

**Comparative studies of life histories of sex breeding and parthenogenetic rock
lizards (*Darevskia* Arribas, 1999)**

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Statement

As an author of this doctoral thesis, I confirm that this compilation thesis is my original work and does not include the materials of other authors that have already been published, accepted for publication, or submitted for a degree, which have not been cited in an accepted manner.

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აბსტრაქტი

Darevskia-ს გვარის კავკასიური კლდის ხვლიკები მრავალფეროვანი ჯგუფია, რომელშიც ვხვდებით როგორც ორსქესიან, ასევე, პართენოგენეზულ (ერთსქესიან) სახეობებს. სწორედ ამ ჯგუფში დაფიქსირდა ხმელეთის ხერხემლიანების პირველი პართენოგენეზული ფორმა.

სადოქტორო კვლევა მიზნად ისახავდა შეგვესწავლა განსხვავებები ერთსქესიანი (*Darevskia dahli*, *Darevskia armeniaca*) და ორსქესიანი (*Darevskia portschinskii*, *Darevskia valentini*) კავკასიური კლდის ხვლიკების სასიცოცხლო ცილკების მნიშვნელოვან განზომილებებს შორის იმ პოპულაციებში, სადაც ისინი თანაარსებობენ.

კვლევის მიზანი იყო დაგვედგინა: 1. მოქმედებს თუ არა ტემპერატურა, ტენიანობა, ნიადაგის ზედაპირის სხვა რაოდენობრივი მახასიათებლები და წყლის მუდმივი წყაროდან დაშორება განსხვავებულად *D. dahli* და *D. portschinskii*-ის წვრილმასშტაბიან სივრცულ განაწილებაზე; 2. განაპირობებს თუ არა რეპროდუქციული სტრატეგია (პართენოგენეზი თუ ორსქესიანი გამრავლება), ზრდასრული მდედრის სხეულის ზომა, ჰაბიტატის სიმაღლე ზღვის დონიდან, ჰაბიტატის ლანდშაფტი და/ან ამ ფაქტორებს შორის ურთიერთქმედება მდედრის მიერ ფარდობითი ენერგიის ინვესტირებას გამრავლების ერთ სეზონის განმავლობაში, კვერცხების რაოდენობასა და ფარდობით ზომას; 3. რომელი ფენოტიპური ვარიაციებია გამოწვეული გენეტიკური კომპონენტით; 4. ადგილობრივი ადაპტაციების და მშობელ სახეობათა ფენოტიპების ზეგავლენა პართენოგენეზული სახეობების თავის ფორმიაზე.

კვლევის ფარგლებში შევავროვეთ მონაცემები ზედაპირის ტემპერატურისა და ტენიანობის შესახებ, დაფიქსირებული სახეობების სქესი, ასაკი, სხეულის სიგრძე, მდედრის სხეულის ზომა და წონა კვერცხის დადებამდე და დაების შემდეგ, ასევე, კვერცხების წონა და მაქსიმალური დიამეტრი. შევისწავლეთ მოფორმეტრიული ცვალებდობა ტრადიციული 2დ და 3დ გეომეტრიული მოფორმეტრიის საშუალებით. შეგროვებული მონაცემები დამუშავდა სხვადასხვა სტატისტიკური მეთოდით.

კვლევის შედეგებმა აჩვენა, რომ: 1. მიუხედავად პოტენციური კონკურენტული ურთიერთქმედებისა, ერთსქესიანი (*D. dahli*) და ორსქესიანი (*D. portschinskii*) სახეობები ერთმანეთს არ ერიდებიან მიკროჰაბიტატის მასშტაბით, თუმცა ამ უკანასკნელი სახეობების ინდივიდები ფიქსირდებიან როგორც ოპტიმალურ, ასევე სუბოპტიმალურ ჰაბიტატებში, ნაკლებად არიან დამოკიდებული წყლის წყაროსთან სიახლოვეზე და უფრო თანაბრად არიან განაწილებულნი სივრცეში და დროში, ვიდრე პარტენოგენეზული სახეობები; 2. *D. armeniaca* დებს შედარებით მეტ რესურსს რეპროდუქციაში, ვიდრე მისი მამისეული *D. valentini*. თუმცა არ ვხვდებით იგივე სურათას *D. dahli*-სა და *D. portschinskii*-ს წყვილში. ორივე შემთხვევაში, პარტენოგენეზული სახეობები მისდევდნენ გამრავლების r-სტრატეგიას (ასეთი ორგანიზმები ხასიათდებიან შედარებით სწრაფი გამრავლებით და შთამომავლობის დიდი რაოდენობით იმისთვის რომ არასტაბილურ გარემოში გადარჩნენ) რაც გვაძლევს საშუალებას ვარაუდოთ, რომ გარემო მათთვის ნაკლებად სტაბილურია, ვიდრე მათი მშობელი სახეობებისთვის. 3. *D. portschinskii*-ს ჰქონდა უფრო მაღალი ინდივიდუალური და პოპულაციათაშორისი ფენოტიპური და გენოტიპური მრავალფეროვნება, ვიდრე *D. dahli*-ს. *D. portschinskii*-ს . მხოლოდ ორსქესიან სახეობებში პოპულაციებს შორის ფენოტიპური განსხვავებები კორელირებდა გეოგრაფიულ დისტანციებთან: 4. შედარებით დიდტანიან ხვლიკებში (*D. armeniaca*-*D. valentini*), მიუხედავად რეპროდუქციული სტრატეგიისა, ვხვდებით უფრო დიდ და მაღალ თავებს ვიდრე შედარებით პატარა ტანიანებში. ამასთან, ერთსქესიან სახეობებს ახასიათებთ წაგრძელებული და სუსტი ყბები.

დასკვნის სახით, შესწავლილ პარტენოგენეზულ სახეობებს აქვთ რამდენიმე ერთობლივი ეკოლოგიური მახასიათებელი, რაც მათ განასხვავებს ორსქესიანი მშობლებისგან: მათი ევოლუციური სტრატეგია უკეთესად არის ადაპტირებული არასტაბილურ გარემოსთან, უფრო სწრაფი რეპროდუქცია ეხმარება მათ წარმატებაში, ვიდრე ფენოტიპური ადაპტაცია კონკრეტულ გარემო პირობებთან, ასევე, ისინი არ ხარჯავენ დიდ ენერგიას სახეობისშიდა შეჯიბრში, რაც შეიძლება იყოს მათი სუსტი ყბების მიზეზი, რომელიც არ არის ადაპტირებული აგრესიულ ქცევასთან.

Abstract

The Caucasian rock lizards of the genus *Darevskia* is a diverse group, including both bisexual and parthenogenetic species. They are the first terrestrial vertebrates in which the parthenogenetic forms were found.

The research aimed to explore the differences between important dimensions of the life history of the unisexual (*Darevskia dahli*, *Darevskia armeniaca*) and bisexual (*Darevskia portschinskii*, *Darevskia valentini*) Caucasian rock lizards in populations where they coexist.

Within the framework of the research, we investigated the following questions: 1. Do temperature, humidity, quantitative surface characteristics and distance from a permanent water source differentially affect the fine-scale spatial distribution of *D. dahli* and *D. portschinskii*?; 2. Do reproductive mode (bisexual vs. parthenogenetic), adult female body size, sea level, habitat landscape, or interactions among these factors determine the relative energy investment in a single reproduction, the number and relative size of eggs?; 3. Which phenotypic variations in rock lizards have a strong genetic component and can be used in population genetic studies?; 4. Are local adaptations or intermediate phenotypes between parental species more influential in the evolution of head shape variation in parthenogens?.

Under the research, we collected data of surface temperature and humidity, recorded the species' sex, age, body length, the female's body size and weight before and after egg-laying, as well as the weight and maximum diameter of the eggs. We examined morphometric variability using traditional 2D and 3D geometric morphometrics. The collected data was analyzed using various statistical methods.

The results of the research showed that: 1. Despite potential competitive interactions, unisexual (*D. dahli*) and bisexual (*D. portschinskii*) species do not avoid each other on the microhabitat scale; the individuals of the later species were found in both optimal and suboptimal habitats,

being less dependent on the closeness of water source and were more evenly distributed in space and time than the parthenogenetic species; 2. Out of two parthenogenetic species, only *D. armeniaca* invests relatively more resources into reproduction than its paternal *D. valentini*. This is however not the case for *D. dahli* and *D. portschinskii*. In both cases, the parthenogenetic species tended to follow a r-strategy of reproduction, suggesting that the environment is less stable for them than for their parental species. 3. *D. portschinskii* had higher individual and between-population phenotypic diversity than *D. dahli*. Phenotypic differences between populations of *D. portschinskii* correlate with the geographic distances and pairwise fixation indices based on the analysis of the genetic markers. This correlation significantly strengthened when employing corrected Q_{st} (Phenotypic variance, partially influenced by genetic factors) values to assess the heritability of the traits; 4. Certain characteristics of head shape associated with body size and habitat climate, high mountain species (*D. armeniaca*–*D. valentini*) demonstrate larger and deeper heads with stronger jaws regardless of reproductive strategies. Both parthenogenetic species exhibit more elongated jaw areas, more flat heads, and weaker jaws than females of their parental species.

In conclusion, studied parthenogens have several joint ecological characteristics that differing them from bisexual parents: their evolutionary strategy is better adapted to unstable environments, faster reproduction helps them to flourish rather than phenotypic adaptation to specific environmental conditions, as well as they do not invest much in the interspecific competition, which can be a reason for their weaker jaws, not adapted for aggressive behavior.

Keywords: *Darevskia*, niche division, life history, morphometrics

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Abbreviations

3D—three dimensional

CPA—central preanal scale

CPCA—categorical principal component analysis

CT—central temporal scale

FP—number of femoral pores

PCA—principal component analysis

PCR—polymerase chain reaction

PPO3—post—postorbital scale

STR—the number of individuals analyzed for microsatellite profile

TM—tympanal scal

General Introduction

Lizards are classical objects for studying ecological and evolutionary research for several reasons: their reproductive strategies are highly variable, their spatial distributions are species-specific, and they show very high, both intra- and interspecific phenotypic variations (Camargo et al., 2010). Those were the reasons why we chose Caucasian rock lizards as our research objects.

Caucasian rock lizards are small-bodied (snout–vent length – 4.5–8.5 cm in adult individuals), rock-dwelling lizards, that belong to a monophyletic genus *Darevskia*, which includes 42 described species (7 parthenogenetic forms) from the Caucasus Mountain region (Tarkhnishvili and Iankoshvili, 2023). *Darevskia* mainly occupy similar habitats, with different preferences in humidity, temperature, and elevation. Spatial distributions of bisexuals and their daughter species partly overlap (Darevsky, 1967; Tarkhnishvili et al., 2010; Tarkhnishvili, 2012, Petrosyan et al., 2019; Petrosyan et al., 2020; Barateli et al., 2021).

According to studies, parthenogenetic vertebrates typically arise through repeated interspecific hybridization events (Murphy et al., 2000; Freitas et al., 2019). Parthenogenesis is a form of asexual reproduction in which an egg develops into an embryo without fertilization (Wininger, 2004). This process can occur in both plants and animals, including some invertebrates such as aphids, and vertebrates such as certain reptiles and fish.

Two main hypotheses explain how interspecific hybridization leads to parthenogenetic offspring: the balance hypothesis (BH) and the phylogenetic constraint hypothesis (PCH) (Freitas et al., 2019):

- The BH suggests that parthenogenetic animals arise from hybrids of two species that are different enough to disrupt normal reproduction, but not so different that the hybrids can't survive or reproduce. There's a certain "window" of genetic difference in which this happens. If the species are too similar or too different, parthenogenetic offspring won't occur (Moritz et al, 1989, 1992).

- The PCH states that asexual offspring result from hybrids of species with specific genetic factors that allow them to reproduce without mating. These factors could come from either parent. This hypothesis suggests that hybrids of certain combinations of species are more likely to produce asexual offspring.

Unisexual species initially have highly heterozygous genotypes, and the maintenance of this initial diversity allows many parthenogenetic vertebrates to persist and evolve (Tarkhnishvili et al., 2020b; Avise, 2015; Vrijenhoek and Parker, 2009; Schön et al., 2009).

Parthenogenesis has certain advantages, such as allowing species to reproduce in the absence of males and allowing rapid population growth (Tarkhnishvili et al., 2010; Tarkhnishvili, 2012; Barateli et al., 2022). However, it also has disadvantages, including reduced genetic diversity and lack of adaptability to changing environments.

The Caucasian rock lizards are the first group of terrestrial vertebrates in which parthenogenetic species have been found. Two of the three main *Darevskia* clades were involved in the hybridization that gave rise to parthenogenetic rock lizard species: the maternal “caucasica” and paternal “rudis” clades (Petrosyan et al., 2020; Tarkhnishvili et al., 2020b; Freitas et al., 2022). After hybridisation between two maternal (*D. mixta* and *D. raddei*) and two paternal (*D. valentini* and *D. portschinskii*) sexual species originated seven nominal parthenogenetic species (*D. armeniaca*, *D. dahli*, *D. unisexualis*, *D. rostombekowi*, *D. uzzelli*, *D. bendimahiensis*, *D. sapphirina*) (Fig. 1) (Ianchukov et al., 2022). Understanding the mechanisms of parthenogenesis and its evolutionary implications provides valuable insights into biodiversity and species adaptive strategies.

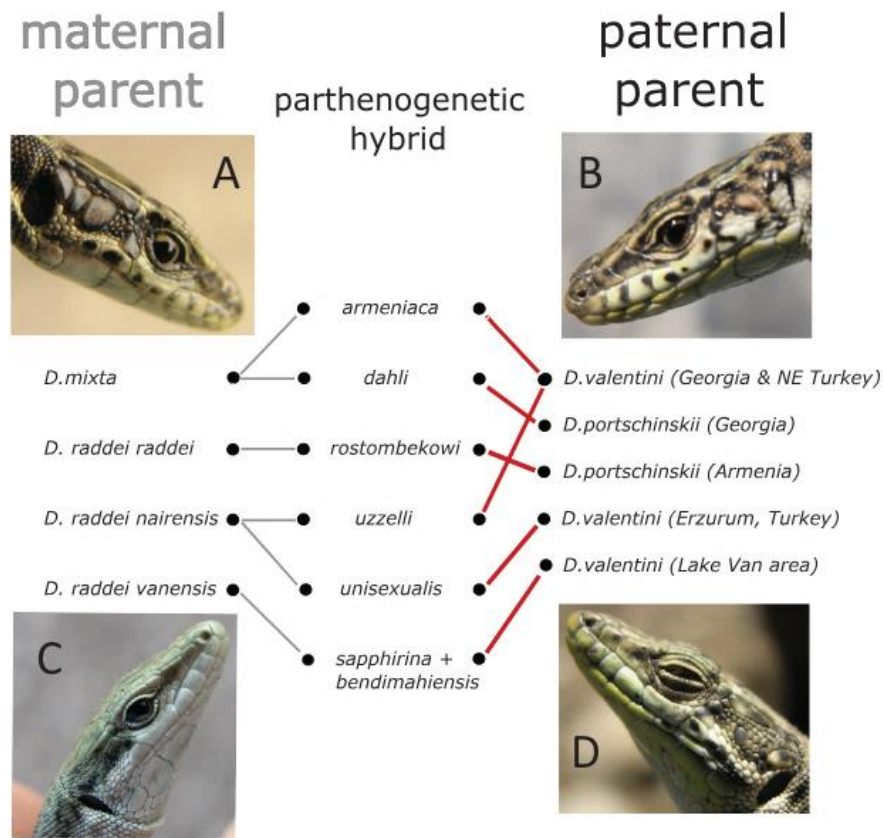


Fig. 1. Schematic origin of parthenogenetic taxa in the genus *Darevskia* (Figure 1 from Ianchukov et al., 2022).

Within the PhD research framework, we studied niche division, reproductive effort, genetic and morphological heritability, and head shape divergence of the parthenogens and their parental bisexual species in Georgia (in the locations where they coexist) and divided the research topics into four chapters. Our study primarily focused on two hybrid parthenogenetic species, *D. dahli* (Fig.2 A) (Darevsky, 1957) and *D. armeniaca* (Fig.2 B) (Méhely, 1909) and their paternal species, *D. portschinskii* (Fig.2 C) (Kessler, 1878) and *D. valentini* (Fig.2 D) (Boettger, 1892), which commonly share habitats with the daughter parthenogens (Murphy et al., 2000; Tarkhnishvili et al., 2010). Additionally, maternal species such as *D. mixta* (Méhely, 1909) and closely related *Darevskia obscura* (Lantz and Cyrén, 1936) were included in some aspects of the research.



Fig. 2. Research Objects (A–*Darevskia dahli*, B–*Darevskia armeniaca*, C–*Darevskia portschinskii*, D–*Darevskia valentini*).

Within this study, we divided parthenogenetic and paternal species into two groups: 1. small fine line between sex and unisexuality: the phylogenetic constraints on parthenogenesis in lacertid lizards–bodied *D. dahli* and its paternal species *D. portschinskii*, coexist in the mountain forest belt of central Georgia, 800–1200 m a.s.l. (Fig. 3) (Tarkhnishvili et al., 2010). Adult body length of both species varies between 5.4–5.8 cm.; 2. Relatively bigger–bodied, *D. armeniaca* and its paternal species *D. valentini*, coexist at the elevations 1900–3100 m a.s.l. (Fig. 3) (Galoyan et al., 2019; Barateli et al., 2022). The body of these species is larger than that of the previous pair, with body length varying between 6.1–6.4 cm. *Darevskia mixta*, an endemic species of Georgia (Gabelaia et al., 2017; Petrosyan et al., 2020), is distributed from 300–2100 m a.s.l. (Ciobanu et al., 2003; Tarkhnishvili, 2012; Gabelaia et al., 2015), average body length varies between 4.9–6.3 cm. (Darevsky, 1967).

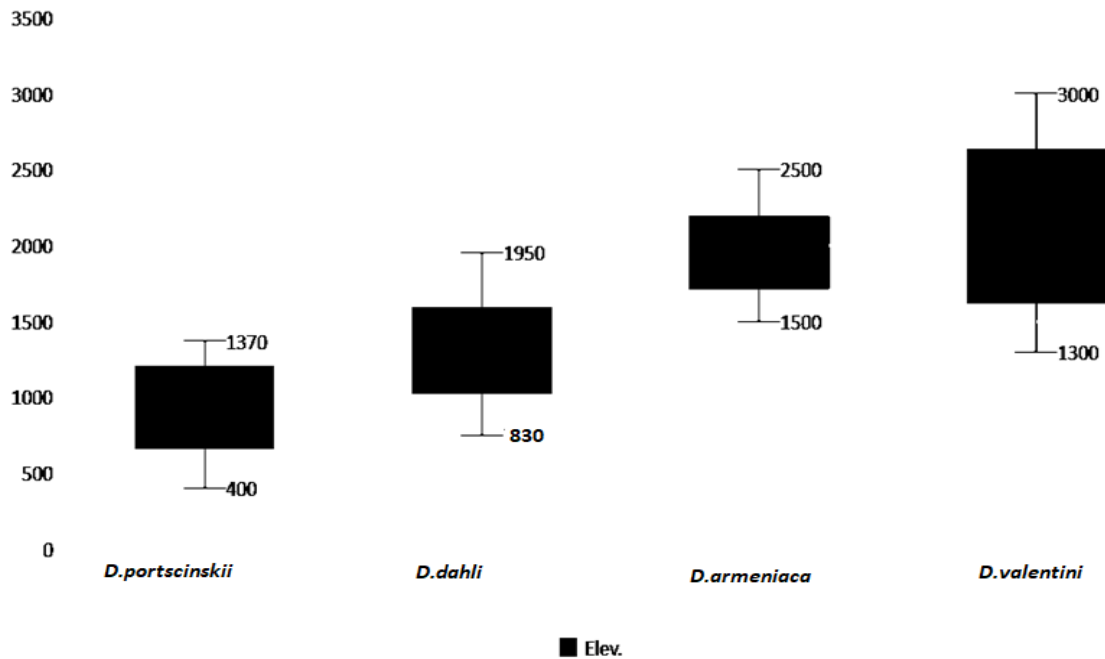


Fig. 3. Altitudinal distributions of the studied species (*Darevskia portschinskii*, *Darevskia dahli*, *Darevskia armeniaca*, *Darevskia valentini*).

ზოგადი შესავალი

ხვლიკები კლასიკური ობიექტები არიან ეკოლოგიური და ევოლუციური კვლევების შესასწავლად მათი ცვალებადი რეპროდუქციული სტრატეგიების, სივრცითი განაწილებისა თუ მაღალი სახეობათ შიდა თუ შორისი ფენოტიპური ვარიაციების გამო (Camargo et al., 2010). სწორედ მათი მრავალფეროვნება იყო მიზეზი იმისა, რომ კვლევის ობიექტად შევარჩიეთ კავკასიური კლდის ხვლიკები.

კავკასიური კლდის ხვლიკები პატარა ტანიანი ხვლიკები არიან (სხეულის საშუალო ზომა – 4,5–8,5 სმ) და ძირითადად კლდოვან ჰაბიტატებში ბინადრობენ. ისინი მიეკუთვნებიან მონოფილურ გვარს *Darevskia*, რომელიც მოიცავს 42 სახეობას, აქედან 7 პართენოგენეზული ფორმაა (Tarkhnishvili and Iankoshvili, 2023). *Darevskia*-ს გვარის ხვლიკები ძირითადად მსგავს ჰაბიტატებს იკავებენ, თუმცა განსხვავებულ ტენიანობას, ტემპერატურასა თუ სიმაღლეს ზღვის დონიდან ანიჭებენ უპირატესობას. ორსქესიანი და მათი შვილი, ერთსქესიანი სახეობების სივრცითი განაწილება ნაწილობრივ გადაფარავს ერთმანეთს (Darevsky, 1967; Tarkhnishvili et al., 2010; Tarkhnishvili, 2012; Petrosyan et al., 2019; Petrosyan et al., 2020; Barateli et al., 2021).

პართენოგენეზული სახეობები წარმოიქმნენ მრავალჯერადი სახეობათშორისი ჰიბრიდიზაციის შედეგად (Murphy et al., 2000; Freitas et al., 2019). პართენოგენეზი არის რეპროდუქციის ასექსუალური ფორმა, რომელის დროსაც კვერცხუჯრედი ვითარდება ემბრიონად განაყოფიერების გარეშე (Wininger, 2004). ეს პროცესი შეიძლება მოხდეს როგორც მცენარეებში, ასევე ცხოველებში, მათ შორის ზოგიერთ უხერხემლოებში, როგორიცაა ბუგრები, და ხერხემლიანებში, როგორიცაა ზოგიერთი ქვეწარმავალი და თევზი.

