

Original Article

Love bites: males of lizards prefer to mate with conspecifics, but do not disdain parthenogens

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ABSTRACT

Parthenogenesis has been discovered in a great number of reptiles, indicating that parthenogens can outcompete bisexual species and hybridize with them. This raises intriguing questions: how do parthenogenetic species coexist with gonochoristic relatives, and what outcome does this coexistence produce? To answer these two questions, we studied relationships between male rock lizards, *Darevskia valentini*, and parthenogenetic *Darevskia armeniaca*. We compared social and sexual reactions of males to females of their own species and parthenogenetic *D. armeniaca* and vice versa. We found that females of both species basked together with males and demonstrated mostly submissive behaviour. Females of *D. valentini* were less aggressive towards males in the mixed-species population than in the single-species population, suggesting female competition for males. The proportion of successful and unsuccessful mating attempts with females of bisexual and unisexual species was equal, and we found no significant differences in male reactions to bisexual and unisexual females in sympatry. The proportion of females bearing mating scars on their venters and the number of these scars per female were greater in the bisexual species. This indicates that females of the bisexual species were more attractive than parthenogenetic females as mating partners to males. We found no triploid hybrids among the offspring of parthenogenetic females that mated with males in the mixed population. Our findings suggest that parthenogenetic species might outcompete bisexual species by mating with males and integrating into the social system.

Keywords: *Darevskia*; competition; mate choice; rock lizards; parthenogenesis; sympatry

INTRODUCTION

Parthenogenetic reproduction occurs among reptiles in a number of different taxa (Avisé 2008, Arakelyan *et al.* 2023) and often appears in the hybrids between different species of the same genus (Cuellar 1977). Rock lizards (*Darevskia* spp.) are a diverse group comprising some 40 species, among which seven parthenogenetic lineages are present (Arakelyan *et al.* 2023). Genetic and cytogenetic studies have confirmed their hybrid origin from two sexual species belonging to different phylogenetic clades (Murphy *et al.* 2000, Vergun *et al.* 2014, Freitas *et al.* 2016, Spangenberg *et al.* 2017, Yanchukov *et al.* 2022). Ecological niches of parthenogens and parental bisexual species of rock lizards overlap broadly (Petrosyan *et al.* 2019), and they often coexist within secondary overlap areas (Petrosyan *et al.* 2019, Arakelyan *et al.* 2008). Such

an overlap of the distribution area has been registered for other parthenogens, such as *Aspidoscelis* (Cuellar 1979, Bateman *et al.* 2010) and *Teius* (Cacciali *et al.* 2016). Triploid and tetraploid hybrids appear as a result of hybridization between parthenogenetic females and males of parental species (Arakelyan *et al.* 2008, Abrahamyan *et al.* 2014). The presence of such hybrids indirectly demonstrates socio-sexual integration of parthenogens into the population of bisexual relatives. Interspecies mating behaviour in nature has been supported previously by visual observations for males of *Darevskia valentini* with females of *Darevskia armeniaca* and *Darevskia unisexualis* (Galoyan 2013). Mating scars on the venters of lacertid females occur after male mating attempts (Martin *et al.* 2003) and confirm sexual interest of males towards females. Carretero *et al.* (2018) attempted to analyse the number of

mating scars on the venters of parthenogenetic females in a hybrid zone in Kuchak, Aragatsotn Province in central Armenia, where a sexual species (*D. valentini*), two parthenogens (*D. armeniaca* and *D. unisexualis*), and triploid hybrids occur (Carretero *et al.* 2018). They demonstrated that the number of females of the bisexual species was 20 times less than that of the parthenogens. Owing to the low number of females of the sexual species, males had limited options and mated with unisexual heterospecifics.

To understand whether parthenogenetic females compete with sexual females for the males, we tested male preferences in the areas where the number of conspecific females is close to that of parthenogenetic females. Here, we assume that parthenogenetic females can attract males of parental species, bringing sexual and social attention of males to themselves and thereby reducing the frequency of intraspecific mating. To test this hypothesis, we compared intersexual intra- and interspecific reactions and analysed the number of mating scars on the venters of females from three different colonies of rock lizards from northern Armenia. One colony comprised only sexual *D. valentini*, and two included sympatric parthenogenetic *D. armeniaca* and gonochoristic *D. valentini*.

MATERIALS AND METHODS

Fieldwork

Three sites in northern Armenia near Mets Sepasar village were chosen (Fig. 1) and sampled in 2014, 2016, 2021, and 2022. The first site (N 41° 1' 52.1", E 43° 49' 46.4"), called the 'Hill',

was in an open meadow 500 m from the left bank of Ashotsk River and inhabited only by a sexual species, *D. valentini*, with a local population density of >400 individuals/ha (Galoyan *et al.* 2019a). The second study site, called the 'Bridge', was on an old, stone bridge over the confluence of the Ashotsk and Akhurian Rivers (N 41° 1' 49.115", E 43° 49' 15.7"). Here, parthenogenetic *D. armeniaca* and gonochoristic *D. valentini* coexist at high population densities (Galoyan *et al.* 2019a). The third sampling site, called the 'Riverside', was 2 km above the flow of these rivers from the old bridge on the right bank of the Ashotsk River (N 41° 2' 13.5", E 43° 50' 27.9").

Lizards were captured by noose or by hand during fieldwork. Adult females were easily distinguished from males by their smaller heads, longer trunks (Darevsky 1967), femoral pores, vivid coloration, and absence of hemipenes. For each individual, we measured the snout–vent length (SVL) and tail length with an accuracy of ±0.5 mm. In the late mating season (mid-June), we counted the number of mating scars on the abdomens of females (Fig. 2).

The dorsal coloration of parthenogenetic *D. armeniaca* varied; we categorized these lizards as 'brown', 'intermediate', or 'green' (Fig. 3). Adult female *D. valentini* were bright green, with brown medial stripes (Fig. 3).

