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ORIGINAL ARTICLE

Friend-or-foe? Behavioural evidence suggests interspecific discrimination leading to low probability of hybridization in two coexisting rock lizard species (Lacertidae, *Darevskia*)

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Abstract

The parthenogenetic rock lizard *Darevskia rostombekowi* is considered to be the product of hybridization between female *Darevskia raddei* and male *Darevskia portschinskii*. These two species coexist within several secondary contact areas; however, no trace of their hybridization has been previously reported. We conducted focal observations of individually marked lizards in 2017 and 2018 to establish if there is behavioural isolation between these species. We demonstrate that individuals distinguish between lizards of the same and different species. Individuals of both studied species interacted regularly, but we found no evidence of males of either species using interspecific interference competition for resources or for females. Neutral reactions prevailed in the reactions of males to the individuals of the different species; aggressive or submissive behaviour was more common towards males of the same species. The differences in reactions of males to females of the same and different species were less clear and interspecific social interactions were almost as common as intraspecific interactions. Interspecific male mating behaviour was rare and unsuccessful; only a single female with heterospecific copulation marks on the body was found. The mating period in both species overlapped broadly in late May and early June; body size (SVL) in females of both species was equal. Hence, we suggest that the selection of the sexual partner and preference of the conspecific partner for the social and sexual contacts is the most plausible explanation of the revealed behavioural asymmetry in intra- and interspecific relationship.

Significance statement

Here, we present the first description of the social and sexual behaviour of two bisexual species of rock lizard belonging to the *Darevskia* genus. Most works devoted to the study of reticulate evolution in lizards have used genetic and morphological approaches rather than behavioural observations. The study of the proximal mechanisms of evolution is important in understanding how evolution occurs. The results indicate that even in ecologically similar species, social and sexual selection tend towards separation of the species rather than mixing, although they are able to coexist peacefully.

Keywords Social behaviour · Hybridization · *Darevskia* · Reproduction barrier · Species recognition

Introduction

Of the more than 30 species of rock lizard (*Darevskia* spp.), seven are parthenogenetic. Their origin and role in reticulate evolution of the complex have been studied mostly from a genetic and cytogenetic point of view (Cuellar 1974; Borkin and Darevsky 1980; Vrijenhoek 1989; Cole and Townsend 1990; Darevsky 1995; Grismer and Grismer 2010; Freitas et al. 2016; Spangenberg et al. 2017). As a result, there is an extensive body of data supporting the hypothesis of origin of each parthenogenetic species from hybridization between different bisexual taxa of the same genus (Darevsky et al. 1985; Cole et al. 1988; Moritz et al.

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1992; Fu et al. 2000a, b; Murphy et al. 2000; Grismer et al. 2014).

The possibility of hybridization between different bisexual species is supported under laboratory conditions (Danielyan 1965; Darevsky et al. 1985; Darevsky 1995). Although in some experiments only sterile hybrids of the first generations were bred (Vrijenhoek 1989), other experiments had more promising results. The first artificial breeding of a unisexual fish belonging to the genus *Poeciliopsis* was reported by Schultz (1973), and the first parthenogenetic lizard *Aspedocelis* (Teidae) was obtained fairly recently by Cole et al. (2014). These experiments support the potential of interspecific hybridization to lead to the origin of new species of vertebrates, including parthenogenetic ones. However, there is a lack of both genetic and experimental data on the relationships and interactions of heterospecific individuals to each other in natural habitats. Moreover, the proximal mechanisms resulting in hybridization between different related species remain poorly studied (Wirtz 1999; Rosenthal 2013).

Interspecific mating and hybridization generally occur within the areas of secondary species contacts. This is supported by studies of *Aspedocelis* (Dessauer et al. 2000), *Podarcis* (Pinho et al. 2009), *Vipera* (Tarroso et al. 2014) and other reptiles (Axtell 1972; Jenssen 1977; Jančúchová-Lásková et al. 2015; Panov and Zykova 2016). In some cases of secondary contacts, gene flow between species is absent (Gabirot et al. 2010). Likewise, parthenogenetic lizards often coexist and mate with the males of parental species (Darevsky et al. 1985; Taylor et al. 2001; Danielyan et al. 2008; Jančúchová-Lásková et al. 2015). Hence, range overlap is a key precondition for hybridization.

Two types of syntopy between bisexual species of rock lizard are described by Darevsky (1958). For the first type, the area of distribution of one species overlaps the area of another species. For instance, a small number of *D. valentini* (paternal species of *D. unisexualis*) individuals have been found within the range of *D. raddei nairensis* (maternal species) in the area of Lake Sevan, Armenia (Danielyan 1965; Darevsky et al. 1985). However, no hybrids were recorded there, most likely due to different timing of reproduction and gonadal ripening (Danielayn 1965). The second type of species coexistence is characterised by almost equal numbers of individuals of both syntopic species (Arakelyan 2012).