არსებობს ორი ჰიპოთეზა, რომელიც ხსნის, თუ როგორ იწვევს სახეობათშორისი ჰიბრიდიზაცია პართენოგენეზული შთამომავლობის წარმოშობას: ბალანსის ჰიპოთეზა (BH) და ფილოგენეტიკური შეზღუდვის ჰიპოთეზა (PCH):

- BH თანახმად, პართენოგენეზული ორგანიზმები წარმოიქმნებიან ორი განსხვავებული, მაგრამ არა ზედმეტად განსხვავებული სახეობის ჰიბრიდიზაციის

შედეგად. თუ სახეობები ძალიან მსგავსია ან ძალიან განსხვავებული, პარტენოგენეზული ფორმის ჩამოყალიბება ვერ მოხდება.

- PCH მიხედვით, ასექსუალური/პარტენოგენეზული შთამომავლობა წარმოიქმნება ისეთი სახეობების ჰიბრიდიზაციის შედეგად, რომლებსაც აქვთ სპეციფიკური გენეტიკური ფაქტორები, რომლებიც მათ საშუალებას აძლევს გამრავლდნენ შეჯვარების გარეშე.

პარტენოგენეზს აქვს გარკვეული უპირატესობები, როგორცაა სახეობის გამრავლება მამრების არარსებობის შემთხვევაში და პოპულაციის სწრაფი ზრდა (Tarkhnishvili et al., 2010; Tarkhnishvili, 2012; Barateli et al., 2022). თუმცა, მას აქვს ასევე უარყოფითი მხარეები, მათ შორის გენეტიკური მრავალფეროვნების სიმცირე და გარემოს ცვლილებებთან ადაპტაციის უნარის ნაკლებობა. მართალია, ერთსქესიან სახეობებს ღარიბი გენოფონდი აქვთ, თუმცა ახასიათებთ მაღალი ჰეტეროზიგოტულობა, რაც საშუალებას აძლევთ გადარჩნენ და განვითარდნენ (Tarkhnishvili et al., 2020b; Avise, 2015; Vrijenhoek and Parker, 2009; Schön et al., 2009).

კავკასიური კლდის ხვლიკები ხმელეთის პირველი ხერხემლიანები არიან, სადაც პარტენოგენეზული სახეობები დაფიქსირდა. *Darevskia*-ს გვარში განარჩევენ სამ ძირითად კლადას: caucasica, rudis და saxicola. პარტენოგენეზული კლდის ხვლიკის სახეობების წარმოშობაში მონაწილეობდნენ დედისეული „caucasica“ და მამისეული „rudis“ ჯგუფები (Petrosyan et al., 2020; Tarkhnishvili et al., 2020b; Freitas et al., 2022). ორ დედა (*D. mixta* და *D. raddei*) და ორ მამა (*D. valentini* და *D. portschinskii*) სქესობრივ სახეობებს შორის ჰიბრიდიზაციის შედეგად წარმოიშვა შვიდი პარტენოგენეზული სახეობა (*D. armeniaca*, *D. dahli*, *D. unisexualis*, *D. rostombekowi*, *D. uzzelli*, *D. bendimahiensis*, *D. sapphirina*) (Ianchukov et al., 2022) (ფიგ.1). პარტენოგენეზის მექანიზმების და მისი ევოლუციური შედეგების შესწავლა მნიშვნელოვან ინფორმაციას გვაძლევს ბიომრავალფეროვნების და სახეობების ადაპტაციური სტრატეგიების შესახებ.

სადოქტორო კვლევის ფარგლებში, შევისწავლეთ ნიშების გაყოფა, რეპროდუქციული ძალისხმევა, გენეტიკური და მორფოლოგიური მემკვიდრეობა, და თავის ფორმის ცვალებადობა პარტენოგენეზსა და მათ ორსქესიან მშობელ სახეობებს შორის

თანაარსებობის პირობებში, საქართველოში. საკვლევი სახეობები იყვნენ: *D. dahlī*–დალის ხვლიკი (ფიგ.2 A) (Darevsky, 1957) და *D. armeniaca*–სომხური ხვლიკი (ფიგ.2 B) (Méhely, 1909) და მათი მამა სახეობები, *D. portschinskii*–მტკვრის ხვლიკი (ფიგ.2 C) (Kessler, 1878) და *D. valentini*–ვალენტინის ხვლიკი (ფიგ.2 D) (Boettger, 1892), რომლებიც ჰაბიტატებს იზიარებენ პართენოგენულ მემკვიდრეობთან (Murphy et al., 2000; Tarkhnishvili et al., 2010). დამატებით, დედა სახეობა *D. mixta*–აჭარული ხვლიკი (Méhely, 1909) და ახლო მონათესავე *Darevskia obscura* (Lantz და Cyrén, 1936) ჩართულნი იყვნენ კვლევის გარკვეულ ასპექტებში.

კვლევის მიზანი იყო პართენოგენული და მათი მშობელი, სქესობრივი სახეობების ადაპტაციური სტრატეგიების შედარება.

Chapter 1. Fine-scale analysis of habitat occupancy by Kura lizard (*Darevskia portschinskii*) and its daughter parthenogenetic form (*Darevskia dahl*)

The comparison of ecological niches between closely related species is a complex issue due to the effect of spatial and time settings on ecological preferences. Interspecific competition, forcing character displacement and organismal evolution (Grant, 1972; Pfennig and Pfennig, 2010), can shift the spatial distribution and daily activities of species (Schoener, 1983; Abrams, 1986; Pianka, 2011; Carvalho and Cardoso, 2020). Remote sensing studies (Tarkhnishvili et al., 2010; Petrosyan et al., 2020) have shown that the spatial distribution of these species is influenced by different thermal and humidity preferences. In addition, the presence of *D. dahl* can negatively affect *D. portschinskii* within shared habitats, suggesting possible interspecific competition (Tarkhnishvili et al., 2010). To gain deeper insight into these dynamics, it is important to understand how these species share resources at the microgeographic scale. Galoyan (2011, 2013) showed that differences in habitat use may be related to differences in territorial behavior (both males and females of bisexual *Darevskia* species may be territorial, while the unisexuals show reduced territoriality).

In this study, we investigated whether *D. dahl* and *D. portschinskii*, coexisting species, occupy different niches in a small, semi-isolated habitat. We proposed two hypotheses for their different habitat use: mutual avoidance to reduce competition and differences in territorial behavior. Alternatively, habitat requirements may differ for reasons unrelated to competition.

Material and methods

We conducted long-term stationary observations of lizards in the vicinity of Kojori (1.648939°N, 44.683445°E) (Fig. 4). The study area consists of a gentle rocky slope covered with rare shrubbery, bordered by a broad-leaved forest, and edged with a small brook. Elevations in the area range from 1003 to 1042 m. a.s.l. Observations were conducted between April and October of 2017, 2018, and 2019.

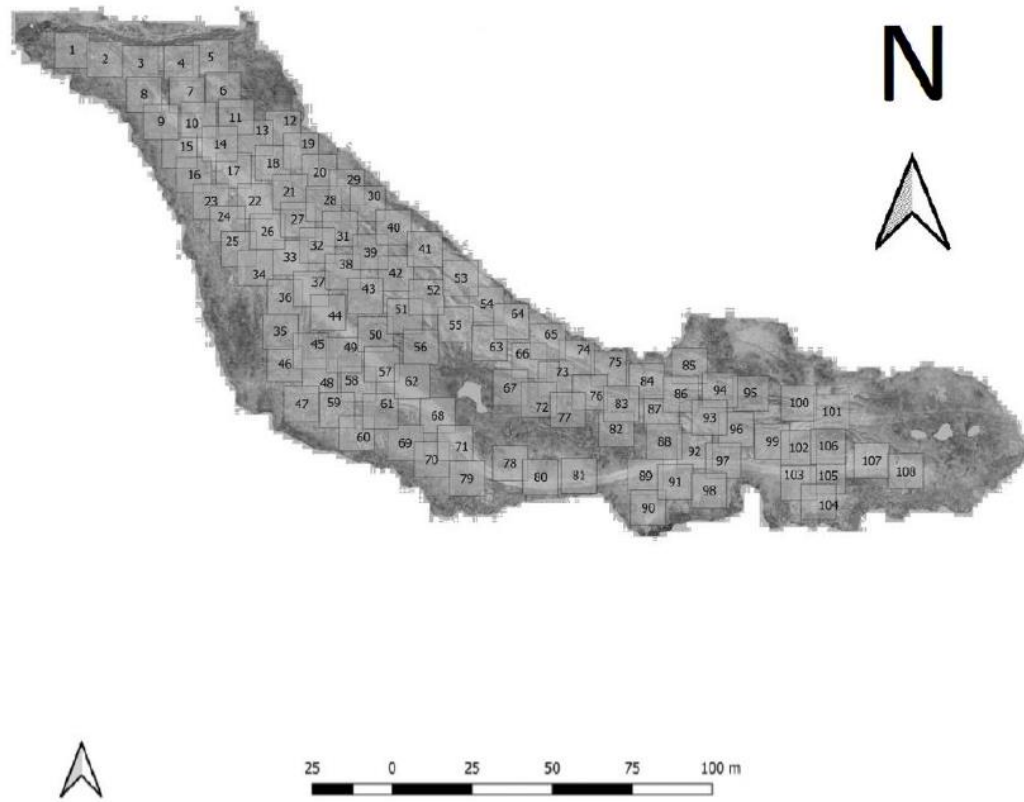


Fig. 4. The number of studied plots (Figure 2 from Barateli et al., 2021).

The study area was divided into 108 squared plots, each measuring 10×10 meters. Longitude, latitude, and altitude were measured for each plot, and the data were processed into a polygon shapefile. Aerial photos of the surface were captured using drone cameras to create orthoimages for mapping the surface types.

During 27 field sessions, we collected data of land surface temperature (LST) and land surface humidity (LSH) twice within specific timeframes: between 11:00 AM and 1:00 PM, and between 5:00 PM and 7:00 PM. These measurements were taken at the geometric center of each of the 108 plots.

For each observed individual, we recorded age (adult or juvenile), species (*D. dahli* or *D. portschinskii*), sex (for bisexual species), time of day, and plot number. All individuals with the

distance from the back surface of the hip to the tip of the snout exceeding 5 cm were treated as adults.

To create the orthoimages for mapping surface types (Boulders, Forest, Grass, Gravel, Leafy Ground, Rock) first, we took aerial photos and processed them in the Agisoft PhotoScan software (Agisoft, L. L. C. “Agisoft PhotoScan User Manual: Professional Edition, Version 1.4”, 2018).

All calculations were done using IBM SPSS v. 23.0 and Excel 2010 for Windows.

Results with discussion

The results of the study lead us to question why environmental conditions, such as surface type, proximity to water sources, and air temperature, affect the occurrence of *D. dahli* stronger than that of *D. portschinskii*. One of the possible explanations might be territoriality, which forces *D. portschinskii* individuals to expand into plots with suboptimal conditions. This territorial behavior, combined with inherited ecological differences, could account for the observed variations between *D. portschinskii* and its parthenogenetic species.

Another intriguing consequence of territoriality is the differential habitat preferences observed between territorial and parthenogenetic species. Territorial species, *D. portschinskii* are often found in suboptimal habitats and times, while parthenogenetic species typically exhibit an absence of territoriality and prefer more optimal areas. This finding appears to contradict earlier suggestions by Tarkhnishvili et al. (2010), that the negative impact of *D. dahli* on *D. portschinskii* is stronger than vice versa.

Despite these differences, a substantial overlap in spatio-temporal niches between *D. dahli* and *D. portschinskii* suggests the potential for interspecific competition. However, within the study area, preferred locations for the lizards partly overlap, with *D. dahli* exhibiting high density near the brook where *D. portschinskii* are almost absent. Interestingly, both species show increased density in plots with optimal terrain and cover conditions, even away from the brook. Microhabitat similarities between the two species appear to outweigh potential competition, suggesting that ecological differences are unlikely the result of character displacement.

Instead, differences in temperature and humidity optima, as well as historical range limitations, may play a more significant role. Previous observations and modeling results indicate that the matrilineal ancestor, *D. mixta*, is limited to mesophytic forests and uplands of central and western Georgia and is adapted to more humid habitats than *D. portschinskii*. *D. dahli*, exhibiting phenotypic features intermediate between *D. portschinskii* and *D. mixta*, displays a dependency on water sources and rocky surfaces.

Although the species do not actively avoid each other on a microhabitat scale, macro-geographic analyses reveal mutual negative impacts. Certain locations with equally suitable climates show a negative correlation between the abundance of each species.

Overall, the study suggests that negative interspecific interactions do not necessarily lead to niche shifts or character displacement. Instead, long-lasting coexistence between species like *D. dahli* and *D. portschinskii* may be attributed to metapopulation dynamics and a source-sink spatial structure. This structure facilitates re-population from neighboring source locations, compensating for negative population trends and hindering character displacement and reinforcement in multispecies assemblages. The coexistence of *D. dahli* and *D. portschinskii* in the study location for over 53 years further supports this conclusion.

თავი 1. მტკვრის ხვლიკის (*Darevskia portschinskii*) და მისი შვილი პართენოგენეზული ფორმის (*Darevskia dahli*) ჰაბიტატის დეტალური/წვრილმასშტაბიანი ანალიზი

ეკოლოგიური ნიშების შედარება მონათესავე სახეობებს შორის კომპლექსური საკითხია ეკოლოგიურ პრეფერენციებზე სივრცითი და დროითი გავლენების გამო. კვლევებმა აჩვენა, რომ ტემპერატურამ და ტენიანობამ შეიძლება გავლენა მოახდინოს *Darevskia*-ს სახეობების სივრცულ განაწილებაზე. სახეობათაშორისი კონკურენცია მნიშვნელოვან როლს თამაშობს ეკოლოგიური ნიშების ფორმირებაში. ამან შეიძლება გამოიწვიოს ფენოტიპური ცვალებადობა კონკურენციის პირობებში და ასევე, ორგანიზმებში ევოლუციური ცვლილებები. მაგალითად, *D. dahli*-ს არსებობამ შეიძლება ნეგატიურად იმოქმედოს *D. portschinskii*-ს სივრცულ განაწილებაზე თანაარსებობის პირობებში, რაც მიუთითებს მათ შორის შესაძლო კონკურენციაზე.

ამ დინამიკის უკეთ გასაგებად მნიშვნელოვანია გავიგოთ, თუ როგორ იზიარებენ მონათესავე *Darevskia*-ს სახეობები რესურსებს მიკროგეოგრაფიულ მასშტაბში. კვლევებმა აჩვენა, რომ ჰაბიტატის გამოყენებაში/დაკავებაში სხვაობა შესაძლოა დაკავშირებული იყოს ტერიტორიული ქცევასთან. ორსქესიანი *Darevskia*-ს სახეობების მამრები და მდედრები ავლენენ ტერიტორიულ ქცევას, ხოლო ერთსქესიანები—ნაკლებად.

კვლევის ფარგლებში ჩვენ შევისწავლეთ, იკავებენ თუ არა *D. dahli* და *D. portschinskii* სხვადასხვა ეკოლოგიურ ნიშებს თანაარსებობის პირობებში მცირე, ნაწილობრივ იზოლირებულ ჰაბიტატში. ჩვენ გამოვყავით ორი ჰიპოთეზა, რომელიც ხსნის მათ მიერ ჰაბიტატის გამოყენებას: 1. ურთიერთ აცილება/არიდება კონკურენციის შესამცირებლად და მეორე, პოტენციური განსხვავებები ტერიტორიულ ქცევაში.

კვლევა ჩატარდა კოჯორში, თბილისის მახლობლად, კლდოვან ჰაბიტატში, რომელიც აქა-იქ დაფარულია ბუჩქებით, გარშემორტყმულია ფართოფოთლოვანი ტყით და პატარა ნაკადულით. საკვლევი ტერიტორია დაყავით 108 ნაკვეთად, თითოეული 10x10 მეტრი ზომის (ფიგ. 4). თითოეული ნაკვეთისთვის დავაფიქსირეთ გრძედი, განედი და სიმაღლე და შევქმენით პოლიგონის შეიპფაილი GIS-ში.

27 საველე გასვლის განმავლობაში, დღეში ორჯერ ვზომავდით მიწის ზედაპირის ტემპერატურას და ტენიანობას თითოეული ნაკვეთის ცენტრში. ქამატებით ვიწერდით დაფიქსირებული ინდივიდის სახეობას, სქესს, ასაკს, დროსა და ნაკვეთის ნომერს. ნებისმიერი ხვლიკი, რომლის სხეულის სიგრძე იყო 5 სმ-ზე მეტი, კლასიფიცირდა როგორც ზრდასრული.

ზედაპირის სხვადასხვა ტიპების (ლოდები, ტყე, ბალახი, ხრეში, ფოთლიანი მიწა, კლდე) განსასაზღვრად გადავიღეთ საჰაერო ფოტოები დრონის გამოყენებით.

ყველა გამოთვლა ჩატარდა IBM SPSS v. 23.0-ისა და MS Excel 2010-ის გამოყენებით.

კვლევამ აჩვენა მცირედ განსხვავებული ეკოლოგიური პრეფერენციები დალისა და მტკვრის ხვლიკებს შორის. მტკვრის ხვლიკი უპირატესობას ანიჭებს შედარებით მშრალ და წყლიდან დაშორებულ ჰაბიტატს, ხოლო დალის ხვლიკი – კლდოვან ჰაბიტატს და წყალთან სიახლოვეს. ამასთან, *D. portschinskii* ტერიტორიული ქცევის გამო მთლიანად ითვისებს ტერიტორიას და ინდივიდები იძულებულნი არიან შეეგუონ იმ ადგილს, რომელიც მათ ერგოთ, სრულად აითვისონ ის, რაც არის ხანგრძლივ დროზე გათვლილი სტრატეგია. რაც შეეხება ერთსქესიანებს, რომელთათვის გარემო არის ნაკლებად სტაბილური, ისინი ცდილობენ ოპტიმალური გარემო პირობების მქონე ნაკვეთი დაიკავონ და აითვისონ მაქსიმალურად, იმის ხარჯზე რომ მათ არ აქვთ ტერიტორიული ქცევა.

საერთო ჯამში, *D. dahli* აქტიურად ეძებს ოპტიმალურ პირობებს, მაშინ როცა *D. portschinskii* ადაპტირდება ჰაბიტატში არსებულ ცვალებად პირობებთან.

Chapter 2. Reproductive effort of unisexual and bisexual rock lizards (genus *Darevskia*)

Organisms with multiple reproductive cycles must allocate energy between growth, survivorship, and reproduction (Stearns, 1977; 1992; Congdon, 1989; Macip-Ríos et al., 2017). Due to limited resources, finding a balance between investment in survivorship and reproduction is essential (Babich Morrow et al., 2021). Life history theory, which explains how natural selection influences organisms to optimize survival and reproduction, emphasizes these variables as key factors in increasing an animal's fitness (Stearns, 1992; Roff, 1992; Stearns et al., 2000; Fabian and Flatt, 2012).

In lizards with multiple reproductive cycles and the absence of parental care, an increase in energy expenditure increases the potential number of viable offspring during one season but decreases the probability that a female will survive to the next season (Qualls and Shine, 1998). For the same female body mass and reproductive effort, an increase in the number of eggs is associated with a decrease in egg size. Egg size potentially increases the probability of individual egg survival to hatching and juvenile survival within the first few months of life (Sinervo, 1990; Levitan, 2006). Lizards are commonly studied to understand trade-offs within life history theory due to variation among conspecific populations and closely related species. Previous studies show that lizard reproductive effort, reproductive period, clutch size, and egg size are strongly dependent on environmental variables (Shine and Elphick 2001; Brandt and Navas, 2011; Menezes and Rocha, 2014). In addition to environmental variables, female body size plays a crucial role in determining the reproductive variables of lizards (Vrcibradic and Rocha 2011; Winck and Rocha 2012; Menezes and Rocha, 2014). Females with larger bodies can produce larger and heavier eggs (Brandt and Navas, 2011; Menezes and Rocha, 2014).

Another reason lizards are compelling subjects for the study of trade-offs is the existence of bisexual and parthenogenetic taxa within certain groups. Research examining the influence of reproductive strategies on reproductive efforts in parthenogenetic and bisexual species has shown different results. For example, Schall (1978) analyzed data from five sympatric species in

southwest Texas, two of which reproduce parthenogenetically, and showed no significant differences in clutch size, egg size, or clutch/body weight ratio. In contrast, Congdon et al. (1978), based on a study of two bisexual and two parthenogenetic *Aspidoscelis* species from Arizona, concluded that unisexual species tend to have larger clutches, increase the clutch size more rapidly with body size, produce lighter eggs, and have higher clutch/body caloric ratios than closely related bisexual species, regardless of body size differences between species.

In this study, we compared the life history components of two parthenogenetic and three bisexual species of Caucasian rock lizards (*Darevskia* Arribas, 1999) from three locations in Georgia. The parthenogens and their paternal, sympatric species differ in body size and preferred altitudes. Our purpose was to examine if and how the mode of reproduction (sexual breeding vs parthenogenetic) affects the reproductive characteristics of a female (including reproductive effort, egg size, egg number, or the trade-off among these parameters), and whether this effect depends on the other variables, such as body size of a female, altitude of a habitat.

Material and methods

In this study, we included both parthenogenetic species and their patrilineal ancestors and added a closely related, *Darevskia* species, *D. obscura*, which also coexists with *D. armeniaca* (Darevsky, 1967; Tarkhnishvili, 2012). In May-June of 2019, 2021, and 2022, 87 gravid female individuals of five species of rock lizards were collected from three locations in Georgia (Kojori, Saghamo Lake, Riv. Kirkhbulaki) (Table 1) and separately placed in plastic containers containing moss and soil. Each female was measured using calipers and weighed before and after egg deposition. Similarly, we measured the number, weight, and maximum diameters of eggs in a clutch.

Table 1. Sample sizes (gravid female lizards) and key characteristics of the sampling location of six species of *Darevskia* (Table 1 from Barateli et al., 2022).

Species	Location	Easting	Northing	Alt. a.s.l.	Sample size	Rep. mode
<i>D. dahli</i>	Kojori	44.68°	41.64°	1120	17	unisexual
<i>D. portschinskii</i>	Kojori	44.68°	41.64°	1120	16	bisexual
<i>D. armeniaca</i>	Sagamo Lake	43.73°	41.29°	2015	11	unisexual
<i>D. armeniaca</i>	Riv.Kirkhbulakhi	43.73°	41.29°	1715	15	unisexual
<i>D. valentini</i>	Sagamo Lake	43.73°	41.29°	2015	15	bisexual
<i>D. obscura</i>	Riv.Kirkhbulakhi	43.73°	41.29°	1715	13	bisexual

Both females and juveniles were released back into their natural habitat after a few weeks to ensure minimal disturbance to their environment.

