

# Hybrids of *Darevskia valentini*, *D. armeniaca* and *D. unisexualis* from a sympatric population in Armenia

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**Abstract.** A unique hybridization zone of rock lizard species of genus *Darevskia*, with diploid ( $2n$ ), triploid ( $3n$ ) and tetraploid ( $4n$ ) individuals, occurs in the mountain steppe of central Armenia. Our long-term monitoring has showed an increasing birth rate of triploid hybrids in this mixed population. Among these hybrids, the frequency of captured males with fully developed reproductive systems and presumably fertile females also increased significantly. Consequently, intensive microevolution has taken place in this sympatric population. The morphological, ecological, cytological, histological, and parasitological characteristics of the hybrid *Darevskia* are presented here and compared with parental species.

**Keywords:** Armenia, diploid, hybrid zone, lizards, triploid and tetraploid individuals.

## Introduction

Four parthenogenic and four bisexual species of Caucasian rock lizard from genus *Darevskia* Arribas, 1997 (Arribas, 1999) are widespread on the territory of Armenia. The distributions of different species of rock lizards often overlap (Darevsky, 1967; Darevsky, Kupriyanova and Uzell, 1985). Such contact zones are quite common between two or three parthenogenetic species. The bisexual species of *Darevskia* can also be found sympatrically. The sympatric zones between parthenogenetic and bisexual species are especially interesting because natural hybridization between parthenogenetic lizards and males of the bisexual species occurs (Darevsky, 1967; Darevsky and Danielyan, 1968; Darevsky, Kupriyanova and Uzell, 1985; Darevsky, 1995; Darevsky and Danielyan, 2001; Danielyan, 2003). Such zones of hybridization exist in mountainous regions of central Armenia at elevations from 1800 to 2000 m above sea level. The contact zones are usually small areas on the periphery of the ranges of partheno-

genetic and bisexual species. Opportunities for hybridization occur in river canyons along which bisexual species penetrate the mountains and enter the ranges of parthenogenetic forms (Darevsky, Kupriyanova and Uzell, 1985). Generally, in such contact zones, hybrids comprise 5-12% of the population (Darevsky et al., 1973; Darevsky, Kupriyanova and Uzell, 1985; unpublished data of Danielyan).

One highly interesting zone of hybridization was discovered in 1994 in the vicinity of Kuchak village (Aragatsotn region) on the eastern slopes of Mt. Aragatz in central Armenia. In this zone, the parthenogenetic species *D. armeniaca*, *D. unisexualis* and bisexual species *D. valentini* occur in sympatry (Danielyan et al., 1999). The number of hybrids that appear annually in this sympatric zone is extremely high. The majority of them are triploid sterile females ( $3n = 57$ ) (Darevsky and Kulikova, 1964), however, male hybrids and intersexual individuals were also found. Currently, the Kuchak population is a unique hybridization zone of rock lizard species that includes diploid ( $2n$ ), triploid ( $3n$ ) and tetraploid ( $4n$ ) individuals.

The Kuchak population has been a subject of multidisciplinary studies since the time of its discovery and some studies on these lizards have already been published (MacCulloch et al., 1995; Kupriyanova, 1999; Arakelyan, 2002). In

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addition to the more data on the Kuchak population, the morphological, ecological, cytological, histological and parasitological studies of *Darevskia* hybrids and parental species are summarized in this paper. The evolutionary potential for hybridogenic speciation by Caucasian rock lizards is also discussed.

## Materials and methods

The Kuchak population is located in central region of Armenia on the northeastern slope of Aragats Mountain near Kuchak village. This study was conducted between 1994 and 2006 (table 1). All specimens were captured within a few hectares of one another. Parthenogenetic *D. unisexualis* and *D. armeniaca*, the bisexual *D. valentini*, and their hybrids of *D. valentini* × *D. unisexualis* and *D. valentini* × *D. armeniaca*, were captured with a noose and were palpated to assess their reproductive condition. Hybrids were identified according to their color pattern and scutellation. The specimens used for comparative studies were sampled simultaneously. Chloroform gas was used for euthanasia of a few lizards for cytological, histological and skeletochronological studies. The animals which were used for morphological, parasitological and cytometrical studies, were measured, blood was taken from the tail for smears and then released.

### Morphological analyses.

The hybrids ( $n = 33$ ) and their parental species (*D. valentini*  $n = 30$ , *D. unisexualis*  $n = 23$ , and *D. armeniaca*  $n = 16$ ) were studied for seven pholidosis characters adopted for rock lizards by Darevsky (1967) and measured for snout-vent length (SVL) using calipers. The meristic scalation characters included DORS (the number of dorsal scales in a single row around the midbody); VENT (the number of abdominal scales along the midline on the left side of the body); GUL (the number of gular scales on the midline); FPOR (the number of femoral pores); SCG (the number supraciliar granules located between the supraoculars and superciliary scales); PA (the number of large preanal scales);

CTT (the number of scales in a single row between central temporal and tympanicum shields). The comparative analyses of characters have been performing with univariate methods.

### Skeletochronological study

The data on body sizes of hybrids ( $n = 34$ ) and their parents (*D. valentini*  $n = 10$ , *D. unisexualis*  $n = 13$ , and *D. armeniaca*  $n = 10$ ) is represented for each age group. The age of lizards was determined by skeletochronological techniques, based on counting the annual bone layers. Following a technique adopted by Smirina (1974) and Castanet and Smirina (1990), the transverse sections were prepared from middle of diaphysis of the femur bone and stained with Ehrlich haematoxylin.

### Analyses of erythrocytes

The sizes of erythrocytes of three adult individuals of each diploid parental *D. valentini* and *D. unisexualis* species, triploid male and female hybrids, and a single specimen of tetraploid hybrid, were studied. Giemsa-stained smears of blood were used in the measurements of erythrocytes. The program ImageJava1.36b was used for automatic determination of sizes of erythrocytes on digitized images. The blood cells were examined under light microscope with teleheader (Nikon Digital Camera COOLPIX4500) at magnification of ×200 (objective ×20, eyepiece ×10).

### Chromosomal analyses

Chromosome smears were prepared from the bone marrow, spleen, intestine, and testes tissues ( $n = 11$ ) according to the method described by Macgregor and Varley (1986). The colchicine solution (0.1%) was used intraperitoneally. The cells were treated by hypotonic KCl solution (0.56%), fixed in an ice-cold acetic acid–methanol solution (1 : 3). The chromosomes were stained with 5% Giemsa in phosphate buffer at pH 6.8. The chromosome complements were detected by observation of 50 metaphase plates for each individual. Homologous chromosome pairs were identified according to their related length (Levan et al., 1964).

**Table 1.** Number of hybrids and of parental species in samples.

Collecting date	<i>D. valentini</i> × <i>D. unisexualis</i>	<i>D. valentini</i> × <i>D. armeniaca</i>	<i>D. valentini</i> ♂♀	<i>D. unisexualis</i> ♀♀	<i>D. armeniaca</i> ♀♀
06.07.1994	8	2	9	11	0
21.05.1997	14	5	6	25	4
21.04.1999	12	6	8	15	2
18.07.1999	12	4	2	12	10
08.06.2003	14	2	4	8	6
28.04.2004	17	4	8	6	5
28.05.2006	7	2	3	5	2
Total	84	25	40	82	29

### Histological analyses

The gonads of adult hybrids ( $n = 6$ ) and parental species ( $n = 6$ ) were studied histologically. Comparative samples were collected simultaneously and had approximately similar body length. The paraffin slides were prepared according to classical microtechnical method (Romeis, 1958; Kiseli, 1962). For light microscopy the ovaries and testis of lizards were fixed in Bouin's solution. After fixation, the tissues were washed, dehydrated, cleared and embedded in paraffin. The tissues were serially sectioned at 5-10  $\mu\text{m}$ , then were deparaffinized and stained in Ehrlich haematoxylin and eosin.

