ISSN 1062-3590, Biology Bulletin, 2025, Vol. 52:234. © Pleiades Publishing, Inc., 2025.
Russian Text © The Author(s), 2024, published in Zoologicheskiy Zhurnal, 2024, Vol. 103, No. 11, pp. 60–74.

Genome Size Variation in Diploid and Polyploid Rock Lizards of the Genus *Darevskia* (Lacertidae, Squamata)

S. N. Litvinchuk[a, b, *], N. D. Aksyonov[a], L. J. Borkin[c], I. V. Doronin[c], V. O. Erashkin[d], and A. A. Kidov[d]

[a] *Institute of Cytology, Russian Academy of Sciences, Saint Petersburg, 194064 Russia*
[b] *Dagestan State University, Makhachkala, 367025 Republic of Dagestan, Russia*
[c] *Zoological Institute, Russian Academy of Sciences, St. Petersburg, 199034 Russia*
[d] *Russian State Agrarian University, Timiryazev Moscow Agricultural Academy, Moscow, 127550 Russia*
**e-mail: litvinchukspartak@yandex.ru*

Received June 30, 2024; revised July 23, 2024; accepted July 23, 2024

Abstract—Study of genome size variation in cells of vertebrates using DNA flow cytometry makes it possible to determine polyploid individuals precisely, which is extremely important when studying the processes of reticulate speciation. In addition, in many groups of vertebrates, closely related species often differ in the nuclear DNA content. Therefore, the purpose of our study was to explore the variability of genome size and ploidy in populations of 29 species and subspecies of rock lizards of the genus *Darevskia*, as well as their hybrids. As a result of this study, the range of variability in individuals of different ploidy (91% of diploid and 9% of triploid individuals) was established not to overlap. Among diploid species, no correlation was found between the nuclear DNA content and phylogenetic relationships, geographic coordinates, altitude, average annual temperatures, and precipitation. Representatives of all studied species complexes (with the exception of *D. adjarica*) had approximately the same limits of variability. Two species (*D. derjugini* and *D. saxicola*) show significant intraspecific variability. Comparisons of the genome size of parthenogenetic and bisexual species generally revealed no noticeable differences between them. Studies of triploid hybrids have shown that their genome size as a whole roughly corresponds to the sum of the average size of the diploid genome of the maternal parthenogenetic species and the haploid genome of the paternal species. The variability of genome sizes within samples of triploid hybrids was, on average, slightly higher than in most parthenogenetic species, but somewhat lower than in bisexual species. This paper discusses the peculiarities of reticulate speciation in this group of animals.

Keywords: nuclear DNA content, DNA flow cytometry, polyploidy, reticulated speciation, reptiles
DOI: 10.1134/S1062359025700463

INTRODUCTION

Polyploidy is a poorly understood phenomenon, occasionally encountered in vertebrates, in which an increase in the chromosome set, a multiple of the haploid one, is observed (Mason and Pires, 2015; Dar and Rehman, 2017). Usually, two categories of polyploids are distinguished: allopolyploids, which are hybrids, and autopolyploids, which are individuals that have several sets of chromosomes of the same species (Borkin et al., 1996). Among reptiles, polyploidy is relatively common: triploid lineages have been identified in approximately 20 species complexes from seven families (Bogart, 1980; Kearney et al., 2009; Trifonov et al., 2015; Abdala et al., 2016; Stöck et al., 2021). The origin of these triploid lineages is usually associated with interspecific hybridization and parthenogenetic reproduction (Kearney et al., 2009). Sometimes triploid parthenogenetic females cross with males of diploid bisexual syntopic species, which can result in tetraploid individuals. Such cases have been noted in American lizards of the genus *Aspidoscelis* Fitzinger 1843 from the family Teiidae Gray 1827 (Hardy and Cole, 1998; Lutes et al., 2011; Cole et al., 2014, 2017).

In addition to triploid parthenogenetic lines, nonhybrid mixoploidy and random spontaneous autotriploidy are occasionally found in reptiles. Thus, diploid-triploid mosaicism is observed in populations of the twist-necked turtle *Platemys platycephala* (Schneider 1792) of the family Chelidae Gray 1831 and in the Chilean lizard *Liolaemus chiliensis* (Lesson 1830) from the family Liolaemidae Frost et Etheridge 1989. It is possible that mosaicism plays an important role in sex determination in these animals (Bickham et al., 1985; Lamborot et al., 2006; Bickham and Hanks, 2009). A mixture of diploid and triploid somatic cells was also found in a parthenogenetic female Central American lizard *Lepidophyma flavimaculatum* Duméril 1851 from the family Xantusiidae Baird 1858 (Bezy, 1972), and spontaneous autotriploidy in the Australian leaf-tailed gecko *Saltuarius cornutus* (Ogilby 1892) from the family Carphodactylidae Kluge 1967 (Pensabene et al., 2024).

Mountain or rock lizards of the genus *Darevskia* Arribas 1999 (family Lacertidae Bonaparte 1831) are currently represented by 41 species, of which seven are parthenogenetic (Arakelyan et al., 2023; Uetz, 2024). These are small lacertids that inhabit mainly mountain forest, grassy, and rocky biotopes in western Asia and southeastern Europe from the Balkans to the Kopet-dag Mountains. Most species have relatively small ranges (https://www.lacerta.de/AS/Home.php).

It has been proven by various methods that parthenogenetic species arose through hybridization between bisexual "parental" species (Uzzell and Darevsky, 1974, 1975; Borkin and Darevsky, 1980; Moritz et al., 1992; Murphy et al., 2000; Girnyk et al., 2018). Various authors date their appearance to the Pleistocene or even the Holocene (Darevsky et al., 1985; Moritz et al., 1992; Freitas et al., 2016; Yanchukov et al., 2022). In areas where the ranges of bisexual and parthenogenetic species of rock lizards in the Caucasus overlap, hybridization can occur, which sometimes results in triploid individuals (Darevsky et al., 1978). They are usually sterile and can be female, male, or hermaphrodite (Darevsky et al., 1989). Sometimes these triploids are able to produce offspring, which can lead to the emergence of extremely rare tetraploid hybrids (Danielyan et al., 2008; Freitas et al., 2019; Arakelyan et al., 2023).

Karyological methods are generally used to determine ploidy in eukaryotic organisms. However, they are quite labor-intensive. Therefore, in recent decades, precise DNA flow cytometry has become widespread (Rozanov and Vinogradov, 1998). It allows one to determine the amount of nuclear DNA (= genome size) in one measurement in a huge number of cells. It should be noted that, in many groups of vertebrates, closely related species often differ in the amount of nuclear DNA, which makes it possible to perform species identification easily in each individual using this method (Biriuk et al., 2016; Dufresnes et al., 2019, 2019a; Borkin, Litvinchuk, 2022).

We are aware of several papers (Chilingaryan and Pavlov, 1961; Darevsky and Kupriyanova, 1982; Darevsky et al., 1989; Darevsky et al., 1991; Ochkalova et al., 2022) containing information on the amount of nuclear DNA in some species of the genus *Darevskia*. However, the results presented in them were obtained using different methods and are therefore poorly comparable. Measuring the genome size in vertebrate cells using precise DNA flow cytometry allows for accurate identification of polyploid individuals (Litvinchuk et al., 2010; Biriuk et al., 2016; Litvinchuk et al., 2018, 2019), which is extremely important when studying the processes of reticulate speciation.

Over 40 years, we have accumulated a large amount of genome size data from different populations and species of the genus *Darevskia*. Thus, the aim of our study was to investigate the variability of nuclear DNA quantity and ploidy in populations of rock lizards and their hybrids using the method of DNA flow cytometry.

MATERIALS AND METHODS

In the period 1984–2024, we studied the variability of genome size in 273 individuals of 29 species and subspecies, as well as their hybrids from 82 localities from Abkhazia, Armenia, Azerbaijan, Georgia, Iran, Russia, Turkey, and South Ossetia, as well as in three laboratory hybrids *D. chlorogaster* ♀ × *D. caspica* ♂ (Table 1, Fig. 1). Selective capture and preliminary determination of triploid hybrids in field conditions were carried out by I.S. Darevsky. Division of the genus *Darevskia* into species complexes was carried out according to data from phylogenetic studies (Arribas, 1999; Ahmadzadeh et al., 2013; Doronin, 2015; Tarkhnishvili et al., 2020). However, it is important to note that the inter- and intraspecific structure of representatives of this genus is not yet sufficiently developed. Therefore, our assignment of some populations to certain subspecies (for example, *D. derjugini* (Nikolsky 1898)) requires further confirmation.

