacrylamide gel in tris-borate buffer, pH 8.3 (Sambrook et al., 1989). After electrophoresis the gel was dried on a glass plate (Garoff and Ansorge, 1981) and radioautographed onto an RT-1 film (Svema, Russia).

Results

Gel profiles reveal that DNA taxonprints of all rock lizards and related forest species (\textit{Lacerta praticola} and \textit{L. derjugini}) are very similar, but most of the species can be diagnosed with selected enzymes. \textit{MspI}, \textit{Sau3AI}, and \textit{Hin6I}-taxonprints of these species are nearly identical, while \textit{HindIII}, \textit{AsuI}, \textit{TaqI}, \textit{Hinfl}, \textit{EcoRI}+\textit{HindIII}, and \textit{Csp6I}-taxonprints reveal some differences among some of the species. The representatives of some other genera of \textit{Lacertidae} — \textit{Podarcis} and \textit{Eremias} — share some bands with \textit{Lacerta} species, and the number of shared bands is larger in \textit{Lacerta} and \textit{Podarcis} species. A detailed description of relationships among bisexual \textit{Lacerta} will be published elsewhere.

\textit{Csp6I}-taxonprints of Caucasian "archaeolacertas" demonstrate obvious differences among \textit{L. saxicola} complex species (fig. 1). The subgroup \textit{mixta-valentini-portschinskii-}
\textit{armeniaca(p)-dahli(p)-saxicola(darevskii)-derjugini} (lanes 4-8, 17, 25), subgroup \textit{raddei-}
\textit{nairensis-unisexualis(p)-rostombekovi(p)-uzzelli(p)} (the latter one was tested in other experiments) (lanes 9-13) and subgroup \textit{rudis-saxicola(lindholmi)-caucasica(daghestani-}
\textit{praticola} (lanes 14-16, 18, 24) (intensity of 150 bp band of the last group being much less) could be separated. Control species \textit{L. viridis} (19), \textit{L. agilis} (20-22) and \textit{L. vivipara} (23) may be differentiated from the latter subgroup by the weak 160 bp band and by the absence of band 155 bp. \textit{Lacerta vivipara} has at least three more specific bands (135, 92, and 75 bp). Taxonprints of \textit{Podarcis taurica} and \textit{muralis} (1-3), having eight common bands with \textit{Lacerta}, differ from them by five unique bands. \textit{Lacerta raddei} and \textit{L. nairensis} cannot be distinguished from each other in the experiments with \textit{Csp6I}.

The \textit{raddei} subgroup (lanes 9-13) has the most significant variation. There are three additional bands in every species of this subgroup, two of which (85 and 60 bp) are unique and the third one (150 bp), being common for all rock lizards, seems to have two bands instead of one band in the \textit{mixta} subgroup. Three parthenogenetic species (\textit{unisexualis},
Figure 1. Csp61-taxonprints. 1 - Podarcis t. taurica; 2, 3 - P. m. muralis; 4 - Lacerta p. portschinskii; 5 - L. dahlia; 6 - L. m. mixta; 7 - L. armeniaca; 8 - L. v. valentini; 9 - L. unisexualis; 10 - L. r. raddei (E); 11 - L. r. raddei (G); 12 - L. r. raddei (Kh); 13 - L.rostombekovi; 14 - L. rudis chechenica; 15 - L. r. obscura; 16 - L. saxicola lindholmi; 17 - L. s. darevskii; 18 - L. caucasica daghestanica; 19 - L. v. viridis; 20 - L. agilis agilis; 21 - L. a. boemica; 22 - L. a. chersonensis; 23 - L. v. vivipara; 24 - L. praticola pontica; 25 - L. d. derjugini. m - the marker fragments of DNA in base pairs. The arrows show the discriminating bands.
uzzelli, and rostombekovi) contain specific DNA fractions shown also in raddei and nairensis, which are hypothesized to be parental species in the hybridogeneous origin of the parthenoclines (table 2).

The discrimination between mixta and raddei-nairensis subgroups may be seen also in HindIII-, TaqI-, AsuI-, HinfI-, and EcoRl+HindIII-taxonprints (not presented). In the EcoRI+HindIII-taxonprints Lacerta mixta, L. armeniaca(p), L. dahli(p), L. saxicola (darevskii), L. caucasica (daghestanica), L. praticola (pontica) and L. derjugini have a prominent specific band (155 bp) and also three other bands shared in all the species studied (200, 165 and 85 bp).