### Parasitological study

Blood parasites were examined on smears of blood ( $n = 82$ ). Blood smears were fixed by absolute methanol and stained with Giemsa (Beyer, 1968; Beyer and Sidorenko, 1984). Blood parasites were examined microscopically and counted. The abundance of blood parasites for specimens was expressed as the rate of infected erythrocytes.

### Data analysis

Statistical analysis of data was performed by Statistica 6.0 Software package. We used one-way analyses of variances (ANOVA) to determine univariate differences among hybrids and their parental species. Significant ANOVAs were followed by Turkey HSD multiple comparison test to identify specific pairs of significantly different samples.

## Results and Discussion

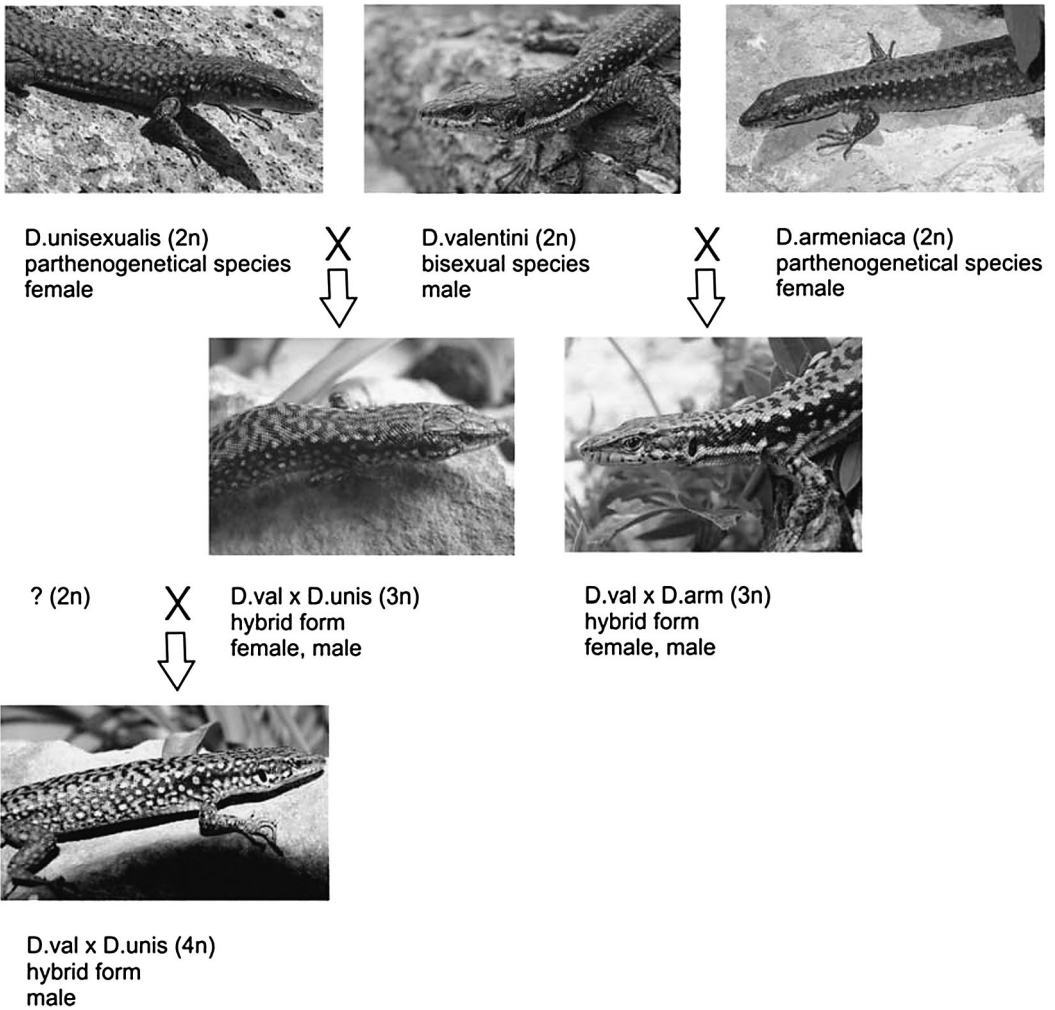
The studied hybridization zone is located on the eastern slopes of Aragats Mountain in central Armenia approximately 5 km south of the Aparan town (Aragatsotn region) and 0.5 km north of Kuchak village (40°31'N, 44°23'E; 1920 m), directly next to the main republic road. The habitat is mountain steppe with gentle slopes of volcanic origins, covered by grass and bushes. Grassland vegetation is dominated by *Festuca*, *Stipa*, *Carex*, *Trifolium*, and the prevailing bush is dog-rose (*Rosa* spp.). Rock lizards typically inhabit stony heaps of volcanic origin, rock piles, and large fragments of lava that are quite common in the mountain steppe zone. Local people used this territory as a pasture. The climate is generally moderate, with cool summers (average temperature +20°C), and cold winters (average temperature -10°C). The rock lizards appear in this site after hiber-

nation at the middle of April until the end of October.

Approximately 60% of the population is represented by *D. unisexualis*, about 10% is comprised of *D. armeniaca*, and 30% of *D. valentini* (Chi-square = 3.0, 2 df,  $P = 0.22$ ). The habitats of the sympatric species are slightly different from each other. Newly arisen hybrid lineages may have ecological requirements intermediate between those of their bisexual parents, restricting them to "hybrid" habitats (Moritz et al., 1992). Parthenogenetic lizards *D. unisexualis* prefer to live on bedrocks and large fragments of lava, whereas the bisexual *D. valentini* occupies stony heaps with shrubs and grassy vegetation. *D. armeniaca* in this zone are mainly confined to artificial constructions and waistlands along the road. On the whole, the habitats of parthenogenetic *Darevskia* differ slightly from those of closely related bisexual forms. However, they tend to inhabit sites with colder, dryer, or more variable climates than their bisexual relatives (Darevsky, Kupriyanova and Uzell, 1985).

The studied zone of sympatry between parthenogenetic *D. unisexualis*, *D. armeniaca*, and bisexual *D. valentini* is very narrow and does not exceed 500 m<sup>2</sup>. *D. valentini* is adapted to high mountains and can be met on the southern slopes of Mountain Aragats, in mountain steppes and alpine zone at altitudes ranging from 1900 to 2700 m meets. *D. armeniaca* and *D. unisexualis* occur on northern slopes of M. Aragats at elevation 1700-2000 m.

The intensive process of hybridization is taking place in the sympatric zone of the Kuchak population (figs 1, 2). The number of hybrids there exceeds 35% of the mixed population. The percent of hybrid individuals in other mixed populations is about 7-12% (Darevsky and Danielyan, 2001). Furthermore, there is evidence that the percent of hybrids in Kuchak population is increasing over time. The percentage of hybrids was 33% in 1994, 35% in 1997, 41% in 1999, 47% in 2003, and 52% in 2004 (fig. 2).

