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Mixed-ploidy clutches in parthenogenetic *Darevskia dahli* (Sauria: Lacertidae) from a hybrid zone

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Abstract. Hybridization between parthenogenetic females and heterospecific males may generate polyploid offspring and contribute to reticulate evolution in Caucasian rock lizards. We analyzed 30 offspring from ten clutches of parthenogenetic *Darevskia dahli* females showing evidence of mating with *D. portschinskii* in a hybrid zone in northern Armenia. Genotyping of offspring from all clutches using three microsatellite loci identified seven triploid hybrids. One clutch consisted entirely of triploids, whereas three contained both diploid and triploid offspring. Fertilization appears to occur only in some eggs within a clutch. Triploid production was positively associated with female body size. The low frequency of successful triploid formation may limit further polyploid and reticulate evolution in *Darevskia*.

Keywords. Caucasian rock lizards, hybridization, triploid-diploid offspring, reticulate evolution.

The Caucasian rock lizards of the genus *Darevskia* Arribas, 1999, which includes both sexual and parthenogenetic species, are a classic model for studying hybridization and reticulate evolution. All parthenogenetic forms are of hybrid origin (Arakelyan et al., 2023). The geographic ranges of parthenogenetic females, which reproduce clonally without males, often overlap with those of their sexual relatives, resulting in hybrid zones in areas of syntopy (Darevsky, 1967; Petrosyan et al., 2020; Arakelyan et al., 2023). Previous studies have demonstrated that triploid and, occasionally, tetraploid hybrids can arise through repeated hybridization between parthenogenetic females and sexual males (Darevsky and Kulikova, 1962; Darevsky, 1967; Darevsky et al., 1973; Danielyan et al., 2008). The occurrence of polyploid offspring highlights the need to better understand the reproductive outcomes of interactions between parthenogenetic and sexual forms. In the present study, we examined how often reproductive interactions of parthenogenetic *D. dahli* with *D. portschinskii* result in triploid hybrids and whether diploid and triploid offspring can occur within the same clutch.

Field sampling was conducted in a sympatric zone of the parthenogenetic rock lizard *Darevskia dahli* and the sexual species *D. portschinskii* near Amrakits village, northern Armenia (40.99° N, 44.42° E). Two field expeditions were carried out from 28 May to 16 June 2023 and from 21 May to 13 July 2025. A total of 11 gravid *D. dahli* females showing visible ventral bite marks characteristic of mating attempts were collected from among more than 300 females examined. Females were maintained at the A.N. Severtsov Institute of Ecology and Evolution and laid 33 eggs under laboratory conditions (Table 1). Twenty-six produced live hatchlings, four unhatched eggs contained embryos suitable for DNA analysis (designated E in Table 1), and three eggs that died early were excluded. Thus, ploidy was determined for 30 offspring or embryos.

Microsatellite genotyping was performed using three nuclear loci (Du215, Du281, and Du323) previously applied to characterize clonal diversity in *D. dahli* (Vergun et al., 2014).

PCR amplification followed published primers and conditions (Korchagin et al., 2007; Badaeva et al., 2008; Girnyk et al., 2021). Offspring showing only maternal alleles at all informative loci were classified as diploid (2n), whereas those carrying an additional non-maternal allele at one or more loci were classified as triploid (3n) hybrids.

All eleven *D. dahli* females with mating marks were diploid. Among their 30 offspring, seven were triploid hybrids and 23 were diploid (Table 1). Triploids were produced only by four females, where three clutches contained both diploid and triploid siblings, whereas one contained only triploids. Thus, offspring ploidy varied both among and within clutches.

Spearman's rank correlations showed that female body length (SVL) was not significantly associated with clutch size ($\rho = 0.510$, $P = 0.132$), but was positively correlated with both the number of triploid offspring ($\rho = 0.709$, $P < 0.05$) and their proportion within the clutch ($\rho = 0.695$, $P < 0.05$). Larger females therefore produced more triploid offspring and a higher proportion of triploids within their clutches. Although the sample size was small, larger females tended to produce both more triploid offspring and a higher proportion of triploids, while clutch size itself was not significantly related to female size. This suggests that the pattern is not simply because larger females lay more eggs. Female body size may therefore influence fertilization success, although whether this is related to age, reproductive condition, egg characteristics, or mating behaviour remains unclear.

The occurrence of mixed-ploidy clutches indicates that parthenogenetic development and heterospecific fertilization can occur within the same reproductive episode. We suggest that diploid offspring developed parthenogenetically from unfertilized eggs, whereas triploids resulted from fertilization of unreduced diploid eggs by haploid *D. portschinskii* sperm (Dedukh et al., 2024). Similar mixed-ploidy offspring were reported by Darevsky and Kulikova (1962) in *D. armeniaca* from a sympatric zone with *D. valentini*. This is particularly important in the evolutionary context of *Darevskia*, whose parthenogenetic species are stable diploids

(Kupriyanova, 2010; Spangenberg et al., 2020, 2021). Although fertile triploid individuals have occasionally been reported (Arakelyan et al., 2025), triploid *Darevskia* hybrids are predominantly sterile (Darevsky, 1966; Darevsky et al., 1973, 1985), limiting their contribution to subsequent generations. In our population, females with visible mating marks were also rare, representing only 11 of more than 300 examined *D. dahli* (about 4%). Of these, only four produced triploid offspring, which accounted for 23.3% (7/30) of all offspring examined. This suggests that successful triploid formation is considerably less frequent than reproductive interactions indicated by mating marks. Together, the low frequency of triploid formation and the predominant sterility of triploid hybrids may represent an important bottleneck in further reticulate and polyploid evolution in *Darevskia*, helping to explain why higher-ploidy forms arise only rarely in this group of reptiles.

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Ethics approval and consent to participate

Permit for the collection of lizards was issued by the Ministry of Environment of the Republic of Armenia (No. 3/29.7/2593-2025). Animal experimental procedures were conducted in accordance with the regulations of the Animal Ethics Committee of the IEE RAS (protocol No. 123, 2025).

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Table 1. Offspring ploidy and snout–vent length of *Darevskia dahli* with visible copulation marks.

Offspring ploidy	Female SVL, mm (ID)	No. of diploid offspring (2n) (IDs)	No. of triploid offspring (3n) (IDs)
Diploid only	63 (2022)	4 (2054, 2051, 2052, 2053)	0
	61 (1355)	2 (2415, 2414)	0
	63 (5021)	1 (2044)	0
	67/62 (5014/5015)*	7 (2100, 2110, 2112, 2113, 2224, E1, E2)	0
	63 (5133)	1 (5305)	0
	66 (5114)	3 (5310, 5311, 5312)	0
Triploid only	70 (5025)	0	3 (1542, 2123, 2122)
Mixed	66 (3003)	2 (2103, 2104)	1 (2105)
	65 (5124)	1 (5243)	1 (5244)
	65 (5131)	2 (E3, E4)	2 (5334, 5335)

*The row with female IDs 5014/5015 lists seven offspring because they were from mixed clutches.