The specific bands for L. mixta and two parthenogenetic armeniaca and dahli exist in HindIII-taxonprints (fig. 2, lanes 4-6); two specific bands (arrows) for portschinskii

**Figure 2.** HindIII-taxonprints. 1, 2 - Podarcis m. muralis; 3 - Lacerta p. portschinskii; 4 - L. dahli; 5 - L. m. mixta; 6 - L. armeniaca; 7 - L. v. valentini; 8 - L. unisexualis; 9 - L. n. nairensis; 10 - L. r. raddei (E); 11 - L. r. raddei (G); 12 - L. r. raddei (Kh); 13 - L. rostombekovi; 14 - L. n. nairensis; 15 - L. saxicola darevskii; 16 - L. caucasica daghestanica; 17 - L. praticola pontica; 18 - L. d. derjugini. The designations as in fig. 1.
(lane 3), valentini (lane 7), and armeniaca, dahli and unisexualis (lanes 4, 6, 8) are shown too. Thus parthenoclines armeniaca and dahli possess some specific bands both of mixta and portschinskii or valentini; parthenocline unisexualis has no mixta-specific band, but does possesses bands diagnostic for portschinskii or valentini. It must be

Figure 3. Hinf-taxonprints. 1 - Podarcis t. taurica; 2, 3 - P. m. muralis; 4, 5 - L. p. portschinskii; 6 - L. v. valentini; 7 - L. m. mixta; 8 - L. armeniaca; 9 - L. dahli; 10 - L. r. raddei (E); 11 - L. r. raddei (G); 12 - L. r. raddei (Kh); 13 - L. rostombekova; 14 - L. n. nairensis; 15 - L. unisexualis; 16 - L. rudis chechenica; 17 - L. r. obscura; 18 - L. saxicola lindholmi; 19 - L. s. darevskii; 20 - L. praticola pontica; 21 - L. d. derjugini. The designations as in fig. 1.
emphasized that *rudis* (*chechenica*) (lane 14) has *portschinskii/valentini* specific bands too.

The experiments with *Asul* revealed that the triade *mixta-armeniaca*(p)-*dahli*(p) plus *praticola* has a specific band of 160 bp. *Lacerta caucasica* (*daghestanica*) has a specific band which runs a little faster. In some other details the taxonprints of species of "archaeolacertas" are nearly indistinguishable and share more than ten common bands.

*Hinfl*-taxonprints show specific bands (150 and 155 bp) for *portschinskii* and *valentini* (4-6) (not *rudis*, 16-17), but none of the assumed parthenogenetic descendants of *valentini* and *portschinskii* (fig. 3, lanes 9, 13, 15) contain these bands.

*Taql*-taxonprints contain one band (87 bp) characteristic for *raddei*, *nairensis* and related parthenoclines, and some specific bands for *L. caucasica*, *L. praticola*, and *L. derjugini*, whereas none of these are present in any parthenoclines.

*Mspl*-taxonprints show very close relationships among all *L. saxicola* complex species and their obvious difference from *Podarcis taurica*.

**Discussion**

Summarizing briefly the evidence in favour of hybrid origin of the parthenogenetic rock lizard species of the Caucasus, it is worth to concentrate on some points requiring more experimental proofs.

1. The obvious morphological similarity of *armeniaca*(p) with *mixta* and *valentini*. Before discovery of parthenogenesis in lizards, *valentini* and *armeniaca* were considered conspecific (Darevsky, 1967). The hybrid hypothesis was based upon morphological similarity of other parthenogenetic species with some bisexual ones.
2. *Lacerta armeniaca*(p) has histocompatibility protein genes of *mixta* and *valentini*: skin grafts of both bisexuals survive when transplanted to *armeniaca* for much longer time than on *raddei* (Danielyan, 1981). Cross grafting of *valentini* and *mixta* lead to fast rejection of transplants.
3. *Lacerta armeniaca*(p) is heterozygous at some allozyme loci and possesses both *mixta* and *valentini* alleles (Uzzell and Darevsky, 1975).
4. *Lacerta armeniaca*(p) karyotype contains the male sex chromosome, which was discovered in *valentini* (Kupriyanova, 1989).
5. An artificial hybridization of *mixta* and *valentini* is possible; hybrids look like *armeniaca*, although they seem to be sterile (Danielyan, 1981).
6. The range of *Lacerta armeniaca*(p) is localized between the ranges of supposed parental species.
7. PCR-RAPD patterns of all the parthenogenes and their supposed bisexual relatives revealed shared bands, and more similarity in triades designated in table 2 (Grechko et al., 1993a).
Collectively these facts support the hybrid origin hypothesis, although each of them, taken separately, leaves room for doubt. The main limitation is the lack of controls with similar rock lizard species. As our data suggest, saxicola (darevskii), rudis (chechenica), praticola (pontica) and derjugini ideally should be included in the analysis. These species were not studied in the early experiments of Uzzell and Darevsky (1977) with allozyme technique.