The amount of nuclear DNA was measured using DNA flow cytometry. Blood was collected from the autopsied lizard tail tip into a Versene solution (phosphate buffer containing 0.7 mM EDTA, pH = 7.3–7.7; Biolot, St. Petersburg, Russia). The cells were stored at 4–6°C. The blood cells to be tested were mixed with reference cells, which were male house mouse splinocytes (*Mus musculus* Linnaeus 1758) (laboratory line C57B1, average genome size is 6.80 pg by Bianchi et al., 1983) or blood cells of the grass frog (*Rana temporaria* Linnaeus 1758) from Leningrad oblast of Russia (10.32 pg by Borkin et al., 2001). The total concentration of cells in the suspension was approximately 10^6 cells/mL. To 500 μL of the reference cell mixture and 500 μL of the test species cell mixture, 5.5 μL of a 0.3 M aqueous solution of $MgCl_2$ were added successively, 5.0 μL of 1% aqueous Triton X-100 solution and 5.0 μL of 1% aqueous ethidium bromide (EB; for measurement on CytoFLEX), or a mixture of 2.5 μL of 1% aqueous olivomycin solution and 5.0 μl of 1% aqueous solution of ethidium bromide (EB + OM; for measurement on a laboratory model of a flow cytometer). The standard staining duration ranged from 10 to 60 min at room temperature (~21°C).

DNA flow cytometry was conducted using a CytoFLEX flow cytometer, Beckman Coulter, Inc., CA (EB) and/or a laboratory model of a flow cytometer (EB +OM) developed at the Institute of Cytology, Russian Academy of Sciences (St. Petersburg, Russia; https://patents.google.com/patent/SU1056008A1/ru), based on a Lumam-I1 fluorescence microscope (Lomo, St. Petersburg, Russia) with a mercury lamp as a light source.

The genome size in relative units was determined for each individual as the ratio of the average value of

Table 1. Collection sites, coordinates, altitude, sample size (*n*), and genome size variability in rock lizards of the genus *Darevskia*

No.	Taxon	Location	Coordinates, degrees N, E	Altitude, m above sea level	*n*	Genome size, pg	
						Mean ± SD (CV)	Range
Complex *D. caucasica*							
1	*alpina*	Mount Dzykhva, Abkhazia	43.217, 41.150	1500	1	3.21	–
2	*alpina*	Kamyshanaya Polyana, Adygea, Russia	44.105, 40.017	1450	1	3.25	–
3	*alpina*	Mount Zakan, Karachai-Cherkessia, Russia	43.708, 40.800	2000	1	3.31	–
Total for the species *D. alpina*					3	3.26 ± 0.05 (1.4)	3.21–3.31
4	*caucasica caucasica*	Lechinkai, Kabardino-Balkaria, Russia	43.533, 43.367	800	1	3.24	–
5	*caucasica caucasica*	Tamisk River gorge, North Ossetia–Alania, Russia	42.917, 44.183	950	12	3.20 ± 0.02 (0.7)	3.18–3.26
Total for the subspecies *D. c. caucasica*					13	3.21 ± 0.02 (0.8)	3.18–3.26
6	*caucasica vedenica*	Kharachoi, Chechnya, Russia	42.883, 46.117	1500	3	3.32 ± 0.04 (1.3)	3.28–3.36
Total for the species *D. caucasica*					16	3.23 ± 0.05 (1.7)	3.18–3.36
7	*dagestanica*	Khupri, Dagestan, Russia	42.203, 45.845	1758	4	3.23 ± 0.07 (2.1)	3.17–3.33
8	*derjugini abchasica*	Sukhum, Abkhazia	43.000, 41.044	20	1	3.47	–
9	*derjugini abchasica*	Verkhnyaya Gumista River gorge, Abkhazia	43.183, 41.017	550	7	3.42 ± 0.03 (1.0)	3.37–3.47
10	*derjugini abchasica*	Buru Ridge, Abkhazia	43.233, 41.100	1400	2	3.38 ± 0.02	3.37–3.39
Total for the subspecies *D. d. abkhazica*					10	3.42 ± 0.04 (1.1)	3.37–3.47
11	*derjugini derjugini*	Bakuriani, Georgia	41.733, 43.533	1660	1	3.25	–
12	*derjugini derjugini*	Chobiskhevi, Georgia	41.768, 43.318	1225	2	3.21 ± 0.00	3.21–3.21
Total for the subspecies *D. d. derjugini*					3	3.22 ± 0.03 (0.8)	3.21–3.25
Total for the species *D. derjugini*					13	3.37 ± 0.09 (2.7)	3.21–3.47
13	*mixta*	Akhaltsikhe, Georgia	41.925, 43.486	800	1	3.31	–
14	*mixta*	Ertso Lake, South Ossetia	42.467, 43.751	1720	5	3.28 ± 0.03 (0.9)	3.25–3.33
Total for the species *D. mixta*					6	3.28 ± 0.03 (1.0)	3.25–3.33

Table 1. (Contd.)

No.	Taxon	Location	Coordinates, degrees N, E	Altitude, m above sea level	*n*	Genome size, pg	
						Mean ± SD (CV)	Range
Complex of *D. chlorogaster*							
15	*caspica*	Kandovan, Iran	36.590, 51.390	205	5	3.34 ± 0.03 (0.9)	3.31–3.37
16	*chlorogaster*	Piran, Azerbaijan	38.683, 48.633	602	2	3.43 ± 0.03	3.41–3.44
17	*chlorogaster*	Siaku, Azerbaijan	38.594, 48.788	30	4	3.40 ± 0.03 (1.0)	3.37–3.45
18	*chlorogaster*	Sym, Azerbaijan	38.500, 48.650	500	2	3.26 ± 0.00	3.26–3.27
19	*chlorogaster*	Vilyash-chai River gorge, Azerbaijan	38.969, 48.532	230	2	3.27 ± 0.03	3.25–3.29
Total for the species *D. chlorogaster*					10	3.35 ± 0.08 (2.4)	3.25–3.45
Complex of *D. parvula*							
20	*adjarica*	Akhaltsikhe, Georgia	41.925, 43.486	800	1	3.64	–
Complex *D. praticola*							
21	*pontica*	Staroshcherbinovskaya, Krasnodar krai, Russia	46.644, 38.657	10	1	3.25	–
22	*pontica*	Krepostnaya, Krasnodar krai, Russia	44.703, 38.693	74	10	3.22 ± 0.02 (0.7)	3.19–3.28
23	*pontica*	Psebai, Krasnodar krai, Russia	44.098, 40.771	685	1	3.23	–
24	*pontica*	Khadzhokh, Adygea, Russia	44.309, 40.189	407	2	3.22 ± 0.01	3.21–3.22
25	*pontica*	Kamyshanaya Polyana, Adygea, Russia	44.168, 40.041	1200	1	3.30	–
26	*pontica*	Sovkhoznyi, Adygea, Russia	44.545, 40.150	255	1	3.22	–
27	*pontica*	Mount Strizhament, Stavropol krai, Russia	44.808, 42.068	500	2	3.33 ± 0.00	3.33–3.34
Total for the species *D. pontica*					18	3.24 ± 0.04 (1.4)	3.19–3.34
28	*praticola hyrcanica*	Gadazydakhi point, Azerbaijan	38.467, 48.583	1510	1	3.25	–
29	*praticola loriensis*	Dilijan, Armenia	40.738, 44.837	1316	2	3.37 ± 0.00	3.37–3.38
30	*practicola practicola*	Staropavlovskaya, Stavropol krai, Russia	43.833, 43.617	330	3	3.28 ± 0.04 (1.1)	3.24–3.31
31	*practicola practicola*	Krasnokumskoe, Stavropol krai, Russia	44.184, 43.507	250	3	3.31 ± 0.04 (1.2)	3.27–3.33
32	*practicola practicola*	Shaumyansky, Stavropol krai, Russia	44.161, 43.523	254	1	3.29	–

Table 1. (Contd.)