For statistical analysis, we calculated several derivative reproductive variables from the original measurement. We used statistical methods (one-way ANOVA, General Linear Model (GLM)) to infer: 1. significant differences between the populations in the studied reproductive variables 2. the influence of the predictors on each dependent reproductive variable separately, across the species and populations.

Analysis was conducted using IBM SPSS software (2021).

Results with discussion

In brief, our results demonstrate: 1. big-bodied females exhibit greater relative investment into single reproduction events (heavier clutches related to the female body weight and more eggs); 2. the differences in egg shape between big-bodied (round) and small-bodied (elongated) species; 3. among parthenogenetic species, *D. armeniaca* (but not *D. dahli*) invests a larger portion of its body mass, and deposits heavier eggs relative to its body size, in comparison with its patrilineal ancestor;

4. from the sample locations, larger individuals of *D. dahli* deposit more eggs and larger individuals of *D. portschinskii* deposit heavier eggs; 5. *Darevskia obscura* allocates more resources in a single reproduction than two other bisexual species and it deposits heavier eggs.

Parthenogenetic species typically show more rapid reproduction compared to their parental counterparts and are often found in less stable environments (Barateli et al., 2021). If we consider unisexual species as 'weed' animals (Wright and Lowe, 1968) they follow r-strategies when the environment is unstable and the reproductive resources are allocated to maximize the number of offspring, whereas bisexual species follow K-strategies, prioritizing investment in single, viable offspring. *D. armeniaca* allocates relatively more resources into a single reproduction than *D. valentini*, which can be linked to its occurrence in less stable environments for the adults than the latter species. Our results indicate that bisexual species focus on producing single, viable offspring.

Egg size within our study group is less dependent on female size than egg number. This observation suggests the existence of minimum and maximum thresholds for egg development and successful reproduction. In particular, smaller species such as *D. dahli* and *D. portschinskii* are depositing elongated eggs in no way affect juvenile survival, this is because eggs have a certain lower threshold size for juveniles to develop normally, but if a small-bodied female will have a high diameter of an egg may harm a female's ovaries.

When comparing environmental stability for two species, it's important to consider it across different life stages: 1. the stability of the environment for eggs and juveniles, and 2. the stability of the environment for adults. Drawing on the insights of Wilbur's paper 'Complex Life Cycles' (1980), we know that these stages present challenges and opportunities for adaptation. If the adult environment of species A is less stable than that of species B, it is logical for species A to change its reproductive strategy. In such circumstances, organisms of species A may invest relatively more energy in a single reproductive event, resulting in a higher relative clutch mass. This strategic adjustment, as discussed by Wilbur (1980), serves as an adaptive response to environmental instability, increasing the likelihood of reproductive success despite difficult conditions. Furthermore, if the environment is less stable for eggs and juveniles of species A compared to

species B, a similar strategic shift is expected. In line with Wilbur's research, species A would be expected to lay more, but smaller, eggs per clutch. This strategy optimizes the offspring's chances of survival in an environment of increased unpredictability during early developmental stages (Wilbur, 1980).

თავი 2. პართენოგენეზული და ორსქესიანი კლდის ხვლიკების (გვარ *Darevskia*) რეპროდუქციული ძალისხმევა

ხვლიკები, ისევე როგორც მრავალი სხვა ცოცხალი ორგანიზმი, რომელსაც სიცოცხლის განმავლობაში მრავალჯერადი რეპროდუქციული ციკლი ახასიათებთ, იძულებულნი არიან ენერგია ზრდას, გადარჩენასა და რეპროდუქციას შორის გადაანაწილონ. იმის გამო რომ გარემოში რესურსები შეზღუდულია, მათ უწევთ ენერგიის ამ სამ კატეგორიას შორის გადანაწილება. სასიცოცხლო ციკლების შესახებ თეორია გვეხმარება გავიგოთ, თუ როგორ აოპტიმიზებენ ორგანიზმები ენერგიის განაწილებას მეტი რეპროდუქციული წარმატების მოსაპოვებლად.

მდედრი ხვლიკის მიერ, გამრავლების ერთი სეზონის განმავლობაში ენერგიის ინვესტიციის მატება ზრდის სიცოცხლისუნარიანი შთამომავლობის პოტენციალს, მაგრამ ამცირებს მისი გადარჩენის ალბათობას შემდეგ სეზონამდე (Qualls and Shine, 1998). იმავე სხეულის მასისა და რეპროდუქციული ძალისხმევის პირობებში, კვერცხების რაოდენობის ზრდა უკავშირდება კვერცხის ზომის შემცირებას. კვერცხის დიდი ზომა პოტენციურად ზრდის კვერცხის გადარჩენის ალბათობას გამოჩეკვამდე, ასევე ახალშობილთა გადარჩენის ალბათობას–ცხოვრების პირველ თვეებში (Sinervo, 1990; Levitan, 2006).

კვლევებმა აჩვენა, რომ ხვლიკების რეპროდუქციული ძალისხმევა, რეპროდუქციული პერიოდი, კვერცხის რაოდენობა და კვერცხის ზომა დამოკიდებულია გარემო ფაქტორებზე (ტემპერატურა, ტენიანობა, მიწის საფარი) (Shine and Elphick 2001; Brandt and Navas, 2011; Menezes and Rocha, 2014). გარემო ფაქტორებთან ერთად, მდედრის სხეულის ზომა მნიშვნელოვან როლ თამაშობს ხვლიკების რეპროდუქციული ცვლადების განსაზღვრაში (Vrcibradic and Rocha 2011; Winck and Rocha 2012; Menezes and Rocha, 2014). უფრო დიდი სხეულის მქონე მდედრები უფრო დიდ და მძიმე კვერცხებს დებენ (Brandt and Navas, 2011; Menezes and Rocha, 2014). ამასთან, რეპროდუქციულ სტრატეგიას (პართენოგენეზი თუ ორსქესიანი გამრავლება) ზოგიერთი კვლევის მიხედვით აქვს ზეგავლენა რეპროდუქციულ დანახარჯებზე, თუმცა ზოგიერთი კვლევის მიხედვით–არა

(Schall 1978). მაგალითად, Congdon et al. (1978), ერთ და ორსქესიან *Aspidoscelis* სახეობში, პართენოგენეზი, უფრო დიდ კლაჩებს (კვერცხდების ერთი ციკლის განმავლობაში დადებული კვერცხების ერთობლიობა) დებენ, თუმცა პატარა ზომის კვერცხებს.

ჩვენი მიზანი იყო გამოგვეკვლია, თუ და როგორ მოქმედებს გამრავლების რეჟიმი (პართენოგენეზი თუ ორსქესიანი გამრავლება) მდედრის რეპროდუქციულ მახასიათებლებზე (მათ შორისაა რეპროდუქციული ძალისხმევა, კვერცხის ზომა, კვერცხების რაოდენობა, და ამ პარამეტრების ინტერაქცია), და არის თუ არა ეს გავლენა დამოკიდებული სხვა ცვლადებზე, როგორიცაა მდედრის სხეულის ზომა თუ ჰაბიტატის სიმაღლე ზღვის დონიდან.

კვლევის ფარგლებში შეგროვდა 87 მაკე მდედრი სამი სხვადასხვა ლოკაციიდან: კოჯორი, საღამოს ტბა და მდინარე კირხულაკი, სამი წლის განმავლობაში – 2019, 2021 და 2022 (ცხრილი 1). საკვლევი ობიექტები დაყავით ორ ჯგუფად, შედარებით პატარა ტანიანი (*D. dahli*–*D. porstchinskii*) და შედარებით დიდტანიანი (*D. armeniaca*–*D. valentini*) ხვლიკები. თითოეული მდედრი განთავსდა ცალ–ცალკე პლასტმასის კონტეინერში. ვზომავდით მდედრის სხეულის სიგრძეს და ვწონდით მის მასას კვერცხის დადებამდე და დადების შემდეგ. ასევე, გავზომეთ თითოეულ კვერცხების გროვაში კვერცხების რაოდენობა, წონა და მაქსიმალური დიამეტრი. სტატისტიკური ანალიზისთვის ერთცვლადიანი ANOVA და ზოგადი ხაზოვანი მოდელი (GLM) გამოვიყენეთ. ანალიზი ჩატარდა IBM SPSS (2021) პროგრამული უზრუნველყოფის გამოყენებით.

კვლევის შედეგებმა აჩვენა, რომ შედარებით დიდი ზომის მდედრები დებენ მეტ რესურსს გამრავლების ერთ სეზონზე, დებენ უფრო მძიმე კვერცხის გროვებს და მეტი რაოდენობის კვერცხებს. ამასთან, დიდი სხეულის მქონე სახეობები მრგვალ კვერცხებს დებენ, ხოლო პატარა სხეულის მქონე სახეობები – წაგრძელებულ კვერცხებს.

D. armeniaca და *D. valentini* -ს შედარების შედეგად აღმოჩნდა, რომ სომხური ხვლიკი გამრავლების ერთ სეზონზე უფრო მეტ ენერგიას დებს რეპროდუქციაში, ვიდრე მისი მამა მშობელი სახეობა – ვალენტინის ხვლიკი, რითიც ამცირებს ალბათობას იმისა, რომ გადარჩეს შემდეგი რეპროდუქციული სეზონისთვის და ისევ გამრავლდეს. ქვენ შეგვიძლია ვივარაუდოთ, რომ ზრდასრულ სტადიაზე, მდედრისთვის გარემო პირობები

არის ნაკლებად სტაბილური და ცდილობს მაქსიმალურად მეტი ენერგია ჩადოს ერთ გამრავლებაში. ორსქესიანი ვალენტინის ხვლიკი კი ენერგიის ინვესტირებას ანაწილებს მრავალ რეპროდუქციულ სეზონზე.

დალისა და მტკვრის ხვლიკების შემთხვევაშიც ჩვენ ვხვდებით სტაბილურ და არასტაბილურ გარემოს, თუმცა მათ მცირედ განსხვავებული სტრატეგია ახასიათებთ. ამ შემთხვევაში, ორსქესიანი სახეობა დებს უფრო დიდ კვერცხებს, რაც მიუთითებს იმაზე, რომ მისი ინვესტიცია მაქსიმალურად ფოკუსირებულია თითოეულ კვერცხე, თითოეულ ახალშობილზე. დალის ხვლიკი კი დებს ბევრ, თუმცა პატარა კვერცხებს, რაც ნიშნავს იმას, რომ გარემო სტაბილურია ზრდასრულებისთვის, მაგრამ არა კვერცხებისთვის და ახალშობილებისთვის. შესაბამისად, მისი სტრატეგიაა დადოს რაც შეიძლება მეტი კვერცხი და გაზარდოს შანსი, იმისა რომ ამ კვერცხებიდან ზოგი მაინც გადარჩეს.

Chapter 3. Estimating phenotypic heritability of sexual and unisexually reproducing rock lizards (genus *Darevskia*)

Genetic drift and differential selection play an important role in the case of divergence of natural populations (Wright, 1931). According to population geneticists, an excess of phenotypic variation over genetic variation estimated by neutral genetic markers indicates the presence of differential selection (Merilä, 1997; Lynch et al., 1999; Merilä & Crnokrak, 2001; DeFaveri and Merilä, 2013). Phenotypic variance, which supposedly is related to genetic factors is commonly referred to as Q_{st} (Merilä and Crnokrak, 2001; Whitlock, 2008; Miller et al., 2008), but the total phenotypic variance of organisms is related to both genetic and non-genetic/environmental factors. Therefore, analyzing phenotypes without considering heritability may lead to biased conclusions (Leinonen et al., 2006; Brommer, 2011; DeFaveri and Merilä, 2013). Some authors suggest using the term P_{st} to measure simple phenotypic differentiation (Leinonen et al., 2006; Brommer, 2011; DeFaveri and Merila, 2013) and distinguish it from "true" Q_{st} , which includes the heritability of the phenotypes under study.

Estimating heritability in wild populations is challenging. Experimental methods require a precise understanding of the genetic relationships among individuals with different traits and are typically conducted in controlled laboratory settings (Falconer, 1996; Wray and Visscher, 2008; Whitlock, 2008). In contrast, field-based approaches are more probabilistic and often don't address the sources of phenotypic diversity. When comparing differences in phenotype and genotype, field studies often assume a strong correlation between estimates of genetic variation within populations and differences between populations, although this isn't always the case (Leinonen et al., 2013).

Lizard groups that include parthenogenetic taxa, which co-exist with their parental species, provide possibilities to analyze the sources of diversity without running labor-intensive crossing experiments. These taxa, such as lizards of the Caucasian genus *Darevskia* (Darevsky, 1967; Danielyan et al., 2008; Tarkhnishvili et al., 2010, 2017; Tarkhnishvili, 2012) and North American *Aspidoscelis* (Lowe and Wright, 1966; Reeder et al., 2002; Sullivan et al., 2013), are natural clones

characterized by lack of genetic recombination. Their phenotypic variation can be caused by environmental factors and de novo mutations but, different from sexually reproducing species, the factor of recombination is excluded. Therefore, phenotypic variation in sexually reproducing populations typically exceeds that observed in closely related parthenogenetic clones from the same location. This difference can be used as an indicator of heritability.

For this study, we described phenotypic variation in the bisexual and unisexual rock lizards, *D. portschinskii* and *D. dahli* from six river valleys (RVs) in eastern Georgia, where they coexist. The research aimed to use the natural system to avoid bias during the analysis of the heritability of individual phenotypic characters and comparison of genetic and phenotypic variation in both taxa to estimate the difference between total and genetically determined phenotypic variation.

Material and methods

Under this study, we collected *D. dahli* and *D. portschinskii* individuals from six river valleys of western Georgia and photographed temporal and anal areas of 186 individuals of *D. dahli* and 101-*D. portschinskii*. In further analyses, only females of *D. portschinskii* were used which reduced the total sample size of this species to 54 (Table 2).

Table 2. Numbers of individuals used for phenotypic analysis and scored microsatellite genotypes. RV–the name and the number of River Valley. PH–the number of females analyzed for phenotype; STR–the number of individuals analyzed for microsatellite profile. DD –*D. dahli*, DP–*D. portschinskii* (Table 1 from Tarkhnishvili et al., 2020a).

RV	DD (PH)	DP(PH)	DD (STR)	DP (STR)
Khrami (1)	74	4	11	6
Algeti (2)	34	7	7	8
Kojori (3)	18	6	13	9
Vere (4)	17	6	5	4

RV	DD (PH)	DP(PH)	DD (STR)	DP (STR)
Mtskheta (5)	30	18	11	8
Tana (6)	13	13	10	5
TOTAL	186	54	57	40

For scalation analysis, we selected 21 scalation traits (Fig. 5), which vary in both taxa. Thirteen out of 21 traits were numeric or ordinal. The character descriptions included the number, absence/presence, displacement, and/or shape of the specific character or their intersections.

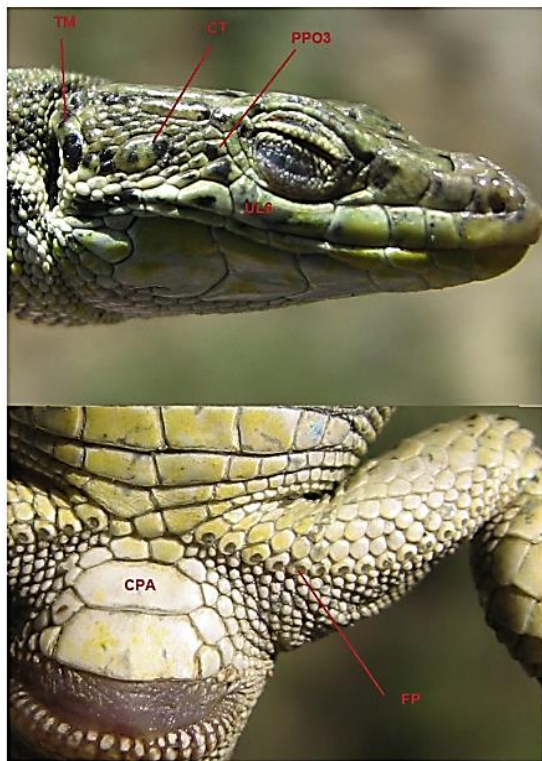


Fig. 5. Temporal and preanal areas of *Darevskia* lizards, with scales used in the analyses indicated (Figure 2 from Tarkhnishvili et al., 2020a).

The analysis of microsatellite genotypes

For comparison of the phenotypic variation using neutral genetic markers, a microsatellite of five nuclear tetra nucleotide repeats locus Du215, Du281, Du418, Du47, and Du323 (Korchagin et al., 2007). PCR reaction QIAGEN Multiplex PCR Kit was performed. To visualize genotypes we used

Genemapper v3.5 software (PerkinElmer, Waltham, MA, USA). Every locus of each individual was run two to four times to control for allelic dropout and false allele amplification.

Statistical analysis

We calculated the geometric center of each River Valleys and evaluated the distance among the RVs by connecting their geometric centers with straight lines, and following lines along roadways and used Mantel Tests (software zt; Bonnet and Van de Peer, 2002) for calculating the correlation coefficients, for each of the studied taxa, between Pst (phenotypic distances) and geographic distances among the geometric centers of RVs and with cost distances (along roads) among the centers of RVs (both for *D. portschinskii* and *D. dahli*); between Qst and the geographic distances (*D. portschinskii* only); between Pst and Rst based on the STR profiles (both species), and between Qst and Rst (fixation index) (*D. portschinskii* only). Finally, between Pst and Rst with the averaged climatic differences among the RVs (Euclidean distance), based on 19 bioclimatic variables of the respective locations.

Results with discussion

In summary, 1. *D. portschinskii* showed higher and stronger phenotypic as well as genotypic variations among individuals and between–population than *D. dahli*; 2. the phenotypic distances between six populations of *D. portschinskii* but not *D. dahli* significantly correlated with geographic distances between the locations and pairwise fixation indices based on the analysis of the genetic markers. This correlation substantially increased when, instead of the original phenotypic distances, the corrected Qst values were used to assess the heritability of the characters.

The correlation between spatial diversification of neutral genetic markers and morphological traits is well documented, but despite this, researchers often overlook the heritability of phenotypic components when reporting this correlation. However, phenotypic divergence that does not explicitly consider heritability can substitute for genetically determined diversification of phenotypes (Qst) only if some robust assumptions are met (Whithlock, 2008; Brommer, 2011;

Leinonen et al., 2006, 2013). In addition, distinguishing between environmental and genetic variation can be challenging due to various factors such as varying heritability between populations and epistatic effects.

Some authors suggest the "common-garden" principle for evaluating heritability. Estimating heritability experimentally is resource-intensive, and prior knowledge of heritability may not be sufficient to calculate "true" Q_{st} in new environments. Parallel analysis of asexually and sexually reproducing organisms can help overcome these challenges by allowing heritability to be measured *in situ*. On the other hand, application of this method can lead us to difficulties: 1. Fixed phenotypic differences among unisexual and bisexual species, which is common for *Darevskia*. Parthenogenetic *D. dahli* is an interspecific hybrid (Uzzell and Darevsky, 1975; Darevsky et al., 1985; Murphy et al., 2000) and exhibits combined characteristics of both parental species, which potentially contributes to its genetic diversity. However, this additive genetic diversity would increase rather than decrease genetic diversity in the parthenogen and does not explain its lower genetic variation compared to the paternal species. For the analysis we excluded fixed phenotypic differences between *D. dahli* and *D. portschinskii* and used broadly variable characteristics in both forms and, hence, their variability was comparable. If we consider *D. dahli* individuals as descended from more than one hybridization event and reject previous studies (Tarkhnishvili et al., 2017), we can explain some phenotypic variation in this form; 2. Slight habitational and microclimatic differences in the same river valleys may influence the embryo development stage, which according to results is an unimportant factor, and 3. Coding of the variable (Some characters that significantly vary are difficult to code as numeric or ordinals, and they should be treated as nominal categorical variables).

D. dahli and *D. portschinskii* share similar patterns of variation, but *D. portschinskii* shows stronger variation in all traits (13 numeric and ordinal variables with PCA and CPCA axes) examined. This suggests a substantial genetic component in the variation of *D. portschinskii*, preanal scale size, and the number of upper labials showed high heritability. In contrast, variation in *D. dahli* appears to be largely influenced by the environment.

Results show, that in *D. portschinskii* scalation traits (6 numeric and ordinal) and 1st and 2nd PCA show greater variation among the river valleys than within those, in *D. dahli*, between-group variation was found only for the 2nd PCA axis and five characters describing the size of the central temporal and anal scales. This suggests that environmental effects may contribute to differences between widely separated populations. The lack of correlation between phenotypic plasticity on selection implies that the variation within the norm of reaction would not substantially influence the spatial distribution of variation.

The correlation between genetic differentiation and geographic distance suggests that migration rate is a key factor in genetic differentiation (Wright, 1931; Weir, 1996). The presence of a significant correlation between fixation index and geographic distance supports an isolation-by-distance model. This suggests that populations as it is shown in this study have reached a drift-migration equilibrium over time. Phenotypic distances in *D. portschinskii* (but not *D. dahli*) correlate with both fixation index and geographic distances. Corrected Q_{st} values show an increased correlation with geography, indicating that migration is an important force determining both genetic and phenotypic differentiation in these lizards. To put it differently, the inherited variation in the scalation pattern results in a spatial distribution pattern similar to that of neutral genetic markers. Consequently, this suggests that accurately assessed Q_{st} values derived from the analysis of easily measurable phenotypic traits can be used to estimate relative migration rates when analyzing more than two populations of the same species.

Wright (1931) showed that if population differentiation is driven solely by genetic drift, the Q_{st} value should be equal to the fixation index. However, in the case of *D. portschinskii*, all pairwise Q_{st} values significantly exceeded the fixation indices based on microsatellite loci. On average, the Q_{st} value was five times higher than the fixation index for individual pairwise comparisons.

This suggests a substantial role for diversifying selection in the diversification of *D. portschinskii* populations. This pattern is consistent with the findings of Merila and Conkrak (2001), suggesting that while genetic and morphological characters are often correlated, Q_{st} typically exceeds the fixation index. However, it's difficult to interpret this observation straightforwardly. Stronger diversifying selection between the most geographically distant populations would only be

expected if there were a correlation between climatic differences and geographic distance, which is not observed in this study. Diversifying selection would typically decrease the correlation between geographic and genetic distances, but in this study, the correlation coefficients of geographic distance with the fixation index and with Q_{st} are roughly equal.

The correlation of a trait with environmental conditions is strong evidence for natural selection. In this dataset, only two characters show a relatively high (>0.3) correlation with climatic differences. One of these traits, CPA, is strongly influenced by genetics, while the other, TMUT, is less influenced by genetics. The combination of these traits is significantly related to differences in climate in *D. portschinskii*, not to differences in geography. This suggests that the relationship between traits and distance between locations isn't due to differences in climate between these locations and selection based on climate.