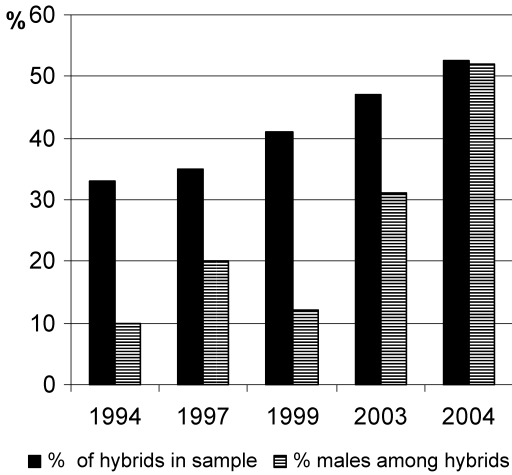


**Figure 1.** Hybridization schemes of bisexual *D. valentini* and parthenogenetic *D. unisexualis*, *D. armeniaca*.

Nineteen fertilized parthenogenetic females with male's jaw marks on their abdomens were collected in 1997 and their eggs were incubated in the laboratory (Danielyan, unpublished). From 22 hatchlings, eight (36.3%) were triploid hybrids. The success of hybridization in this population should be attributed to the optimal time of maturation of gonads in both parental species.

The ratio of hybrid *D. valentini* × *D. unisexualis* to *D. valentini* × *D. armeniaca* in the studied mixed population is approximately 4 : 1 (Chi-square = 2.0, 1 df,  $P = 0.16$ ). In an experiment conducted by Darevsky and Danielyan

(1968) many hybrids were obtained from an artificial hybrid zone created by introducing males of *D. valentini* into isolated population of *D. unisexualis* and *D. armeniaca*. Judging by size of the marks left by jaws of males on the bellies of females during copulation, the larger males *D. valentini* mated more frequently with females of *D. unisexualis*, which are relatively larger than *D. armeniaca*. Also, males of *D. valentini* are more attracted to females of *D. unisexualis* with brightly colored bellies (grey or white) because their ventral coloration is very similar to that of *D. valentini* females. In addition, *D. unisexualis* have marked lateral blue



**Figure 2.** The percentage of hybrids and males among hybrids between 1994-2004.

spots which play important role in mating behavior as it was shown by López, Martín and Cuadrado (2004) on *Iberolacerta monicola*

Usually *D. valentini* shares common range more often with *D. armeniaca* (six known sites) than with *D. unisexualis* (two sites, including Kuchak population). The higher success of hybrids forming in Kuchak population may also explain the high percent of *D. unisexualis* that more frequently mates with *D. valentini*, than *D. armeniaca*.

### Morphological comparisons

Seven meristic scalation characters have shown variation within and among the parental species and their hybrids from studied population (table 2). Hybrids *D. valentini* × *D. unisexualis* differed significantly ( $P < 0.05$ ) from paternal species in four characters: dorsal scales at the middle of the body (DORS), gular scales on the midline (GUL), femoral pores (FPOR), supraciliar granules (SCG), when hybrids *D. valentini* × *D. armeniaca* differed significantly both from *D. valentini* species in dorsal scales at the middle of the body (DORS) and from *D. armeniaca* in supraciliar granules (SCG) followed by Turkey's HSD test. Other compared pairs are not significantly different.

Initially, hybrids individuals were recognized in nature by their comparatively large size and brighter coloration. All *D. valentini* × *D. unisexualis* hybrids have quite wide contact between the rostral and frontonasal shields, typical for maternal species. The coloration of bellies in hybrids is usually dull creamy or white like those of *D. unisexualis*, but occasionally individuals with yellowish color are found. In *D. valentini* the ventral coloration varies from dull white to greenish-yellow or pale lemon yellow. The hybrids of *D. valentini* × *D. armeniaca* can be distinguished by two equal scales, one under another between central temporal and tympanic shields and by yellowish color of their bellies. The belly of *D. armeniaca* is usually yolk yellow.

Although the hybrids combine morphological characters of both parental forms, the impact of unisexual maternal form is stronger than that of bisexual paternal one (our data, Darevsky and Kulikova, 1962). Variability of the pattern of scutellation, cranium size, and cranial indices have been analyzed for *D. valentini* × *D. armeniaca* hybrids and their parental forms (Shimansky, 1969). Hybrids share five maternal and three paternal scutellation characters out of the 22 analyzed. The mean hybrid index for scale characters of the hybrids is 15% compared to the maternal species, and 72% compared to the paternal species. On the whole, the cranial size of hybrid individuals is closer to that of paternal species, because the head of male rock lizards is much larger (Darevsky, Kupriyanova and Uzell, 1985). The larger head sizes of hybrids can be explained by polyploidy resulting from fusion of the diploid female and haploid male pronuclei. Consequently, maternal hereditary information prevails in triploid hybrids, which receive two sets of chromosomes from mother and one from the father (Darevsky and Kulikova, 1962, 1964).

### Age, growth, and longevity

The data of snout-vent length (SVL) of triploid hybrids and their parental species distributed

**Table 2.** Characters of pholidosis (means  $\pm$  SE, range limits) of two types of hybrids *D. valentini*  $\times$  *D. unisexualis*, *D. valentini*  $\times$  *D. armeniaca* and their parents *D. valentini*, *D. unisexualis*, *D. armeniaca* where: DORS: dorsal scales along a transversal line at the middle of the body; VENT– abdominal scales along the midline on the left side of the body; GUL – gular scales on the midline; FPOR – femoral pores; SCG – supraciliary granules; PA – large preanal scales, CTT – scales between central temporal and tympanicum shields.

Characters	<i>D. valentini</i> ♀♂ (n = 30)	<i>D. unisexualis</i> ♀♀ (n = 23)	<i>D. armeniaca</i> ♀♀ (n = 16)	<i>D. val</i> $\times$ <i>D. un</i> hybrid (n = 24)	<i>D. val</i> $\times$ <i>D. arm</i> hybrid (n = 9)
DORS	46.89 $\pm$ 0.21 42-52	51.84 $\pm$ 0.36 49-53	43.87 $\pm$ 0.22 41-47	49.30 $\pm$ 0.52 45-51	44.20 $\pm$ 0.42 40-46
VENT	26.03 $\pm$ 0.33 24-27	26.99 $\pm$ 0.19 26-28	25.93 $\pm$ 0.24 23-27	27.07 $\pm$ 0.29 27-28	25.98 $\pm$ 0.26 24-27
GUL	24.8 $\pm$ 0.44 21-27	28.49 $\pm$ 0.28 26-30	22.31 $\pm$ 0.28 19-26	28.73 $\pm$ 0.43 27-30	23.90 $\pm$ 0.17 21-28
FPOR	16.93 $\pm$ 0.23 14-20	18.08 $\pm$ 0.17 16-21	15.63 $\pm$ 0.14 14-17	18.28 $\pm$ 0.36 16-21	16.11 $\pm$ 0.18 15-19
SCG	7.56 $\pm$ 0.23 5-10	11.09 $\pm$ 0.24 8-13	4.59 $\pm$ 0.19 2-6	10.69 $\pm$ 0.36 9-12	6.75 $\pm$ 0.25 4-11
PA	1.05 $\pm$ 0.05 1-2	1.92 $\pm$ 0.05 1-2	1.60 $\pm$ 0.13 1-3	1.33 $\pm$ 0.12 1-3	1.16 $\pm$ 0.03 1-2
CTT	2.33 $\pm$ 0.11 1-3	2.64 $\pm$ 0.09 2-4	1.00 $\pm$ 0.00	2.40 $\pm$ 0.05 2-3	1.68 $\pm$ 0.26 1-3

**Table 3.** Number of lizards, mean  $\pm$  SE, limits range of body length (SVL) in the age groups of hybrids *D. valentini*  $\times$  *D. unisexualis*, *D. valentini*  $\times$  *D. armeniaca* and their parents *D. valentini*, *D. unisexualis*, *D. armeniaca* from Kuchak.