The lizards from the Riverside colony were marked individually by toe clipping (Tinkle and Woodward 1967) and by colourful beads sewn with a nylon line through the skin (Muth and Fisher 1989). This allowed us to distinguish between individuals

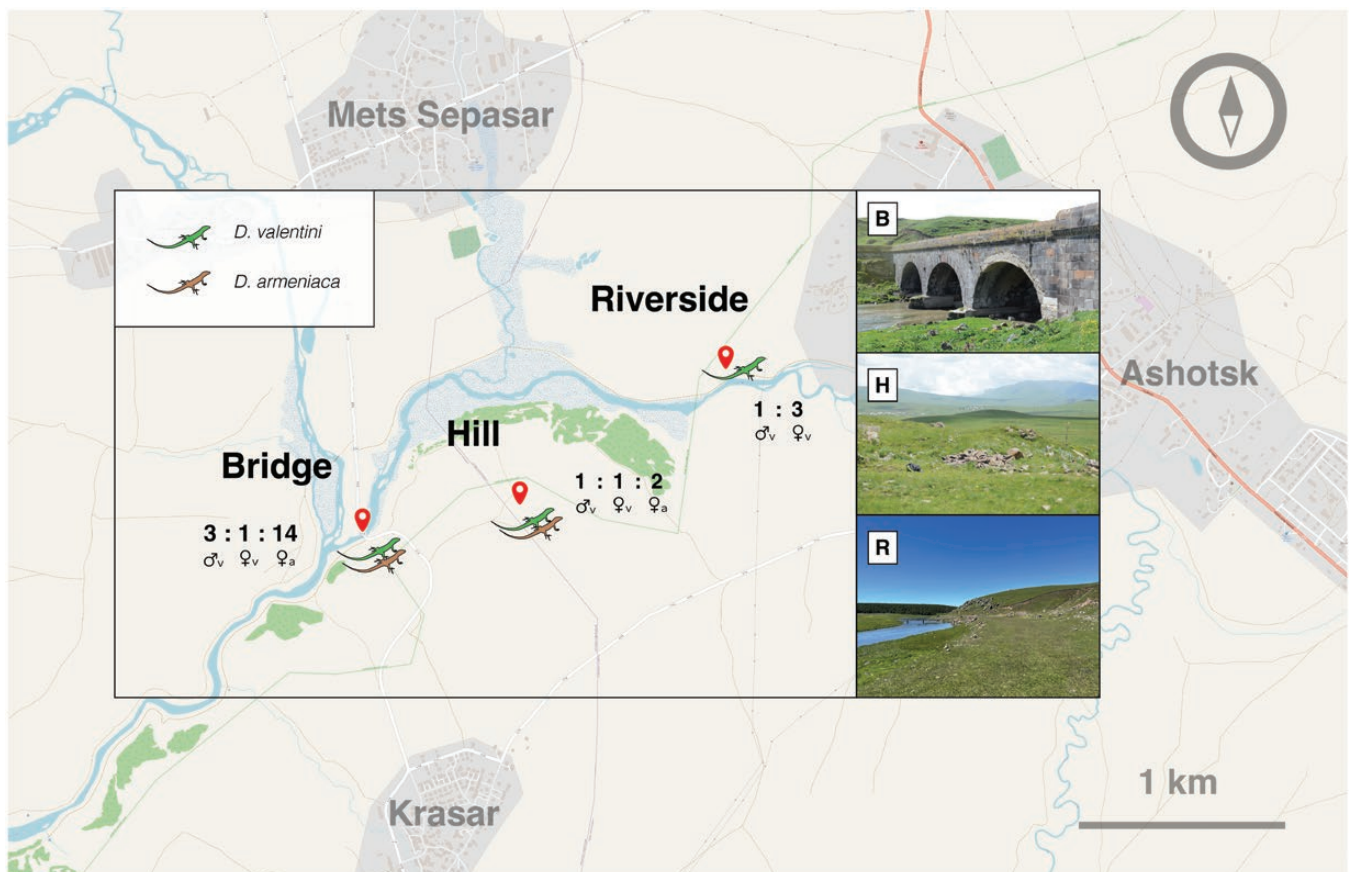


Figure 1. The layout of the three sampling sites and the proportion of the species and sexes comprising the studied lizard colonies. Abbreviations: a, *Darevskia armeniaca*; B, Bridge (two species); H, Hill (single species); R, Riverside (two species); v, *Darevskia valentini*.



Figure 2. Male *Darevskia valentini* detain females for ≤ 40 min for mating. During this time, they may bite the right or left flank of the female. A, mating with female *D. valentini*. B, mating with female *Darevskia armeniaca*. C, mating scars on the venter of female *D. valentini*.

during focal observation of the lizards. Animals marked with either or both of these methods survived for many years (Galoyan 2013; Galoyan *et al.* 2019b). Lizards were released at the point of capture after all manipulations. Individuals of either species with an SVL of >60 mm were considered adults (Galoyan *et al.* 2019a). In the Hill colony, a total of 132 mature *D. valentini* (55 males and 77 females) were captured and individually marked in 2014; 151 mature *D. valentini* (102 females and 49 males) were captured and individually marked in 2016, and 63 lizards (44 females and 19 males) were analysed for the presence of mating scars. In the sympatric Bridge colony, we captured 10 female and 29 male *D. valentini* and 82 females of *D. armeniaca* in 2021 to count mating scars and determine the proportion of individuals. In the Riverside colony, we captured and marked 29 female and 35 male *D. valentini*, in addition to 136 parthenogenetic *D. armeniaca*, in 2022.

Visual observations

We visually observed individually marked lizards during favourable weather conditions between 08:00 and 18:00 h. The observations occurred when the lizards were most active. Focal observations were performed by one or two observers positioned ~ 5 –10 m away from the lizards; when the lizards became accustomed to the presence of the observers, this distance was decreased. To minimize disruption to the lizards, observers moved slowly and took care not to disturb them. Over time, the observed individuals ceased deviating from their normal behaviour when approached or when observers passed by.

During the observation period, the observer selected a focal lizard and closely observed it for 10–15 min before switching to another individual. The observer documented the location, contacts between individuals (e.g. social interactions, successful and unsuccessful mating attempts), and routine behaviour. When