თავი 3. სქესობრივად და უსქესოდ გამრავლებადი კლდის ხვლიკების (გვარ *Darevskia*) ფენოტიპური მემკვიდრეობის შეფასება

სქესობრივად გამრავლებად სახეობებს აქვთ უფრო მდიდარი გენეტიკური ფონდი რეკომბინაციის გამო, ვიდრე პართენოგენეზულ სახეობებს, შესაბამისად ფენოტიპური ვარიაციების ფართო სპექტრი. პართენოგენეზული სახეობები, რომლებიც კლონები არიან და დაბალი გენეტიკური მრავალფეროვნებით ხასიათდებიან, ცვალებადობენ, ძირითადად, გარემო ფაქტორებისა და შემთხვევითი მუტაციების შედეგად. თანაარსებობის პირობებში, პართენოგენეზული სახეობების მორფოლოგიური ცვალებადობის დიაპაზონი უფრო ვიწროა, ვიდრე მშობელი ორქესესიანი სახეობებისა. ფენოტიპური ვარიაციების განსხვავება სქესობრივ და პართენოგენეზულ სახეობებს შორის მემკვიდრეობის ინდიკატორად შეიძლება იყოს გამოყენებული—თუ რამდენად არის გარკვეული მორფოლოგიური ნიშნები გენეტიკით განპირობებული, და არა მხოლოდ გარემოს ფაქტორებით.

სქესობრივად და უსქესოდ გამრავლებადი კლდის ხვლიკების შესწავლის მიზანი იყო დაგვედგინა გენეტიკის როლი მორფოლოგიური მახასიათებლების ფორმირებაში მათი ფენოტიპური და გენოტიპური ვარიაციების შედარებითი ანალიზის საფუძველზე. ჩვენ შევადარეთ გენეტიკური და ფენოტიპური ცვალებადობა დალისა და მტკვირის ხვლიკების პოპულაციებს შიგნით და შორის სხვადასხვა ლოკაციებიდან.

კვლევის ფარგლებში შეგროვდა 186 დალის და 101 მტკვირის ხვლიკის (ორივე სქესი) ფოტო და გენეტიკური მასალა 6 ხეობიდან (ხრამი, ალგეთი, კოჯორი, ვერე, მცხეთა, ტანა) (ცხრილი 2). დეტალური ანალიზისთვის, მამრი ინდივიდების ამოღების შედეგად, მტკვირის ხვლიკების სინჯები შემცირდა 54-მდე. მორფოლოგიური ცვალებადობის დონის დასადგენად, ჩვენ შევისწავლეთ 21 მახასიათებლის (ქერცლის) რაოდენობა, არსებობა/არარსებობა, განლაგება და ფორმა (ფიგ. 5), ხოლო გენეტიკური ანალიზისთვის, მიკროსატელიტური ლოკუსების ამპლიფიკაციისთვის (GATA repeats), გამოვიყენეთ ხუთი პრაიმერების წყვილი (Korchagin et al. 2007). პჯრ რეაქცია ჩატარდა

QIAGEN Multiplex PCR Kit გამოყენებით (qiagen.com # 206143) ორ მულტიპლექს რეაქციაში.

სტატისტიკური ანალიზისთვის გამოვთვალეთ მდინარის ხეობების გეომეტრიულ ცენტრები და გავზომეთ მანძილები ცენტრებს შორის სწორი ხაზებისა და გზების გამოყენებით.

კვლევის შედეგების მიხედვით, *D. portschinskii*-ს პოპულაციებს შიგნით და შორის ფენოტიპური და გენოტიპური ვარიაციები უფრო მაღალი და ძლიერი იყო, ვიდრე *D. dahli*-ს შემთხვევაში; 2. *D. portschinskii*-ს ექვსი პოპულაციის ფენოტიპური მანძილები მნიშვნელოვნად კორელირებდა გეოგრაფიულ მანძილებთან, მაგრამ *D. dahli*-ს შემთხვევაში ასეთი კორელაცია არ აღინიშნა.

ორსქესიან სახეობებში გენოტიპური სხვაობა პოპულაციებს შორის უკავშირდება გეოგრაფიულ მანძილებს. ასევე, გენეტიკური ცვალებადობა უკავშირდება გარემო პირობებს, მაგალითად, სიმაღლეს ზღვის დონიდან, რაც ნიშნავს იმას, რომ სვხდასხვა ეკოლოგიურ პირობებში, განსხვავდება გენოტიპი ორსქესიანი სახეობებისა და ამას თან სდევს ფენოტიპური განსხვავებაც. ჩვენ ვასკვნით, რომ ორსქესიანი სახეობები ბუნებრივი გადარჩევის გზით ეგუებიან თავიანთ ჰაბიტატს. ერთსქესიან სახეობებში, გენეტიკური ცვალებადობა არის დაბალი, და არ ასახავს ეკოლოგიური პირობების განსხვავებას. პართენოგენეზში არის გარკვეული ფენოტიპური ცვალებადობა, რომელიც უკავშირდება გარემო ფაქტორებით ინდუცირებულ ცვალებადობას. ეს ყოველი მიუთითებს იმაზე, რომ ერთსქესიანი სახეობები ბუნებრივი გადარჩევის გზით ვერ ეგუებიან ადგილობრივ პირობებს და მათი სტრატეგიაა, რაც შეიძლება სწრაფად გამრავლდნენ და მაქსიმალურად აითვისონ ის ადგილები, სადაც არის მათთვის ოპტიმალური პირობები.

Chapter 4. Head shape divergence between parthenogenetic and their paternal bisexual rock lizards in sympatry

Head shape is an important trait in lacertid lizards, influencing various aspects such as bite force capacity, prey handling efficiency, microhabitat preferences, and even respiratory and brain protection mechanisms (Herrel et al., 2004; Barros et al., 2011; Verwaijen et al., 2002; Oliveira et al., 2023). Furthermore, environmental factors such as elevation and temperature have been observed to influence head morphology, e.g. head length and shape in certain *Podarcis* lizards (Ortega et al., 2019; Alagić et al., 2021), as well as geographic gradients affecting head shape in the lacertid *Gallotia galloti* on the island of Tenerife (Thorpe and Baez, 1987).

In addition to environmental influences, phylogenetic relationships play a significant role in head shape variation (Openshaw and Keogh, 2014; De Meyer et al., 2019). Related species tend to show similarities in head morphology, a phenomenon referred to as phylogenetic signal in morphological characters (Blomberg et al., 2003; Tarkhnishvili et al., 2020c). However, when closely related species with similar ecological niches coexist, competition or enhancement can drive morphological divergence, resulting in greater divergence compared to allopatric regions (Taylor, 1965; McDowall, 1998; Wijesundara and Freed, 2018). This divergence is evident in a variety of taxa, including the *Paragalaxias* fish of submontane Tasmania (McDowall, 1998), the *Rana macrocnemis* complex brown frog of the Caucasus (Tarkhnishvili et al., 2001), and the *Zosterops* songbird of Sri Lanka (Wijesundara and Freed, 2018).

Certain groups of lizards exhibit both parthenogenetic and bisexual forms (Lowe and Wright, 1966; Darevsky, 1967; Moritz, 1991; Adams et al., 2003; Tarkhnishvili et al., 2010; Petrosyan et al., 2020). Parthenogenetic species, often of hybrid origin, have genetic contributions from both maternal and paternal ancestors. However, it remains uncertain whether parental species continue to contribute genes to parthenogenetic lineages after the initial hybridization (Tarkhnishvili et al., 2020b), and whether this hybrid origin is reflected in the morphology of daughter species. Interestingly, parthenogenetic daughter species may not exhibit intermediate morphological characteristics despite their mixed genetic heritage (Heiser et al., 1965; Bemis et

al., 1970; Pembleton and Baker, 1978). For example, *Aspidoscelis tessellatus* (formerly–*Cnemidophorus*) is more similar in size to its maternal species *A. gularis*, despite greater sympatry with the paternal species *A. tigris* (Parker, 1979).

Since parthenogenetic and their parental (in this case paternal) rock lizard species coexist and share genes from both maternal and paternal ancestors, we tried to find out the relative importance of genetic inheritance and environmental conditions on head shape in *D. armeniaca* and *D. dahli*. Specifically, if local adaptations play an important role, one would expect parthenogens to be more similar to females of the paternal species, with which they commonly coexist, than to females of the maternal species, which never share the habitat with the parthenogen. On the other hand, the phenotype of parthenogens may be intermediate between the two parental species, or it may show some peculiarities common to parthenogenetic lineages but absent in bisexual forms. For this purpose, we analyzed the head shapes of samples of both parthenogens (*D. dahli* and *D. armeniaca*), two paternal species from the same localities (*D. portschinskii* and *D. valentini*, respectively), and their common maternal species, *D. mixta*, from several localities in Georgia.

Material and methods

For morphological analysis, we collected 60 adult female individuals from three locations (Table 3) and each lizard was securely placed in a plastic tube and photographed from multiple angles, 36 times from a perspective of 90° to the 11 midline and 36 times from a 45° perspective. AgiSoft PhotoScan Pro v.2.0.2 was used to generate 3D models of the head surface from the aligned images, and 54 homologous landmarks were digitized (Fig. 6). The 3D coordinates were exported and analyzed using RStudio (R 4.2.0, 2022).

Table 3. Sample sizes and key characteristics of three sampling locations of 5 species of *Darevskia* (Table 1 from Barateli et al., 2024, MS under revision, see attached draft).

	Location	Easting	Northing	Altitude	Female
<i>D. dahli</i>	Kojori	44.683337°	41.648891°	1120	13
<i>D. portschinskii</i>	Kojori	44.683337°	41.648891°	1120	11
<i>D. armeniaca</i>	Sagamo Lake	43.730600°	41.293600°	2015	16
<i>D. valentini</i>	Sagamo Lake	43.730600°	41.293600°	2015	15
<i>D. mixta</i>	Rveli	43.416361°	41.871489°	824	5

Statistical analysis

We used several functions to process and analyze the landmark data. After estimating missing landmarks and aligning them, we checked for symmetry and outliers. To visualize differences in head shape, we performed PCA and generated landmark wireframes. We then performed a Procrustes Anova to test for significant differences between species. To account for differences in body size, we repeated the test with body size as a covariate. All analyses were performed in R studio (R 4.2.0, 2022) using packages such as 'geomorph' and 'RRPP'.

All calculations were done in the R studio (R 4.2.0, 2022).

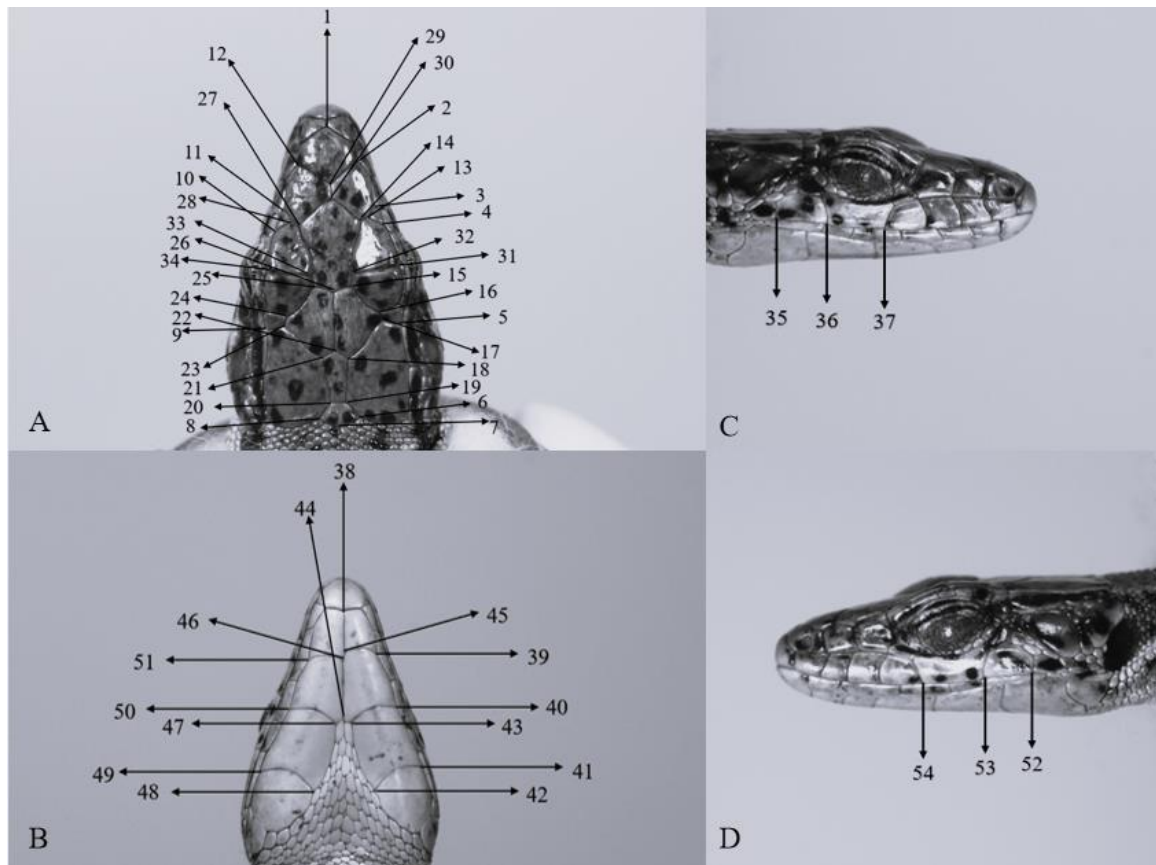


Fig.6. Digitized three-dimensional landmarks. Names of the scales are labeled with white text, landmarks are shown with black numbers (see description of the scales and landmarks in Supporting Information, Appendix S1). A–dorsal view of the head; B–ventral view of the head; C–view of the right profile of the head; D–view of the left profile of the head. The specimen in the photograph is *Darevskia armeniaca* (Figure 3 from Chapter 4, Barateli et al., 2024).

Discussion with results

In summary, our results reveal several key observations: 1. There is no clear evidence of mixed genetic ancestry reflected in the head shape of the parthenogens. However, they show a stronger affinity to females of the maternal species than to the paternal species; 2. In addition, certain aspects of head shape are associated with body size and/or habitat climate, high mountain species tend to have larger body sizes and deeper heads with stronger jaws, regardless of breeding mode; 3. Both parthenogen species have narrower and more elongated jaw areas with flatter mandibles, which makes their jaws weaker compared to the females of their respective parental species. For example, *D. armeniaca* from Sagamo Lake have stronger jaws and deeper heads than species from

lower elevations, but their jaws are weaker (narrower) compared to females of the paternal species, *D. valentini*, also from Sagamo Lake.

The morphological variation observed in *Darevskia* lizard species is influenced by a combination of genetic factors, such as gene drift and mutation, and environmental influences, such as temperature, humidity, and food availability (Runemark et al., 2010; Oliveira et al., 2023). Adams (2011) suggests that despite shared selective pressures, unique genetic patterns may be driving distinct head-shape evolution in *Plethodon* salamanders.

In the case of *Darevskia* lizards, if environmental factors significantly influence the head shape, we might expect parthenogens to show greater similarity to females of the paternal species, with which they commonly coexist, than to females of the maternal species, with which they do not share habitats. Previous morphological studies of parthenogenetic hybrids suggest that unisexual species often exhibit intermediate morphological traits, resembling either both parental species or one more closely (Espeche et al., 2023). For example, parthenogenetic *Teius suquiensis* shows intermediate morphological traits compared to bisexual *T. teyou* and *T. oculatus*, while parthenogenetic *Cnemidophorus tessellatus* shows greater similarity to its maternal species than to its paternal species (Espeche et al., 2023; Parker, 1979).

The overall shape, relative size, and depth of the head, as well as the size of the jaws, are closely correlated with the body size of individuals, which is strongly influenced by climate and altitude (Darevsky, 1967). Indeed, body size tends to increase with elevation as previous studies on lizards show it. According to Bergmann's rule, this trend is typical for larger individuals found in colder climates due to a lower surface-to-volume ratio, resulting in better heat retention (Ortega et al., 2019; Pincheira-Donoso et al., 2008).

Although we observed slight differences in morphometric aspects between parthenogenetic and sexually reproducing species, we can hypothesize that these differences may be related to the territorial behavior of bisexual species, wider jaws, indicating a stronger bite, contribute to successful territorial defense (Galoyan, 2011; Galoyan, 2013; Tsellarius, 2016; Barateli et al., 2021). Additionally, studies have highlighted the relationship between wider jaws and enhanced bite force (Kohlsdorf, 2008; De Meyer, 2019).

Procrustes ANOVA analysis indicates a greater similarity of parthenogenetic head shape to maternal ancestry, particularly in *D. armeniaca*. This suggests a significant maternal effect on morphology, which is supported by studies in other squamates. Maternal influence, rather than simple Mendelian inheritance, may determine body size and behavior. In addition, differences in territorial behavior between parental species may contribute to variations in head shape. Further behavioral observations are needed to clarify these findings. Overall, while some traits distinguish parthenogens from their bisexual ancestors, others are associated with body size and habitat elevation. However, differences in reproductive biology, such as the absence of territorial behavior in parthenogens, may overshadow the effects of intermediate genetics or habitat type on head shape.

თავი 4. თავის ფორმის დივერგენცია პართენოგენეზულ და მათ მამა მშობელ ორსქესიან კლდის ხვლიკებს შორის სიმპატრიაში

თავის ფორმა გავლენას ახდენს ხვლიკის ბიოლოგიის სხვადასხვა ასპექტზე, მათ შორის კბენის ძალაზე, ნადავლის დაჭერაზე თუ ჰაბიტატის შერჩევაზე. გარემო ფაქტორებს, როგორიცაა ტენიანობა და ტემპერატურა, ასევე, აქვთ ზეგავლენა ხვლიკების თავის ფორმის ფორმირებაზე, როგორც ეს სხვადასხვა კვლევებმა აჩვენა (Thorpe and Baez, 1987; Ortega et al., 2019; Alagić et al., 2021).

გარემო ფაქტორების ზემოქმედების გარდა, ფილოგენეტიკურ კავშირებს აქვს მნიშვნელოვანი გავლენა ხვლიკის თავის ფორმის ცვალებადობაზე (Openshaw and Keogh, 2014; De Meyer et al., 2019). მიუხედავად იმისა, რომ მონათესავე სახეობებს ხშირად აქვთ თავის მსგავსი ფორმა, კონკურენციამ შეიძლება გამოიწვიოს მნიშვნელოვანი განსხვავებების ჩამოყალიბება თანაარსებობის პირობებში.

რადგან პართენოგენეზული და მათი მშობელი (ამ შემთხვევაში მამა) კლდის ხვლიკების სახეობები თანაარსებობენ და ასევე, აქვთ გენები ორივე, როგორც დედა, ასევე, მამა წინაპრებისგან, ჩვენ მიზანი იყო გაგვეჩვენა გენეტიკური მემკვიდრეობისა და გარემო ფაქტორების ზემოქმედება დალისა და სომხური ხვლიკების თავის ფორმის ცვალებადობაზე. კონკრეტულად, თუ ლოკალური ადაპტაციები მნიშვნელოვან როლს თამაშობენ, შეიძლება ველოდოთ, რომ პართენოგენები უფრო მეტად მსგავსები იქნებიან მამის სახეობის მდედრებისა, რომელთანაც ისინი თანაარსებობენ, ვიდრე დედის სახეობის მდედრების, რომელთანაც ჰაბიტატებს არ იზიარებენ. მეორეს მხრივ, პართენოგენების ფენოტიპი შეიძლება იყოს შუალედური ორ მშობელ სახეობას შორის, ან აჩვენოს გარკვეული განსხვავებული თვისებები, რომლებიც არ გვხვდება სქესობრივ ფორმებში. ამისთვის, ჩვენ გავაანალიზეთ როგორც პართენოგენების (*D. dahli* და *D. armeniaca*), ასევე მათი მამა (*D. portschinskii* და *D. valentini*) და საერთო დედა (*D. mixta*) მშობელი სახეობების, თავის ფორმები რამდენიმე ლოკაციიდან საქართველოში.

კვლევის ფარგლებში ჩვენ შევაგროვეთ სამი სხვადასხვა ლოკაციიდან (კოჯორი, რველი, სადამოს ტბა) 60 ზრდასრული მდედრი ინდივიდი (ცხრილი 3). თითოეული ხვლიკი

ცოცხლად განთავსდა პლასტმასის მილში. ჩვენ გადავიღეთ 72 ფოტო: 36 სურათი 90° კუთხით და 36 სურათი 45° კუთხით, რომელიც შემდეგ გამოვიყენეთ 3D მოდელების შესაქმნელად. ამ 3D მოდელებიდან, ჩვენ შევარჩიეთ 54 ჰომოლოგიური ნიშანსვეტი (landmark) (ფიგ.6).

თავის ფორმის განსხვავებების ვიზუალიზაციისთვის, გამოვიყენეთ პრინციპული კომპონენტების ანალიზი (PCA) და სახეობებს შორის მნიშვნელოვანი განსხვავებების შესასწავლად—Procrustes Anova.

ყველა ანალიზი შესრულდა RStudio-ში (R 4.2.0, 2022) პაკეტების 'geomorph' და 'RRPP' გამოყენებით.

კვლევის შედეგად დაგინდა, რომ პართენოგენების თავის ფორმა უფრო მეტად ჰგავს დედა სახეობის მდედრების, ვიდრე მამა სახეობისას თავის ფორმას. თავის ფორმა დაკავშირებულია სხეულის ზომასთან და ჰაბიტატის კლიმატთან. მაღალი მთის, დიდტანიან (სომხური და ვალენტინის ხვლიკები) სახეობებს აქვთ უფრო მაღალი თავები ძლიერი ყბებით, გამრავლების რეჟიმის მიუხედავად. პართენოგენებს აქვთ ვიწრო და უფრო წაგრძელებული ყბები ბრტყელი ქვედა ყბებით, რაც მათ ყბებს სუსტს ხდის მშობელი სახეობის მდედრებთან შედარებით. მაგალითად, *D. armeniaca*-ს სადამოს ტბიდან აქვთ უფრო ძლიერი ყბები და მაღალი თავები, ვიდრე *D. dahlī*-ს, მაგრამ სუსტი ყბები ვიდრე მამა სახეობის, *D. valentini*-ის მდედრებს.