Species	Age group				
	3	4	5	6	7
<i>D. val</i> $\times$ <i>D. un</i>	6; 67.7 $\pm$ 1.2 62-70.5	10; 71 $\pm$ 0.5 68-74	7; 76.3 $\pm$ 0.9 73-80	2; 75 71-79	0
<i>D. val</i> $\times$ <i>D. arm</i>	1; 67	0	2; 71.2 $\pm$ 1.8 70-72	5; 75 $\pm$ 1.8 70-78	2; 75 $\pm$ 1.1 73-77
<i>D. valentini</i>	0	3; 65.7 $\pm$ 2.4 61-68	5; 71.0 $\pm$ 2.2 69-73	2; 74 73-75	0
<i>D. unisexualis</i>	0	6; 56.5 $\pm$ 0.7 55-60	6; 67.1 $\pm$ 1.3 60-70	0	0
<i>D. armeniaca</i>	1; 50	3; 59.0 $\pm$ 2.3 55-63	5; 61.6 $\pm$ 1.2 59-66	1; 65	0

by age groups come from our own published studies (Arakelyan, 2002) and cited here in table 3. According to Arakelyan (2002), hybrids and their parental species have shown similar character of growth, though the growth rate of hybrids exceeds those of their parents, especially during first three years of their life. Then there is a reduction in growth, especially after the third hibernation (time of maturity of parthenogenetic and bisexual species) and it becomes comparable to the growth rate of parental species. As can be seen from table 3, the average SVL in age groups of hybrids ex-

ceed those of lizards from samples of parental species and closer to paternal forms. In addition to distribution of SVL in age groups, significant ( $P < 0.05$ ) distinctions were found between hybrids and their parents in average thickness of periosteal femoral bone measured on sections taken from the narrowest part of diaphysis where correlations between the diameter of the femur at the diaphysis level and SVL was high ( $R = 0.84$ ) As a general rule, the factors contributing to the large body sizes of hybrids are polyploidy, and probably, their sterility.

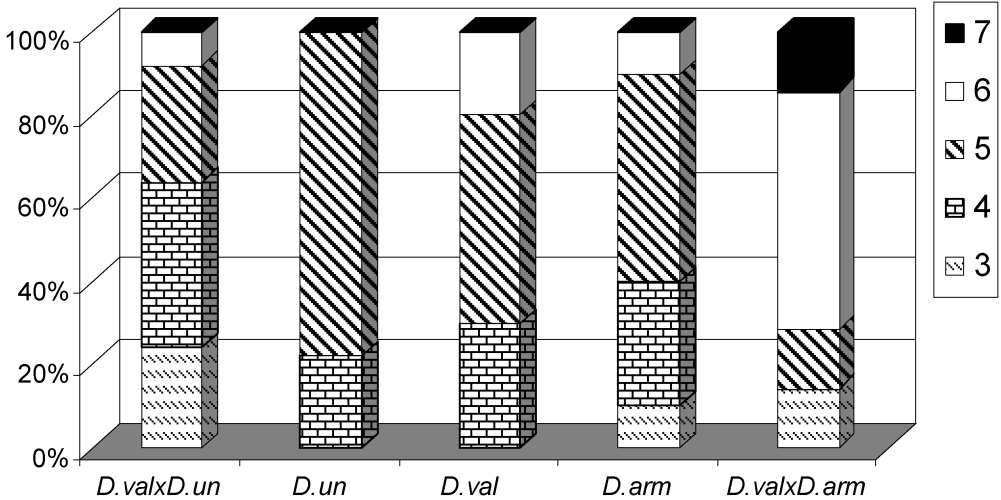


Figure 3. Age distribution of hybrids and their parents.

In studied samples the oldest lizard for *D. valentini* × *D. unisexualis* was 6 years old, whereas that of *D. valentini* × *D. armeniaca* – 7 years old. The maximum age of individuals of parental species in the studied samples was 6 years. Age distributions in the samples of adult lizards from studied population have shown larger proportion of older individuals of hybrids, as compared to their parental species (fig. 3).

Our recent study of age of tetraploid hybrid of *D. valentini* × *D. unisexualis* has shown that it was four-year-old lizard with SVL = 68 mm, which is considered to be small if compared with the body size of triploid hybrids in this age group.

#### Erythrocyte size analyses

The results of cytometrical analyses of erythrocytes of diploid parental species and triploid, tetraploid hybrids from studied population are shown in fig. 4. The size of erythrocytes of triploids exceeds that of diploids by 25-30%. The difference between them is significant ( $P < 0.001$ ). Surprisingly, the red blood cells of tetraploid male hybrid were 10% smaller than those of triploid male hybrids ( $P < 0.001$ ). In terms of red blood cell size, tetraploid male exceeds diploid males by 22% ( $P < 0.001$ ). We

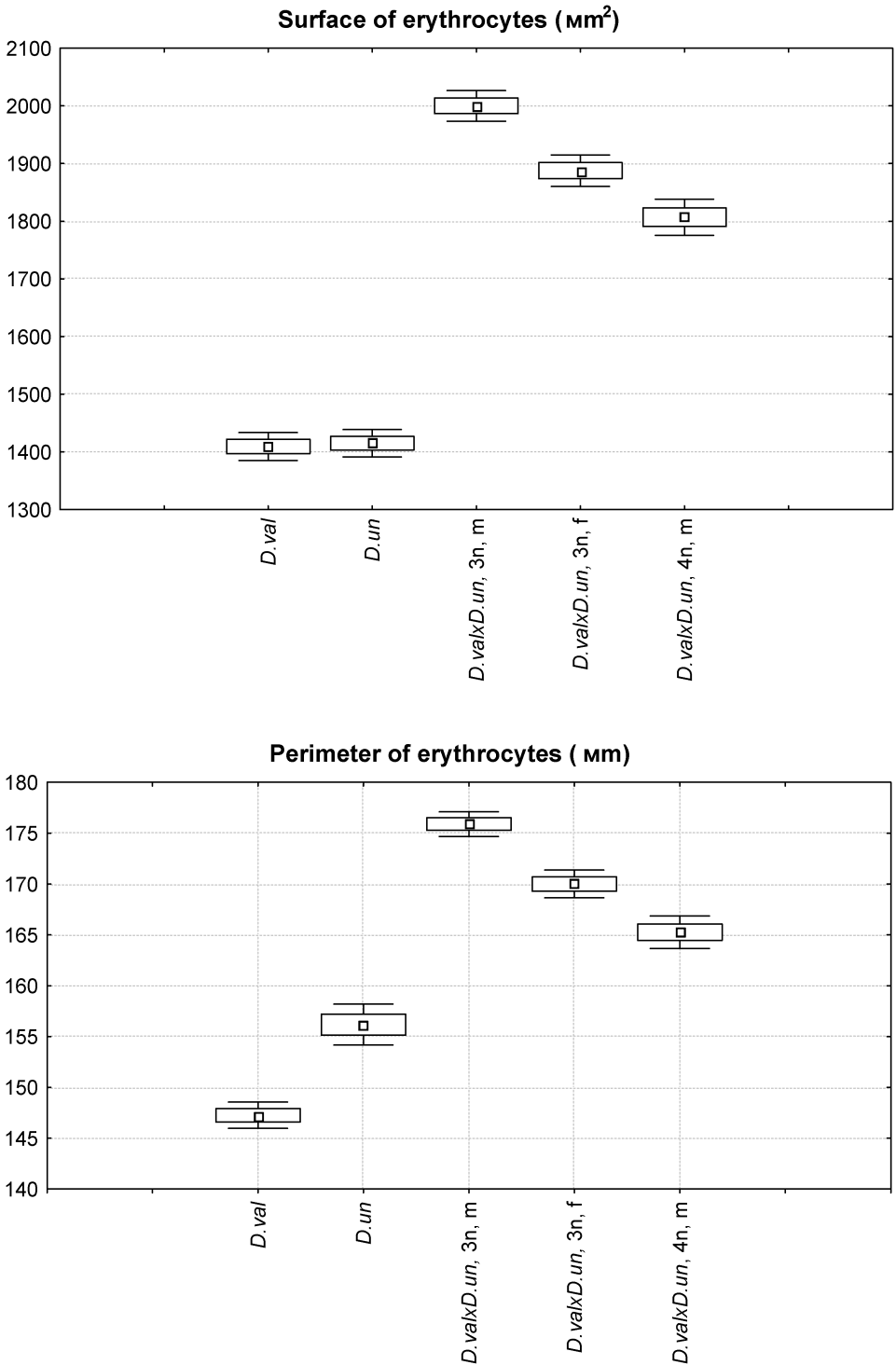
have also found significant difference between sizes of erythrocytes of triploids males and females ( $P < 0.001$ ) where the cells of hybrid males were approximately by 6% larger than the cells of hybrid females.