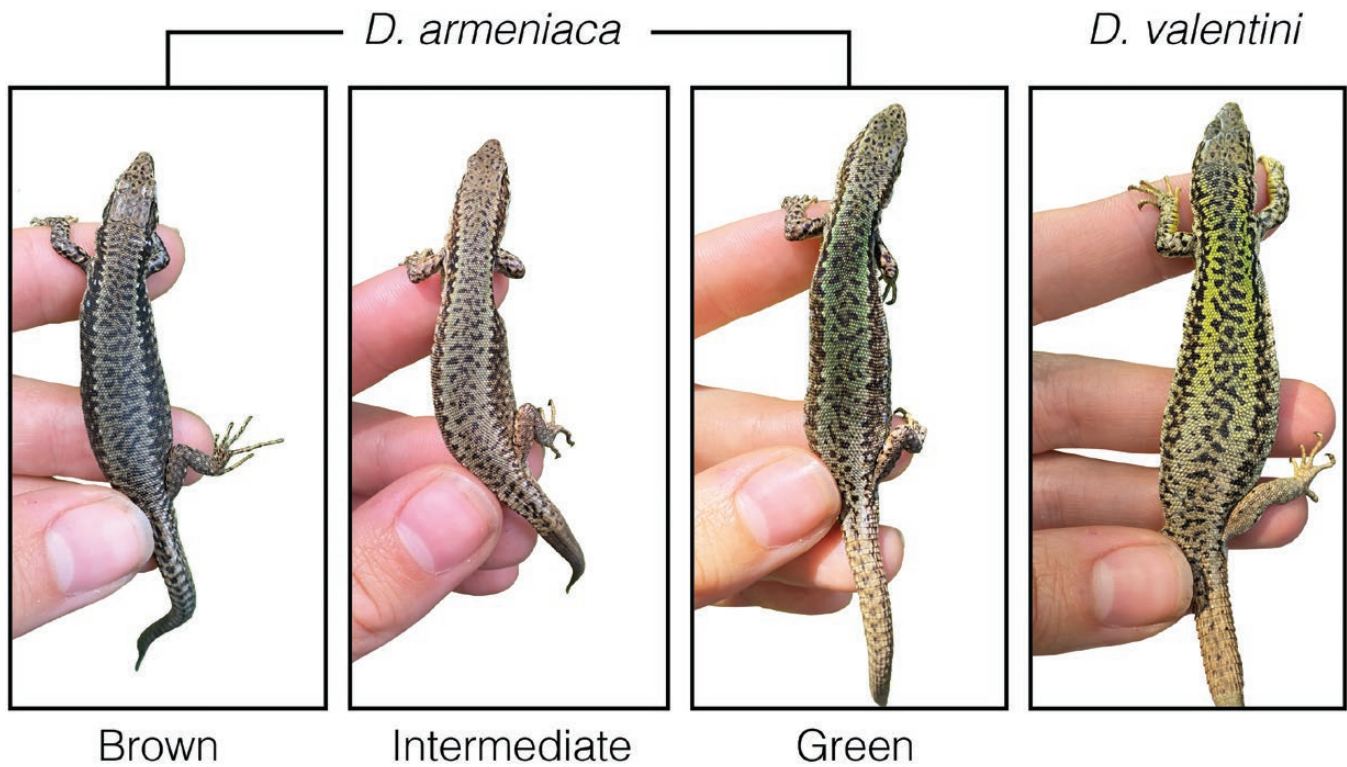


Figure 3. Dorsal coloration of parthenogenetic female *Darevskia armeniaca* and gonochoristic female *Darevskia valentini* from the studied population.

possible, these observations were recorded using a Nikon D600 or D750 camera with Nikkor 70-200/2.8 objective. Additional observations were recorded in a field notebook. All records and videos were reviewed and processed at the end of each observation day by the same person. In total, the observation durations were 144 h in 2014 and 2016 for the allopatric Hill site with only *D. valentini* and 65 h in 2022 for the Riverside site, where *D. valentini* coexisted with *D. armeniaca*.

Male–female relationships

In our study, we defined an interaction as an encounter between two or more individuals within a certain distance (typically ~1 m) if they could see each other clearly, without any obstructions, such as rocks, grass, or logs. We recorded a total of 352 intersexual contacts in *D. valentini* during 2 years (2014 and 2016) in the Hill site and 95 intersexual interactions in 2022 in the Riverside colony, where *D. armeniaca* coexisted with *D. valentini*.

To analyse these interaction patterns, we described the reactions of the interacting individuals. Each contact began with a direct reaction, where one individual (marked ‘A’) would respond to the presence of another individual (‘B’) before individual B switched from routine activities, such as moving or foraging, to social behaviours. Then, a reciprocal reaction would occur in response to the initial direct reaction (Tsellarius *et al.* 2008). It is worth noting that intersexual interactions were often initiated by males (Verbeek 1972), with females typically responding to the behaviour of the males.

During these interactions, we observed several different types of social reactions. Neutral behaviour was recorded when there was no noticeable change in behaviour from either individual

when lizards approached each other within the interaction distance and were able to see each other clearly. Agonistic behaviour encompassed both aggression and submission. Aggression was characterized by threatening displays, such as limb extension, dorsum curving, body flattening, and gular pouch engagement, often accompanied by short steps around the encounter. Aggressive behaviour also included chasing and biting. Submission involved circumduction (slow or fast rotation of the forelimb) and avoidance, which included a gradual retreat from the encounter area before physical contact with the other individual occurred or in response to the neutral behaviour of the other individual. Flee entailed a swift departure from the encounter area. Orientation behaviour was observed when individuals approached each other, often accompanied by tongue licking or nudging with the head. Affiliation was a type of acceptance behaviour, which included joint basking in physical contact or in proximity to each other, typically within a few centimetres. It was not uncommon for individuals to overlap their bodies with each other; one lizard would climb onto the back of another and vice versa.

In terms of sexual behaviour, mating attempts were documented. They often included chasing and forced restraint of a female. Females typically tried to escape and sometimes responded aggressively by biting the males. Non-aggressive mating was frequently preceded by affiliation. A mating attempt was considered successful if it culminated in copulation and unsuccessful if it did not result in copulation.

The number of observed contacts differed greatly between study sites and between individuals, both males and females. The total dataset included 351 observations of 34 individually

marked males, 74 individually marked females, a single unmarked male, and 15 unmarked females at the Hill site; and 93 observations of 32 marked *D. armeniaca* females, 16 unmarked *D. armeniaca* females, 13 marked and 10 unmarked *D. valentini* females, and 28 marked and one unmarked male at the Riverside site.

Statistical analysis

Statistical analysis and visualization were performed in RSTUDIO (v.2022.12.0 + 353) using *ggplot2* (Wickham 2016) with *ggp*, *tidyverse* (Wickham et al. 2019), *wPerm*, and *smpplot2* (Min 2021) packages.

Sex ratio deviations within the studied colonies were calculated with a binomial test. We chose the conservative Tukey's *post hoc* test. Percentages are given with percentage standard errors. We estimated the relationship between SVL and the number of mating scars with Spearman rank correlation and compared the distributions of female SVL according to female dorsum coloration using the Kruskal–Wallis test.

To neutralize the effect of these differences when comparing distributions of behavioural reaction types between species and colonies, we randomly selected a subset from a larger dataset and compared the proportions of interaction types between this subset and the smaller dataset. Each dataset included a single behavioural reaction of each individual. This operation was performed 100 times for each analysis for both sexes.