Darevskia raddei and *D. portschinskii* are proposed to be parental for *D. rostombekowi* (Fu et al. 2000b; Murphy et al. 2000; Ryskov et al. 2017). To date, the ranges of these parental species are mostly allopatric. One contact zone between them has been found in the vicinity of the village of Gosh in Armenia, where about 9% of individuals of *D. portschinskii* occur within the range of *D. raddei* (Arakelyan and Danielyan 2014). However, a study of allozymes in *D. raddei*, *D. portschinskii*, and plausible hybrids between them revealed no evidence of hybridization in this area (Uzzel and Darevsky 1973). In contrast to the population in Gosh, a large zone of syntopy, representing this second type of hybrid zone, was found for *D. portschinskii* and *D. raddei* in 2004 in the vicinity of Zuar village in Artsakh (Arakelyan 2012). Again, no hybrids have been discovered here (Galoyan et al. 2019).

According to the hypothesis of hybridogenous speciation, the parental species for *D. rostombekowi* (*D. raddei* and *D. porschinskii*) should lack interspecific reproductive barrier. However, their stable coexistence within the secondary contact zones and the absence of any evidence of hybridization suggests the existence of such a barrier. Similar bimodal hybrid zones with some, but not absolute, isolation have been reported for *Podarcis* (Pinho et al. 2009). Our goal was to determine if there are any behavioural mechanisms of reproductive isolation between *D. raddei* and *D. portschinskii* in the area of their coexistence. We suggest that the lizards' reactions to individuals of the same and different species must be different if they can distinguish between species and if some prezygotic reproduction barriers are present. Otherwise, the males of both species should mate with females of both species and compete for them with males of both species. Therefore, our aim was to analyse the type and frequency of contacts between two coexisting species of rock lizard, which are parental to *D. rostombekowi* in the area of their sympatry.

Methods

Study species

According to Arribas (1999), *D. portschinskii* (Kessler 1878) belongs to the 'rudis' clade, while *D. raddei* (Boettger 1892) is part of the 'raddei' clade within the *Darevskia* genus. Both species are diurnal lizards distributed to the south of the Great Caucasus, and are found up to an elevation of 1500 m.a.s.l. for *D. portschinskii* and 3200 m.a.s.l. for *D. raddei*.

Both studied species have a slim and relatively small body, with a flattened head well-adapted to living among rocks and squeezing within narrow crevices. SVL of *D. portschinskii* reaches 67 mm and SVL of *D. raddei* reaches 69 mm (Darevsky 1967; Arakelyan et al. 2011). Males of *D. raddei* are larger than females and have similar body size to *D. portschinskii* (Darevsky 1967). Body proportions are different between sexes for both species: males have wider and more robust heads and females have longer bodies (Arakelyan et al. 2011; Dehghani et al. 2014). Dorsum coloration is brown (greenish in adult males) with dark indistinct blotches and two wide flank bands in *D. portschinskii*; brown with reticulate dark pattern in *D. raddei*. Belly is light or bright yellow for *D. portschinskii* and green-yellow for *D. raddei*. Most species of rock lizards, including *D. raddei* and *D. portschinskii*, become sexually mature adults after their third winter, when their SVL exceed 50 mm (Darevsky 1967; Arakelyan 2001).

In general, *D. raddei* prefers dryer and sunlit habitats, while *D. portschinskii* inhabits rocky habitats along rivers and in deciduous forests (Arakelyan et al. 2011). In some areas, both species tend to occupy the same rocky steppes (Uzzel and Darevsky 1973).

Study area

The survey area was located near the village of Zuar in the south-eastern most part of the Lesser Caucasus Mountains at a height of 1500 m.a.s.l. on the rocky southern slopes, comprising the easternmost part of the range for both *D. portschinskii* and *D. raddei.* Here, deep river valleys lie between watersheds, where rock lizards occupy the rocky outcrops along the roads and trails within the gorges, as well as the rocks and stony heaps on the slopes and the edges of the watersheds covered with deciduous oak forests and grass meadows.

A rocky plot of about 4000 m^2 in the area of the village was chosen as the survey area. A 2.5-m high stony wall and the remains of an old abandoned building were within the study site. There was also a meadow with a few trees (birch, medlar, plum and hornbeam). Here, the lizards mostly occupied the walls, using the cracks between stones as shelter, as well as wall debris and stony heaps, although they also moved from one rocky patch to another crossing the vast areas of grassland.

Field work

The field survey covered the period between 23 May to 27 June 2017 (27 working days) and 9 May to 21 July 2018 (33 working days). According to our observations, this timing covers the reproduction period of both species in the studied area. Mating period in *D. portschinskii* starts in mid-May and ends in early June, and it starts in late May and ends in late June in *D. raddei* (ESM: Table S1). Adult lizards (snout vent length [SVL] > 50 mm) were captured within the study area by hand or by noose. Each lizard was individually marked using a combination of two colour beads sewn through the skin fold on the dorsum or flank caudal area using nylon line (Fisher and Muth 1989). This method makes it possible to observe and distinguish individuals from a distance. In addition, photographic identification was used to identify individuals. The described marking technique has no negative impact on the lizard's behaviour (Tsellarius and Tsellarius 2001; Husak and Fox 2003; Nicholson and Richards 2011; Galoyan 2013, 2017).