Our cytometrical analysis of the size of erythrocytes of hybrids and parental species supports earlier findings that triploid hybrid individuals can be easily distinguished by the size of their red blood cells that in these hybrids are on the average one-third larger than those of the diploid parental forms (Darevsky and Krasilnikov, 1965). As a rule, the amphibian and reptilian erythrocytes contain nuclei and their size is positively correlated to their DNA content.

Polyploidy usually leads to an increase in average cell size (Szarski, 1970; Morris, 1984). However, our triploid hybrids have larger sizes of cells than the discovered tetraploid hybrid. Comparative study of lizards with different ploidy levels can shed light to this phenomenon.

#### Comparison of parasitic infestation

Four of the five forms (or species) of blood parasites of genus *Karyolysus* (Sporozoa, Coccidia, Adeleida, Haemogregarinidae), that were previously described by Krasilnikov (1967), Beyer (1968a, b) and Beyer and Selivanova (1969), have been found on blood smears of lizards both



**Figure 4.** Surface area ( $\mu\text{m}^2$ ) and perimeter ( $\mu\text{m}$ ) measurements of erythrocytes of 3n, and 4n males and female *D. valentini*  $\times$  *D. unisexualis* hybrids and their parents ( $\square$  mean,  $\square$   $\pm\text{SE}$ ,  $\text{—}$   $\pm 1.96\text{SE}$ ).



**Table 4.** Extensiveness (*E.i*) and intensity (*I.i*) of invasion by blood parasites of genus *Karyolysus* in peripheral blood of rock lizards from Kuchak.

Species	Sex	<i>E.i</i> %	<i>I.i</i>		
			<i>Lim</i>	Mean ± SE	<i>SD</i>
<i>D. valentini</i>	both	100	0-20	2.74 ± 0.33	3.30
	males	100	0-20	2.96 ± 0.37	3.32
	females	100	0-14	1.86 ± 0.71	3.18
<i>D. unisexualis</i>	females	0	0-0	0.00 ± 0.00	0.00
<i>D. armeniaca</i>	females	21	0-3	0.13 ± 0.03	0.43
Hybrids	both	88	0-20	2.30 ± 0.22	3.42
	males	100	0-20	3.98 ± 0.43	4.29
	females	92	0-17	1.40 ± 0.19	2.17

hybrids and paternal species. Our parasitological observation of blood smears has shown that all studied hybrid lizards ( $n = 25$ ) with exception for one adult and two subadult individuals, contain blood parasites of genus *Karyolysus*. The percent of infected lizards (extensiveness of invasion, *E.i*) and average percent of erythrocytes infected by blood parasites (intensity infected *I.i*) are presented in table 4. The blood of all samples of the paternal species *D. valentini* ( $n = 19$ ) were infected. However, blood of the both maternal species *D. armeniaca*, *D. unisexualis* were relatively clean, which contradicts the “Red Queen” hypothesis (Moritz et al., 1984). No infected erythrocytes were noticed on the smears of individuals of *D. unisexualis* ( $n = 19$ ) from studied samples. Among 19 individuals of *D. armeniaca* only four infected lizards were detected. The level of infections by blood parasites in hybrids does not differ from that of bisexual (paternal) species ( $P = 0.27$ ) and significantly differ from parthenogenetic (maternal) species ( $P < 0.001$ ).

Our comparative study of the intensity of erythrocyte invasion by blood parasites in males and females of *D. valentini* has not shown significant difference depending on the sex of the host ( $P = 0.87$ ), whereas the ratio between hybrid males and hybrid females was significantly different ( $P < 0.001$ ).

A positive relationship ( $r = 0.19$ ) was revealed between the body size of *Darevskia* lizards and parasite intensity. A similar depen-

dence was also noted by Guégan et al. (1992), Amo, Lopez and Martin (2005) and Sluys et al. (2006) on other species of animals. Moreover, during mating season, the incidence of parasite infection was greater in males than in females, probably due to immunosuppressive effects of testosterone (Uller and Olson, 2003; Amo, Lopez and Martin, 2005). The question why parthenogenetic lizards are less susceptible to blood parasites than bisexual species and hybrids calls for future study.

#### Cytogenetic study

The results of karyotypic study of adult hybrid females, males and intersexual individuals from Kuchak population are presented in table 5. Diploid sets of chromosomes of parental species are made of 38 chromosomes ( $NF = 38$ ). According to our study and the literature (Kupriyanova, 1986, 1989, 1999) the karyotypes of maternal parthenogenetic species *D. unisexualis* and *D. armeniaca* have 18 pairs of acrocentric macrochromosomes, a pair of microchromosomes and *wZ* heteromorphic sex chromosomes, where “*w*” denotes microchromosome and “*Z*” – acrocentric macrochromosome. The males of paternal species *D. valentini* have macro- and microchromosomes complements similar to those of maternal species and *ZZ* acrocentric sex chromosomes.

Our study of hybrids has shown the majority of mitotic metaphase plates of triploid female hybrids consist of 57 chromosomes (fig. 5-1). The 15% of plates in our sample had incomplete (aneuploid) chromosome set. The karyotype of these females include 53 acrocentric macrochromosomes and 4 microchromosomes ( $3n = 57$ ,  $NF = 57$ ). All chromosomes are possibly grouped into 19 triplets. First three triplets are represented by the three largest chromosomes, the next three triplets by medium-sized chromosomes, and the following 12 triplets group by the smallest chromosomes. The 18th triplet consists of two acrocentric chromosomes and one microchromosome. According to the literature, chromosomes from the 18th

**Table 5.** Data on chromosomal sets of hybrids *D. valentini* × *D. unisexualis* from Kuchak.

Sex, ploidy	Karyotype	NF	Sex chromosomes	<i>n</i>	Tissues studied	Number of studied mitotic/meiotic cells	Percentage and number of chromosomes in metaphase plates
Female, 3 <i>n</i>	51A + 3m	57	wZZ	4	bone marrow, spleen	200 mitotic	85% 3 <i>n</i> = 57 15% 50, 54, 55, 60
				1	spleen	50 mitotic	80% 3 <i>n</i> = 57 20% 50, 54, 55, 60
Male, 3 <i>n</i>	51A + 3m	57	wZZ	2	bone marrow, spleen	170 mitotic	15% 46, 25% 50, 47% 59 13% 60
				1	spleen, testes	50 mitotic 50 meiotic	70% 3 <i>n</i> = 57 10% 38-48 20% 50-60 70% 3 <i>n</i> = 57
Intersexual hybrid, 3 <i>n</i>	51A + 3m	57	wZZ	1	spleen, testes	50 mitotic 60 meiotic	10% 38-48 20% 50-60  70% 3 <i>n</i> = 57
				1	spleen, testes	50 mitotic 50 meiotic	10% 38-48 20% 50-60
Male, 4 <i>n</i>	71A + 4m	76	wZZZ	1	spleen, testes	50 mitotic 50 meiotic	60% 4 <i>n</i> = 76 40% 40-70

triplet represent sex chromosomes of wZZ type (Kupriyanova, 1994, 1999). Metaphase plates of the other two females were comprised of 46 to 60 chromosomes. These females are again considered triploid individuals, because the numbers of their chromosomes is more than diploid and less than tetraploid chromosomal set.