No.	Taxon	Location	Coordinates, degrees N, E	Altitude, m above sea level	*n*	Genome size, pg	
						Mean ± SD (CV)	Range
33	*practicola practicola*	Vladikavkaz, North Ossetia–Alania, Russia	42.995, 44.690	780	1	3.30	–
Total for the subspecies *D. p. praticola*					8	3.30 ± 0.03 (0.9)	3.24–3.33
Total for the species *D. practicola*					11	3.31 ± 0.04 (1.3)	3.24–3.38
Complex of *D. raddei*							
34	*raddei nairensis*	Mount Hatis, Armenia	40.217, 44.650	1550	8	3.27 ± 0.01 (0.4)	3.25–3.30
35	*raddei nairensis*	Yerevan, Armenia	40.167, 44.483	950	3	3.27 ± 0.04 (1.1)	3.23–3.30
36	*raddei nairensis*	Bjni, Armenia	40.448, 44.624	1641	3	3.37 ± 0.02 (0.6)	3.36–3.40
37	*raddei nairensis*	Lchashen, Armenia	40.511, 44.951	1916	3	3.33 ± 0.04 (1.1)	3.29–3.37
Total for the subspecies *D. r. nairensis*					17	3.30 ± 0.05 (1.4)	3.23–3.40
38	*raddei raddei*	Sevkar, Armenia	41.017, 45.133	940	7	3.30 ± 0.05 (1.4)	3.25–3.37
39	*raddei raddei*	Tilyakant, Azerbaijan	38.950, 48.500	460	2	3.28 ± 0.00	3.28–3.28
40	*raddei raddei*	Sym, Azerbaijan	38.489, 48.641	480	2	3.20 ± 0.04	3.17–3.23
Total for the subspecies *D. r. raddei*					11	3.28 ± 0.06 (1.7)	3.17–3.37
Total for the species *D. raddei*					28	3.29 ± 0.05 (1.5)	3.17–3.40
Complex of *D. rudis*							
41	*bithynica tristis*	Abant Lake, Turkey	40.609, 31.289	1331	6	3.38 ± 0.02 (0.5)	3.36–3.41
42	*portschinskii nigrita*	Stepanovan, Armenia	41.014, 44.383	1357	4	3.48 ± 0.06 (1.7)	3.40–3.54
43	*rudis chechenica*	Verkhnii Lars, North Ossetia–Alania, Russia	42.750, 44.617	1600	1	3.36	–
44	*rudis chechenica*	Patsa River gorge, South Ossetia	42.366, 43.889	1100	5	3.41 ± 0.01 (0.3)	3.40–3.43
Total for the subspecies *D. r. chechenica*					6	3.40 ± 0.02 (0.7)	3.36–3.43
45	*rudis obscura*	Dabadzveli, Georgia	41.768, 43.332	1522	1	3.18	–
46	*rudis obscura*	Akhaltsikhe, Georgia	41.925, 43.486	800	1	3.40	–
Total for the subspecies *D. r. obscura*					2	3.29 ± 0.15	3.18–3.40
Total for the species *D. rudis*					8	3.37 ± 0.08 (2.3)	3.18–3.43

Table 1. (Contd.)

No.	Taxon	Location	Coordinates, degrees N, E	Altitude, m above sea level	*n*	Genome size, pg	
						Mean ± SD (CV)	Range
47	*valentini*	Mount Hatis, Armenia	40.217, 44.650	1550	4	3.34 ± 0.05 (1.6)	3.31–3.42
Complex of *D. saxicola*							
48	*arribasi*	Ertso Lake, South Ossetia	42.467, 43.751	1720	8	3.37 ± 0.10 (2.9)	3.27–3.51
49	*brauneri brauneri*	Verkhnyaya Gumista River gorge, Abkhazia	43.183, 41.017	550	5	3.19 ± 0.11 (3.4)	3.01–3.28
50	*brauneri brauneri*	Buru Ridge, Abkhazia	43.233, 41.100	1400	1	3.24	–
51	*brauneri brauneri*	Bzyb River gorge, Abkhazia	43.241, 40.396	80	1	3.22	–
52	*brauneri brauneri*	Ochamchira Ridge, Abkhazia	42.920, 41.554	220	3	3.27 ± 0.01 (0.4)	3.25–3.28
53	*brauneri brauneri*	Tuapse, Krasnodar krai, Russia	44.100, 39.050	100	1	3.15	–
54	*brauneri brauneri*	Novomikhaylovskii, Krasnodar krai, Russia	44.250, 38.850	60	5	3.26 ± 0.02 (0.5)	3.23–3.27
55	*brauneri brauneri*	Sochi, Krasnodar krai, Russia	43.563, 39.761	80	1	3.18	–
Total for the species *D. brauneri*					17	3.23 ± 0.07 (2.1)	3.01–3.28
56	*lindholmi*	Cape Fiolent, Sevastopol, Russia	44.516, 33.474	25	4	3.44 ± 0.05 (1.4)	3.37–3.47
57	*lindholmi*	Angarskii Pass, Crimea, Russia	44.717, 34.344	490	1	3.41	–
Total for the species *D. lindholmi*					5	3.43 ± 0.04 (1.3)	3.37–3.47
58	*saxicola*	Nizhnyaya Yermolovka, Karachai-Cherkessia, Russia	43.741, 41.503	1128	2	3.28 ± 0.03	3.26–3.30
59	*saxicola*	Nizhnyaya Teberda, Karachai-Cherkessia, Russia	43.630, 41.868	1058	3	3.32 ± 0.01 (0.4)	3.30–3.33
60	*saxicola*	Chegem River gorge, Kabardino–Balkaria, Russia	43.383, 43.183	1200	1	3.03	–
61	*saxicola*	Kislovodsk, Stavropol krai, Russia	43.950, 42.733	800	4	3.55 ± 0.02 (0.5)	3.53–3.56
62	*saxicola*	Nikitino, Krasnodar krai, Russia	43.959, 40.676	1400	7	3.18 ± 0.03 (0.9)	3.13–3.21
Total for the species *D. saxicola*					17	3.29 ± 0.16 (4.9)	3.03–3.56

Table 1. (Contd.)

No.	Taxon	Location	Coordinates, degrees N, E	Altitude, m above sea level	*n*	Genome size, pg	
						Mean ± SD (CV)	Range
Parthenogenetic species of hybrid origin							
63	*armeniaca* (*mixta* ♀ × *valentini* ♂)	Ankavan, Armenia	40.633, 44.483	1980	1	3.31	–
64	*armeniaca* (*mixta* ♀ × *valentini* ♂)	Sevkar, Armenia	41.017, 45.133	940	15	3.39 ± 0.04 (1.3)	3.31–3.44
65	*armeniaca* (*mixta* ♀ × *valentini* ♂)	Privolnoye, Armenia	41.139, 44.395	1771	4	3.28 ± 0.01 (0.4)	3.26–3.29
66	*armeniaca* (*mixta* ♀ × *valentini* ♂)	Dilijan, Armenia	40.739°C 44.846	1264	5	3.36 ± 0.04 (1.1)	3.31–3.41
Total for the species *D. armeniaca*					25	3.36 ± 0.06 (1.7)	3.26–3.44
67	*dahli* (*mixta* ♀ × *portschinskii* ♂)	Sevkar, Armenia	41.017, 45.133	940	2	3.35 ± 0.00	3.35–3.35
68	*dahli* (*mixta* ♀ × *portschinskii* ♂)	Stepanovan, Armenia	41.014, 44.383	1357	7	3.39 ± 0.03 (0.8)	3.36–3.43
69	*dahli* (*mixta* ♀ × *portschinskii* ♂)	Tumanyan, Armenia	41.000, 44.650	850	3	3.35 ± 0.00 (0.1)	3.35–3.35
70	*dahli* (*mixta* ♀ × *portschinskii* ♂)	Dilijan, Armenia	40.739°C 44.846	1264	2	3.37 ± 0.07	3.33–3.42
Total for the species *D. dahli*					14	3.38 ± 0.03 (1.0)	3.33–3.43
71	*rostombekowi* (*r. raddei* ♀ × *portschinski* ♂)	Sevkar, Armenia	41.017, 45.133	940	3	3.35 ± 0.01 (0.2)	3.34–3.35
72	*unisexualis* (*r. nairensis* ♀ × *valentini* ♂)	Mount Hatis, Armenia	40.217, 44.650	1550	12	3.33 ± 0.01 (0.2)	3.32–3.35
73	*unisexualis* (*r. nairensis* ♀ × *valentini* ♂)	Kuchak, Armenia	40.520, 44.381	1880	1	3.37	–
74	*unisexualis* (*r. nairensis* ♀ × *valentini* ♂)	Unknown locality, Armenia			9	3.34 ± 0.04 (1.3)	3.27–3.43
Total for the species *D. unisexualis*					22	3.34 ± 0.03 (0.9)	3.27–3.43
Natural hybrid triploids							
75	*armeniaca* × *r. nairensis*	Hrazdan, Armenia	40.500, 44.767	1750	1	4.99	–
76	*armeniaca* × *valentini*	Mount Tezh, Armenia	40.617, 44.467	1950	4	5.04 ± 0.12 (2.4)	4.94–5.20
77	*dahli* × *portschinskii*	Stepanovan, Armenia	41.014, 44.383	1357	1	5.08	–
78	*dahli* × *r. nairensis*	Kojori, Georgia	41.667, 44.700	1410	1	5.02	–
79	*rostombekovi* × *r. raddei*	Gosh, Armenia	40.717, 45.000	1300	2	5.18 ± 0.08	5.12–5.24
80	*unisexualis* × *r. nairensis*	Ltap, Armenia	40.450, 45.067	1970	1	4.97	–
81	*unisexualis* × *r. nairensis*	Hrazdan, Armenia	40.500, 44.767	1750	7	4.97 ± 0.02 (0.3)	4.95–4.99

Table 1. (Contd.)