Most lizards were marked in the first days after emergence from their winter shelters and newcomers were marked as we encountered them. We measured the SVL of each individual using callipers with an accuracy of 0.5 mm and photographed the dorsal pattern of the lizard for future identification.

Visual observations

Visual observations of individually marked lizards were performed between 08:00 and 18:00 h on days with favourable weather conditions, when the lizards were active. Focal observations were made by one or two observers from 5 to 10 m or closer, when the lizards had become habituated to the presence of the observer. It was not possible to record data blind because our study involved focal animals in the field. We moved slowly to minimise any disturbance to the lizards. After several days, observed individuals paid less attention to the observer (did not switch from routine behaviour when the observer approached or could pass near the observer). The observer chose a focal lizard and observed the individual for 10–15 min, before switching to another individual. The location and contacts, including successful or unsuccessful mating attempts, and foraging behaviour, were registered and recorded using a Nikon D600 camera whenever possible, or were written down in the field book. All records and videos were processed at the end of each observation day, by the same person. The total observation time was 141 h in 2017 and 164 h in 2018.

Interactions and relationship

Individual ranges overlapped broadly among the observed individuals of both species and lizards had the opportunity to interact with individuals of both species. We considered an interaction to be an encounter between two or more individuals within the reaction distance, which is less than or around 1 m for rock lizards (Galoyan 2013), if the individuals are clearly able to see each other (there are no rocks or logs between potential contactors). In total, we observed 227 contacts in 2 years (Table S2). We did not have enough data for contacts between females for analysis. Interspecific contacts were as common as intraspecies contacts (Table 1). We constructed a social network based on all interactions observed in 2017 using the 'igraph' and 'network' packages in R (Csardi and Nepusz 2006).

Each contact started with a direct reaction, e.g. (the reaction of individual 'A' to individual 'B' before B switched from routine (moving, foraging, etc.) to social behaviour.

Table 1 Matrix of observed interactions between marked individuals

		Males	
		D. raddei	*D. portschinskii*
Male	*D. raddei*	44 (37.3%)	55 (46.6%)
	D. portschinskii		19 (16.1%)
Female	*D. raddei*	48 (46.2%)	10 (9.6%)
	D. portschinskii	14 (13.5%)	32 (30.7%)

Reciprocal reaction was generated in response to the direct reaction (Tsellarius et al. 2008). We also examined neutral interactions, which occurred if the lizards approached each other to the reaction distance and could see each other but did not change their behaviour. Males often initiate intersexual interactions (Verbeek 1972), whereas females mostly react as a response to the reaction of the particular male. Hence, when analysing intersexual contacts, we only took into account male reactions to the females and excluded females' reactions. We also only used interactions in which both interactors were identified and marked.

The following types of social reaction during the interactions between individuals were described: neutral, agonistic (aggressive and submissive), orientation and friendly behaviour. *Neutral behaviour* was defined as the absence of any reaction to the conspecific (no change in behaviour, Video S1). Agonistic behaviour included aggression and submission. *Aggression* was characterised by 'threat' with the individual extending its limbs, curving the dorsum, flattening the body flanks and gular pouch and moving with short steps often around the encounter; 'attack' involved chasing and biting the second contactor (Video S1, S2). *Submissive behaviour* comprised 'submission', represented by circumduction (slow or fast forelimb rotation), often with slow retreat from the encounter area before actual contact with the partner happened, or in the case of a neutral reaction from the partner; 'flee' entailed leaving the encounter area promptly. *Orientation* was characterised by 'approach to the partner', often with tongue licking. The approaching individual could also push the partner with the tip of the head. *Friendly behaviour* was characterised by jointly basking in a physical contact or in one or two centimetres from each other. Individuals could also overlap each other: one lizard gets on the back of the other and then goes further; we termed this a friendly reaction (Video S3).

Sexual behaviour included courtship and mating attempts, which could differ depending on the relationship between the particular individuals (Tsellarius et al. 2016). Sexual harassment and mating attempts included chasing and forced restraint of a female. Usually, females tried to escape and could respond aggressively to the male's harassment (biting the male, Video S4). Non-aggressive mating was often preceded by joint basking with physical contact (friendly behaviour). We termed a mating attempt successful if it ended with copulation and unsuccessful if not.

Statistical analysis

The distribution of the body lengths (SVL) was tested for normality using the Shapiro-Wilk normality test. Medians of SVL are reported with minimum and maximum measurements for the lizards from the studied settlement within each species and sex; the comparison among individuals in different years and species was performed with unpaired Wilcoxon test.