ჩვენ ვვარაუდობდით, რომ ერთსქესიანი სახეობების თავის ფორმა მსგავსი ან შუალედური იქნებოდა მათი დედა ან მამა მშობელი სახეობების თავის ფორმებისა. ლუმცა კვლევის მიხედვით დადგინდა, რომ პართენოგენებს, განსხვავებით მათი მშობელი სახეობებისა, აქვთ სუსტი ყბები. რასაც ვუკავშირებთ ტერიტორიული ქცევის გაქრობას. ტერიტორიული ქცევის პირობებში ისინი პოპულაციას სწრაფად ვერ გაზრდიან, შესაბამისად შემცირდება შანსი ოპტიმალური პირობების დაკავებისა, და ასევე, კონფლიქტის შემთხვევაში, მათი სუსტი ყბები არ იქნებოდა უპირატესი. მათი სტრატეგიაა მაღალი კონცენტრაციით დაიკავონ ოპტიმალური ჰაბიტატი და მაქსიმალურად გამოიყონ იგი როგორც საკვებისთვის, თავშესაფრისთვის, ასევე, გასამრავლებლად და მაქსიმალურად სრწაფად გაიფანტონ და დაიკავონ ოპტიმალური

პირობებს მქონე ლოკაციები, და ზოგჯერ გაიმარჯვონ კიდევაც კონკურენციაში თავიანთ მშობელ სახეობებთან.

General conclusion

The studies presented here cover various aspects of the comparative ecology of the parthenogenetic lizards and their sexually reproducing parental species. These include microhabitat use, specifics of the life cycle, phenotypic and genetic diversity, and peculiarities of head morphology. However, all these studies help to characterize the differences between the parthenogens and sexual breeders. Both parthenogens tend to adapt to the r-strategy of population dynamics. They are relatively more fertile, which suggests that the adult environment is relatively unstable; they more effectively use those limited areas in space and time with optimal conditions than sexual breeders, thanks to the lack of territorial behavior; they disperse faster, and don't form isolation-by-distance population genetic pattern, typical for old, well-established geographic populations. Finally, their strategy includes fast utilization of available resources, not impeded by intraspecific competition, which is reflected in weaker heads not adapted for battles with individuals of the same species. As a result, parthenogens more effectively use environmental conditions than closely related sexual breeders, at least until macroecological conditions, including climate, remain more or less stable. We can only suppose that sharp changes in environment would give an advantage rather to sexual breeders, where genetic diversity is much higher due to the recombination of alleles.

დასკვნა

სადოქტორო კვლევა მოიცავს პართენოგენეზული ხვლიკებისა და მათი სქესობრივად გამრავლებად მშობელი სახეობების შედარებითი ეკოლოგიის სხვადასხვა ასპექტს: მიკროჰაბიტატის გამოყენებას, სასიცოცხლო ციკლის სპეციფიკას, ფენოტიპურ და გენეტიკურ მრავალფეროვნებას, თავის მორფოლოგიის თავისებურებებს. თუმცა, ყველა ამ კვლევის მიზანი იყო დაგვედგინა განსხვავებები ერთ და ორსქესიან სახეობებს შორის. ორივე პართენოგენი მისდევს r-სტრატეგიის, რაც გულისხმობს იმას, რომ: 1. ისინი შედარებით უფრო მაღალი ნაყოფიერებით ხასიათდებიან და გარემო ზრასდრულთათვის შედარებით არასტაბილურია; 2. ისინი უფრო ეფექტურად იყენებენ ოპტიმალურ პირობებს, ვიდრე სქესობრივად გამრავლებადი სახეობების, ტერიტორიული ქცევის არარსებობის გამო; 3. ისინი უფრო სწრაფად ვრცელდებიან და არ ქმნიან მყარ სივრცულ პოპულაციურ სტრუქტურას; 4. მათი სტრატეგია ოპტიმალური რესურსების სწრაფი უტილიზაციაა, რასაც ხელს არ უშლის სახეობის შიდა კონკურენცია. ეს აისახება სუსტ ყბებში რომლებიც არ არიან შეგუებული ბრძოლებისთვის შიდასახეობრივი კონკურენციის პირობებში. შედეგად, პართენოგენები უფრო ეფექტურად იყენებენ გარემოს პირობებს, ვიდრე ახლო მონათესავე სქესობრივად გამრავლებადი სახეობები, იქამდე მაინც, სანამ მაკროეკოლოგიური პირობები, მათ შორის კლიმატი, მეტნაკლებად სტაბილურია. ჩვენ შეგვიძლია ვივარაუდოთ, რომ გარემოს მკვეთრი ცვლილებები უპირატესობას მიანიჭებს სქესობრივად გამრავლებად სახეობებს, სადაც გენეტიკური მრავალფეროვნება ბევრად უფრო მაღალია ალელების რეკომბინაციის გამო.

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Chapter 1. Fine-scale analysis of habitat occupancy by Kura lizard (*Darevskia portschinskii*) and its daughter parthenogenetic form (*Darevskia dahl*i)

Fine-scale analysis of habitat occupancy by Kura lizard (*Darevskia portschinskii*) and its daughter parthenogenetic form (*Darevskia dahli*)

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Abstract

Two species of rock lizards, the parthenogenetic *D. dahli* and the sexually reproducing *D. portschinskii*, coexist in a rocky outcrop in an area of ca. 1 ha, in the vicinity of Tbilisi, Georgia; the location has been well-known since the middle 1960s. The population density of the parthenogenetic lizard is five times higher than that of the sexual breeder. We studied the distribution of active lizards in space and time over three consecutive years, during the spring and autumn activity periods, to explore spatial and temporal differences between the species on a fine spatial scale. We studied the influence of temperature, humidity, and quantitative characteristics of the surface and the distance from permanent water source on the spatial distribution of *D. dahli* and *D. portschinskii*. *Darevskia portschinskii* was less dependent on the distance from the water source and more evenly distributed in space and time than *D. dahli*. Despite potential competitive interactions, the species did not avoid each other on the microhabitat scale, suggesting that the observed ecological differences are not caused by a niche shift. More individuals of the sexual breeder than individuals of the parthenogen were found in suboptimal habitats. This feature may increase the evolutionary success of *D. portschinskii* in a long-term perspective.

Key Words

competition, ecological niche, parthenogenesis, rock lizards, territoriality

Introduction

Comparing ecological niches of closely related species is a challenging task. A species' spatial distribution and activity dynamics depend on its ecological preferences. Simultaneously, the realized niche in Hutchinson's (1957) sense is shaped by competitive interactions, both inter- and intraspecific. In the case of the presence of a competitor, the space occupied by a population can be shifted, as well as the activity dynamics (Schoener 1983; Abrams 1986; Pfenning and Pfenning 1992; Pianka 2011; Carvalho and Cardoso 2020). This process affects empirically estimated tolerance limits and response curves (Huey and Stevenson 1979; Kassahn et al. 2009) can be different,

dependent on the species composition in a biological community (Petren et al. 1993; Pianka 2011; Frishkoff et al. 2015). In general, competition is a significant force causing character displacement and, hence, organismal evolution (Grant 1972; Pfenning and Pfenning 2010).

Parthenogenetic lizards have hybrid origins, and they commonly coexist with their ancestral bisexual forms (Darevsky 1967; Bakradze 1977; Trofimov 1981; Wright and Vitt 1994; Kearney et al. 2009; Tarkhnishvili et al. 2010; Tarkhnishvili 2012; Galoyan 2013). Coexisting parthenogens and their ancestral species are phenotypically similar, they consume similar food, and most of them occupy rocky habitats (Darevsky 1967; Wright and Vitt 1994; Tarkhnishvili 2012). The offspring of sexually breeding males and

parthenogenetic females are usually sterile (Freitas et al. 2019; Tarkhnishvili et al. 2020). Hence, the parthenogens and their ancestral species can potentially compete for space or food; niche shift and character displacement may be an important force in their phenotypic evolution.

One of such hybrid parthenogenetic lizards is *Darevskia dahli* from the Lesser Caucasus Mountains, the patrilineal ancestor of which is the bisexual *Darevskia portschinskii* and the matrilineal ancestor is *Darevskia mixta* (Murphy et al. 2000). The extent of occurrence of *D. dahli* and *D. portschinskii* almost coincides, and their habitats overlap: *D. portschinskii* is found at altitudes 400–1,370 m. a.s.l., and *D. dahli* at higher altitudes between 830–1,950 m. a.s.l. (Tarkhnishvili et al. 2010). A higher altitudinal range is typical for unisexual species compared to their bisexual relatives (Haag and Ebert 2004) and may help avoid competition (Glesener and Tilman 1978; Kearney et al. 2005; Vrijenhoek and Parker 2009). However, the species coexist throughout a broad mountain forest belt and are commonly found in the same location (Bakradze 1977).

Remote sensing analysis showed that the shift of the spatial distribution of the two species is related to different thermal preferences and to different preferred humidity: *D. portschinskii* prefers higher temperatures than *D. dahli*, and the distribution of *D. dahli* is limited to the areas where annual rainfall level is above a certain threshold (Tarkhnishvili et al. 2010; Petrosyan et al. 2020). Simultaneously, Tarkhnishvili et al. (2010) showed that, within equally suitable habitats, the presence of *D. dahli* might negatively affect *D. portschinskii*: the latter species was less abundant or absent if *D. dahli* was also present in equally suitable locations (Tarkhnishvili et al. 2010), a fact suggesting the importance of interspecific competition. It is unclear whether competition may explain niche shift and character displacement in *D. dahli* and *D. portschinskii*.

For deeper insight into this question, it is essential to understand how the species share space and other resources on the microgeographic scale and whether competition for the resources or behavioral interactions shape the realized niche of either species. Here, several interacting processes may play a role. Galoyan (2011, 2013) showed that both males and females of some sexually reproducing *Darevskia* species may be territorial, whereas the parthenogens show decreased territoriality. One should expect that territorial species do not concentrate so much in areas with optimal living conditions as the species with less expressed territoriality (Brown 1969; Fretwell 1969; Partridge 1978; but see Stamps 1991); this may potentially reduce the mutual negative impact of *D. portschinskii* and *D. dahli*. Simultaneously, populations of parthenogenetic species grow faster than those of sexual breeders (Maynard-Smith 1998) and hence may rapidly expand into sink habitats (*sensu* Pulliam 1988); as a result, the differences in the observed niche dimensions may depend on this spatial dynamics and not on the different ecological preferences.

Here, we examined whether ecological niches of *D. dahli* and *D. portschinskii* are displaced on a microgeo-

graphic scale (within a semi-isolated habitat covering ca. 1 hectare) and what can be the reason for the differences (if any): type of territorial behavior, population density, or competition. We tested two complementary hypotheses: (1) the differences in the habitat use are related to mutual avoidance of the coexisting species, and/or (2) the differences are due to differential territorial behavior; alternatively, habitat requirements might not differ or differ for reasons independent of the intra- or interspecific competition.

Materials and methods

Study area

A rock outcrop near Tbilisi (Georgia) was selected as a study site. This area is described in previous papers (Darevsky 1967; Bakradze 1977; Trofimov 1981; Tarkhnishvili et al. 2010) and presents a good model for long-term stationary observations of the lizards. The area is located at the vicinity of Kojori (1.648939°N, 44.683445°E) (Fig. 1). It is a gentle rocky slope covered with a rare shrub, surrounded by a forest dominated by oriental hornbeam (*Carpinus orientalis*), rocky oak (*Quercus petraea*), and maples (*Acer campestre*, *A. letum*) and edged with a small

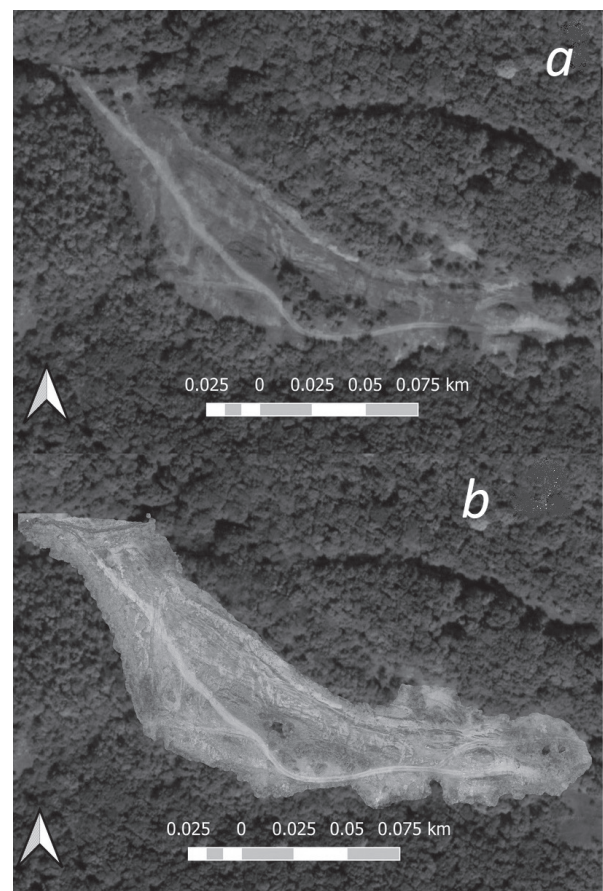


Figure 1. Study area. **a:** satellite image downloaded from Google Earth Pro (2020); **b:** Orthophoto image prepared with the drone (see the methods section). The arrow points in the northern direction. The brook borders the rocky habitat from the north.

brook from the north. The slope has northwestern exposure, and the elevations vary between 1003–1042 m a.s.l. (Fig. 1). The observations were conducted between April and October of 2017, 2018, and 2019; the full data are presented in Suppl. material 1: Table S1.

Description of environmental variables

We divided the study area into 108 squared plots with a surface of 10×10 m each (Fig. 2a; Suppl. material 1: Table S1). For each square, longitude, latitude, and altitude were measured using Garmin GPS (Geographic Positioning System) MAP 64 and then adjusted manually. Geographic Positioning System data were processed in the QGIS 3.12 software and turned into a polygon shapefile (Fig. 2b). DJI Mavic 2 Pro camera drone was used for taking aerial photos of the surface (distance from the ground 60 m). Georeferencing of aerial images was performed using Stonex S9III+ GPS (<https://www.stonex.it/project/s9iii/>). Aerial photos were then processed in the Agisoft PhotoScan software to create Orthoimage and DEM (Digital Elevation Model; Agisoft, L. L. C. “Agisoft PhotoScan User Manual: Professional Edition, Version 1.4”, 2018). We used orthoimage for mapping surface types (Boulders, Forest, Grass, Gravel, Leafy Ground, Rock) manually (Fig. 2c). Digital Elevation Model was resampled in two more spatial resolutions (original resolution 0.03 m/pixel and resampled resolution 0.5 m/pixel; 1 m/pixel). We used SAGA’s surface morphometry tool in QGIS 3.12 for estimating slope and convergence index from the three mentioned resolutions of DEM.

Standard deviation, average and maximal values of slope and convergence index in each sample plot were calculated using the Zonal Statistics tool in QGIS 3.12. The slope represents the inclination angle to the horizontal surface in degrees; the maximum value of slope (MSlope), characterizing each plot, was used in final calculations. The convergence index calculates an index of convergence/divergence regarding overland flow (2004–2016 QGIS Development Team). Our study used the standard deviation of convergence index (SDCI), which measures the unevenness of the rocky surface or density of clefts that the lizards are using as hiding shelters.

In each field session (27 field sessions altogether – Suppl. material 1: Table S1), for each of the 108 plots, the land surface temperature (LST, °C) and land surface humidity (LSH, %) was recorded twice – between 1100AM and 1300 PM and between 1700 and 1900 PM. The measurements were taken in the geometric center of each square plot. The temperature was measured with General Tool MMD7NP; the humidity was measured with Etekcity Lasergrip 774. Ambient temperature from <https://www.accuweather.com/> was recorded during each field session. Altogether 2916 observations (field survey/ plot) were conducted during the study period. During each observation, 0–10 individual lizards were recorded.

For each sighted individual, we recorded: age (adult or juvenile); species (*D. dahli* vs *D. portschinskii*); sex (for *D. portschinskii*); time of the day and the number of the plot. All individuals with the distance from the back surface of the hip to the tip of snout exceeding 5 cm were treated as adults.

Statistical procedures: analysis of habitat use in space and time

The numbers of active *D. dahli* and *D. portschinskii* were recorded for each of 108 plots 100 m^2 each, during each of 27 field sessions. Hence, altogether we had 2,916 observations for each species. Surface temperature and humidity were scored for most of these observations. The entire observation table is shown in the appendix (Suppl. material 1: Table S1). In 18 plots, the lizards were never recorded, and in 35 plots, fewer than three lizards of either species were recorded during the entire period of observations. In the rest of the plots (55 altogether), the number of observed *D. dahli* during one session varied between 0–11, and the number of observed *D. portschinskii* between 0–3.

Because males of *D. portschinskii* might have spatial distribution different from females and juveniles of the same species, due to potentially more expressed territorial behavior, we first tested significance in differences in distribution between *D. dahli*, males of *D. portschinskii*, and females + juveniles of *D. portschinskii* across the 108 plots, using Kolmogorov-Smirnov Lambda test. Since the differences were significant for different species but insignificant for different ages and sexes of *D. portschinskii*, we clumped together all individuals of the latter species for further analyses.

In order to evaluate the significance of each of the factors that potentially influence the presence and activity of the lizards (the number of recorded individuals at a plot during a given session; NL; the analysis separately ran for *D. dahli* and a unified sample of *D. portschinskii*) we applied generalized linear mixed models (GLMM). Because of the excess of NL equal to zero, before applying this method, we removed from the dataset all plots where fewer than three lizards were recorded during the entire period of the study, reducing the number of observations to 1,483. This was done to achieve better correspondence of the NL distribution throughout the plots and sessions to Poisson distribution, recommended for this kind of data. The plots were set as “subjects” and the sessions as “repeated measures.” Target (NL) was set to Poisson distribution with the logarithmic link function. Nine predictors (year, date, time of the day, surface type, humidity and temperature of the ground surface, averaged air temperature, slope, and convergence index) were set as fixed factors; the session combined with the plot was set as a random factor. Covariance type was set to “Variance component” for the subject and to “first-order autoregressive” for the random component (assuming autocorrelation between the sessions close in time).

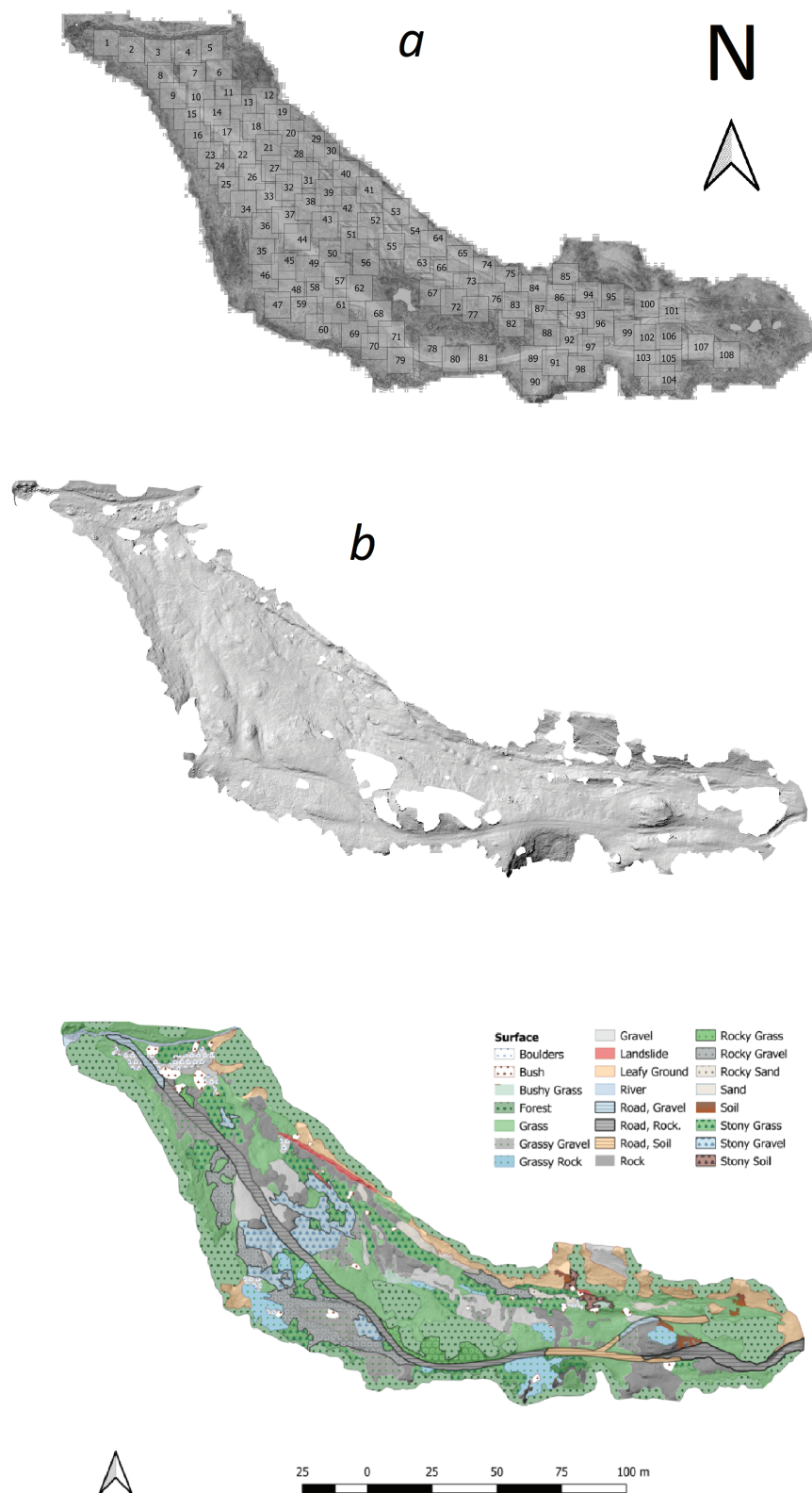


Figure 2. a: the numbers of studied plots; b: DEM image (0.003 m/ pixel); c: surface types (see the text for the explanations).

Classification tree analysis (CHAID algorithm) was an additional method applied for inferring predictive models for both species. In this analysis, we included the occurrence of a potential competitor (PC) in the set of predictors to infer whether and how the presence of PC may

influence the occurrence of the target species, given similar environmental conditions. In this analysis, we used a complete dataset (2,916 observations, including those on the plots where the lizards were never recorded). Parent and Child nodes were set to 100 and 50. Significance lev-

els for splitting nodes and merging categories were set to 0.05, growth limits to 2 levels, the maximum number of iterations to 10,000, and Chi-square statistics calculated using likelihood ratio. For validation, the entire dataset was randomly separated into the training (75%) and test (25%) datasets; squared correlation (R^2) between the observed mean in the training and test datasets was used as an index of the model quality.

Estimation of niche overlap

MacArthur and Levins (1967) suggested several baselines for the analysis of competition and niche displacement. They offered to use an asymmetric coefficient of niche overlap,

$$O_{21} = \sum_{i=1}^n p_{2i} p_{1i} / \sum_{i=1}^n (p_{2i})^2.$$

In this equation, O_{21} is the number of individuals of species 2 whose resources are consumed by a single individual of species 1; p_{1i} is the index of the utilization of resource category i by species 1. We considered a single observation as a “resource category” and estimated p_{xi} as NL for species x in this observation. Before that, we conducted this procedure separately for individual plots and survey sessions and estimated the “overall” overlap indices as the product of the two latter ones (Schoener 1974; Colwell and Futuyma 1971; Hurlbert 1978). Furthermore, we used the symmetric Pianka’s index of niche overlap (Hurlbert 1978).