No.	Taxon	Location	Coordinates, degrees N, E	Altitude, m above sea level	*n*	Genome size, pg	
						Mean ± SD (CV)	Range
Total for *D. unisexualis* × *D. r. nairensis*					8	4.97 ± 0.02 (0.3)	4.95–4.99
82	*unisexualis* × *valentini*	Kuchak, Armenia	40.520, 44.381	1880	8	5.05 ± 0.10 (2.1)	4.95–5.22
Laboratory diploid hybrids							
83	*chlorogaster* × *caspica*	Haneh-e-Asyab, Iran Kandovan, Iran	38.380, 48.760 36.590, 51.390	160 205	3	3.40 ± 0.01 (0.4)	3.38–3.41

the peak of the studied cells on the DNA histogram to the peak for the reference species. To analyze the measurement results, the CytExpert 2.0 (Beckman Coulter) and BARS (Institute of Cytology, Russian Academy of Sciences) programs were used. Because of the GC specificity of olivomycin, the genome size estimates obtained by staining nuclei with a mixture of this dye and ethidium bromide were overestimated. Therefore, we multiplied them by an experimentally calculated coefficient, which, on average, for lizards of this genus was equal to 0.871. Some other details of the method have been described previously (Rozanov and Vinogradov, 1998; Borkin et al., 2001).

To assess the influence of climate conditions on the genome size among populations of diploid rock lizard species, we used the mean annual temperatures and precipitation (climate data for ~1950–2000) from the WorldClim 2.1 database (Fick and Hijmans, 2017).

RESULTS

When studying the genome size of rock lizards, we identified 251 (91%) diploid and 25 (9%) triploid individuals (Table 1). The range of variability in individuals with different ploidy did not overlap (3.01–3.64 and 4.94–5.24 pg, respectively; Fig. 2). Among diploid species, we did not identify any sexual differences. No reliable correlation between the amount of nuclear DNA in populations and the geographic latitude (r^2 = 0.0002, df = 1.60, p = 0.92), longitude (r^2 = 0.21, df = 1.60, p = 0.26), altitude (r^2 = 0.00001, df = 1.60, p = 0.98), the average annual temperatures (r^2 = 0.002, df = 1.60, p = 0.71), and the amount of precipitation

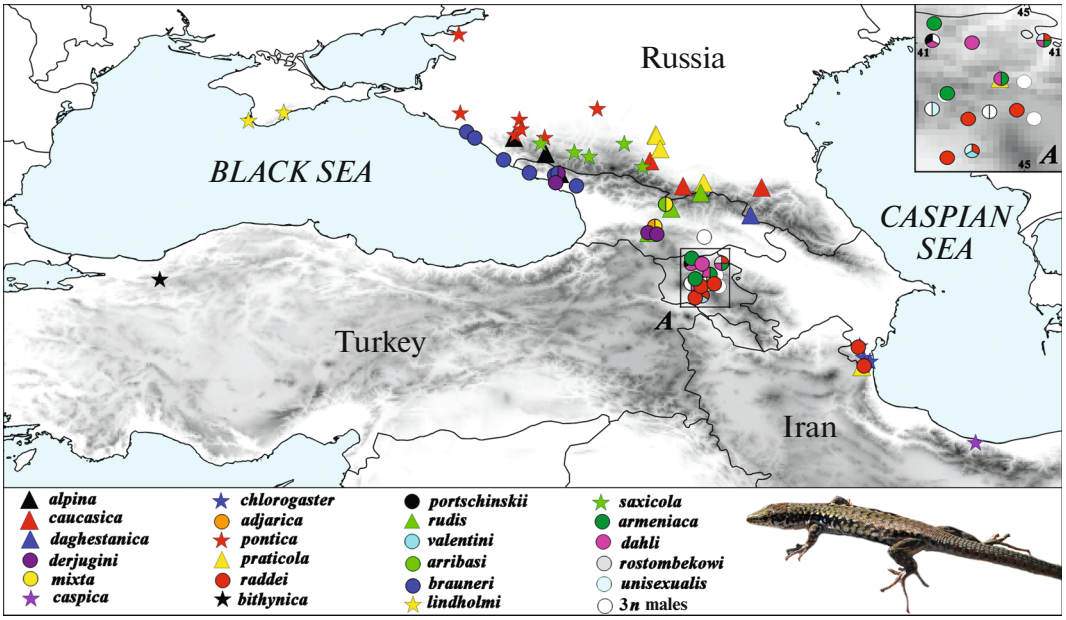

Fig. 1. Map of localities of rock lizards of the genus *Darevskia*. The insert in the upper right corner (a) covers the territory of northern Armenia. There is a photo in the lower right corner of *D. lindholmi* from Cape Fiolent (Sevastopol).

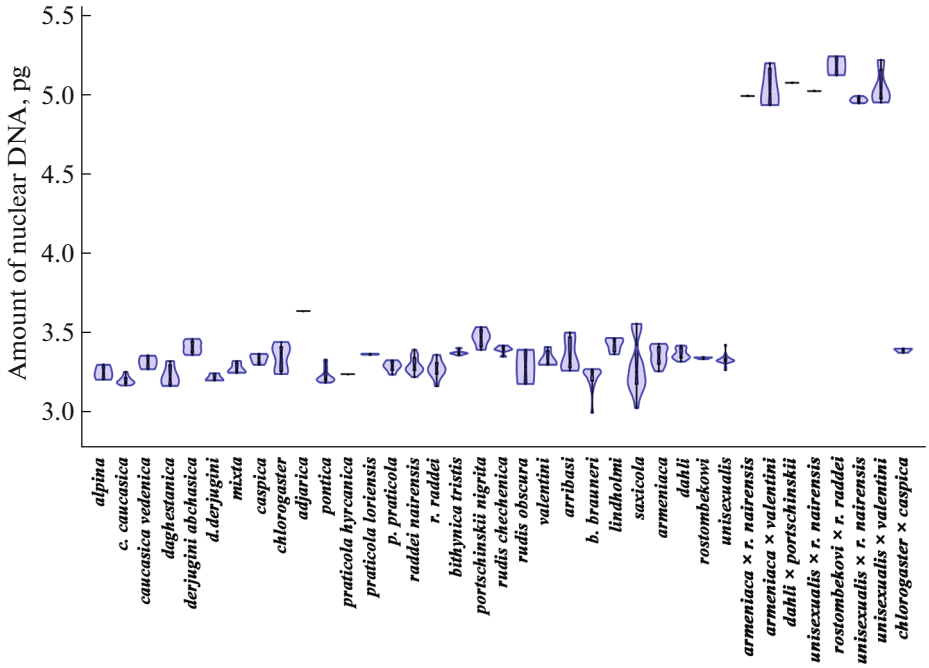

Fig. 2. Variability (violin-plots) of the amount of nuclear DNA in different species, subspecies, and interspecific hybrids of rock lizards of the genus *Darevskia*.

($r^2 = 0.04$, $df = 1.60$, $p = 0.11$) was detected. The highest altitude species of the genus, *D. alpina* (Darevsky 1967), was practically no different by genome size from the lowland *D. pontica* (Lantz et Cyrén 1918) and *D. praticola* (Eversmann 1834). There were no differences between species living in areas with different moisture conditions.

No relationship was observed between the genome size and the phylogenetic relationships of species. Representatives of all complexes studied had approximately the same limits of variability (3.01–3.56 pg). The only exception was *D. adjarica* (Darevsky et Eiselt 1980) (3.64 pg; Fig. 2). Significant intraspecific variability was noted in a number of species. Thus, all the samples we studied that belonged to the subspecies *D. derjugini abchasica* (Bischoff 1982) were characterized by elevated genome size values (3.37–3.47 pg). They differed sharply not only from the nominative subspecies (3.21–3.25 pg), but also from other species of the complex (Table 1, Fig. 2). Also, increased intraspecific variability was noted within *D. saxicola* (Eversmann 1834). The population from Kislovodsk (3.53–3.56 pg) significantly differed in elevated values from other populations of this species (3.03–3.33 pg; Fig. 2).

All three laboratory hybrids between closely related species *D. chlorogaster* (Boulenger 1908) and *D. caspica* Ahmadzadeh, Flecks, Carretero, Mozaffari, Böhme, Harris, Freitas et Rödder 2013 were found to be diploid. Their average genome size (3.40 pg) was slightly higher than that of their parent species (3.34 and 3.35 pg). This small deviation (1.6%) may be due to the fact that the measurement of the amount of nuclear DNA in hybrids and their parental species was carried out at different times and with different individuals of the reference species. This could have significantly influenced the results of our measurements, since the genome size of, for example, *Rana temporaria* from Leningrad oblast, used as a reference standard, can vary from 10.0 to 10.5 pg (our unpublished data).