We developed three data sets for the reactions of each individual from the pool of observed reactions. Each of the data sets included only one reaction of a focal individual to another individual belonging to one of four categories (male of the same species, male of the different species, female of the same species and female of the different species). Each data set included randomly selected reactions for each focal individual to the individual from the particular category. For instance, if we had three reactions of male A to the females of the same species, we three times randomly selected one of these reactions using a random selection command in the R package. This method allowed us to avoid the effect of a bias of contacts for particular individuals. Then, we compared the distribution of reaction frequencies among the datasets using the chi-square (χ^2) test to reveal the absence of differences in the distributions of reactions. The final dataset for analysis included medians of reactions for the three datasets (Table S2). We calculated the proportion of the reactions of males of both species to the males and females of both species and compared the distribution of reactions using unpaired Wilcoxon test.

The average copulation time was estimated based on fully observed copulations ($N = 9$) and given with the standard error (SE). The Excel of the 2007 Microsoft Office was used for making the database and 'ggplot2' 3.1.0. data visualisation package in R for making plots.

Data availability

All data generated or analysed during this study are included in this published article and its supplementary information files.

Results

Settlement composition

We marked 56 *D. raddei* and 40 *D. portschinskii* (Table S3) in 2017 and 2018. The number of adult and mature animals captured and marked in 2017 comprised 35 *D. raddei* (12 females, 23 males) and 28 *D. portschinskii* (13 females, 15 males). The number of individuals marked in 2018 comprised 37 *D. raddei* (9 recaptured and 9 new males; 9 recaptured and 6 new females) and 28 *D. portschinskii* (11 recaptured, 1 registered, but not recaptured and 7 new males; 8 recaptured and 2 new females). Hence, in both species, there were fewer females than males.

We found no significant differences in body length (SVL) of adult lizards belonging to the same species and sex between years (unpaired Wilcoxon test, $p > 0.05$). The median body length (SVL) of adult females for *D. raddei* was 55 mm (min = 51, max = 64 mm, $N = 27$) and 55 mm (min = 50, max = 60 mm, $N = 23$) for *D. portschinskii*. We found no significant difference in SVL between adult females of the studied species were revealed (unpaired Wilcoxon test, $p > 0.05$, Fig. 1, Table S4). The median SVL of adult males of *D. portschinskii* was 54 mm (min = 50, max = 59 mm, $N = 33$). We found no significant differences between adult male and female body length of the same species (unpaired Wilcoxon test, $p > 0.05$); however, SVL of adult males of *D. portschinskii* was significantly smaller than this in adult females of *D. raddei* (unpaired Wilcoxon test, $W = 254.5$, $p < 0.01$, Fig. 1, Table S4). The largest body size was in males of *D. raddei* (median = 58 mm, min = 50, max = 63 mm, $N = 41$) and it was significantly larger than in males of *D. portschinskii* (unpaired Wilcoxon test, $W = 253$, $p < 0.01$).

Relationships between individuals

In total, we observed 118 M–M, 104 M–F and only 5 F–F interactions (Table 1). Lizards of both species shared space, and the social network depicts regular interactions between individuals of both species (Fig. 2).

Male *D. raddei* reacted differently to the males of the same and different species (unpaired Wilcoxon test, $W = 10$, $p < 0.01$, $N = 18$ and 11, Fig. 3a). The reactions of male *D. portschinskii* to conspecific and heterospecific males also differed significantly (unpaired Wicoxon test, $W = 88$, $p < 0.01$, $N = 8$ and 11, Fig. 3b). The same reactions of the same individuals towards conspecific and heterospecific males confirm it (*D. portschinskii*: Wilcoxon signed-rank test, $V = 0.0$, $N = 7$, $p = 0.02$; *D. raddei*: Wilcoxon signed-rank test, $V = 55.0$, $N = 10$, $p = 0.005$). Neutral reactions prevailed when males of two different species met and even rare friendly reactions with physical contact (joint basking) were observed (Fig. 3). The reactions of males of the same species were mostly aggressive or submissive (flee or contact avoidance).

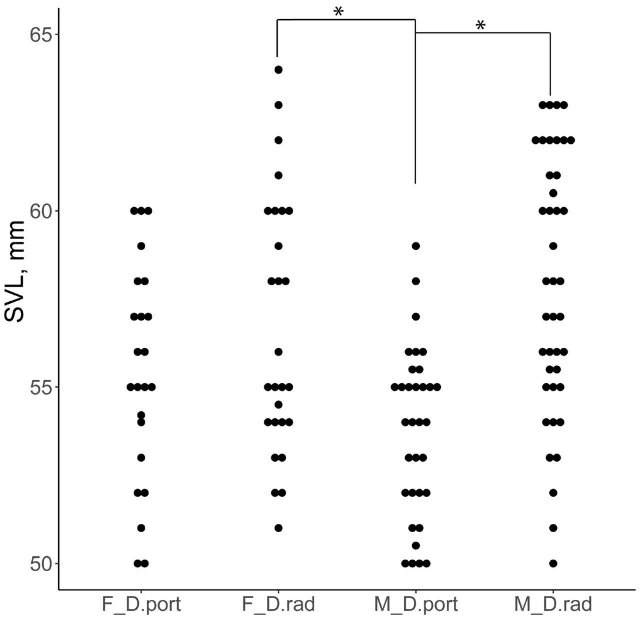


Fig. 1 Body size (SVL) of adult males (M) and females (F) of *D. raddei* ($N = 68$) and *D. portschinskii* ($N = 56$), marked during the study period in 2017 and 2018 years. The significant differences are marked with *