All calculations were done using IBM SPSS v. 23.0 and Excel 2010 for Windows.

Results

Response on the individual environmental variables

Darevskia dahli were recorded at 78 out of 108 studied plots, and *D. portschinskii* at 40 plots (Fig. 3). The average number of the total records per one plot was: 5.62 (SD = 9.07) for *D. dahli*, 0.44 (SD = 1.07) for males of *D. portschinskii*, and 0.43 (SD = 0.90) for females and juveniles of *D. portschinskii*. Kolmogorov-Smirnov statistic $D_{n,m}$ reached 0.382 and 0.345 ($P < 0.001$) when comparing spatial distribution of *D. dahli* with males and with females + juveniles of *D. portschinskii*, respectively. Simultaneously, $D_{n,m}$ reached 0.089 ($P > 0.2$) when comparing spatial distribution of males with that of females + juveniles of *D. portschinskii*. In conclusion, males, females and juveniles of *D. portschinskii* do not differ significantly in the use of space, and simultaneously the differences between the studied species are highly significant.

Both Pearson correlation and Spearman rank correlation coefficients showed a significant negative correlation of the number of records of *D. dahli* with the distance from

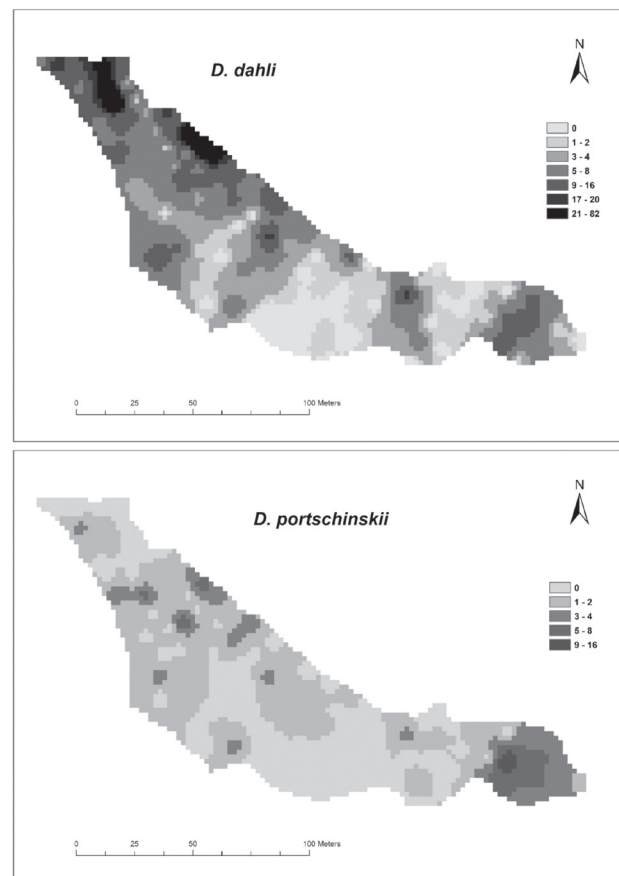


Figure 3. Spatial distribution of *D. dahli* and *D. portschinskii* in the studied rocky outcrop (aggregated data on the lizard sightings throughout the entire study period).

the brook, surface humidity, and convergence index, and positive correlation with an average slope of the plot (Suppl. material 1: Table S1). For *D. portschinskii*, correlation with the distance from the brook was positive (Fig. 4a), whereas correlation with surface humidity was negative. The abundance of *D. dahli* (but not that of *D. portschinskii*) decreased with the distance from the brook. In the part of the study area most distant from the brook, the abundance of both species increased, however (Fig. 4a). Both species were more abundant in 2017 than in the two following years, although in *D. portschinskii* the differences were insignificant (Fig. 4b). NL of *D. dahli* decreased when the air temperature measured before the afternoon was below 13 and above 21 °C; in *D. portschinskii* the dependence was less clear (Fig. 4c). The peak of activity of both species was in the last days of May (Fig. 4d). *Darevskia dahli* preferred the plots where the average slope index was around 6 and avoided flat areas (Fig. 4f). Finally, both species depended on the presence of rocky surface (Fig. 4e). The convergence index did not show any trend.

The analysis based on the assumption that the individual plots are “subjects”, individual sessions are “repeated measures”, and NL has Poisson distribution with Log function, suggested a significant effect of all predictors, except surface temperature and the convergence index on the occurrence of *D. dahli*, and presence of temporal

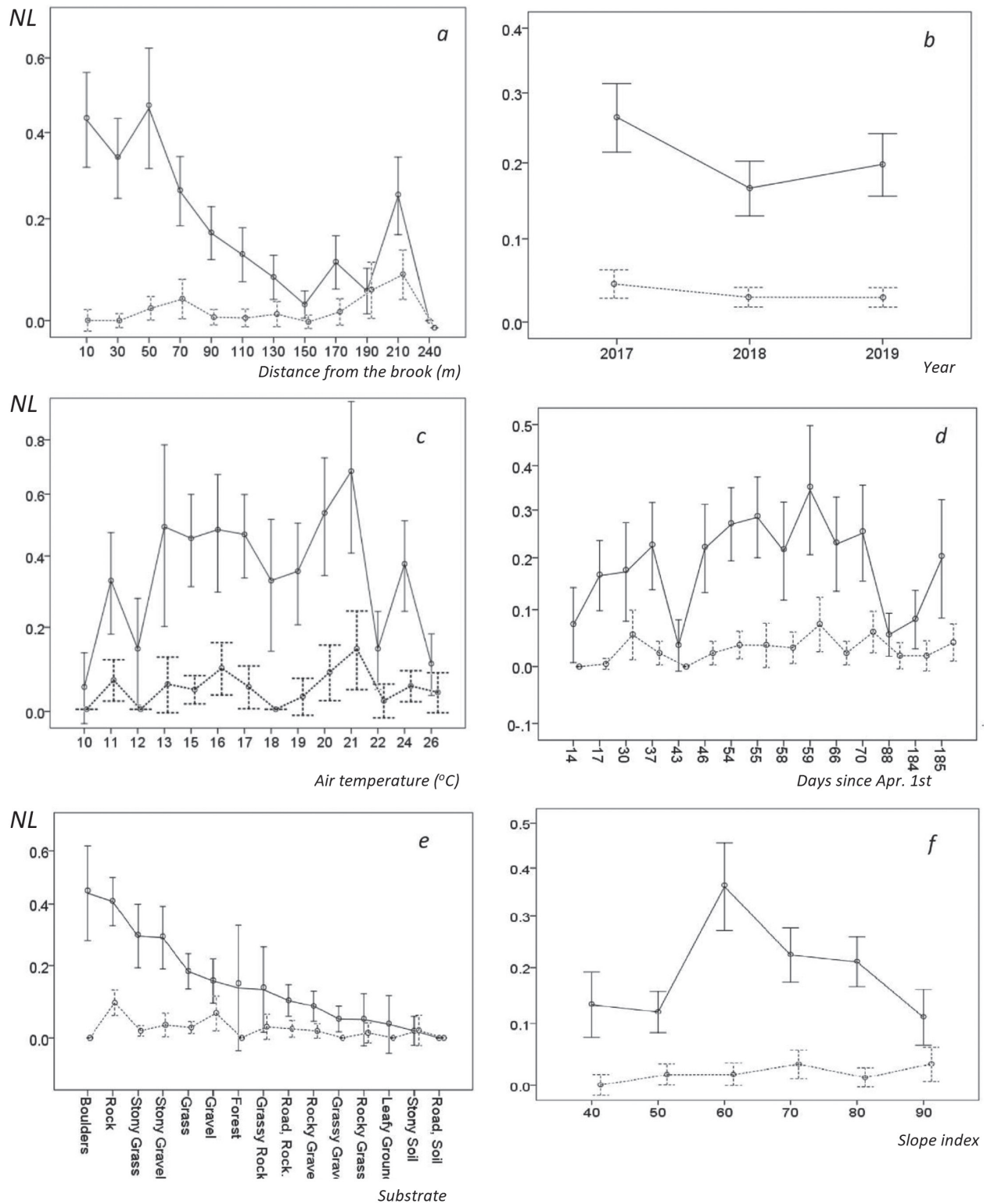


Figure 4. Dependence of the average and 95% confidence intervals for the number of recorded individuals (NL) of *D. dahli* (solid lines) and *D. portschinskii* (dashed lines) at a plot during a given session on: **a:** distance from the brook (m), **b:** year, **c:** air temperature (°C), **d:** days passed after 1st April, **e:** substrate type and **f:** average slope index on the 3 cm pixel scale (averaged for a plot).

autocorrelation explaining the occurrence dynamics (Table 1). This species avoided forest and preferred rock as the surface; the occurrence decreased with surface humidity and increased with the slope steepness. The occurrence

of *D. portschinskii* increased on the gravel and rock surface and with distance from the brook. None of the other predictors significantly affected the abundance or activity of this species (Table 1).

Table 1. The outcome of the GLMM analysis*.

Source of variance	Type	F (DD)	F (DP)	df1	df2	Sig (DD)	Sig (DP)
Corrected model		22.34	2.18	20	1.17	<0.001	0.002
Distance from the brook	Fixed in space	9.75	8.79	1	1.17	0.002	0.003
Surface type	Fixed in space	3.66	1.59	11	1.17	<0.001	0.097
Convergence Index	Fixed in space	0.87	0.21	1	1.17	0.352	0.645
Average Slope	Fixed in space	6.03	0.39	1	1.17	0.014	0.535
Year	Fixed in time	30.90	0.67	2	1.17	<0.001	0.513
Date	Fixed in time	28.99	1.32	1	1.17	<0.001	0.250
Air Temperature	Fixed in time	10.17	0.03	1	1.17	<0.001	0.864
Surface Humidity	Varying	16.00	1.50	1	1.17	<0.001	0.221
Surface Temperature	Varying	2.89	0.01	1	1.17	0.089	0.960
Coefficients (for significant predictors only)							
		b (DD)	b (DP)			Sig(DD)	Sig(DP)
Surface = forest		-2.764	N/A			0.004	N/A
Surface = grassy rock		-1.269	N/A			0.050	N/A
Surface = rock		0.765	0.070			0.012	0.016
Surface = Gravel		N/A	0.126			N/A	0.002
Distance from the brook		-0.006	0.001			0.002	0.003
Date (days from 1 April)		-0.013	N/A			<0.001	N/A
Surface Humidity		-0.025	N/A			<0.001	N/A
Air Temperature		-0.089	N/A			0.001	N/A
Average Slope		0.022	N/A			0.014	N/A

*DD – *Darevskia dahli*, DP – *D. portschinskii*; AIC – Akaike Information Criterion; F – f criterion; df – degrees of freedom; Sig – significance (P); b – regression coefficient.

Classification tree

R² value estimated from the node means of the training and test CHAID trees for *D. dahli* was 0.859 (17 nodes), and for *D. portschinskii* 0.575 (11 nodes).

The classification tree showed 3–4 variables that significantly influenced NL of *D. dahli* (Suppl. material 2: Fig. S1a). The primary variable separating the observations into five categories was the distance from the brook. Given the same distance from the brook, the NL (*D. dahli*) increased on the plots where rocky surface dominated, and the average slope was higher (≥ 65). The occurrence of *D. portschinskii* was not among significant factors, inferred by the analysis, which influences the occurrence of *D. dahli*.

The distance from the brook was also a primary variable affecting NL for *D. portschinskii*. Simultaneously, given the same distance from the brook, NL (*D. portschinskii*) positively correlated with NL (*D. dahli*), suggesting that similarity of the requirements to the environmental conditions, except distance from the permanent water source, outweigh potential negative interactions between the two species.

Niche overlap indices

The asymmetric index of niche overlap calculated for the utilization of space and time was: for dp/dd 0.543, and for

dd/dp 0.053. If we ignore time and only count the overlap in space use, the respective coefficients would be 2.69 (dp/dd) and 0.10 (dd/dp). Symmetric Pianka's niche overlap index reached 0.224 for individual observations and 0.487 for individual plots irrespective of the observation time.

Discussion

Competition is a significant force, able to lead to one of the competitor's extinction if the differences between the utilized resources are insufficient (Gauze 1934; MacArthur 1970; Pianka 1976; Şahin et al. 2020), or to niche shift and character displacement, if the adaptive strategy is mutual avoidance of the competing species (Grant 1972; Pianka 1976). The distance between the resource utilization curve peaks should exceed the average standard deviation of the curves to prevent competitive exclusion if resources are limited (MacArthur and Levins 1967; Begon et al. 1996). There is also a simpler definition: competition between the individuals of different species should not exceed competition among the conspecifics (Angilletta et al. 2013). Are the observed ecological differences between coexisting *D. portschinskii* and its daughter parthenogen, *D. dahli*, driven by inter-specific competition? Our results reject this hypothesis.

Remote sensing analysis (Tarkhnishvili et al. 2010; Petrosyan et al. 2020) suggests that summer temperature and rainfall determine different altitudinal ranges for *D. dahli* and *D. portschinskii*. However, at a fine-scale, these species did not react differentially to temperature and humidity of the surface, probably because of the high variability of these factors. Our analysis suggests that the most critical environmental predictors influencing the fine-scale spatial distribution of adult lizards include the distance from the permanent water source, steepness of the slope, and presence of the rocky surface. *Darevskia dahli* relies more on the physical closeness to the water source than *D. portschinskii* (also reported in Bakradze 1977). The former species is also more selective to the slope steepness, air temperature, surface humidity, and surface type. The presence of a permanent water source determines the availability of water for lizards, similar to the rainfall level on the macrogeographic scale (Tarkhnishvili et al. 2010; Petrosyan et al. 2020), whereas measured surface humidity quickly changes in time and does not determine the suitability of the location, albeit affecting the activity of the individuals (GLMM results).

Why do environmental conditions, including surface type and characteristics, proximity of water source, and air temperature, affect the occurrence of *D. dahli* more markedly than that of *D. portschinskii*? Tsellarius (Tsellarius and Tsellarius 2003; Tsellarius et al. 2016) and Galoyan (2011) showed that in bisexual *Darevskia*, territorial behavior is an important factor influencing spatial distribution. Adults of *D. braueri* from the northwestern Caucasus, both males, and females, defend the individual

territory from the conspecific outsiders (Galoyan 2013). In contrast, the parthenogens do not show territorial behavior, and their spatial distribution depends solely on environmental conditions (Trofimov 1981; Galoyan 2013). We did not conduct behavioral observations on *D. portschinskii* and cannot conclude whether territorial behavior is typical for females of this species. However, there are no significant differences in spatial distribution between the males and females of *D. portschinskii*. These facts explain the less aggregated distribution of active *D. portschinskii*, related to *D. dahli*, in space and time: the latter is concentrated in space with optimal conditions, whereas the bisexual species utilizes space and time more uniformly. The variance of the distribution of *D. portschinskii* along the environmental gradients was higher than that of *D. dahli*. The territoriality of *D. portschinskii* forces the individuals to expand on plots with suboptimal conditions. Hence territoriality, together with the inherited ecological differences, explains the observed differences between *D. portschinskii* and its daughter parthenogen.

Territoriality has another interesting consequence: the territorial species, which is more commonly found in suboptimal habitats and time, may better adapt to changing conditions, if previously optimal parts of the habitat will be lost. Hence, the advantage of sexual reproduction, along with the well-known factor of genetic diversity (White 1970; Case and Bender 1981; Kondrashov 1982; Maynard Smith 1998; Bell 2008), also favors diversity of ecological conditions in a habitat, which is substantially lower in the parthenogenetic form. Case (1990) suggests that parthenogens are weaker competitors than their sexually reproducing relatives; an additional reason for this can be the absence of territoriality and high concentration in optimal areas. This seemingly contradicts an earlier finding suggesting that the negative effect of *D. dahli* on *D. portschinskii* is stronger than vice versa (Tarkhnishvili et al. 2010); however, more effective use of suboptimal habitats may have a long-term positive effect challenging to record in a short-term perspective, similar to the advantage of sexual breeders associated with the ability of recombination.

In spite of these differences, the overlap of spatio-temporal niches between *D. dahli* and *D. portschinskii* is substantial. This may potentially cause interspecific competition (MacArthur and Levins 1967). If competition exists, it can be strongly asymmetric; because of the exceeding abundance of *D. dahli*, the latter species can have a more substantial impact on *D. portschinskii* than vice versa. The niche overlap by itself cannot either support or reject the presence of competition (Holt 1987). However, one can infer active mutual avoidance of potential competitors by applying residual analysis (Tarkhnishvili et al. 2010) or by analyzing the decision-making tree (this study). This analysis helped us to exclude mutual avoidance of the two studied species. Within the studied area, preferred locations for the lizards partly overlapped. On the plots close to the brook, the density of *D. dahli* was very high, and *D. portschinskii* were almost absent. In plots with optimal terrain and cover conditions, the density of both species increased even away from the brook. Within an equally

suitable area, *D. portschinskii* tended to occur simultaneously and in the same plots where *D. dahli* also presents. Hence, given the same parts of the studied outcrop, microhabitat similarities of the two species are more important than the potential competition, and therefore, ecological differences hardly can be the result of character displacement. Why do the differences occur?

These differences, which are related to the temperature and humidity optima, reported here and in earlier observations and modeling results (Darevsky 1967; Bakradze 1977, Tarkhnishvili et al. 2010; Petrosyan et al. 2020) are instead related to the different ranges of *D. portschinskii* and the matrilineal ancestor of *D. dahli*, *Darevksia mixta*. The range of *D. mixta* is limited to mesophytic forests and uplands of central and western Georgia (Darevsky 1967; Tarkhnishvili 2012; Gabelaia et al. 2015). This species is adapted to more humid habitats than *D. portschinskii* (Petrosyan et al. 2020). *Darevksia dahli* has phenotypic features intermediate between *D. portschinskii* and *D. mixta*, being more dependent on the water source than the former one and more dependent on the rocky surface than the latter.

Although the species do not avoid each other on the microhabitat scale, on the macrogeographic scale the mutual negative impacts are obvious (Tarkhnishvili et al. 2010). In certain locations with an equally suitable climate, the abundance of each species negatively correlates with the abundance of the other. The reason for this is not clear. It may be competition for food: rock lizards eat arthropods of appropriate size (Darevsky 1967; Tselarius and Tselarius 2001; Galoyan et al. 2019). Like other ectothermic organisms feeding on small invertebrates (e.g., Kuzmin and Tarkhnishvili 1992), they hardly can seriously affect the abundance of insects; rather, a high concentration of animals may decrease the hunting success. Competition for space is probably a more inclusive explanation of the negative interactions: it influences the accessibility on food and shelters. An additional (and perhaps even more important) reason for the negative impact of *D. dahli* on *D. portschinskii* includes mating attempts of the males of the former species with the parthenogen. Commonly, reinforcement favors pre-zygotic isolation and, hence, decrease risk of unsuccessful mating with non-conspecific individuals (Dobzhansky 1937; Endler 1986). However, parthenogenetic *Darevksia* commonly mate with their patrilineal ancestors (Bakradze 1977; Trofimov 1981; our observations). Sterile triploid hybrids are relatively common, and sometimes even have developed gonads (Danielyan et al. 2008). In spite of the latter observation, no ongoing gene flow was ever shown (Darevsky and Kulikova 1962; Spangenberg et al. 2017; Freitas et al. 2019), and even if it could happen, this would be an extremely rare event (Tarkhnishvili et al. 2017, 2020). Many adult *D. dahli* have tracks of mating bites. We could hypothesize that common mating of *D. portschinskii* with parthenogenetic females may decrease their reproductive success.

This study shows that negative interspecific interactions do not necessarily trigger niche shift and character displacement and that the simultaneous presence of negative interactions and ecological differences between closely

related species cannot be taken as an argument for character displacement. The studied species do not actively avoid the competitor, which suggests that their long-lasting coexistence is a result of metapopulation dynamics and sources-sinks type of spatial structure (Pulliam 1988), where the rates of re-population from neighboring source locations compensate the negative trends in population dynamics. The populations of *D. dahli* and *D. portschinskii* have coexisted in the studied location for at least 53 years (Darevsky 1967), and during all this time, the abundance of *D. dahli* in the studied habitat exceeded several times the abundance of *D. portschinskii* (Trofimov 1981; Tarkhnishvili et al. 2010; this paper). In conclusion, we suggest that a complex spatial structure not only helps species to survive a competitive pressure but also impedes character displacement and reinforcement in multispecies assemblages.

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Supplementary material 1

Table S1

Authors: Natia Barateli, David Tarkhnishvili, Giorgi Iankoshvili, Giorgi Iankoshvili, Nikoloz Dvali, Zurab Janiashvili

Data type: Microsoft Excel table, full data of the records and physical conditions

Explanation note: Full record for the three years of the observation. Pl – the number of a plot (see Fig. 2a). dd – the number of observed active of *D. dahli*, dp – the number of observed *D. portschinskii*, Hum – surface humidity index, Temp – surface temperature, Air.Temp – air temperature, Con-stdev – convergence index, Slope_max – average slope, Time of Day: M – early afternoon, N – before sunset, Surface – surface type as shown in Fig. 2c, N – northing, E – easting of the center of a plot.

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Supplementary material 2

Figure S1

Authors: Natia Barateli, David Tarkhnishvili, Giorgi Iankoshvili, Giorgi Iankoshvili, Nikoloz Dvali, Zurab Janiashvili

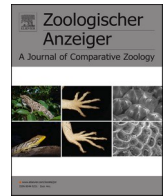
Data type: PNG figure, classification tree describing habitat preferences of the studied species

Explanation note: Classification tree (=decision tree), describing relation of occurrence of *D. dahli* (dd) and *D. portschinskii* (dp) to the most important environmental variables. Method – CHAID; Maximum depth – 2 steps; minimum size of parental node – 100; minimum size of child node – 50; Validation: 75% training observations, 25% test observations. The total number of observation – 2917. Test tree is shown. Variables/ predictors: dd – number of observed *D. dahli*; dp- number of observed *D. portschinskii*; Dis. From River in Meters – the distance of a plot from the brook; slmax – average slope at a plot; cst – convergence index; Surface – surface type (see Fig. 2c).

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Chapter 2. Reproductive effort of unisexual and bisexual rock lizards (genus *Darevskia*)



Reproductive effort of unisexual and bisexual rock lizards (genus *Darevskia*)

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ABSTRACT

We compared the life history components of small and large-bodied unisexual (*Darevskia dahli* and *Darevskia armeniaca*) and bisexual (*Darevskia portschinskii*, *Darevskia valentini*, and *Darevskia obscura*) Caucasian rock lizard species. Research objectives were to determine whether reproductive mode (bisexual or parthenogenetic), female body size, altitude and landscape of the habitat, or interaction of these factors determine the relative investment of energy into a single reproduction, number, and relative size of eggs.