Comparison of the genome size of diploid parthenogenetic species of hybrid origin with bisexual species of this genus generally did not reveal any significant differences between them. The amount of nuclear DNA in parthenogenetic species, as a rule, did not go beyond the limits of variability in the parental species (3.26–3.44 pg and 3.17–3.54, respectively; Table 1). However, when comparing the average values, the genome size of parthenogenetic species sometimes coincided with the average obtained for the parental species and sometimes differed slightly from them. Thus, the average genome size of *D. dahli* (Darevsky 1957) was equal to 3.38 pg, and in the parents *D. mixta* and *D. portschinskii* (Kessler 1878), it was 3.28 and 3.48 pg, respectively; in D. *rostombekovi* (Darevsky 1957) – 3.35 pg, and *D. r. raddei* (Boettger 1892) and *D. portschinskii,* 3.28 and 3.48 pg; *D. unisexualis* (Darevsky 1966), 3.34 pg, and *D. raddei nairensis* (Darevsky 1967) and *D. valentini,* 3.30 and 3.34 pg; *D. armeniaca* (Méhely, 1909), 3.36 pg, and *D. mixta* and *D. valentini,* 3.28 and 3.34 pg. It is important to note that the measurement of the amount of nuclear

DNA in parthenogenetic and parental species was carried out at different times and with different individuals of the reference standard species, which could have influenced the appearance of such relatively small differences from the average, such as, for example, in *D. armeniaca* (up to 1.5%).

A study of triploid hybrids showed that the size of their genome roughly corresponds to the sum of the average diploid genome size of the maternal parthenogenetic species and the haploid genome of the paternal species. Thus, triploid hybrid *D. armeniaca* × *D. r. nairensis* had an average genome size of 4.99 pg, and the sum of the parental genomes was 5.01 pg; in the hybrid *D. armeniaca* × *D. valentines* 5.04 with the estimated 5.03 pg, respectively; *D. dahli* × *D. portschinskii* (5.08 and 5.12 pg); *D. dahli* × *D. r. nairensis* (5.02 and 4.99 pg); *D. unisexualis* × *D. r. nairensis* (4.97 and 4.99 pg); *D. unisexualis* × *D. valentini* (5.05 and 5.01 pg). The only exception were hybrid triploids *D. rostombekovi* × *D. r. raddei*, in which the amount of nuclear DNA was significantly (by 3.7%) higher than the calculated values (5.18 versus 4.99 pg).

The genome size variability within triploid hybrid samples (CV = 0.3–2.4%) was, on average, slightly higher than that of most diploid parthenogenetic species (0.2–1.7%), but slightly lower than that of bisexual species (0.5–4.9%).

DISCUSSION

Comparison of our results with data from other authors showed that the use of different methods can yield very different results. Thus, Chilingaryan and Pavlov (1961) determined the genome size in blood cells of rock lizards (then classified as belonging to the genus *Lacerta* Linnaeus 1758) according to the method of Dische (1931). They found that the average amount of nuclear DNA in parthenogenetic *D. armeniaca* equals 6.05 pg, bisexual *D. valentini* ("*Saxicola terentijevi*") was 6.30 pg; and their triploid hybrid was 13.29 pg. These values turned out to be much higher than the estimates we obtained in this work (the average for *D. armeniaca* was 3.36 pg; *D. valentini,* 3.34 pg; and their triploid hybrid, 5.04 pg). The large difference between the results can be explained by the imperfection of the method of Dische used by these authors.

Darevsky and Kupriyanova (1982) measured the genome size in erythrocytes of three individuals of rock lizards (Stepanovan, Armenia) using the Feulgen reaction (Feulgen and Rossenbeck, 1924). The average amount of nuclear DNA in parthenogenetic *D. armeniaca* was equal to 106.3 conventional units; in bisexual *D. portschinskii* was 108.2; and in the triploid hybrid *D. dahli* × *D. portschinskii* was 140.0. If we recalculate these units to the average genome size we obtained (3.36 pg for *D. armeniaca*), then the amount of nuclear DNA in *D. portschinskii* will be equal to 3.42 pg and in the triploid hybrid, 4.43 pg. These values turned out to be quite close to the data we obtained for the first species (3.48 pg), but differed greatly (by 14%) from those we obtained for these hybrids (5.08 pg). This is probably due to the fact that the measurements were carried out under different staining conditions (the cells of each individual were stained on a separate slide) and without comparison with the cells of the reference standard species. With this experimental design, the results of nuclear DNA quantity measurements are usually inaccurate.

A later study of the genome size using DNA flow cytometry in rock lizards was conducted on four individuals from the valley of the Marmarik River in Armenia (Darevsky et al., 1989). One individual parthenogenetic *D. armeniaca,* two triploid hybrids *D. unisexualis* × *D. valentini,* and one putative hybrid between parthenogenetic species *D. unisexualis* and *D. armeniaca* were studied (in parthenogenetic species, fertile males may occasionally appear (Darevsky and Kupriyanova, 1982)). All individuals studied were collected in one locality, in which *D. valentini* was previously artificially introduced (Darevsky and Danielyan, 1968). Later, these same authors (Darevsky et al., 1991) additionally studied the genome size of one individual *D. unisexualis* from the same location. All measurements were made on the same instrument base (laboratory model cytometer) and using the same reagents as in our work. Therefore, the data obtained in this paper (*D. armeniaca* 0.57 × 0.871 × 6.8 = 3.38 pg; *D. unisexualis* 3.38 pg; triploid hybrids *D. unisexualis* × *D. valentini,* 5.21 pg) are very close to our results (Table 1).

Recently, Ochkalova et al. (2022) presented data on nuclear genome sequencing of *D. valentini* and estimated the size of the haploid genome of this species at 1.46 Gb. This estimate differs somewhat from our results, since, if we use the formula proposed by Dolezel et al. (2003), the diploid genome size of this species should be equal to 2.99 pg, which is obviously (11.1%) lower than our estimate (3.34 pg). Such a noticeable difference can be caused by a number of reasons, but two can be suggested as the main ones.

First, the estimated amount of nuclear DNA in the reference species used in our study may be overestimated. For example, we believe that the average genome size of a male *Mus musculus* is 6.8 pg (Bianchi et al., 1983). But some authors estimated the size of the haploid genome of this species at 2.5 Gb (i.e., 2n = 5.11 pg; https://www.ncbi.nlm.nih.gov/datasets/genome/GCA_029233825.1/), which is significantly lower (by 28.4%) than our estimate.

Secondly, genome size estimates based on sequencing data can vary significantly among different authors. For example, the amount of nuclear DNA in the haploid genome of the three grass frog individuals sequenced to date was estimated at 3.7 Gb (https://www.ncbi.nlm.nih.gov/datasets/genome/GCA_ 905171725.1/),

4.1 Gb (https://www.ncbi.nlm.nih.gov/datasets/genome/GCF_905171775.1/), and 4.3 Gb (https://www.ncbi.nlm.nih.gov/datasets/genome/GCA_009802015.1/). These significant differences (up to 15%) are likely due to difficulties in accounting for the number of repeating elements and the use of different software.

It is important to note that, when using DNA flow cytometry and ethidium bromide as a dye, it is possible to avoid the difficulties that arise when comparing data obtained by different methods. Our studies confirmed that using this method allows us to determine ploidy reliably in each of the rock lizard individuals studied. We previously obtained similar results when studying diploid-polyploid complexes, for example, in amphibians (Borkin et al., 2004; Litvinchuk et al., 2010; Biriuk et al., 2016; Litvinchuk et al., 2018, 2019).

The homogeneity in genome size variability among diploid subspecies and species of rock lizards is noteworthy: 3.01–3.64 pg in bisexual taxa and 3.26–3.44 pg in parthenogenetic hybrid species. There are no major differences between subspecies, species, and even species complexes, or between bisexual and parthenogenetic species.

No geographic variation (correlation of genome size with geographic coordinates) could be detected, despite the large territory covered by our samples, including the entire Caucasus region, the Crimea, Turkey, and Iran (Table 1, Fig. 1). As an example, we note that such a correlation exists, for example, in tailed amphibians in the family Salamadridae Goldfuss 1820 (Litvinchuk et al., 2007).

We also found no correlation with the altitude of the habitats, although the range of altitudes from which the samples were taken extended from sea level to 2000 m. There is no obvious dependence of the genome size on ecological and geographical conditions, for example, on the degree of humidity of the climate, although some species clearly gravitate towards more humid, and others, towards drier habitats. The literature discusses a possible relationship between genome size and environmental temperature in vertebrates (Canapa et al., 2020), as well as environmental temperature and humidity in insects (Gregory et al., 2013).

The study of the evolutionary relationships between species of rock lizards served as the basis for the development of a universal theory of reticulate (=hybridogenic) speciation (Borkin and Darevsky, 1980). This theory explains the origin of polyploid animal species through hybridization. According to it, the emergence of diploid parthenogenetic lineages as a result of natural interspecific hybridization between closely related bisexual species is the first stage of reticulate speciation. The second stage is the emergence of allotriploid forms as a result of hybridization of maternal diploid parthenogenetic and paternal bisexual species. In the third stage, fertile triploid females mate with males of bisexual species and eventually give rise to a new bisexual tetraploid species.