Mitotic metaphases and meiotic metaphases I and II of one male (fig. 5-2) and two “intersex” hybrids were studied (table 5). Metaphase plates of spermatogonial division included 38, 40, 50, 57, 60 chromosomes. The proportion of triploid cells was 70% of the total investigated cells of spermatogonial division. This hybrids had karyotypes (3*n* = 57, NF = 57) with 53 acrocentric chromosomes and 4 microchromosomes. The intersexes individuals included sex chromosomes like hybrid females (18th triplet have sex chromosomes of wZZ type). The number of cells in diakinetic stages and metaphase

I meiosis of studied triploids was low. Numbers of diakinetic bivalents vary from 19 to 33. In the testis preparations of triploid male and intersexes single spermatides (1-2%) were observed. The formation of mature spermatozooids was not detected.

The one hybrid individual had well developed testes and hemipenises. On the spleen smears of this male, metaphase plates with 65 (30%) and 70-76 (70%) numbers of chromosomes were found. The karyotype was represented by 71 acrocentric chromosomes and 5 microchromosomes (4*n* = 76, NF = 76). So, this was a tetraploid male with wZZZ type sex chromosomes (fig. 5-3).

Sex chromosomes may play a key role in the formation of unisexual species. *Darevskia* have chromosomal mechanism of sex determination and female is the heterogametic sex (Murphy et al., 2000). The karyological study of teiid lizards of genus *Cnemidophorus* has shown that

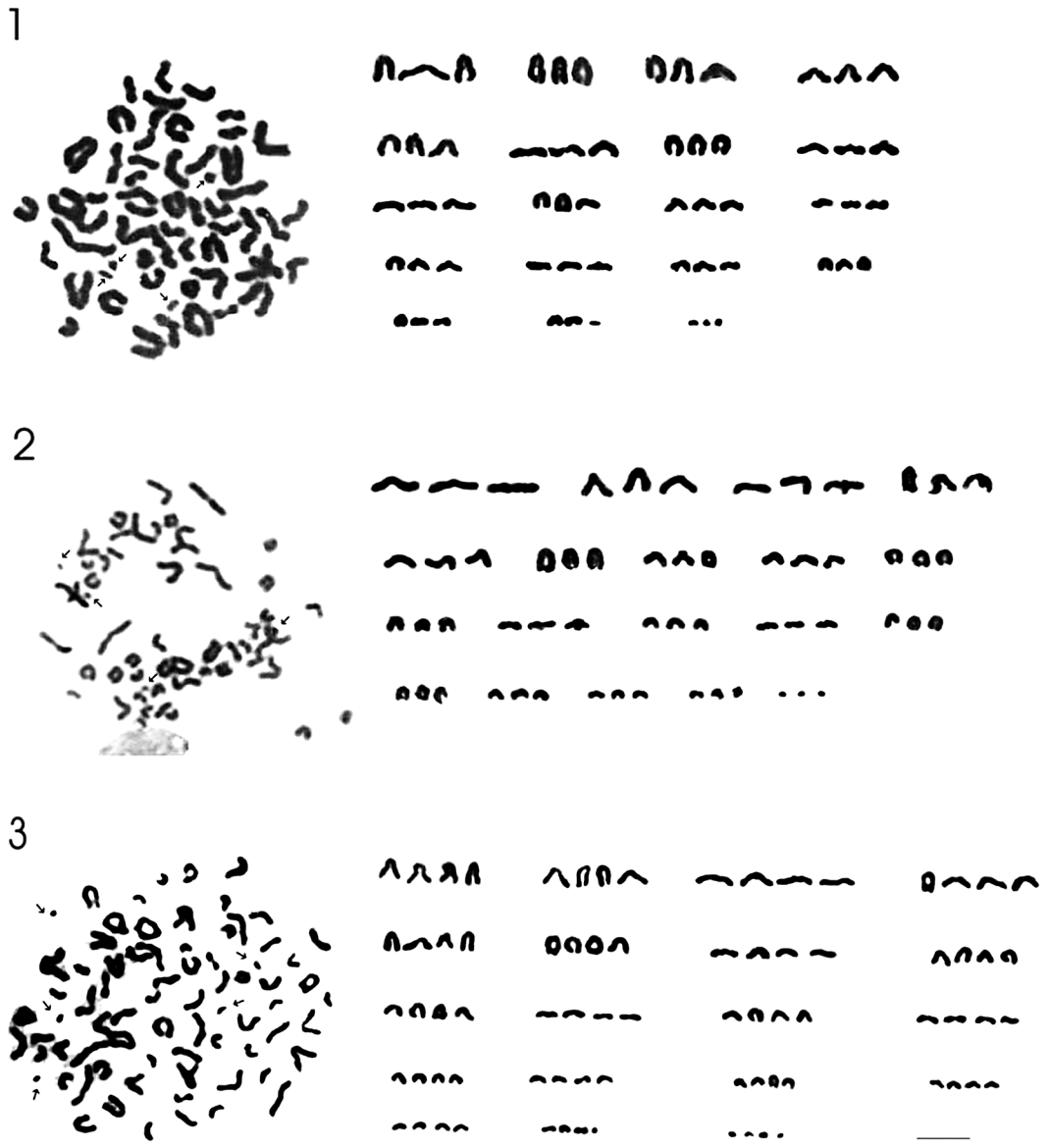


Figure 5. Somatic metaphase and karyotypes (bar equals 10 mkm) of *D. valentini* × *D. unisexualis* hybrids: 1 – triploid female; 2 – triploid male; 3 – tetraploid male.

unlike rock lizards of genus *Darevskia*, their females, are homogametic, whereas the diploid males of *C. tigris* are heterogametic (XY). Probably, the appearance of triploid and tetraploid males of hybrid *C. sonorae* × *C. tigris* (with XXXY sex chromosomes) and females (with XXXX sex chromosomes) take place easier because the actively induced process of the development of heterogametic sex is more compli-

cated than that of homogametic one (Darevsky and Kupriyanova, 1982).

*Reproductive system of hybrids*

Hybrids resulting from mating of parthenogenetic females with males of bisexual species of rock lizards are often sterile triploid females (Darevsky and Kulikova, 1964). However, along with sterile triploid females in the Kuchak popu-

lation, male hybrids, intersexual individuals, and female hybrids with developing follicles and eggs were also detected.