The research revealed the differences between two parthenogenetic species and their paternal bisexual lizards. However, the pattern did not coincide for the two studied lineages i.e., parthenogenetic *D. armeniaca* invests relatively more resources into reproduction than its paternal *D. valentini*. Simultaneously, reproductive effort of parthenogenetic *D. dahli* did not differ from its paternal *D. portschinskii*. Instead, *D. dahli* tended to produce more but lighter eggs than *D. portschinskii*. In both cases, the parthenogenetic form tended to follow a *r*-reproductive strategy, typical for lizards occupying less stable environments than their parental species. However, other interspecific differences such as body size or evolutionary distance shade these differences, and determine the pattern of divergence between a parthenogen and its bisexual relative. In particular, smaller body size constrains increased the reproductive effort, and parthenogenetic species shifted to a balance between egg size and number rather than between resources invested into a single reproduction event and the probability to survive until the next season.

1. Introduction

Organisms with multiple reproductive cycles distribute their energy resources throughout their lifetime, considering growth, survivorship, and reproductive success (Stearns 1977; 1992; Congdon, 1989; Macip-Ríos et al., 2017). According to the life-history theory, explaining how natural selection leads organisms to optimize their survival rates and reproduction (Stearns, 1992; Roff, 1992; Stearns et al., 2000; Fabian and Flatt, 2012), these variables are key factors for increasing the fitness of an animal (Babich Morrow et al., 2021). Because the resources are limited, organisms cannot simultaneously increase individual survival rates and reproductive investment, and the observed balance results from the tradeoffs between those (Babich Morrow et al., 2021). Charnov and his co-authors (Charnov and Downhower 1995; Charnov and Berigan 1991; Charnov 1993; 2002; Babich Morrow et al., 2021) developed three-dimensional metrics to quantify fundamental life-history tradeoffs: lifetime reproductive effort (LRE), relative reproductive life-span (RRL), and relative offspring size (ROS). LRE measures the

proportion of adult mass that a female will allocate to offspring over her lifespan (Charnov, 2002; Babich Morrow et al., 2021); RRL quantifies time to maturity relative to the total amount of time available for reproduction (Charnov, 1993; Charnov, 2002; Babich Morrow et al., 2021); and ROS is the ratio of offspring size to adult size (Millar 1977; Babich Morrow et al., 2021). The other tradeoff in Life-history, one between the number and size of offspring, confirmed to vary at both interspecific and intraspecific levels, coinciding with *r*- and *K*- evolutionary strategies (Stearns, 1989; Tarkhnishvili, 1993; Roff, 2002; Brown and Shine, 2009).

In oviparous vertebrates with multiple reproductive cycles and without expressed parental care, such as most lizards, the tradeoff between life history parameters can be presented in a simple diagram (Fig. 1). An increase of energy share, invested into a single reproduction, increases the potential number of viable offspring during one season but decreases the probability of a female surviving until the next season (Qualls and Shine, 1998). Given the same female body mass and reproductive effort, an increase in the number of eggs is associated with

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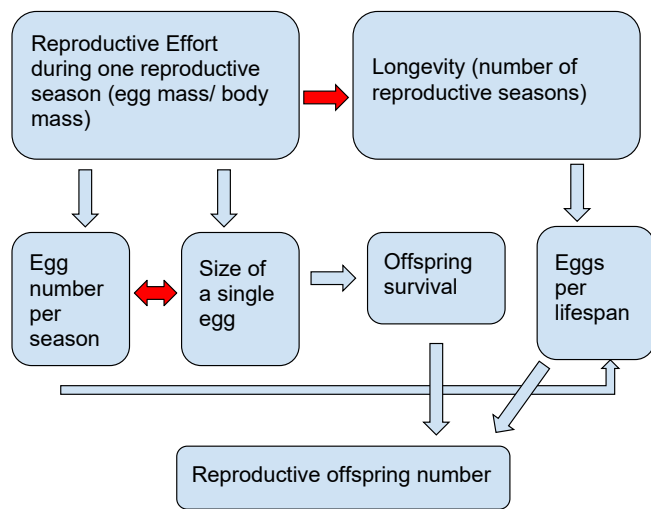


Fig. 1. Interdependence among the components of life history. Reproductive effort increases the number and/or survival of offspring per season but decreases the potential number of reproductive seasons. Increase of an egg size positively affects offspring survival, but negatively - the number of eggs per reproductive season. Egg number and offspring survival both positively affect fitness. Red arrows show negative effects. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

a decrease in egg size. Egg size potentially increases the probability of a single egg to survive until hatching and that of a juvenile to survive in the first months of its life (Sinervo, 1990; Levitan, 2006).

Lizards are classical objects for studying tradeoffs between the components of reproductive strategies. Their reproductive characteristics vary both between conspecific populations and between closely related species. For example, Boretto et al. (2018) described variation of reproductive effort of iguanids from the genus *Phymaturus* depending on the altitude. Inter-population variations in egg size, clutch size, and reproduction frequency is shown for another iguanid, *Sceloporus undulatus* (Tinkle and Ballinger, 1972). In ornate tree lizards (*Urosaurus ornatus*), clutch size depends on the habitats (trees vs rocks) they occupy (Haanel, 2011). In general, reproductive period, clutch size, and egg size of lizards strongly depend on the environmental variables (Shine and Elphick 2001; Brandt and Navas, 2011; Menezes and Rocha, 2014), including temperature (Cruz, 1996; Ramírez-Bautista et al., 2000; Rezende-Pinto et al., 2009; Menezes and Rocha, 2014), rainfall (Rodríguez-Ramírez and Lewis 1991; Ramírez-Bautista et al., 2000; Martelotte et al., 2010; Menezes and Rocha, 2014), habitat type (Haanel, 2011) and food availability (Fitch, 1982; Haanel, 2011). In addition, reproductive variables in lizard populations depend on the female body size (Vrcibradic and Rocha 2011; Winck and Rocha 2012; Menezes and Rocha, 2014). Females with larger bodies may produce larger and heavier eggs (Brandt and Navas, 2011; Menezes and Rocha, 2014), although the effect of body size depends on a specific taxonomic group (Stewart, 1979; Sinervo, 1990; Schwarzkopf, 1992; Doughty 1997). Ljungström et al. (2016) found that the additive genetic component determining the variation of egg size and number within species is minute; hence, the tradeoff between reproductive variables may change during a lifespan of a single animal.

Last but not least, some groups of lizards have both sexual breeding and parthenogenetic taxa, which may also affect the reproductive strategy. Schall (1978) presented the data on five species sympatric in southwest Texas, two of which are parthenogenetically reproducing. The bisexual and unisexual species did not differ in clutch size, egg size, or clutch/body weight ratio, and some interspecific differences in reproductive characteristics were attributable to different body sizes. On the other hand, Congdon et al. (1978), based on the study of two bisexual and two parthenogenetic *Aspidoscelis* from Arizona, concluded

that the unisexual species have larger clutches, increase clutch size faster with body size, have lighter eggs, and higher clutch/body calorie ratios than the closely related bisexual species - irrespective of body size of a species.

In this paper, we compared the components of life history in the two parthenogenetic and three bisexual species of Caucasian rock lizards (*Darevskia* Arribas, 1999) from three locations in Georgia. The parthenogenetic forms ("species") in these locations coexist either with their paternal sexual breeders, or with a closely related bisexual species, but not ancestral to them. The parthenogens and their ancestors differ in body size and preferred altitudes. Our purpose was to explore if and how the mode of reproduction (sexual vs parthenogenetic) affect reproductive characteristics of a female (including reproductive effort, egg size, egg number, or the trade-off among these parameters), and whether this effect depends on the other variables, such as body size of a female, altitude of a habitat, or phylogeny.

2. Materials and methods

2.1. Research organisms

Genus *Darevskia* is a speciose group of small-bodied lizards. Their average lifespan is five to six years (Arakelyan & Danielyan, 2000; Galoyan et al., 2019) and they usually reproduce during several seasons. *Darevskia* differ from each other in the reproductive mode (bisexual vs unisexual), body size (SVL varying between 58 cm) (Darevsky et al., 1985; Galoyan et al., 2019) and preferred altitudinal range (Darevsky et al., 1985; Tarkhnishvili, 2012). Our study organisms were two hybrid parthenogenetic species of the genus from Georgia, *Darevskia dahli* (Darevsky, 1957) and *Darevskia armeniaca* (Méhely, 1909) and their paternal species, *Darevskia portschinskii* (Kessler, 1878) and *Darevskia valentini* (Boettger, 1892), respectively, which commonly share habitats with the daughter parthenogens (Murphy et al., 2000; Tarkhnishvili et al., 2020). Fifth species we studied was *Darevskia obscura* (Lantz and Cyrén, 1936), phylogenetically equidistant from *D. portschinskii* and *D. valentini*.

The first pair of the parthenogen and its ancestor, *D. dahli* and *D. portschinskii*, coexist in the mountain forest belt of central Georgia, 800–1200 m a.s.l (Tarkhnishvili et al., 2010). Adult body length of both species varies between 5.4 and 5.8 cm. The second pair of species, *D. armeniaca* and *D. valentini*, coexist at the elevations 1900–3100 m (Arakelyan et al., 2011; Galoyan et al., 2019). Body of these species is larger than the species of the previous pair, with body length varying between 6.1 and 6.4 cm. There are some locations where *D. armeniaca* coexist with a non-ancestral, *D. obscura*, sympatric with *D. armeniaca* within the elevation range 1500–1800 m a.s.l (Darevsky et al., 1985; Tarkhnishvili, 2012).

2.2. Sampling

In May–June, 2019–2021–2022, females of five species of rock lizards were collected from three locations in Georgia, total sample size - 87 (13–26 individuals per species) (Table 1)). Sample sizes for individual species were modest, however, we considered that catching more lizards could potentially affect the respective populations dynamics if egg incubation was unsuccessful, and further increasing sample size could cover several more years of work. The females in which mature follicles were palpated (all bisexual individuals had mating marks) were placed into plastic containers with moss and soil, daily fed with crickets, and released after the eggs were deposited. Eggs were further kept on slightly humid soil under room temperature (ca. 25–30 °C) until juveniles were hatched (Fig. 2). Every female was measured with calipers and was weighed after capturing and after egg deposition on electronic scales with precision 0.1 g. Each egg was weighted with the same scales not later than in 7–8 h after the deposition, and egg maximum diameter was measured with calipers to precision 0.1 mm. During measuring eggs

Table 1Sample sizes (gravid female lizards) and key characteristics of the sampling location of six species of *Darevskia*.

Species	Location	Easting	Northing	Alt. a.s.l.	Sample size	Rep. mode
<i>D. dahl</i>	Kojori	44.68°	41.64°	1120	17	unisexual
<i>D. portschinskii</i>	Kojori	44.68°	41.64°	1120	16	bisexual
<i>D. armeniaca</i>	Sagamo Lake	43.73°	41.29°	2015	11	unisexual
<i>D. armeniaca</i>	Riv.Kirkhbulakhi	43.73°	41.29°	1715	15	unisexual
<i>D. valentini</i>	Sagamo Lake	43.73°	41.29°	2015	15	bisexual
<i>D. obscura</i>	Riv.Kirkhbulakhi	43.73°	41.29°	1715	13	bisexual

**Fig. 2.** Stages of egg deposition and hatching of individuals of *D. armeniaca* individual at laboratory.

artificial damage was maximally avoided. The weighting procedure was repeated after hatching of the juveniles. Females and juveniles were released after several weeks.

2.3. Statistical analysis

From the original measurements, we calculated several derivative variables, hence increasing the number of the reproductive characteristics of each individual. The characteristics of each individual and egg is given in [supplementary Table A.1](#). The reproductive variables used in the further analysis were: (1) female body mass before (FMB) and (2) after (FMA) egg deposition; (3) female body length from the tip of the snout to the hind angle of hip and body junction (FL); (4) Egg number (EN); (5) Average egg mass in a clutch (AEM); (6) clutch mass (total mass of the eggs; CLM); (7) reproductive effort (RE) calculated as the proportion of clutch mass to female body mass before the egg deposition; (8) Relative egg mass (REM) - proportion of average egg mass to FMB; (9) Egg shape (ESH) - maximum diameter of egg divided on the egg mass.

We assume that the altitude of the location, mode of reproduction (sexual vs parthenogenetic), FMB, FMA, and FL are predictors, whereas all other reproductive variables are dependent on these predictors and on each other.

We tested the significance of the differences between the studied populations, correlation/association between the variables using a combination of simple statistical methods: (1) we used one-way ANOVA to infer significant differences between the populations in the studied reproductive variables. We used post-hoc analysis using Bonferroni method to identify those populations which show significant differences in average values. (2) General Linear Model (GLM) was applied to infer the influence of the predictors on each dependent reproductive variable separately, across the species and populations. (3) we calculated Pearson correlation matrix in order to find the dependencies between the reproductive variables separately for each studied species. All calculations were done in IBM SPSS software (2021).

3. Results

3.1. Interspecific comparisons

Most variables related to reproduction showed significant differences among the study species, with the exception of absolute and relative egg mass, which showed near-significant differences (one-way ANOVA; [Table 2](#)). As expected, *D. armeniaca* and *D. valentini* (both species living at higher altitudes) had longer and heavier bodies, heavier clutches, and more eggs in clutch than *D. portschinskii* and *D. dahl* living at lower altitudes; the differences being mostly significant (see [Table 2](#)). Female *D. obscura* were larger, had more eggs and heavier clutches than *D. portschinskii* and *D. dahl*, hence the differences were species-specific and were not related solely to altitude. Simultaneously, *D. obscura* and *D. armeniaca* (but not *D. valentini*) had significantly heavier eggs than the

Table 2Interspecific differences of reproductive biology traits among six lizard species (genus *Darevskia*) using a One-way ANOVA.

Variable	df within/ between groups	F	P	Post-Hoc (Bonferroni), P < 0.05
FL	5/81	14.044	<0.001	a1>d; a2>d; o>d; v>d; a1>p; a2>p; o>p; v>p
FMB	5/81	22.687	<0.001	a1>d; a2>d; o>d; v>d; a1>p; a2>p; o>p; v>p
FMA	5/81	16.967	<0.001	a1>d; a2>d; o>d; v>d; a1>p; a2>p; o>p; v>p
EN	5/81	13.510	<0.001	a1>d; a2>d; v>d; v>p; o>p
AEM	5/81	5.626	<0.001	a2>d; a2>p; o>d; o>p
AEL	5/81	1.887	0.106	–
CLM	5/81	20.014	<0.001	a1>d; a2>d; o>d; v>d; a1>p; a2>p; o>p; v>p
RE	5/81	5.777	<0.001	a2>d; o>d; a1>p; a2>p; o>p
REM	5/81	4.583	0.001	v>a2; v>p
ESH	5/81	7.096	<0.001	a1>p; a2>p; o>p; o>d

FL - body length of a female, FMB and FMA - female weight before and after the egg deposition, RE - reproductive effort, REM - relative egg mass (proportion of average egg mass to FMB). a1 – *Darevskia armeniaca* (Riv. Kirkhbulakhi), a2 – *D. armeniaca* (Sagamo Lake), d – *D. dahl*, o – *D. obscura*, p – *D. portschinskii*, v – *D. valentini*.

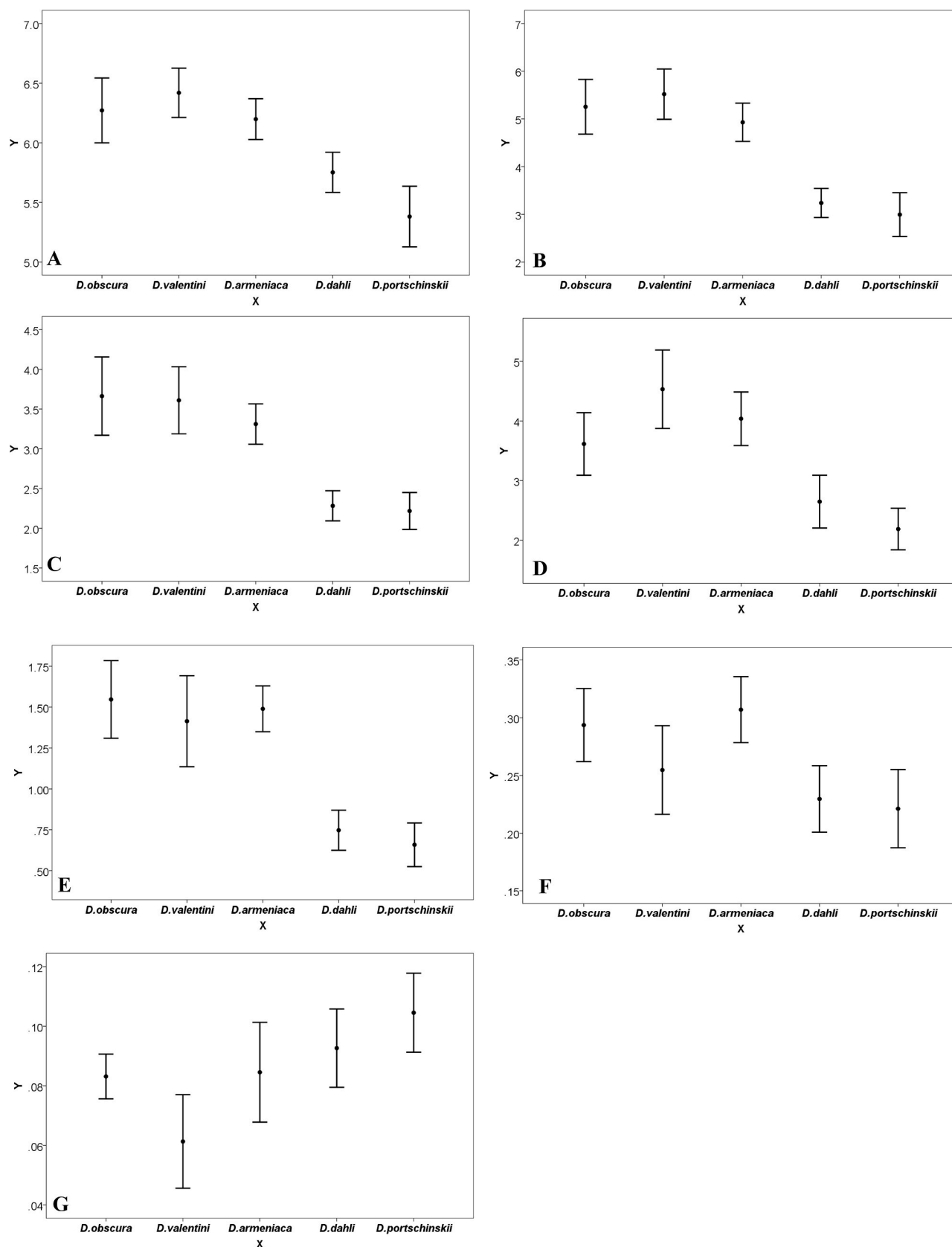


Fig. 3. A–G. Gravid female individuals of five species on X - axis with body lengths (A), body weight before (FMB) (B), body weight after (FMA) (C), number of eggs (EN) (D), clutch weight (CLM) between small and large – bodies species (E), Reproductive effort (RE) (F), Relative egg weight (REM) (G) on Y - axis with 95% confidence Interval.

Table 3

Impact of reproductive mode, altitude, and female body mass on reproductive biology traits. A general Linear Model was applied using reproductive mode and altitude as fixed factors, and female mass as covariate. P values are shown; P values ≤ 0.05 highlighted in bold.

	CLM	RE	AEM	EN	REM	ESH
FMB	0.338	0.040	0.004	<0.001	<0.001	0.149
FMA	0.065	0.185	0.015	0.343	0.010	0.212
FL	0.192	0.117	0.051	0.633	0.107	0.226
Altitude	0.008	0.002	0.009	0.458	0.039	0.004
rep.mode	0.163	0.115	0.927	0.089	0.883	0.412
Alt_repr. mode	0.126	0.095	0.003	0.090	0.010	0.045

FMB and FMA - female weight before and after the egg deposition, FL - body length of a female, rep.mode-reproductive mode, Alt_repr.mode - Altitude with reproductive mode, CLM - total mass of the eggs, RE - reproductive effort, AEM - average egg mass, EN - egg number, REM - relative egg mass, ESH - egg shape.

other three species (Fig. 3A–D). Reproductive effort of these species was also higher than in *D. portschinskii* and *D. dahli*; *D. valentini* was intermediate in this respect (Fig. 3E). Conversely, *D. portschinskii* and *D. dahli* had a higher relative egg mass than the other species, although significantly exceeded only *D. valentini*'s relative egg mass (Fig. 3F). Finally, these two species had more elongated eggs than *D. armeniaca* and *D. obscura*, whose eggs were more round in shape (Fig. 3G).

3.2. Interspecific life history trade offs

Female body mass across species significantly affected the number of deposited eggs and relative egg mass, but not reproductive effort or absolute egg mass (see Table 3). Larger-bodied species deposited more eggs but each egg was lighter in proportion to female body mass. Clutch mass, average and relative egg mass, and reproductive effort were higher in species living at higher altitudes, although the altitude hardly affected these variables directly: simply, the larger lizard species lived at higher altitudes. Reproductive mode by itself influenced egg mass (*D. armeniaca* had heavier eggs than *D. valentini*), and in interaction with the altitude of the location, it had a significant effect on reproductive effort and both average and relative egg mass. Hence, while reproductive mode had an effect on some reproductive variables, the direction of this effect was not consistent and depended on other characteristics such as altitude or the specific evolutionary lineage of the lizard.

3.3. Intraspecific life history trade offs

Within *D. armeniaca*, *D. dahli*, and *D. valentini*, increased female body mass positively correlated with clutch size and egg number (although in *D. portschinskii* and *D. obscura*, the correlation was not significant), and negatively with relative egg mass respectively (see Table 4). Average egg mass was positively related to female body mass in *D. obscura* and *D. portschinskii*, but negatively in *D. armeniaca* (the correlation was not significant in *D. dahli* and *D. valentini*). Average egg mass also negatively correlated with egg number in *D. armeniaca*; but the correlation was not significant in the other lizard species investigated (see Table 4). In *D. armeniaca* and *D. valentini* (but not in three other lizard species) the absolute and relative egg mass increased with reproductive effort. In short, there were obvious patterns of interspecific differences that could not be generalized when comparing species with having variable modes of reproduction i.e., *D. valentini* and especially *D. armeniaca* invested more resources into egg number than in egg size during growth, whereas female *D. portschinskii*, and *D. obscura* invested more into egg size. In *D. armeniaca* this trend was especially strong as heavier females produced more but even lighter eggs. The reproductive effort was not directly associated with female body size, although female *D. armeniaca* and *D. valentini* invested relatively more resources into a single reproduction event by producing heavier eggs, whereas *D. portschinskii*, *D. dahli*, and *D. obscura* produced more eggs.