Indeed, modern research confirms that interspecific hybridization is a widespread phenomenon in many animal groups (Borkin and Litvinchuk, 2013). But, as a rule, hybridization between closely related species of terrestrial vertebrates at the boundaries of their ranges (parapatry) or in zones of their overlap (sympatry) does not lead to the formation of either parthenogenetic lineages or polyploid individuals. For example, in amphibians, discoveries of polyploid individuals in zones of interspecific hybridization of diploid species are extremely rare (Litvinchuk et al., 2016). In reptiles, polyploid individuals have not yet been discovered in the hybridization zones of diploid bisexual species.

However, most of the studied polyploid lineages among vertebrates are of hybrid origin (Litvinchuk et al., 2016; Stöck et al., 2021). This is apparently explained by the fact that they only appear if hybridization occurs between phylogenetically distant species (Dufresnes et al., 2019, 2021). Reproductive isolation between species develops gradually through the accumulation of many "barrier loci", each of which has only a small effect. When species are young, their genomes can easily mix without polyploidy. However, if the divergence time between them is relatively large, then the gene flow between them becomes extremely limited, since many of the genes can no longer function normally in hybrids (Dufresnes et al., 2021).

In this case, the only way out for such hybrids is the transition to one of the types of clonal reproduction, such as parthenogenesis, gynogenesis, creditogenesis, etc. (Litvinchuk, 2021). However, with such methods of reproduction, a rapid accumulation of harmful mutations occurs ("Muller's ratchet"; Muller, 1932), which can lead to significant difficulties in reproduction and ultimately to the extinction of the species. The way out of this situation is the transition to polyploidy (an increase in the number of genomes leads to a decrease in the influence of harmful mutations). For example, in green frogs of the genus *Pelophylax* Fitzinger 1843 testicular anomalies in triploid hybrids are much less common than in diploid hybrids (Litvinchuk, 2018).

However, unlike many other groups of reptiles, obligate triploid lineages have not been found in rock lizards. Only occasionally in the contact zones of parthenogenetic and bisexual species can single polyploid individuals appear, which are usually sterile (Danielyan et al., 2008). This situation is probably due to the fact that this process is only at the beginning of its evolutionary path (Arakelyan et al., 2023). However, it is important to note that, unlike fish and amphibians, not a single tetraploid species has yet been identified among reptiles in natural conditions. Based on this, we can conclude that reptiles (as well as amniotes in general) have some internal (probably genetic) limitations

that prevent the transition to the third and final stage of reticulate speciation.

ACKNOWLEDGMENTS

This article uses field collections and laboratory measurements of I.S. Darevsky (1924–2009), F.D. Danielyan (1938–2021), and Yu.M. Rozanov (1938–2021), without whom this work would not have been possible. The authors are also grateful to K.D. Milto, K.Yu. Lotiev, M.S. Arakelyan, A.A. Ivanov, and K.A. Matushkina for invaluable assistance in conducting field and laboratory research, as well as to D.V. Skorinov and I.A. Martirosyan for valuable comments and review of this paper.

AUTHOR CONTRIBUTION

The authors contributed equally to the writing of this article.

FUNDING

This work was carried out within the framework of the Zoological Institute, Russian Academy of Sciences, topic no. 122031100282–2 (L.J. Borkin, I.V. Doronin) and in part (A.A. Kidov, V.O. Erashkin) at the expense of the Development Program of the Russian State Agrarian University, Timiryazev Moscow Agricultural Academy within the framework of the Strategic Academic Leadership Program "Priority-2030."

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. The compliance of the study with international ethical standards was confirmed by the Commission of the Russian State Agrarian University, Timiryazev Moscow Agricultural Academy on bioethics (protocol no. 1 dated September 6, 2019).

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

REFERENCES

Abdala, C.S., Baldo, D., Juárez, R.A., and Espinoza, R.E., The first parthenogenetic pleurodont iguanian: A new all-female *Liolaemus* (Squamata: Liolaemidae) from Western Argentina, *Copeia*, 2016, vol. 104, no. 2, pp. 487–497. https://doi.org/10.1643/ch-15-381

Ahmadzadeh, F., Flecks, M., Carretero, M.A., Mozaffari, O., Böhme, W., Harris, D.J., Freitas, S., and Rödder, D., Cryptic speciation patterns in Iranian rock lizards uncovered by integrative taxonomy, *PLoS One*, 2013, vol. 8, no. 12, p. e80563. https://doi.org/10.1371/journal.pone.0080563

Arakelyan, M., Spangenberg, V., Petrosyan, V., Ryskov, A., Kolomiets, O., and Galoyan, E., Evolution of parthenogenetic reproduction in Caucasian rock lizards: A review, *Current Zoology*, 2023, vol. 69, no. 2, pp. 128–135. https://doi.org/10.1093/cz/zoac036

Arribas, O.J., Phylogeny and relationships of the mountain lizards of Europe and Near East (*Archaeolacerta* Mertens, 1921 sensu lato) and their relationships among the Eurasian lacertid radiation, *Russ. J. Herpetol.*, 1999, vol. 6, no. 1, pp. 1–22.

Bezy, R.L., Karyotypic variation and evolution of the lizards in the family Xantusiidae, *Contributions in Science*, 1972, vol. 227, pp. 1–29. https://doi.org/10.5962/p.241212

Bianchi, N.O., Redi, C., Garagna, C., Capanna, E., and Manfredi-Romanini, M.G., Evolution of the genome size in *Akodon* (Rodentia, Cricetidae), *J. Mol. Evol.*, 1983, vol. 19, no. 5, pp. 362–370. https://doi.org/10.1007/bf02101640

Bickham, J.W. and Hanks, B.G., Diploid-triploid mosaicism and tissue ploidy diversity within *Platemys platycephala* from Suriname, *Cytogenet. Genome Res.*, 2009, vol. 127, nos. 2–4, pp. 280–286. https://doi.org/10.1159/000297716

Bickham, J.W., Tucker, P.K., and Legler, J.M., Diploid-triploid mosaicism: An unusual phenomenon in side-necked turtles (*Platemys platycephala*), *Science*, 1985, vol. 227, no. 4694, pp. 1591–1593. https://doi.org/10.1126/science.227.4694.1591

Biriuk, O.V., Shabanov, D.A., Korshunov, A.V., Borkin, L.J., Lada, G.A., Pasynkova, R.A., Rosanov, J.M., and Litvinchuk, S.N., Gamete production patterns and mating systems in water frogs of the hybridogenetic *Pelophylax esculentus* complex in north-eastern Ukraine, *J. Zool. Syst. Evol. Res.*, 2016, vol. 54, no. 3, pp. 215–225. https://doi.org/10.1111/jzs.12132

Bogart, J.P., Evolutionary implications of polyploidy in amphibians and reptiles, *Polyploidy*, Lewis, W.H., Ed., Basic Life Sciences, vol. 13, New York: Springer, 1980, pp. 341–378. https://doi.org/10.1007/978-1-4613-3069-1_18

Borkin, L.J. and Darevsky, I.S., Retinal (hybridogenic) speciation in vertebrates, *Zh. Obshch. Biol.*, 1980, vol. 16, no. 4, pp. 485–507.

Borkin, L.J. and Litvinchuk, S.N., Animal hybridization, speciation, and systematics, Proceedings of the Zoological Institute RAS, 2013, vol. 317, no. s2, pp. 83–139.

Borkin, L.J. and Litvinchuk, S.N., In commemoration of Jury M. Rosanov (1938–2021), Current Studies in Herpetology, 2022, vol. 22, no. 3/4, pp. 166–182.

Borkin, L.J., Litvinchuk, S.N., and Rosanov, J.M., Spontaneous triploidy in the crested newt, *Triturus cristatus* (Salamandridae), *Russ. J. Herpetol.*, 1996, vol. 3, no. 2, pp. 152–156.

Borkin, L.J., Litvinchuk, S.N., Rosanov, J.M., and Milto, K.D., Cryptic speciation in *Pelobates fuscus* (Anura, Pelobatidae): evidence from DNA flow cytometry, *Amphibia-Reptilia*, 2001, vol. 22, no. 4, pp. 387–396. https://doi.org/10.1163/15685380152770354

Borkin, L.J., Korshunov, A.V., Lada, G.A., Litvinchuk, S.N., Rosanov, J.M., Shabanov, D.A., and Zinenko, A.I., Mass occurrence of polyploidy green frogs (*Rana esculenta* com-

plex) in eastern Ukraine, *Russ. J. Herpetol.*, 2004, vol. 11, no. 3, pp. 194–213.

Canapa, A., Biscotti, M.A., Barucca, M., Carducci, F., Carotti, E., and Olmo, E., Shedding light upon the complex net of genome size, genome composition and environment in chordates, *Eur. Zool. J.*, 2020, vol. 87, no. 1, pp. 192–202.
https://doi.org/10.1080/24750263.2020.1747558

Chilingaryan, A.A. and Pavlov, E.F., Quantitative measurements of DNA content in blood erythrocyte nuclei in interspecific avian and reptilian hybrids, *Dokl. Akad. Nauk Arm. SSR*, 1961, vol. 32, no. 1, pp. 55–60.