When comparing intersexual reactions of *D. valentini* males and *D. valentini* females between the Hill and Riverside sites, we selected 33 random observations from the Hill dataset with the aforementioned method, because the Riverside dataset contained only 33 observations in which *D. valentini* females participated.

To compare reactions of *D. valentini* males from the Riverside site with *D. valentini* and *D. armeniaca* females, we selected 23 observations in which *D. valentini* males interacted with *D. armeniaca* females, because our data contained observations of 23 males interacting with individually marked *D. armeniaca*. This subset was compared with the whole dataset of 33 observations of *D. valentini* males interacting with *D. valentini* females.

We used the permutation analogue of the χ^2 test of homogeneity implemented in *perm.hom.test* function of the *wPerm* R package to compare the discrete distributions of interaction types. Given that the permutation χ^2 test of homogeneity was performed in 100 replicates in each analysis, we obtained 100 *P*-values as an output. Assuming that the null hypothesis (distributions of interaction types are equal between two samples) is true, we expect 5% of *P*-values to be less than the significance threshold of .05. To estimate the real probability of obtaining a *P*-value of <.05, we performed the exact binomial test with the set of *P*-values. If the observed proportion of *P*-values < 0.05 was significantly >.05, we rejected the null hypothesis.

If the distributions were significantly different, we compared vectors of proportions generated during the previous step for each interaction type with the sign test. When zeroes appeared after subtraction of one vector from another in the sign test procedure, we replaced them with an equal number of -0.001 and 0.001, because the sign test does not calculate the correct *P*-value when zeroes are present.

Subsetting was not required to compare reactions of *D. armeniaca* and *D. valentini* females to males in the Riverside colony. A permutation χ^2 test of homogeneity was performed. Furthermore, we performed Fisher's exact test to compare mating success rates of *D. valentini* males with *D. armeniaca* females (*N* = 13) and *D. valentini* females (*N* = 13) in the Riverside colony. The sample of mating attempts from the Hill colony was larger than that from the Riverside colony (*N* = 71), and the number of observations differed greatly between individuals. Hence, we subsampled this dataset 1000 times such that only one random observation per individual per iteration was included. We then compared the mating success rate calculated for each of these subsamples with that of males mating with *D. valentini* females from the Riverside colony with Fisher's exact test.

Genetic analysis

Microsatellite differentiation of the ploidy was established using the previously developed method (Girnyk et al. 2021) on four *D. valentini*, 17 *D. armeniaca*, and four suggested triploids, which were captured in 2021 from the Bridge colony. We maintained five gravid females of *D. armeniaca* with mating scars on their venters in a small terrarium until they oviposited 12 eggs; then we released them at the place of capture. We incubated the eggs in small boxes filled with sand and sampled tail tips of the hatched offspring. The incubation of all eggs was successful, and the juveniles were released back into the family colony soon after hatching.

DNA was isolated from the lizard tail tips using standard phenol–chloroform extraction method with proteinase K and resuspended in TE buffer, pH 8.0. Three loci (Du281, Du323, and Du47G) were PCR amplified using previously described primer pairs (Korchagin et al. 2007, Girnyk et al. 2017). PCR was performed on 50 ng of DNA in a total volume of 20 μ L using a GenePak PCR Core Kit (Isogene) and 1 μ M of each primer. The reaction conditions were as follows: one cycle of 3 min at 94°C; 30 cycles of 1 min at 94°C, 40 s at the annealing temperature (50°C for Du281, 52°C for Du323, and 54°C for Du47G), and 40 s at 72°C; followed by one cycle of 5 min at 72°C. PCR products (6 μ L) were loaded onto an 8% non-denatured polyacrylamide gel (to separate allelic variants for each locus) and run for 4 h at 280 V. A 100 bp ladder (Fermentas) was used as a size marker. DNA in the gel was stained with ethidium bromide, and the results were visualized on a transilluminator (SIGMA 2202) and subsequently registered in the BioDoc-Analyze system (Biometra, Germany). To confirm the validity of this method, we checked the ploidy in 19 putative triploid hybrids between *D. valentini* and *D. armeniaca* from the population near Sotk village (N40.223894, E46.004368) and revealed seven triploids by the presence of triple stripes on the gel electrophoresis. Tail tips of these individuals were sampled in 2016 by E.A.G.

RESULTS

Composition of the colonies

Parthenogenetic females prevailed in both (Bridge and Riverside) sympatric colonies (Table 1). The sex ratio in *D. valentini* differed among all study sites. Male *D. valentini* prevailed over females of the same species in the Bridge site (binomial test = 0.235;

$N = 17$; $P < .05$), the number of male and female *D. valentini* was equal in the Riverside site (binomial test = 0.525; $N = 40$; $P = .874$), and the single-species Hill colony was female biased (binomial test = 0.733; $N = 45$; $P = .0024$).

Female SVL and mating scars

We found no differences in female SVL between *D. armeniaca* and *D. valentini* from the same sympatric colonies (Dunn's *post hoc* test, Bridge colony $Z = -2.146$, $P = .318$; Riverside colony $Z = .283$, $P = 1$; Table 2). The SVL of *D. valentini* females did not differ significantly among all three colonies (Dunn's *post hoc* test). However, *D. armeniaca* females from the Bridge colony were larger than those from the Riverside colony (Dunn's *post hoc* test, $Z = 3.258$, $P = .011$).

The percentages of parthenogenetic females bearing mating scars on their venters (Table 2) in two sympatric colonies did not differ significantly ($\chi^2 = .005$; d.f. = 1; $P = .942$). Adult female *D. valentini* with mating scars were more common than parthenogenetic female *D. armeniaca* with mating scars in sympatric colonies (Bridge $\chi^2 = 44.477$, d.f. = 1, $P < .0001$; Riverside $\chi^2 = 27.612$, d.f. = 1, $P < .0001$; Table 2).

The number of mating scars per female was greater in bisexual species than in parthenogens (Kruskal–Wallis test, Bridge colony $\chi^2 = 11.496$, d.f. = 1, $P < .0001$; Riverside colony $\chi^2 = 18.594$, d.f. = 1, $P < .0001$).

We did not find any correlation between female SVL and the number of mating scars in females of parthenogenetic *D. armeniaca* (Fig. 4; $\rho = .33$; $P = .002$; $N = 87$); however, we found a positive correlation in females of the bisexual *D. valentini* (Fig. 4B; $\rho = .564$; $P < .0001$; $N = 53$).