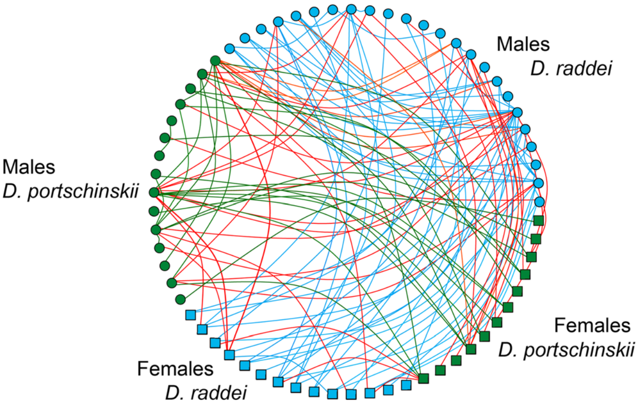


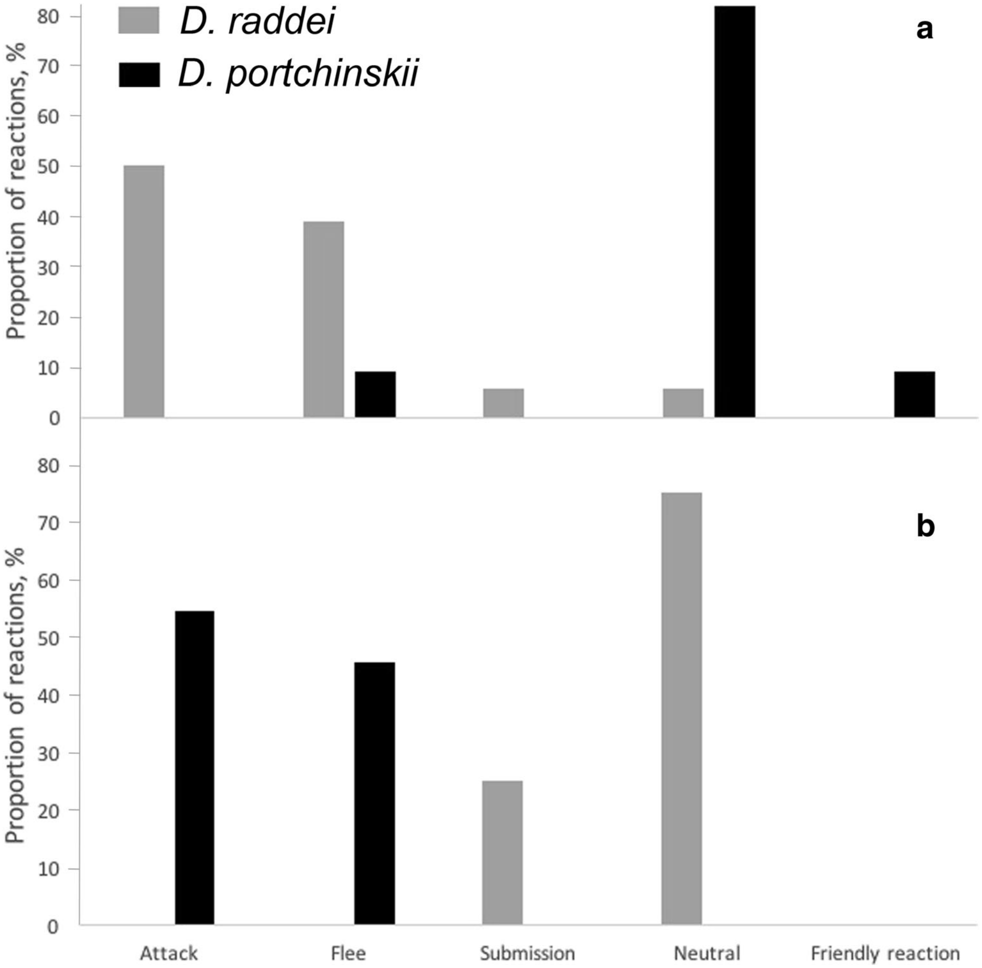
Fig. 2 Interspecific interactions (red lines); interactions between *D. raddei* (green lines) and *D. portshinskii* (blue lines) among adult and subadult males (circles) and females (squares) of *D. raddei* (blue) and *D. portschinskii* (green) in 2017 and 2018 years. $N_{\text{interactions}} = 228$; $N_{D.raddei} = 14$ females and 24 males; $N_{D.portschinskii} = 12$ females and 15 males