Cole, C.J., Taylor, H.L., Baumann, D.P., and Baumann, P., Neaves' whiptail lizard: The first known tetraploid parthenogenetic tetrapod (Reptilia: Squamata: Teiidae), *Breviora*, 2014, vol. 539, pp. 1–19.

Cole, Ch.J., Taylor, H.L., Neaves, W.B., Baumann, D.P., Newton, A., Schnittker, R., and Baumann, P., The second known tetraploid species of parthenogenetic tetrapod (Reptilia: Squamata: Teiidae): Description, reproduction, comparisons with ancestral taxa, and origins of multiple clones, *Bull. Mus. Comp. Zool.*, 2017, vol. 161, no. 8, pp. 285–321.
https://doi.org/10.3099/mcz37.1

Danielyan, F., Arakelyan, M., and Stepanyan, I., Hybrids of *Darevskia valentini, D. armeniaca* and *D. unisexualis* from a sympatric population in Armenia, *Amphibia-Reptilia*, 2008, vol. 29, no. 4, pp. 487–504.
https://doi.org/10.1163/156853808786230424

Polyploidy: Recent Trends and Future Perspectives, Dar, T.-Ul-H. and Rehman, R.-Ul., Eds., New Dehli: Springer, 2017.
https://doi.org/10.1007/978-81-322-3772-3

Darevsky, I.S., Danielyan, F.D., Rozanov, Yu.M., and Sokolova, T.M., Intraclonal mating and its likely evolutionary significance in a group of parthenogenetic species of rock lizards of the genus *Archaeolacerta, Zool. Zh.*, 1991, vol. 70, no. 5, pp. 63–74.

Darevsky, I.S. and Danielyan, F.D., Diploid and triploid progeny arising from natural mating of parthenogenetic *Lacerta armeniaca* and *L. unisexualis* with bisexual *L. saxicola valentini, J. Herpetol.*, 1968, vol. 2, no. 3/4, pp. 65–69.
https://doi.org/10.2307/1563104

Darevsky, I.S., Kupriyanova, L.A., and Bakradze, M.A., Occasional males and intersexes in parthenogenetic species of Caucasian rock lizards (genus *Lacerta*), *Copeia*, 1978, vol. 1978, no. 2, pp. 201–207.
https://doi.org/10.2307/1443551

Darevsky, I.S. and Kupriyanova, L.A., Rare males in parthenogenetic lizard *Lacerta armeniaca* Méhely, *Vertebrata Hungarica*, 1982, vol. 21, pp. 69–75.

Darevsky, I.S., Kupriyanova, L.A., and Uzzell, T., Parthenogenesis in reptiles, *Biology of Reptiles*, Gans, C. and Billet, F., Eds., New York: Wiley, 1985, vol. 15, pp. 411–526.

Darevsky, I.S., Danielyan, F.D., Sokolova, T.M., and Rozanov, Yu.M., Intraclonal mating in the parthenogenetic lizard species *Lacerta unisexualis, Evolution and Ecology of Unisexual Vertebrates*, Dawley, R.M. and Bogart, J.P., Eds., New York: Bulletin of the NY State Museum, 1989, pp. 228–235.

Disshe, Z., Nachweis und Bestimmung der Thymonukleinsaure, Abderhalden, E., *Handbuch der biologischen Arbeitsmethoden*, Wien, 1931, Abt. 5, vol. 2, no. 16, pp. 18–29.

Dolezel, J., Bartos, J., Voglmayr, H., and Greilhuber, J., Nuclear DNA content and genome size of trout and human, *Cytometry, Part A*, 2003, vol. 51, no. 2, pp. 127–128.
https://doi.org/10.1002/cyto.a.10013

Doronin, I.V., Systematics, phylogeny, and distribution of rock lizards supraspecific complexes *Darevskia* (*praticola*), *Darevskia* (*caucasica*), *Darevskia* (*saxicola*), *Cand. Sci. (Biol.) Dissertation*, St. Petersburg: Zoological Institute of the Russian Academy of Sciences, 2015.

Dufresnes, Ch., Mazepa, G., Jablonski, D., Oliveira, R.C., Wenseleers, T., Shabanov, D.A., Auer, M., Ernst, R., Koch, C., Ramírez-Chaves, H.E., Mulder, K.P., Simonov, E., Tiutenko, A., Kryvokhyzha, D., Wennekes, P.L., Zinenko, O.I., Korshunov, O.V., Al-Johany, A.M., Peregontsev, E.A., Masroor, R., Betto-Colliard, C., Denoël, M., Borkin, L.J., Skorinov, D.V., Pasynkova, R.A., Mazanaeva, L.F., Rosanov, J.M., Dubey, S., and Litvinchuk, S., Fifteen shades of green: The evolution of *Bufotes* toads revisited, *Mol. Phylogenet. Evol.*, 2019, vol. 141, p. 106615.
https://doi.org/10.1016/j.ympev.2019.106615

Dufresnes, Ch., Strachinis, I., Suriadna, N., Mykytynets, G., Cogălniceanu, D., Székely, P., Vukov, T., Arntzen, J.W., Wielstra, B., Lymberakis, P., Geffen, E., Gafny, S., Kumlutaş, Yu., Ilgaz, Ç., Candan, K., Mizsei, E., Szabolcs, M., Kolenda, K., Smirnov, N., Géniez, P., Lukanov, S., Crochet, P., Dubey, S., Perrin, N., Litvinchuk, S.N., and Denoël, M., Phylogeography of a cryptic speciation continuum in Eurasian spadefoot toads (*Pelobates*), *Mol. Ecol.*, 2019, vol. 28, no. 13, pp. 3257–3270.
https://doi.org/10.1111/mec.15133

Dufresnes, Ch., Brelsford, A., Jeffries, D.L., Mazepa, G., Suchan, T., Canestrelli, D., Nicieza, A., Fumagalli, L., Dubey, S., Martínez-Solano, I., Litvinchuk, S.N., Vences, M., Perrin, N., and Crochet, P.-A., Mass of genes rather than master genes underlie the genomic architecture of amphibian speciation, *Proc. Natl. Acad. Sci. U. S. A.*, 2021, vol. 118, no. 36, p. e2103963118.
https://doi.org/10.1073/pnas.2103963118

Feulgren, R. and Rossenbeck, H., Mikroskopisch-chemischer Nachweis einer Nucleinsäure vom Typus der Thymonucleinsäure und die- darauf beruhende elektive Färbung von Zellkernen in mikroskopischen Präparaten, *Hoppe-Seyler's Zeitschrift für Physiologische Chemie*, 1924, vol. 135, nos. 5–6, pp. 203–248.
https://doi.org/10.1515/bchm2.1924.135.5-6.203

Fick, S.E. and Hijmans, R.J., WorldClim 2: New 1-km spatial resolution climate surfaces for global land areas, *Int. J. Climatol.*, 2017, vol. 37, no. 12, pp. 4302–4315.
https://doi.org/10.1002/joc.5086

Freitas, S., Rocha, S., Campos, J., Ahmadzadeh, F., Corti, C., Sillero, N., Ilgaz, Ç., Kumlutaş, Yu., Arakelyan, M., Harris, D.J., and Carretero, M.A., Parthenogenesis through the ice ages: A biogeographic analysis of Caucasian rock lizards (genus *Darevskia*), *Mol. Phylogenet. Evol.*, 2016, vol. 102, pp. 117–127.
https://doi.org/10.1016/j.ympev.2016.05.035

Freitas, S.N., Harris, D.J., Sillero, N., Arakelyan, M., Butlin, R.K., and Carretero, M.A., Correction to: The role of hybridisation in the origin and evolutionary persistence of vertebrate parthenogens: A case study of *Darevskia* lizards, *Heredity*, 2019, vol. 123, no. 6, pp. 809–810.
https://doi.org/10.1038/s41437-019-0270-7

Girnyk, A.E., Vergun, A.A., Semyenova, S.K., Guliaev, A.S., Arakelyan, M.S., Danielyan, F.D., Martirosyan, I.A., Murphy, R.W., and Ryskov, A.P., Multiple interspecific hybridization and microsatellite mutations provide clonal diversity in the parthenogenetic rock lizard *Darevskia armeniaca, BMC Genomics*, 2018, vol. 19, no. 1, p. 979.
https://doi.org/10.1186/s12864-018-5359-5

Gregory, T.R., Nathwani, P., Bonnett, T.R., and Huber, D.P.W., Sizing up arthropod genomes: an evaluation of the impact of environmental variation on genome size estimates by flow cytometry and the use of qPCR as a method of estimation, *Genome*, 2013, vol. 56, no. 9, pp. 505–510.
https://doi.org/10.1139/gen-2013-0044

Hardy, L.M. and Cole, C.J., Morphology of a sterile, tetraploid, hybrid whiptail lizard (Squamata: Teiidae: Cnemidophorus), *Am. Mus. Novit.*, 1998, vol. 3228, pp. 1–16.