Female coloration

Among 131 subadult and adult *D. armeniaca* from two colonies (Bridge and Riverside), 59 had brown dorsal coloration, 56 had intermediate dorsal coloration, and 16 had green dorsal

coloration. All immature individuals were brown ($N = 23$) or intermediate ($N = 4$). Mean SVLs of brown, intermediate, and green *D. armeniaca* females were 60.2 ± 4.40 , 63.4 ± 4.01 , and 66.4 ± 3.10 mm, respectively.

Brown *D. armeniaca* females had fewer mating scars than green females (Dunn's *post hoc* test, $Z = -3.967349$; $P = .0001$) and intermediate-coloured females (Dunn's *post hoc* test, $Z = -3.489$; $P < .001$; Fig. 5) of the same species.

Relationships between males and females

Males often initiated affiliation with females of both species (Fig. 6). Frequencies of male reactions to *D. armeniaca* and *D. valentini* females from the sympatric Riverside colony were significantly different (permutation χ^2 test, $P < .0001$). Males reacted neutrally and performed orientation behaviour more frequently when interacting with *D. armeniaca* than with *D. valentini* females (sign test, $P < .0001$ in both cases). Male submission, affiliative behaviour, and mating attempts were observed more often when males interacted with *D. valentini* females than with *D. armeniaca* females in the Riverside colony (sign test, $P < .0001$ in all cases). Male aggression, flee, and avoidance were not observed towards females of either species in the Riverside colony, unlike in the Hill colony (Fig. 6).

Males showed neutral behaviour, avoidance, flee, and orientation behaviour more frequently towards *D. valentini* in the Hill colony than in the Riverside colony (sign test, $P < .0001$ in all cases). Furthermore, males reacted to female *D. valentini* with submission and mating attempts more frequently in the Riverside colony (sign test, $P < .0001$ in both cases). Frequencies of aggressive and affiliative reactions of males towards *D. valentini* females did not differ significantly (sign test, $P = .3682$ and $P = .1933$, respectively) between the Hill and Riverside colonies.

Female reactions towards males were mostly submissive (Fig. 7). The social behavioural repertoire of parthenogenetic females was as full as that in females of bisexual species in sympatry.

Table 1. Percentage of captured lizards in three studied colonies. Proportions are given with standard errors of the proportion.

Colony	Year	♀ <i>Darevskia valentini</i>		♂ <i>Darevskia valentini</i>		♀ <i>Darevskia armeniaca</i>		Number of lizards
		Subadults	Adults	Subadults	Adults	Subadults	Adults	
Bridge	2021	5.0 ± 1.97	3.3 ± 1.63	13.2 ± 3.08	10.7 ± 2.82	22.3 ± 3.97	45.5 ± 4.53	121
Riverside	2022	4.0 ± 1.39	10.5 ± 2.17	8.0 ± 1.92	9.5 ± 2.07	28.0 ± 3.17	40.0 ± 3.46	200
Hill		17.5 ± 4.78	52.4 ± 6.29	11.1 ± 3.96	19.0 ± 4.95			63

Table 2. Number of lizards (N), average snout–vent length (SVL) of adult female bodies, average count of mating scars per female on their venters (MS), and percentage of females with mating scars (MP%) in three sampling colonies. Values are presented as the mean ± SD.

Colony	<i>Darevskia valentini</i>				<i>Darevskia armeniaca</i> ^a			
	N	SVL (mm)	MS	MP%	N	SVL	MS	MP%
Bridge	4	69.5 ± 1.32	7.5 ± .96	100% (4/4)	55	64.9 ± .40	1.47 ± .21	61.8 ± 6.55% (34/55)
Riverside	21	62.8 ± .59	4.10 ± .53	95.2 ± 4.65	56	63.1 ± .35	1.3 ± .27	64.3 ± 4.47%
Hill	33	65.3 ± .67	8.29 ± 1.31	72.7 ± 7.75%	Absent			

^aParthenogenetic species.