The correlation of areas of supposed parental and parthenogenetic species could not be the definitive proof in itself. For example, L. rostombekovi(p) is more similar in terms of mtDNA to Egegnadzor raddei population, in spite of their allopatric distributions, whereas L. raddei Gosh population has less mtDNA similarity with rostombekovi, but are partly sympatric (Moritz et al., 1992).

Hybrid origins of other Caucasian parthenoclines were not verified even to such an extent as described above for L. armeniaca. The suggested relations among bisexual and parthenogenetic species are based mainly on allozyme data of Uzzell and Darevsky (1975), which were considered by these authors as preliminary ones, and on morphological correlations. Moreover, the ideas in favour of hybridogeneous origins of Lacerta parthenoclines were based mainly on comparison with the results of extensive investigations of parthenogenetic Cnemidophorus species, though this analogy may not be correct. It is worth to say that most of them have triploid number of chromosomes (Dessauer and Cole, 1989; Darevsky, 1993); at the same time Caucasian rock lizard parthenoclines are diploid, and rare triploid hybrids of bisexual and parthenogenetic species are not fertile. Therefore the cytogenetic mechanisms of their origin might not be the same. In principle, the evolution of animal parthenogenesis can be different (Bullini, 1994).

These doubts became the reason of our search for new approaches to reinvestigate the problem of hybridisation in Lacerta. The molecular data presented here confirm the hypothesis of hybridogeneous origin of at least three Caucasian parthenogenetic species (armeniaca, dahli and unisexualis); these data are the first direct evidence that genomic DNA of parthenoclines contain the characters of two bisexual species. While this paper was prepared, results of Murphy et al. (pers. comm.) became available; these authors came to the same conclusion on the basis of multilocus allozyme markers.

Lacerta mixta and L. praticola have specific bands in Asul-taxonprints present also in their putative parthenogenetic descendants. The specific band for L. mixta in HindIII-taxonprints is present, besides in parthenogenetic armeniaca and dahli, in L. saxicola (darevskii), praticola (pontica) and derjugini too. Formally, these species might also be considered. However, the preference should be given to L. mixta as a parental species, taking into account Csp6I-, and TaqI-taxonprints for L. praticola and derjugini, and Asul-taxonprints for L. saxicola (darevskii), which discriminate them from mixta-armeniaca-dahli group.

The second parental species for armeniaca and dahli are most likely L. valentini or L. portschinskii, practically indistinguishable by any taxonprints studied to date. Two specific bands for both species in HindIII-taxonprints (fig. 2) are present in armeniaca
and dahli, but not in rostombekovi. *Lacerta rudis* also has both specific HindIII-bands and cannot be excluded from consideration. *Hinfl*-taxonprint bands (fig. 3), which are specific for *valentini* and *portschinskii*, are not present in any parthenogenetic species.

Both bisexual species (*raddei* and *nairensis*), which are not distinguishable by *Csp6I*-taxonprints, may be considered as equally probable parents in any of parthenoclines grouped with these species. However the mtDNA data of Moritz et al. (1992) show that the probable maternal species for *rostombekovi* is *raddei*. Our data showed that the most probable population for hybridization is *raddei* Egegnadzor population (Ryabinin et al., 1996). *Lacerta portschinskii* is not excluded as a paternal species; but for *unisexualis* and *uzzelli* the equivalent paternal species might be *raddei* or *nairensis*. So far, if the maternal species of *uzzelli* is *valentini* (Moritz et al., 1992), our data may be considered in support of *nairensis* (or *raddei*) being the paternal species. The similarity of *unisexualis* and *nairensis* in our experiments correlates with Moritz et al. (1992) data showing that *nairensis* might be the maternal species for *unisexualis*.

Hybridogeneous origin of *rostombekovi* is not clear, as we failed to reveal the specific bands of *valentini* or *portschinskii*, but these experiments should be repeated.

Finally, from a methodological perspective, the absence of shared bands in any interspecies hybrid, which originated thousands of years ago from the relict form of recent species, may not be valid for exclusion of taxa from candidacy for ancestors. Only the presence of such common features can be considered as meaningful in elucidation of what species took part in the process. Therefore the lack of characters of *valentini* and *portschinskii* (in *EcoRI+HindIII*, and *Hinfl*-taxonprints) in their possible descendants should not be considered as rigid proof of exclusion. We were fortunate to identify some shared characters in other taxonprints (*HindIII*) of *valentini* and *portschinskii* and parthenogenetic *armeniaca*, *dahli* and *unisexualis*, but additional enzymes have to be incorporated.

**Acknowledgements.** We are indebted to E. Roitberg, A. Misura, F. Danielyan, S. Sharigin, V. Negmetzianov and O. Arribas who kindly supplied us with some lizard specimens. This work was supported by the Russian State Program "Priority Trends in Genetics" and the Russian Foundation for Basic Research.

**References**


*Received:* August 18, 1996. *Accepted:* March 19, 1997.