In general, male *D. raddei* seemed to be more neutral to the females of *D. portschinskii* than to females of the same species, although unsuccessful mating attempts with females of other species were also recorded (Fig. 4a). The differences in reactions of *D. raddei* males to females of both species were confirmed statistically (unpaired Wilcoxon test, $W = 112$, $N = 16$ and 10, $p < 0.01$), but not in the paired reaction of the same individuals (Wilcoxon signed-rank test, $V = 22.0$, $N = 7$, $p = 0.19$). We found no significant differences in the reactions of *D. portschinskii* males to females of both species (Wilcoxon signed-rank test, $N = 8$ and 11, $p > 0.05$, Fig. 4b). Although we had no statistical support for differences between male reactions to the females of the same and different species, our general impression was that males were searching for contacts with conspecifics and interacted with heterospecifics only if met them by chance.

Mating

Among 23 observed mating attempts, only two were interspecific: we observed two *D. raddei* males chase female *D. portschinskii*. None of these attempts were successful (Table 2). We did not observe any attempts by male *D. portschinskii* to mate with 'wrong' females, although in general they were typically indifferent to female *D. raddei* as well. More than half the recorded intraspecific mating attempts were successful (no differences between *D. portschinskii* and *D. raddei*, chi-square test, $\chi^2 = 0$, $df = 1$, $p = 1$).

Fig. 3 Responses of *D. raddei* males (**a**, $N = 29$) and of *D. portschinskii* males (**b**, $N = 19$) to males of the same and different species. The reactions of the particular category on another category are considered as 100%



On average, mating lasted for 6 ± 1.38 min ($N = 7$, max = 12 min) in *D. raddei*. Only two instances of mating were observed for *D. portschinskii*, both much longer than any in *D. raddei* (26 and 50 min, respectively). The ways in which the males restrained females also differed among species. For *D. portchinskii*, jaw prints were left on the side body flank and for *D. raddei* on the femur. Hence, mating attempts could be distinguished by the position of the jaw prints on a female's body (Fig. 5). We saw no females with jaw prints left by males of the different species within the study area; however, on 13 June 2018, a female of *D. raddei* with femur prints (as from a male of *D. raddei*) and with belly prints (as from a male of *D. portschinskii*) was found 100 m from the 2018 study area.

Discussion

The parthenogenetic rock lizard *D. rostombekowi* is considered to be the progeny of hybrids between *D. raddei* as the maternal species and *D. portschinskii* as the paternal species (Ryskov et al. 2017). If this is true, barriers to reproductive isolation have broken down once. The external morphology and body proportions of both studied species are similar (Fig. 4; Darevsky 1967). This should be a good predictor for niche overlap and use of the same resources (Pianka and Vitt 2003) favouring range overlap and existence of the stable secondary contact zones (Uzzel and Darevsky 1973; Arakelyan et al. 2011). However, no evidence of hybridization between *D. raddei* and *D. portschinskii* has been reported (Uzzel and Darevsky 1973; Galoyan et al. 2019). Hence, range overlap is important, but it is not the only condition for hybridization between the two species.

Male reactions to conspecifics and heterospecifics indicate their ability to distinguish between males of the same and different species. Males are known to be more aggressive than females, and direct competition is expressed due to rather high aggression in males (Ruby 1978). Indeed, interactions between males of the same species were always agonistic (aggressive or submissive) and neutral reactions prevailed during interspecific encounters of males. Only a few aggressive encounters were observed between males of different species. These could have happened if in the case of mistaken identity and/or if a heterospecific male also approached the focal male. Males of different species even basked in physical contact