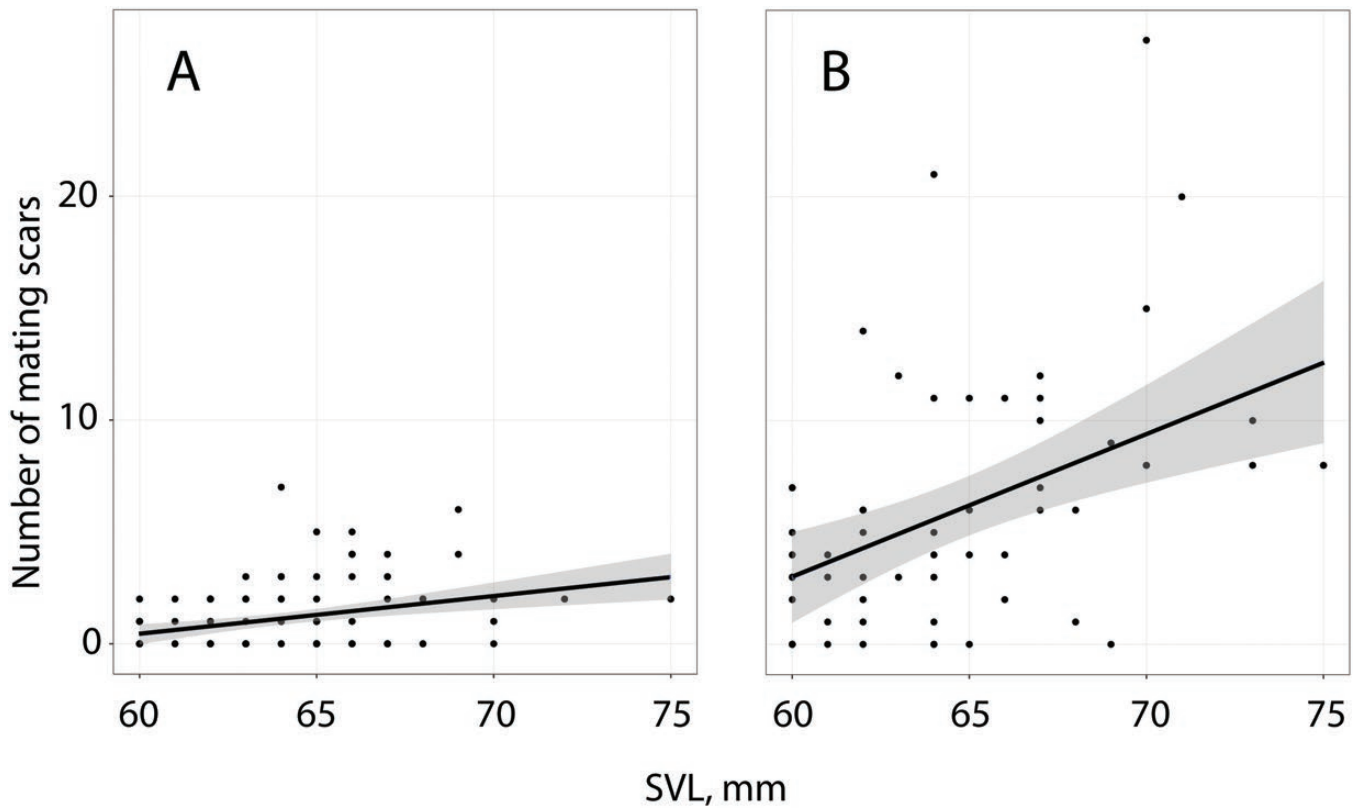


Figure 4. Correlation plots between female snout-vent lengths (SVLs) and the number of mating scars on the venter for parthenogenetic female *Darevskia armeniaca* (A) and females of the bisexual species *Darevskia valentini* (B) from the areas of their coexistence.

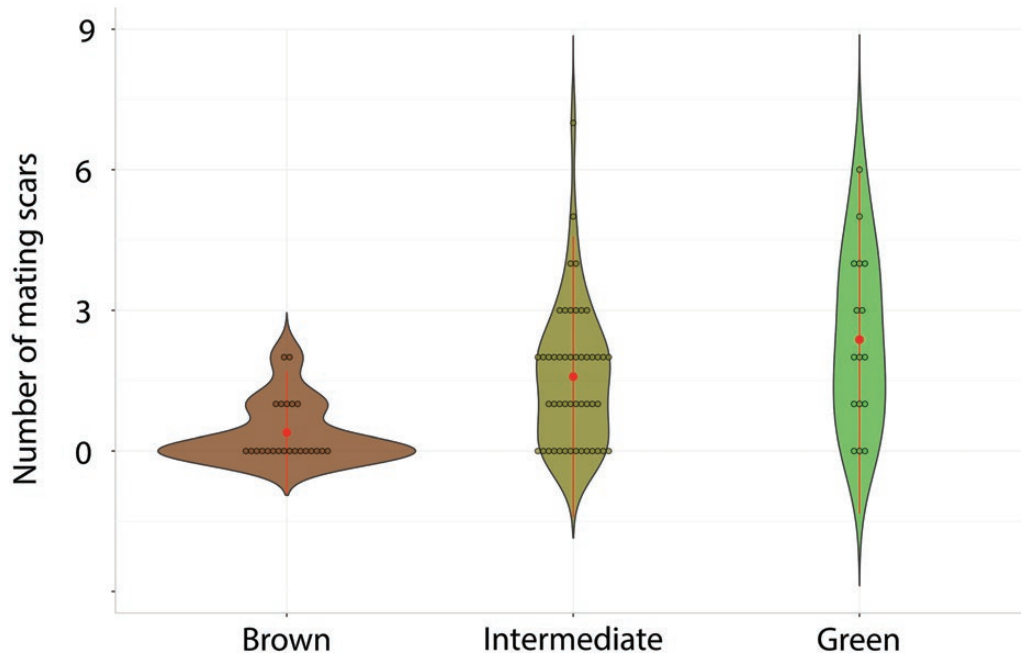


Figure 5. The proportion of mating scars on the venters of parthenogenetic female *Darevskia armeniaca* with brown, intermediate and green dorsal coloration from the sympatric Riverside colony.

There were no differences in *D. armeniaca* and *D. valentini* female reactions to male *D. valentini* in the Riverside colony (permutation χ^2 test, $P = .64$). Female *D. valentini* were more aggressive and less often submissive towards males in the Hill colony

than in the Riverside colony (sign test, $P < .001$ in both cases). Female *D. valentini* from the Hill colony avoided males and performed orientation and affiliative behaviour towards them more frequently than females from the Riverside colony (sign test,