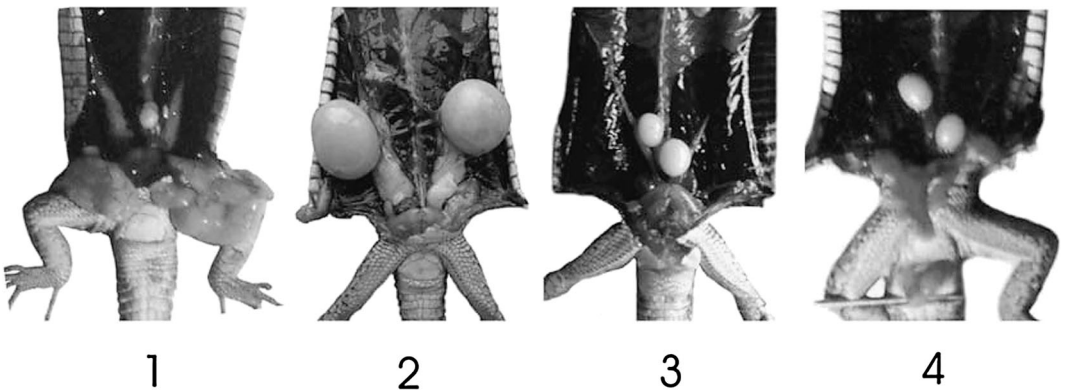
#### *Sterile triploid hybrid females*

The sterility of the most triploids females is caused by gross anomalies in structure and development of gonad (fig. 6-1). The ovary of hybrids differs from cluster ovary of parental species. They are usually smaller in size and lack oocytes. On our histological samples of triploid hybrid females the main part of stroma of the ovary is weakly delineated and the cortex is poorly developed. Some of them have one or two empty false follicles up to 1 mm in diameter (fig. 7-8). The size of hybrid follicles is not increasing during breeding season, because vitellogenesis is not activated. The oviducts have rudimentary structure and are represented by two straight tubs with germinal funnel.

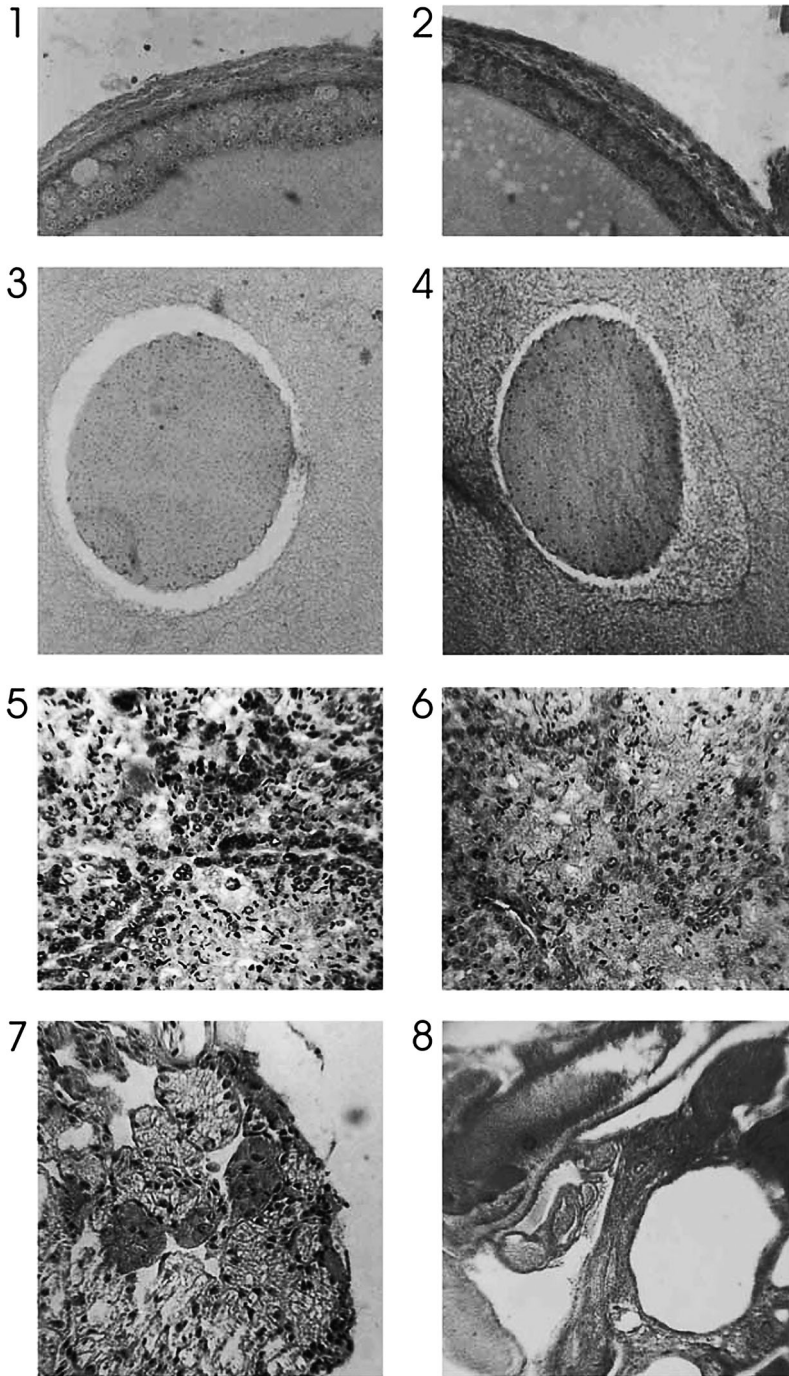
*Presumably fertile hybrid females.* In spite of the hypothesis that in the group of rock lizards hybrid females are usually sterile (Darevsky et al., 1985; Kupriyanova, 1999), we have collected evidence of the rare occurrence of presumable fertile females among *D. valentini* × *D. armeniaca* and *D. valentini* × *D. unisexualis* hybrids in the Kuchak population. The first hybrid female with two eggs in oviducts was discovered in 1999. Then, rare hybrid females with

developing oocytes (fig. 6-2), eggs and corpora lutea were collected in 2000, 2001 and 2006.

The comparative histological analyses of developing oocytes of “fertile” hybrids and their parents show some similarities. The nuclei of hybrids and parental females are similar in size and structure (figs 7-3, 7-4). The ovarian follicle cell layer of hybrid females was complete and well structured (fig. 7-2). However, the surface area of tunica granulose of hybrids was comparatively thinner than that of parthenogenetic females at a similar stage of ovary development (young oocytes were 1.5 mm in diameter). As a result, the number of pyriform cells in tunica granulose of presumably fertile hybrids was less than that of parthenogenetic rock lizards. The larger oocytes (4-6 mm) of presumably fertile hybrids were vitellogenic where the yolk deposition was in progress as in normal oocytes (Arakelyan, 2001). The oviducts were well developed and structured. Given the above observations, the hypothesis of the complete sterility of hybrid females is in question. However, only progeny obtained from female hybrids of Caucasian rock lizards will provide solid evidence for the statement that hybrid females can produce viable and fertile eggs. According to histological study of Taylor et al. (2001) there is no evidence that female hybrids of *Cnemidophorus tessellatus* × *C. tigris marmoratus* can produce viable and fertile eggs.



**Figure 6.** Reproductive system of *D. valentini* × *D. unisexualis* hybrids: 1 – triploid sterile female; 2 – triploid presumably fertile female; 3 – triploid presumably fertile male; 4 – tetraploid presumably fertile male.



**Figure 7.** Gonad histology; 1 – ovarian layers follicle cells of *D. unisexualis*, 2 – ovarian layer follicle cells of presumably fertile hybrid *D. valentini* × *D. unisexualis*, 3 – oocyte nucleus of *D. unisexualis*, 4 – oocyte nucleus of presumably fertile hybrid *D. valentini* × *D. unisexualis*, 5 – testes of *D. valentini*, 6 – testes of hybrid *D. valentini* × *D. unisexualis*, 7 – ovotestes of intersexual hybrid *D. valentini* × *D. unisexualis*, 8 – ovary of sterile hybrid *D. valentini* × *D. unisexualis*.