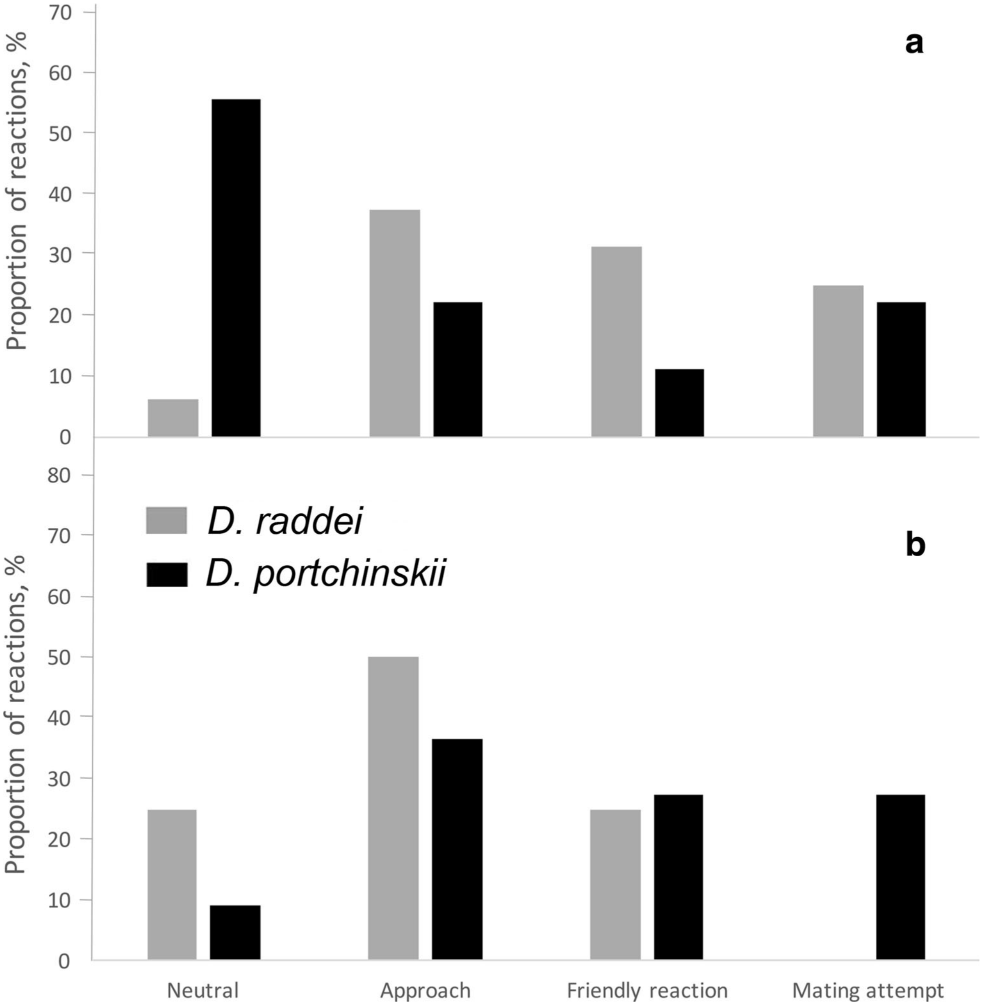


Fig. 4 Responses of *D. raddei* (**a**) males and of *D. portschinskii* (**b**) males to females of the same and different species. The reactions of the particular category on another category are considered as 100%

with each other. Such a high tolerance of males towards heterospecifics of the same sex suggests an absence of interspecific resource competition. The differences in reactions towards conspecifics and heterospecifics cannot be explained by spatial segregation, which is absent in the studied population: individuals of both species interacted regularly. Relatively low number of heterospecific interactions is best explained by the social preferences.

Male reactions to females of the same and different species also differed, although these differences were less distinct than in intrasexual reactions among males. Male *D. raddei* might have chased female *D. portschinskii* (mating attempt), although it is difficult to interpret this unambiguously—we could not tell if these were cases of mistaken identity or whether they did so deliberately. A single female, which was found with interspecific jaw prints, suggests a mating attempt, but we could not say whether this was a successful copulation. Hence, a suitable precondition for hybridization between *D. raddei* and *D. portshicnskii* does exist, but behavioural prezygotic isolation takes place as well. The absence of observations of interspecific mating support this statement.

The ability of a lizard to distinguish between individuals of the same and different species is a well-known phenomenon. *Podarcis*, for instance, react differently to the scent of individuals of the same and different species (Gabirot et al. 2010). Rock lizards may also use visual signals (colour) at a distance for species discrimination. It is no surprise that visual and chemical signals allow lizards to distinguish between species, making them also capable of individual discrimination (Tsellarius and Men’shikov 1994; Lena and de Fraipont 1998; Bull 2000; Chapple and Keogh 2005) and establishing of personalised relationship between individuals in many species of reptiles including rock lizards (Tsellarius et al. 2016).

There are several possible explanations of why males do not mate with females of the other species. On the one hand, species recognition represents a fundamental mating threshold while on the other hand, sexual selection through mate choice may also determine mating success. It has been suggested that body size differences among related species promote reproductive isolation in vertebrates (Bolnick et al. 2006). Successful hybridization is possible between species if the size differences among males and female are minimal and

Table 2 Successful and failed intraspecies and interspecific mating attempts ($N = 23$) among *D. raddei* and *D. portschinskii*. M, males; F, females

M	F	Mating attempts	Successful mating	Percentage success
D. portschinskii	*D. raddei*	0	0	0
D. portschinskii	*D. portschinskii*	6	5	83.3
D. raddei	*D. raddei*	15	9	60.0
D. raddei	*D. portschinskii*	2	0	0

comparable with those for conspecifics (Jančúchová-Lásková et al. 2015). This is the case for the studied species, where females have no significant differences between species in body size (50–60 mm). Hence, we have no reason to suggest body size influences the probability of hybridization between *D. raddei* and *D. portschinskii.*

Morphophysiological features are not the only mechanisms for interspecific isolation, which is often also based on differences in behaviour (Capula 1993). Male *D. raddei* and *D. portschinskii* differed in the way they restrain a female during copulation and its duration, which was longer in *D. portschinskii*.