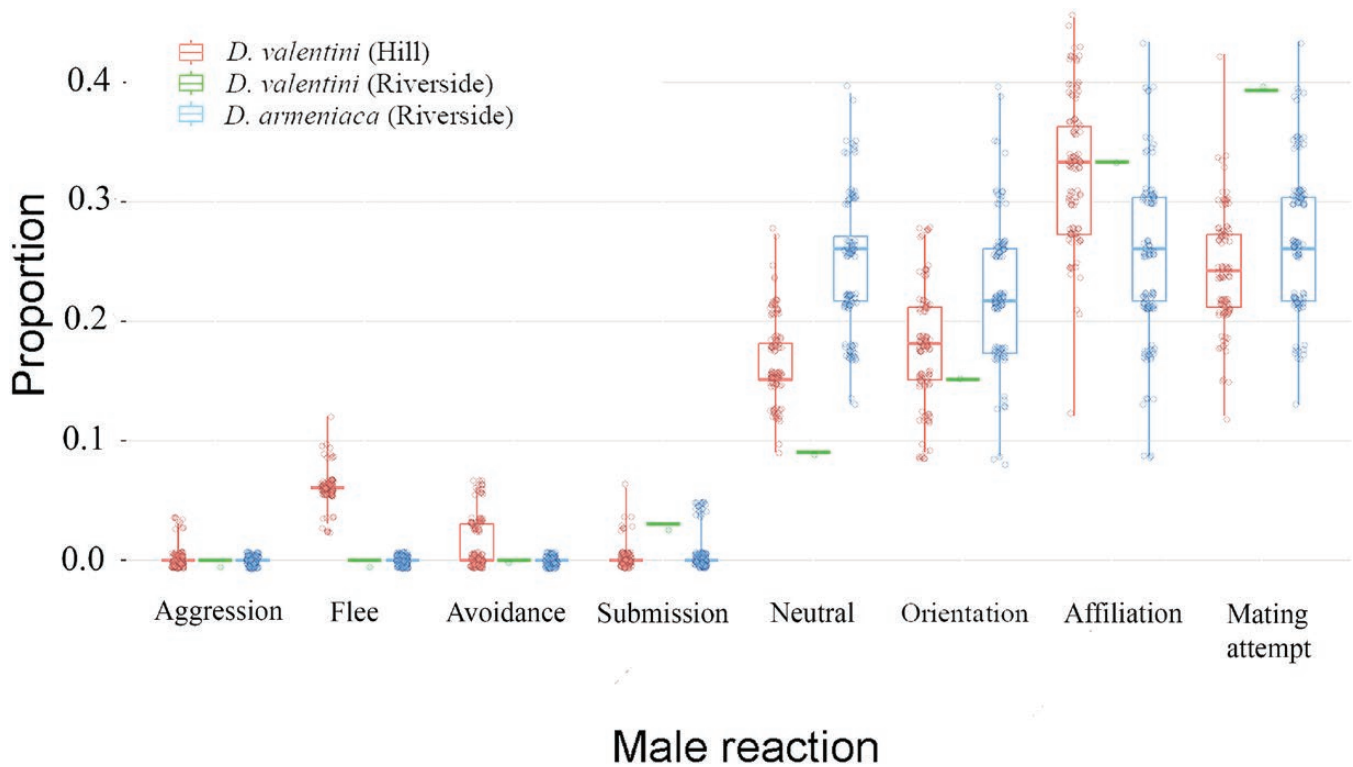


Figure 6. Boxplot showing the proportion of male reactions to females of both species within two studied colonies. Each point represents one iteration of the bootstrap procedure described in the Materials and Methods. Horizontal lines in the boxplots represent the median proportion over 100 bootstrap iterations and the observed proportion in the *Darevskia valentini* Riverside sample, which was not bootstrapped. The box represents the interquartile range, and whiskers represent the total range of the values. Points are jittered to avoid overplotting.

$P < .0001$ in all cases). In contrast, *D. valentini* females from the Hill colony were less likely to flee from males than *D. valentini* females from the Riverside colony ($P < .0001$).

The percentage of observed successful mating attempts with *D. valentini* females was $38.5\% \pm 13.5\%$ ($N = 13$), in comparison to $15.4\% \pm 10.00\%$ ($N = 13$) with parthenogenetic *D. armeniaca* females in the Riverside colony. However, this difference was not significant (Fisher's exact test, $P = .6447$). Among 71 observed mating attempts performed by 19 males from the Hill colony, 16 ($22.5\% \pm 4.96\%$) were successful and concluded with copulation. Proportions of successful and unsuccessful mating attempts with *D. valentini* females were not significantly different between the Hill and the Riverside colonies (1000 times Fisher's exact test, $P > .1937$ in all iterations).

Genetic analysis and ploidy

Four suggested hybrids were determined as *D. valentini* (two males and two females) in the Bridge colony in 2021. However, microsatellite analysis did not reveal any triploids among 44 studied individuals from this colony. There were also no triploid individuals among 12 offspring of five *D. armeniaca* bearing mating scars on their venters.

DISCUSSION

Parthenogenetic rock lizards often coexist with parental bisexual species (Petrosyan *et al.* 2020). The proportion in these multispecies colonies is often biased towards parthenogens, which is supported by our observations (Table 1) and other

studies (Abrahamyan *et al.* 2014). When the number of parthenogenetic lizards is tens of times greater than the number of bisexual species, males often mate with parthenogens (Carretero *et al.* 2018). This could be explained by the scarcity of conspecific females and absence of choice for males of bisexual species. However, as we saw in the Riverside colony, where the number of parthenogenetic females was not so dramatically shifted towards parthenogens, males still frequently contacted and mated with them. The number of mating scars per female in sympatry indicates that males preferred to mate with larger females of their own species; surprisingly, this was not the rule for parthenogenetic females. The fact that male lizards often prefer larger females is well documented (Olsson 1993) and is usually explained by better reproductive success of larger females (Olsson 1993). This is supported by the number of eggs laid by female *D. valentini* from this area: two for 3-year-old females, five or six for 4-year-old females, and more than six for females >5 years old (Galoyan *et al.* 2019a). Parthenogenetic females were less attractive to males regardless of their SVL. The coloration of females might also be an important sexual signal for males, and male mate choice can be affected by female coloration, as in *Acanthodactylus erythrurus*, in which males choose red females (Belliure *et al.* 2018). Adult female *D. valentini* are green; adult females of *D. armeniaca* can be green, pale green, or brown (Fig. 3). The average SVL of green females in *D. armeniaca* was larger than that of brown females; hence, larger and older parthenogenetic females were more attractive to males than younger individuals. The absence of differences in female size of *D. armeniaca* and *D. valentini* from the same colonies demonstrates that males