*Triploid male hybrids.* The natural hybridization between parthenogenetic and bisexual species can also produce triploid male hybrids (Write et al., 1967; Darevsky et al., 1973; Darevsky, Kupriyanova and Bakradze, 1978; Darevsky, Kupriyanova and Danielyan, 1986; Darevsky, Kupriyanova and Uzell, 1985; Darevsky et al., 1989; Taylor et al., 2001). Darevsky and Danielyan (1973) first described male hybrid from a sympatric zone of parthenogenetic *D. rostombekovi* and bisexual *D. raddei* species. Following 1981-1988, 24 specimens of male hybrids were collected from this population. In the Kuchak population, the occurrence of male hybrids is also quite high. Moreover, the proportion of males was increasing during the study period (fig. 2).

Our examination has shown that the majority of hybrid individuals appeared to be typical bisexual males in all details. Most of them had two fully-developed testes (fig. 6-3). Their hemipenes were more or less of the same shape and size as those of bisexual males. Some lizards had two testes: one is well developed, whereas the other (usually the left one) is abnormal.

Different degrees of fertility were observed on histological preparations of male hybrids. All stages of spermatogenesis and mature spermatozoa of hybrid males with well-developed testes were detected. According to the amount of spermatids and mature spermatozoa, all hybrids can be divided into three groups: 1) hybrids with seminiferous tubules containing sperm, as in bisexual males, 2) the sperm were less abundant in comparison with paternal species (fig. 7-6), 3) no sperm were present. Therefore some triploid males were probably reproductive and capable of producing sperm that looked normal. Taylor et al. (2001) have shown that hybrid males of teiid lizards (*Cnemidophorus*) are capable of producing sperm that looked normal.

*Tetraploid male hybrid.* A unique finding was made on April 28<sup>th</sup>, 2004, at Kuchak, when a tetraploid hybrid male was caught and exam-

ined for the first time. This lizard was similar to triploid males in coloration, pattern of dorsal picture and pholidosis. However, karyological analysis has shown tetraploid set of chromosomes of this lizard. This hybrid has two fully developed testes (2.4 × 3.2 mm), well-organized hemipeneses and marked femoral pores (fig. 6-4). The Giemsa-stained smears of testis have shown diakinetic stages of meiosis and middle, late spermatides (60%). Compared with triploid hybrids caught on the same day, the tetraploid male had numerous spermatids and spermatozoa.

The origin of this tetraploid male is unclear. The most possible pattern is a crossing between hybrid triploid female with a male of *D. valentini*. In this case the tetraploid hybrid with *wZZZ* sex chromosomes can arise from mating of triploid females with *wZZ* sex chromosomes with a male of *D. valentini* with *Z* sex chromosome. Another possible scenario is the mating of a hybrid triploid male with a female of *D. valentini*. The female of *D. valentini* has *WZ* sex chromosomes. In this case, the egg with *Z* chromosomes fuses with spermatozoa of triploid hybrid male with *wZZ* sex chromosomes. In any case, the appearance of a tetraploid individual is conclusive evidence of the fertility of either females or males of triploid hybrids. Accordingly, for the genus *Cnemidophorus*, Lowe et al. (1970) discovered a tetraploid male in a mixed population of triploid parthenogenetic *C. sonorae* and diploid *C. tigris*.

*Intersexual triploid hybrids.* In the studied samples four hybrid individuals of *D. valentini* × *D. unisexualis* were detected which had intersexual characteristics. They had “female” oviducts, “male” hemipenes and “ovotestis” gonads. Before our discovery of intersexual individuals (according to the classification of Darevsky and Kulikova, 1962), the hermaphroditic hybrids *D. raddei* × *D. rostombekovi* for rock lizards were described from “Sev-Kar” population in northern Armenia (Danielyan, unpublished). Earlier well-developed oviducts

were observed in a triploid hybrid male studied by Darevsky et al. (1973).

Our histological study of gonads shows that this type of hybrid has ovotestis, because they include both “female” and “male” parts of gonads. The main part of the cortical tissue is thinner tunica albuginea, similar to that of male of bisexual lizards, but 1/5 of the cortical layer looked like a thick tunica vasculosa which is similar to females (fig. 7-7). The medullary tissue is more developed in male gonads, but again the 1/5 fraction of ovotestes includes segments of ovary, which is wedged into the testes tissue. In the tissue of testes poorly developed seminiferous tubules were visible. Only few spermatogonia were present in seminiferous tubules. A similar structure of ovotestes was described by Saint and Ineich (1997) for hybrids between gonochoristic males and parthenogenetic females of the gecko *Lepidodactylus lugubris* in French Polynesia.

#### *Evolutionary consequences*

Fertility of triploid hybrids is a significant prerequisite in reticulate evolution. The universal theory of reticulate speciation (Borkin and Darevsky, 1985) is based upon the following premises: hybridization, unisexuality and polyploidy. The first stage of reticulate speciation is the appearance of diploid parthenogenetical forms as a result of natural interspecies hybridization between closely related bisexual species. Morphological, ecological, cytological, and genetic studies have shown that two parthenogenetical species of genus *Darevskia*, are of hybrid origin (Darevsky and Danielyan, 1968, 1979; Uzell and Darevsky, 1975; Moritz et al., 1992; Grechko et al., 1993; Darevsky, 1995; Fu et al., 2000; Murphy et al., 2000; etc.). The second stage of the scheme is the emergence of allotriploid forms as a result of hybridization of maternal diploid parthenogenetical and paternal bisexual species. On the third stage of speciation, fertile triploid females mate with males of bisexual species and give origin to new tetraploid species (Schultz, 1969;

Borkin and Darevsky, 1980). Consequently, the appearance of fertile females in hybrids of rock lizards can move them away from an evolutionally dead end and give them a chance to reproduce parthenogenetically. As result of changes in environmental conditions, some generations of hybrid females can give rise to triploid species as was shown for the lizards of genus *Cnemidophorus* (Cole, 1975; Cuellar, 1979).

As a rule, polyploid individuals with unpaired set of chromosomes often become sterile. According to Kupriyanova (1999), the bisexual reproduction of triploids males *D. valentini* × *D. unisexualis* from Kuchak has been considered to be impossible because of the problem of equal distribution of three chromosome sets in meiosis. However, Stöeck et al. (2002) report about geographically isolated populations of all-triploid green toads (*Bufo viridis* complex) that reproduce bisexually. On the other hand, appearance of triploid individuals with successful combination of genes is theoretically possible, which can increase their number due to parthenogenetic reproduction. The formation of haploid, diploid, and triploid gametes allows us to speculate future evolutionary pathway of such hybrids. Especially interesting is the case, when a triploid nucleus of hybrid fuses with haploid nucleus of female of paternal species giving rise to a tetraploid form. The result can be second generations of hybrids of both sexes and a return to bisexuality on a polyploidy level. Probably this scenario led to the origin of tetraploid lizards of genus *Cnemidophorus* (Low, 1966; Darevsky, Kupriyanova and Uzell, 1985; Moritz et al., 1989).

Based on the above noted evidences of increasing of percentage of hybrids and the abundance of hybrid males and females with fully developed reproductive systems, it can be inferred that there is an intensive process of hybridization in Kuchak population. Therefore, in this population, suitable conditions can be established for the next stage of the progressive process of speciation by hybridization. Thus,

the occurrence of tetraploid male hybrid with fully developed reproductive system is significant precondition for the opportunity of advancing to the following stages of reticulate evolution in Caucasus rock lizards. Possibly, the speciation by hybridization will give origin to a new polyploid species of rock lizards *Darevskia* in the Kuchak population.

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