Kearney, M., Fujita, M.K., and Ridenour, J., Lost sex in the reptiles: Constraints and correlations, *Lost Sex*, Schön, I., Martens, K., and Dijk, P., Eds., Dordrecht: Springer, 2009, pp. 447–474.
https://doi.org/10.1007/978-90-481-2770-2_21

Lamborot, M., Manzur, M.E., and Alvarez-Sarret, E., Triploidy and mosaicism in *Liolaemus chiliensis* (Sauria: Tropiduridae), *Genome*, 2006, vol. 49, no. 5, pp. 445–453.
https://doi.org/10.1139/g05-122

Litvinchuk, S.N., Testicular anomalies in the hybridogenetic frog *Pelophylax esculentus* (Amphibia: Anura: Ranidae), *KnE Life Science*, 2018, vol. 4, no. 3, pp. 92–96.
https://doi.org/10.18502/kls.v4i3.2109

Litvinchuk, S.N., Speciation and hybridization in Palaearctic amphibians, *Problems of Herpetology*, Dunaev, E.A. and Poyarkov, N.A., Eds., Moscow: Tovarishchestvo Nauchnykh Izdanii KMK, 2021, pp. 170–172.

Litvinchuk, S.N., Rosanov, J.M., and Borkin, L.J., Correlations of geographic distribution and temperature of embryonic development with the nuclear DNA content in the Salamandridae (Urodela, Amphibia), *Genome*, 2007, vol. 50, no. 4, pp. 333–342.
https://doi.org/10.1139/g07-010

Litvinchuk, S.N., Kazakov, V.I., Pasynkova, R.A., Borkin, L.J., Kuranova, V.N., and Rosanov, J.M., Tetraploid green toad species (*Bufo pewzowi*) from the Altay Mountains: The first record for Russia, *Russ. J. Herpetol.*, 2010, vol. 17, no. 4, pp. 290–298.

Litvinchuk, S.N., Borkin, L.J., Skorinov, D.V., Pasynkova, R.A., and Rozanov, Yu.M., Natural polyploidy in amphibians, *Vestnik Sankt-Peterburgskogo Gosudarstvennogo Universiteta. Seriya 3. Biologiya*, 2016, no. 3, pp. 77–86.
https://doi.org/10.21638/11701/spbu03.2016.314

Litvinchuk, S.N., Borkin, L.J., Mazepa, G.A., and Rozanov, Y.M., Genome size and distribution of diploid and polyploid green toads of the genus Bufotes in Uzbekistan and Turkmenistan, *Gerpetologicheskie i ornitologicheskie issledovaniya. Sovremennye aspekty. Posvyashchaetsya 100-letiyu A.K. Rustamova (1917–2005)* (Herpetological and Ornithological Research: Modern Aspects: Dedicated to the 100th Birthday of A.K. Rustamov (1917–2005)), Moscow: Tovarishchestvo Nauchnykh Izdanii KMK, 1917, pp. 88–101.

Litvinchuk, S.N., Skorinov, D.V., Pasynkova, R.A., Kidov, A.A., Matushkina, K.A., Borkin, L.J., and Rozanov, Yu.M., Polyploid speciation in Asian green toads of the genus *Bufotes* (Bufonidae), *Izvestiya Vysshikh Uchebnykh Zavedenii. Povolzhskii Region. Estestvennye Nauki*, 2019, vol. 1, no. 3, pp. 80–93.
https://doi.org/10.21685/2307-9150-2019-1-9

Lutes, A.A., Baumann, D.P., Neaves, W.B., and Baumann, P., Laboratory synthesis of an independently reproducing vertebrate species, *Proc. Natl. Acad. Sci. U. S. A.*, 2011, vol. 108, no. 24, pp. 9910–9915.
https://doi.org/10.1073/pnas.1102811108

Mason, A.S. and Pires, J.Ch., Unreduced gametes: Meiotic mishap or evolutionary mechanism?, *Trends Genet.*, 2015, vol. 31, no. 1, pp. 5–10.
https://doi.org/10.1016/j.tig.2014.09.011

Moritz, C., Uzzell, T., Spolsky, C., Hotz, H., Darevsky, I.S., Kupriyanova, L.A., and Danielyan, F.D., The material ancestry and approximate age of parthenogenetic species of Caucasian rock lizards (*Lacerta*: Lacertidae), *Genetica*, 1992, vol. 87, no. 1, pp. 53–62.
https://doi.org/10.1007/bf00128773

Muller, H.J., Some genetic aspects of sex, *Am. Nat.*, 1932, vol. 66, no. 703, pp. 118–138.
https://doi.org/10.1086/280418

Murphy, R.W., Fu, J., MacCulloch, R.D., Darevsky, I.S., and Kupriyanova, L.A., A fine line between sex and unisexuality: The phylogenetic constraints on parthenogenesis in lacertid lizards, *Zool. J. Linn. Soc.*, 2000, vol. 130, no. 4, pp. 527–549.
https://doi.org/10.1111/j.1096-3642.2000.tb02200.x

Ochkalova, S., Korchagin, V., Vergun, A., Urin, A., Zilov, D., Ryakhovsky, S., Girnyk, A., Martirosyan, I., Zhernakova, D.V., Arakelyan, M., Danielyan, F., Kliver, S., Brukhin, V., Komissarov, A., and Ryskov, A., First genome of rock lizard *Darevskia valentini* involved in formation of several parthenogenetic species, *Genes*, 2022, vol. 13, no. 9, p. 1569.
https://doi.org/10.3390/genes13091569

Pensabene, E., Augstenová, B., Kratochvíl, L., and Rovatsos, M., Differentiated sex chromosomes, karyotype evolution, and spontaneous triploidy in carphodactylid geckos, *J. Hered.*, 2024, vol. 115, no. 3, pp. 262–276.
https://doi.org/10.1093/jhered/esae010

Rozanov, Y.M. and Vinogradov, A.E., Precise DNA cytometry: Investigation of individual variability in animal genome size, *Tsitologiya*, 1998, vol. 40, no. 8/9, pp. 798–800.

Stöck, M., Dedukh, D., Reifová, R., Lamatsch, D.K., Starostová, Z., and Janko, K., Sex chromosomes in meiotic, hemiclonal, clonal and polyploid hybrid vertebrates: Along the 'extended speciation continuum', *Philos. Trans. R. Soc., B*, 2021, vol. 376, no. 1833, p. 20200103.
https://doi.org/10.1098/rstb.2020.0103

Tarkhnishvili, D., Gabelaia, M., and Adriaens, D., Phenotypic divergence, convergence and evolution of Caucasian rock lizards (*Darevskia*), *Biol. J. Linn. Soc.*, 2020, vol. 130, no. 1, pp. 142–155.
https://doi.org/10.1093/biolinnean/blaa021

The Reptile Database, Uetz, P., Ed., 2024. http://www.reptile-database.org. Cited February 28, 2024.

Trifonov, V.A., Paoletti, A., Caputo Barucchi, V., Kalinina, T., O'Brien, P.C.M., Ferguson-Smith, M.A., and Giovannotti, M., Comparative chromosome painting and NOR distribution suggest a complex hybrid origin of triploid *Lepidodactylus lugubris* (Gekkonidae), *PLoS One*, 2015, vol. 10,

no. 7, p. e0132380.
https://doi.org/10.1371/journal.pone.0132380

Uzzell, T. and Darevsky, I.S., Biochemical evidence for the hybrid origin of the parthenogenetic species of the *Lacerta saxicola* complex (Sauria: Lacertidae), with a discussion of some ecological and evolutionary implications, *Copeia*, 1975, vol. 1975, no. 2, pp. 204–222.
https://doi.org/10.2307/1442879

Uzzell, T.M. and Darevsky, I.S., Evidence for a hybrid origin of parthenogenetic species of Caucasian rock lizards of the genus *Lacerta, Zh. Obshch. Biol.*, 1974, vol. 35, no. 4, pp. 553–561.

Yanchukov, A., Tarkhnishvili, D., Erdolu, M., Şahin, M.K., Candan, K., Murtskhvaladze, M., Gabelaia, M., Iankoshvili, G., Barateli, N., Ilgaz, Ç., Kumlutaş, Yu., Matur, F., Çolak, F., Arakelyan, M., and Galoyan, E., Precise paternal ancestry of hybrid unisexual ZW lizards (genus *Darevskia*: Lacertidae: Squamata) revealed by Z-linked genomic markers, *Biol. J. Linn. Soc.*, 2022, vol. 136, no. 2, pp. 293–305.
https://doi.org/10.1093/biolinnean/blac023