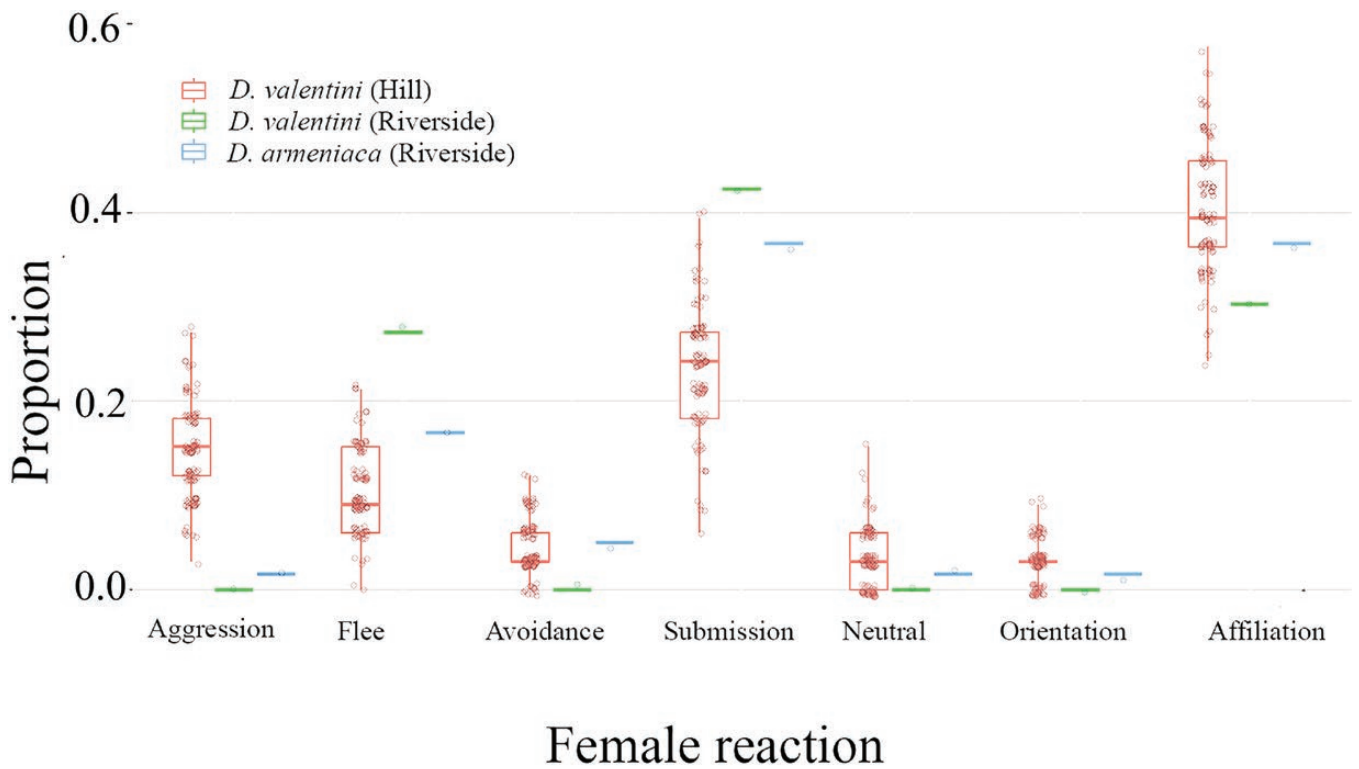


Figure 7. Boxplot with proportion of female reactions to males within two studied colonies. Each point represents one iteration of the bootstrap procedure described above. Horizontal lines represent the median proportion over 100 bootstrap iterations and the observed proportion in the *Darevskia valentini* Riverside and *Darevskia armeniaca* Riverside samples, which were not bootstrapped. The box represents the interquartile range, and the whiskers represent the total range of the values. Points are jittered to avoid overplotting.

preferred to mate with conspecifics not because of their body size but rather because of natural preferences.

Nevertheless, the social roles of parthenogenetic *D. armeniaca* females and bisexual *D. valentini* females from the same colony are similar (Fig. 7). Male reactions to conspecific and heterospecific females (Fig. 6) are different, although not as much as in colonies with other coexisting bisexual species, such as *Darevskia raddei* and *Darevskia portschinskii* (Galoyan *et al.* 2019b). In that case, males mostly ignored or avoided heterospecific females of bisexual species. Heterospecific matings do not occur and intersexual social behaviour is rare in the complex colonies comprising three species of rock lizards (Kropachev *et al.* 2023). In our study, parthenogenetic females attract males as sexual and social partners and behave like conspecific females (Fig. 7). The differences in female reactions of *D. valentini* inhabiting the single-species Hill colony were greater than the differences in female reactions in the Riverside two-species colony (Fig. 7). This is not surprising given that lizards discriminate against heterospecific partners of the same genus in nature (Galoyan *et al.* 2019b) and in experiments (Cooper and Vitt 1986), although our observations demonstrate that parthenogenetic species integrate fully into the social, spatial, and sexual systems of parental species, and colonies become multispecies colonies from an ecological and behavioural point of view.

The most striking behavioural difference among females from the single-species and multispecies colonies was in their aggressiveness levels towards males. It seems that female mate choice of *D. valentini* is based on female aggression, allowing them to choose fitter males. Male rock lizards that are able to establish

social bonds with certain females have more opportunities to mate with them (Tsellarius *et al.* 2017). The competition between males in multispecies populations is reduced if they mate with parthenogenetic females rather than conspecifics. This leads to competition between *D. armeniaca* females and *D. valentini* females for social and sexual partners. Such competition should affect the female behaviour of bisexual species and make them less aggressive towards males to increase mating success and the number of mates per season. This is what we observed in the Riverside colony (Fig. 7).

The number of mating scars per female demonstrated that females often mated several times during the same reproductive season. This was observed previously in rock lizards (Galoyan *et al.* 2019a) and strongly supports that multiple matings increase the genetic variability of lizard offspring (Olsson 1993). This is true for *D. valentini* but not *D. armeniaca* from the studied multispecies colonies. Although hybrids between *D. armeniaca* and *D. valentini* were described in several areas in Armenia (Arakelyan *et al.* 2008, Petrosyan *et al.* 2020), and hybrids between other pairs of parthenogenetic and bisexual species are reported in the literature (Girnyk *et al.* 2021), we discovered none in the vicinity of Mets Sepasar village. Triploid hybrids most probably do not appear in the colonies with parthenogenetic and bisexual species near Mets Sepasar village. However, the presence of hybrids has no impact, owing to their sterility (Saint Girons and Ineich 1992, Arakelyan *et al.* 2008, Spangenberg *et al.* 2017). Male *D. valentini* mate with *D. armeniaca* in vain and do not invest in parthenogenetic offspring. Shifting of male attention from conspecific females to parthenogens would reduce

the fitness of *D. valentini* males. It also might reduce the number of fertilized eggs in females of bisexual species and influence the genetic diversity of the offspring. Ecological outcompeting of bisexual species by parthenogenetic species has already been shown for other pairs of *Darevskia* lizards (Tarkhnishvili *et al.* 2010). We suggest that competition for males might be one of the mechanisms that allows parthenogenetic lizards to outcompete their bisexual relatives.

CONCLUSION

Males prefer to mate with females of bisexual species rather than parthenogenetic females. However, parthenogenetic females integrate into the social and mating systems of parental species of rock lizards and compete with females of bisexual species for males. The presence of competing females affects female behaviour, and the effective role of mate choice based on female aggression is lessened in multispecies colonies. There are no differences in reactions of females to males and vice versa in multispecies colonies. Mating with parthenogenetic females does not necessarily lead to the origin of triploid hybrids, and some physiological mechanisms hinder the origin of triploid hybrids, although they can appear in other circumstances.

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AUTHOR CONTRIBUTIONS

Design of the work – Eduard A. Galoyan; Collected the data – Eduard A. Galoyan, Natalia G. Sopilko, Anna V. Kovalyeva, Oleg D. Nikolaev, Ivan I. Kropachev, Ilya A. Brinev; Genetic analysis – Anastasiya E. Girnyk; Contributed and analysed data – Eduard A. Galoyan, Natalia G. Sopilko, Marine S. Arakelyan, Oleg D. Nikolaev, Ivan I. Kropachev; Wrote the manuscript – Eduard A. Galoyan, Marine S. Arakelyan, Oleg D. Nikolaev, Ivan I. Kropachev, Natalia G. Sopilko.

CONFLICT OF INTEREST

None declared.

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DATA AVAILABILITY

Raw tables and model scripts are available by application to E.A.G.

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