Differences in reproductive cycles have been suggested to be the main isolation mechanism in the area of the secondary contact between *D. valentini* which reproduces in mid-May–early June and *D. raddei nairensis*, which reproduces in mid-June–July (Danielyan 1965). However, this might only partially explain the reproductive isolation between *D. raddei* and *D. portschinskii*, because this reproductive period in both species overlapped broadly in late May and early June, when males of both species were sexually active.

Differences in belly coloration are the most striking external features of the studied species: the throat and belly in *D. raddei* is always green-yellow. As we know, species recognition in some species of lizards is based on coloration (Losos 2009). However, prezygotic isolation exists even among those species, which have similar coloration pattern of dewlap in *Anolis* (Ng et al. 2017).

Morphological and physiological differences between related species are not the only features affecting social and sexual behaviour among the heterospecific individuals. Population density and sex ratio (Emlen and Oring 1977); ecological and social factors (Rosenthal 2013) affect sexual selection and mate choice. Hybridization often occurs in disturbed habitats, where the stable social structure is disrupted (Anderson, 1948). Proximal mechanisms of interbreeding include mate choice, which is based on individual preferences and environmental conditions (Rosenthal, 2013). Dreiss et al. (2010) reported weaker intraspecies sexual selection of males in male-biased populations of *Zootoca vivipara*. Vertebrates accept heterospecific partners if conspecific mates are rare or not available (Willis et al. 2011, 2012). The probability of interaction with the intraspecific sexual partner is

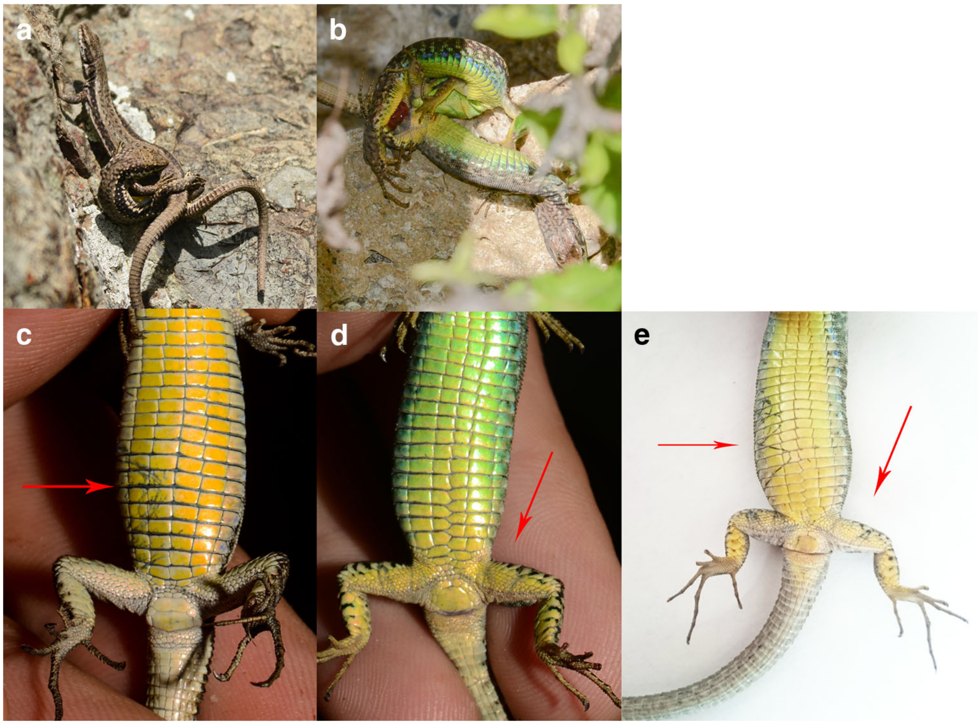


Fig. 5 Mating pattern in *D. portschinskii* (**a**) and *D. raddei* (**b**) and the jaw prints on the female's body after mating for *D. portschinskii* (**c**, on the belly) and *D. raddei* (**d**, on the femur) and a female of *D. raddei* with both types of print (**e**)

significantly reduced, if the sex ratio is biased. It may lead to the less selective mate choice and breeding with individuals belonging to different species (Dreiss et al. 2010). This suggestion is partly supported by the observations of populations comprising parthenogenetic and bisexual rock lizards. Parthenogenetic lizards commonly breed with bisexual species and produce backcrosses (Darevsky et al. 1985; Jančúchová-Lásková et al. 2015), if their number exceeds those of females belonging to bisexual species (Danielyan et al. 2008). For the studied species, this could have occurred under glacial conditions, when different parthenogenetic species appeared (Kearney 2005). The unstable climate and narrowing of the natural habitat areas potentially mixed initially isolated species, what could relax mate preference and interspecific breeding.

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Compliance with ethical standards

Ethical approval The research was approved by the Armenian Ministry of Nature Protection (N5/22.1/51043). All applicable international, national and institutional (Yerevan State and Moscow State Universities) guidelines for the care and use of animals were followed.

Conflict of interest The authors declare that they have no conflicts of interest.

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