4. Discussion

Reproductive characteristics of five lizard species, described in this paper, slightly differ from that described in [Darevsky \(1967\)](#) and [Galoyan et al. \(2019\)](#). The number of eggs per clutch and egg diameter was less than reported by these authors (Table 5). These differences may be associated with the different way of sampling: we measured the eggs deposited a few hours before, whereas [Darevsky \(1967\)](#) measured oocytes from ovaria or eggs found in nature. [Darevsky \(1967\)](#) suggested that Caucasian rock lizards, with few exceptions of particularly big females, deposit only one clutch per season, hence the differences are related to the population specifics rather than to the presence of additional clutches.

There are significant differences in the reproductive parameters of the studied species. We cannot attribute these differences solely to the mode of reproduction, body size, or any other predictor. Rather, there is a complex interaction between the variables associated with a particular species that determine reproductive effort, relative size and number of eggs. It is also important to consider how the need to house females in captivity could have affected some of our variables. In particular, FMA (which could be considered a more independent measure of female body size than FMB) was highly variable and dependent on the condition of the females after egg deposition. Some females were kept longer than the others before the egg deposition, and differences in their feeding rate in captivity may have influenced FMA. Ideally, we would be able to measure female body mass before they were gravid, but because we were not able to obtain that information, we emphasized FMB in our analyses. However, the correlations between our variables and FMA (Table 4) are in the same direction as correlations with FMB.

Our findings can be summarized as the following: (1) Species with larger females produce heavier clutches, deposit more eggs, and invest relatively more resources into a single reproduction than small-bodied species. Besides, the eggs of small-bodied species are elongated, and the eggs of bigger ones are more round; (2) *D. armeniaca* invests larger portion of its body mass in a single reproduction than its paternal *D. valentini*, it deposits heavier eggs relative to its body size, although clutch size or egg size and number don't differ in two species; (3) larger *D. dahli* deposit more eggs than smaller ones, although not heavier eggs. In contrast, larger individuals of its paternal *D. portschinskii* from the same location deposit heavier eggs than the smaller individuals; (4) *D. obscura* invests more resources in a single reproduction than two other sexual breeders, and it deposits heavier eggs.

Our results suggest complex, species-specific relationships between reproductive variables. On the first glance, it is difficult to recognize a clear pattern in this bunch of correlations. However, some of those are easily explained. The concept of *r/K* reproductive strategy, introduced by [MacArthur and Wilson \(1967\)](#) attempts to explain the differences between species dependent on less vs more stable environments. In an unstable environment, a species allocates more resources into the offspring number and hence rapidly reproduces in short periods when the environmental conditions are good (*r* - strategy), whereas in stable environmental conditions allocating more resources in each individual offspring is more favorable ([Pianka, 1970](#)). More precisely, optimization of reproductive strategy considers trade-offs between: total parental investment/reproductive effort (RE), offspring number, and offspring quality ([Tuomi et al., 1983](#); [Morris, 1987](#); [Stearns, 1992](#)). In animals with no parental care, "offspring quality" should be tightly related to egg size: heavier eggs are less dependent on the environmental fluctuations. High RE may decrease the probability of a female to survive until the next reproductive season ([Vitt and Caldwell, 2014](#)), and it may also be associated with a less stable environment.

Parthenogenetic lizards reproduce more rapidly than the sexual breeders; they form larger populations and occupy a larger share of suitable habitats ([Tarkhnishvili et al., 2010](#); [Tarkhnishvili, 2012](#)). [Barateli et al. \(2021\)](#) showed that, for *D. dahli* and *D. portschinskii*, these differences are observed even on microhabitat scale. [Wright and Lowe](#)

Table 4

The correlations between all variables within species. Pearson correlation. Significance coefficient is shown by * (* = <0.05; ** < 0.01; *** < 0.001)

Species		FL	FMA	FMB	AEL	AEM	EN	CLM	ESH	RE	REM
<i>D.armeniaca</i>	FL	1	.764***	.754***	.015	-.125	.590**	.544**	.041	-.109	-.381
	FMA	.764***	1	.918***	.173	-.214	.666***	.545**	.177	-.272	-.520*
	FMB	.754***	.918***	1	.046	-.393*	.829***	.558**	.283	-.354	-.690**
	AEL	.015	.173	.046	1	.109	-.083	.019	.198	-.042	.081
	AEM	-.125	-.214	-.393*	.109	1	-.612**	.267	-.887***	.726***	.920***
	EN	.590**	.666***	.829***	-.083	-.612**	1	.558**	.433*	-.222	-.777***
	CLM	.544**	.545**	.558**	.019	.267	.558**	1	-.427*	.557**	-.005
	ESH	.041	.177	.283	.198	-.887***	.433*	-.427*	1	-.809***	-.790***
	RE	-.109	-.272	-.354	-.042	.726***	-.222	.557***	-.809***	1	.725***
	REM	-.381	-.520**	-.690***	.081	.920***	-.777***	-.005	-.790***	.725***	1
<i>D.dahli</i>	FL	1	.569*	.800***	-.202	-.099	.459	.435	-.012	.066	-.622**
	FMA	.569*	1	.810***	-.153	.235	.415	.576*	-.366	.185	-.437
	FMB	.800***	.810***	1	-.406	-.143	.646**	.584*	-.034	.111	-.790***
	AEL	-.202	-.153	-.406	1	.670**	-.436	-.025	-.219	.210	.610**
	AEM	-.099	.235	-.143	.670**	1	-.385	.250	-.855***	.342	.696**
	EN	.459	.415	.646**	-.436	-.385	1	.791***	.230	.604*	-.754**
	CLM	.435	.576*	.584*	-.025	.250	.791***	1	-.330	.859***	-.332
	ESH	-.012	-.366	-.034	-.219	-.855***	.230	-.330	1	-.321	-.542*
	RE	.066	.185	.111	.210	.342	.604*	.859***	-.321	1	.032
	REM	-.622**	-.0437	-.790***	.610**	.696**	-.754***	-.332	-.542*	.032	1
<i>D.obscura</i>	FL	1	.618*	.750**	-.153	.350	.666*	.769**	-.489	.426	-.589*
	FMA	.618*	1	.903***	-.021	.572*	.265	.493	-.302	-.106	-.631*
	FMB	.750**	.903***	1	-.039	.573*	.478	.713**	-.359	.080	-.757**
	AEL	-.153	-.021	-.039	1	.630*	-.371	.011	.629*	.011	.554*
	AEM	.350	.572*	.573*	.630*	1	-.072	.431	-.027	.042	.084
	EN	.666*	.265	.478	-.371	-.072	1	.864***	-.498	.802**	-.695**
	CLM	.769**	.493	.713**	.011	.431	.864***	1	-.420	.748**	-.580*
	ESH	-.489	-.302	-.359	.629*	-.027	-.498	-.420	1	-.281	.468
	RE	.426	-.106	.080	.011	.042	.802**	.748**	-.281	1	-.138
	REM	-.589*	-.631*	-.757**	.554*	.084	-.695**	-.580*	.468	-.138	1
<i>D.portschinskii</i>	FL	1	.436	.851***	.307	.485	.234	.560*	-.382	-.034	-.356
	FMA	.436	1	.549*	.416	.531*	.102	.522*	-.331	-.011	-.129
	FMB	.851***	.549*	1	.432	.607*	.165	.610*	-.459	-.077	-.363
	AEL	.307	.416	.432	1	.839***	-.296	.403	-.526*	.149	.513*
	AEM	.485	.531*	.607*	.839***	1	-.205	.637**	-.835***	.260	.482
	EN	.234	.102	.165	-.296	-.205	1	.608*	.244	.621*	-.491
	CLM	.560*	.522*	.610*	.403	.637**	.608*	1	-.538*	.710**	.014
	ESH	-.382	-.331	-.459	-.526*	-.835***	.244	-.538*	1	-.294	-.515*
	RE	-.034	-.011	-.077	.149	.260	.621*	.710**	-.294	1	.359
	REM	-.356	-.129	-.363	.513*	.482	-.491	.014	-.515*	.359	1
<i>D.valentini</i>	FL	1	.565*	.755**	.273	-.061	.543*	.335	.243	-.025	-.314
	FMA	.565*	1	.851***	.135	.106	.706**	.678**	-.015	.363	-.189
	FMB	.755***	.851***	1	.022	-.111	.854***	.554*	.168	.131	-.427
	AEL	.273	.135	.022	1	.343	-.263	-.037	.105	-.043	.338
	AEM	-.061	.106	-.111	.343	1	-.485	.506	-.830***	.704**	.936***
	EN	.543*	.706**	.854***	-.263	-.485	1	.434	.309	.035	-.742**
	CLM	.335	.678**	.554*	-.037	.506	.434	1	-.658**	.888***	.214
	ESH	.243	-.015	.168	.105	-.830***	.309	-.658**	1	-.895***	-.762**
	RE	-.025	.363	.131	-.043	.704**	.035	.888***	-.895***	1	.533*
	REM	-.314	-.189	-.427	.338	.936***	-.742**	.214	-.762**	.533*	1

* FL - body length of a female, FMB and FMA - female weight before and after the egg deposition, AEL - average egg length, CLM - total mass of the eggs, RE - reproductive effort, EN - egg number, AEM - average egg mass, REM - relative egg mass (proportion of average egg mass to FMB), ESH - egg shape

(1968) proposed “weed hypothesis”, explaining the distribution of parthenogenetic *Aspidoscelis* and their parental species. Unisexual *Aspidoscelis* are common in the disturbed, quickly changing habitats, and hence the parthenogens “weed” similar to plants which quickly populate the disturbed areas (Baker, 1974; Schall, 1978). In contrast, bisexual species prefer habitats with more stable environments. If we consider unisexual species as “weed” animals, we should expect that the environment they occupy is less stable than the environment of the bisexual species with similar ecology, and hence, they should possess *r* reproductive strategy compared to their parental bisexual forms. We expect that, in comparison to the bisexual species, single reproductive effort of

the parthenogens and relative egg number should be higher (Schall, 1978).

Indeed, *D. armeniaca* from the studied populations invests relatively more resources into a single reproduction than the *D. valentini*, and we associate this with its common presence in less stable environments than the latter species. In contrast to the system *D. armeniaca* - *D. valentini*, parthenogenetic *D. dahli* does not invest more resources in a single reproduction than its paternal species, *D. portschinskii*. However, in *D. dahli* egg number in a clutch (but not egg size) significantly increases with female body size, whereas in *D. portschinskii* female size leads to the growth of egg size. That means, although the bisexual species does not

Table 5Summary of a number of eggs per clutch and egg diameter of six species of *Darevskia*.

Parameter	Authors	Darevsky	Galoyan	Galoyan et al.	This Study	Se-MDE
	Year	1967	2011	2019	2022	
CNEC	<i>D.armeniaca</i>	2–5	>2.5	6	2–6	–
	<i>D.valentini</i>	5	–	–	2–6	–
	<i>D.obscura</i>	–	–	–	2–5	–
	<i>D.dahli</i>	2–5	–	–	1–4	–
	<i>D.portschinskii</i>	2–5	–	–	1–3	–
MDE	<i>D.armeniaca</i>	12–12.5	–	–	13.5	0.0205
	<i>D.valentini</i>	>14	–	–	14.2	0.0333
	<i>D.obscura</i>	12–12.5	–	–	14.05	0.0224
	<i>D.dahli</i>	12–12.5	–	–	14.50	0.0298
	<i>D.portschinskii</i>	12–12.5	–	–	16.35	0.0401

CNEC – counted number of Eggs per clutch; MDE – maximum diameter of eggs (in mm); Se-MDE – Standard error of maximum egg diameter in this study.

invest less resources in a single reproduction than its parthenogenetic daughter form, it invests more resources in producing more viable offspring, whereas the parthenogen produces more offspring at the expense of their potential viability. This correlation suggests a more stable environment for juveniles of *D. portschinskii* than that of its daughter parthenogen.

Our study supports the “weed hypothesis”, but a more unstable environment of the parthenogens may cause either spending more resources for reproduction (long-term adaptation), or in producing relatively heavier eggs (short-term adaptation to unstable juvenile environment). The particular way of r/K divergence between a parthenogen and its paternal species depends on the habitat type and, probably, developmental constraints related to body size. *D. dahli* may not be able to increase reproductive effort above a certain threshold because of energetic limitations related to small body size, although they favor from producing more, even lighter, eggs than *D. portschinskii*.

D. obscura, a bisexual species not parental to the studied parthenogens, invests relatively more resources in a single reproduction than *D. valentini* in a habitat where it coexists with *D. armeniaca*. This observation may suggest that the clear separation between the r - and K -reproductive strategy is easier to infer between closely related species (such as a parthenogen and its parental form) than in more phylogenetically distant ones, and if less related species are compared, species-specific habitat distribution and other ecological features may shade the effect of habitat stability on reproductive strategy.

The reason for this lineage-specific divergence pattern includes multiple factors influencing reproductive parameters, different from habitat stability. This includes the effect of body size, and related phenotypic constraints. Growth of body size in colder climates reflects Bergmann's rule that in some cases applies to squamate reptiles (Olalla-Tárraga et al., 2006; Pincheira-Donoso et al., 2008) and hence, it explains larger bodies of *D. armeniaca* - *D. valentini* pair related to *D. dahli* - *D. portschinskii* pair. Clutch mass and reproductive effort of the large-bodied species, both sexually reproducing *D. valentini* and parthenogenetic *D. armeniaca*, is significantly larger than that of *D. portschinskii* and *D. dahli* respectively; egg size increased with reproductive effort in *D. valentini* and *D. armeniaca* but decreased in *D. portschinskii* and *D. dahli*. Besides, eggs of big-bodied species are more round in shape, whereas the eggs of *D. portschinskii* and *D. dahli* are rather elongated, although average egg mass and reproductive effort do not differ significantly among the species, with the exception of *D. armeniaca*. These facts indicate that small-bodied cannot invest so much resources into a single reproduction as larger-bodied ones, probably because of too high risk of female death associated with a high reproductive expenditures. The individuals with a larger body can deposit more eggs than small-bodied ones, without increasing reproductive effort too much.

Egg size is less dependent on female size than egg number in the studied group. We suppose this is related to a minimum threshold size that allows the eggs of *Darevskia* to develop and a maximum size of egg

that female can successfully produce. The egg laying process is supposedly less traumatic for larger lizards. Vitt and Congdon (1978), Shine (1992) and Du et al. (2005) all suggest that maternal abdominal volume is an important factor limiting clutch size (and egg size) of a reptile of a given body size and shape. The eggs of small-bodied *D. dahli* and *D. portschinskii* are more elongated than this in a large-bodied species, allowing very small lizards to deposit relatively large eggs and hence ensure sufficiently high juvenile survival.

Some previous authors did not find any differences in reproductive characteristics of parthenogens and their sexually breeding relatives in nature. Schall (1978) found that clutch size, egg size and reproductive effort in *Cnemidophorus* (*Aspidoscelis*) are closer between unisexual species and their bisexual ancestors than between different species with the same reproductive mode. Menezes and Rocha (2014) did not find the differences in reproductive characteristics between four bisexuals (*Ameivula ocellifera*, *A. abaetensis*, *A. nativo*, *A. gularittoralis*) and one parthenogenetic (*Cnemidophorus lacertoides*) species from the eastern Brazil. We suppose that, in the differences between the parthenogens and bisexual species do exist, however, the patterns of the differences vary from case to case, dependent on the ecological characteristics of a taxon.

Constraints related to unacceptable energy loss during single reproduction plausibly explain negative correlation of egg number with both absolute and relative egg size. Gaining of mass and energy during growth of a female is transformed into the increasing egg number. This process is discrete and not gradual, and if there is a threshold mass that triggers development of an additional egg (Ljungström et al., 2016), the females whose mass is slightly above the threshold endure a higher mass and energy loss than those below the threshold. This may increase post-reproductive mortality of the females if a clutch size exceeded the threshold; however, the abrupt growth of energy expenditures can be smoothed if the eggs in a larger clutch are lighter.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

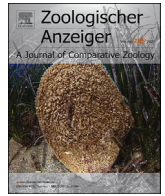
Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jcz.2022.11.002>.

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Chapter 3. Estimating phenotypic heritability of sexual and unisexually reproducing rock lizards (genus *Darevskia*)



Research paper

Estimating phenotypic heritability of sexual and unisexually reproducing rock lizards (genus *Darevskia*)

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ABSTRACT

The difference between phenotypic and genotypic differentiation of conspecific populations is commonly used for detecting natural selection. However, phenotypic variation integrates both genetic and non-genetic components, and this may lead researchers to false conclusions. To avoid bias, the analysis of the heritability of individual phenotypic characters is important, but the means are labor-intensive and require controlled crosses. In this paper, we tried to get around these difficulties by working with a natural system comprised of the coexisting sexually reproducing lizard *Darevskia portschinskii*, and its daughter parthenogenetic form, *Darevskia dahli*. The excess of individual and between-population variation in the sexual form relative to the parthenogen was used as a measure of heritability of each of 21 scalation traits and principal components extracted from their analysis. We compared these data with microsatellite genotypes based on the analysis of five variable loci. We showed that *D. portschinskii* had higher individual and between-population phenotypic diversity than *D. dahli*. Phenotypic differences between populations of *D. portschinskii* (but not *D. dahli*) correlate with both the geographic distances and pairwise fixation indices based on the analysis of the genetic markers. This correlation substantially increased when, instead of the original phenotypic distances, the corrected Qst values are used to assess the heritability of the characters. A similar analysis pattern is recommended for various natural systems with coexisting sexually and asexually reproducing organisms.

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1. Introduction

Genetic drift and differential selection cause divergence of natural populations (Wright 1931). Population geneticists suggest that an excess of phenotypic variation over the genetic variation if the latter is estimated with neutral genetic markers is an argument for the presence of differential selection (Merilä 1997; Lynch et al. 1999; Merilä & Crnokrak 2001; Defaveri & Merilä 2013). Phenotypic variance, some part of which is presumably related to genetic factors, is commonly referred to as Qst (Merilä & Crnokrak 2001; Whithlock 2008; Miller et al. 2008). However, total phenotypic variance integrates both genetic and non-genetic phenotypic plasticity (Dobzhansky 1970; Falconer & Mackay 1996). For this reason, the analysis of phenotype without reference to the heritability of characters may lead to biased conclusions (Leinonen et al. 2006; Brommer 2011; Defaveri & Merilä 2013). Therefore, some

authors suggest using a different term, Pst, for measuring simple phenotypic differentiation (Leinonen et al. 2006; Brommer 2011; Defaveri & Merilä 2013), and distinguish it from 'true' Qst, which incorporates heritability of studied phenotypes.

It is difficult to estimate heritability in the wild. Experimental approaches for estimating heritability are based on the exact knowledge of genetic relationships among individuals with different phenotypes (Falconer & Mackay 1996). This is usually done on 'classical' laboratory organisms and in controlled conditions (Wray & Visscher 2008; Whithlock 2008). Field-based approaches are probabilistic, and usually do not aim to analyze the sources of phenotypic diversity. When comparing phenotypic and genotypic divergence, they usually assume a close relation between Pst and Qst estimates (Leinonen et al. 2013), which is not always accurate.

There are however natural systems that may help to analyze the sources of diversity without running labor-intensive crossing experiments. Those include parthenogenetic taxa, which commonly coexist with their parental species. Well-known examples include

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lizards from the Caucasian genus *Darevskia* (Darevsky, 1967; Danielyan et al., 2008; Tarkhnishvili et al., 2010, 2017; Tarkhnishvili, 2012) and North American *Aspidoscelis* (Lowe & Wright, 1966; Reeder et al., 2002; Sullivan et al., 2013). Parthenogenetic lizards are natural clones. Because they lack genetic recombination, one can assume that their phenotypic variation depends mostly on the direct impact of the environment within the norms of reaction. Theoretically, some genetically based variations can exist due to *de novo* mutations. However, directional selection in parthenogens is improbable, since it would usually request recombination (Fisher 1930; Ghiselin 1974; Maynard Smith 1978; Kondrashov 1988). Thus, phenotypic variation in sexually reproducing populations would exceed that found in a closely related parthenogenetic clone from the same location, and the difference can be used as an indication of heritability; the estimated heritability of individual phenotypic traits can be used for the evaluation of 'true' Q_{st} from the observed phenotypic variation. This approach may help to analyze various processes that are difficult to explore otherwise, such as the relative contributions of selection and genetic drift into genetic and phenotypic variation within and among populations, or identifying characters that are under selective pressure, etc.

We partitioned the sources of phenotypic variation in sexually reproducing rock lizard *Darevskia portschinskii* (Kessler, 1878) by studying phenotypic variation in six river valleys from Eastern Georgia with differences in altitude, land cover, and other environmental variables. In all these valleys *D. portschinskii* coexists with its daughter parthenogen, *Darevskia dahli* (Darevsky, 1957). The latter is a hybrid between males of *D. portschinskii* and females of another rock lizard, *Darevskia mixta* (Méhely, 1909). *D. mixta* is quite distant genetically from *D. portschinskii* (Murphy et al., 2000; Tarkhnishvili, 2012); however, the divergence time between the hybrid parthenogen and its paternal ancestor does not exceed a few tens of thousands of years (Freitas et al. 2016). Vergun et al. (2014) suggest that the most of *D. dahli* descend from a single F1 hybrid between *D. portschinskii* and *D. mixta*, although do not exclude that some individuals may inherit from different hybridization event. However, most of genetic variation that presents in this species both these authors and Tarkhnishvili et al. (2017) attribute to mutations within the clonal lineage.

The authors accounted phenotypic and genetic variation in both *D. dahli* and *D. portschinskii* coexisting in multiple locations of eastern Georgia. *D. mixta* was not included in this study, because its current range does not overlap with that of *D. dahli*, different from the range of the paternal species of the parthenogen. Comparing the genetic and phenotypic variation in both taxa helped us to estimate the difference between the total and genetically determined phenotypic variation. The study showed that phenotypic plasticity may substantially decrease the correlation of phenotype with both selection and genetic drift.

2. Material and methods

2.1. Sampling

We studied individuals of *D. dahli* and *D. portschinskii* from six river valleys (RVs) of Eastern Georgia, where both species are found concurrently (Bakradze 1977; Tarkhnishvili et al. 2010, Fig. 1). For each location, the elevation was recorded (*D. dahli* is found on elevations 750–1800 m, *D. portschinskii* – 500–1300 m; at elevations 750–1300 m both species commonly coexist: Darevsky 1967; Tarkhnishvili et al. 2010).

The temporal and the anal areas of 186 individuals of *D. dahli* and 101 *D. portschinskii* were photographed (Fig. 2). In further analyses, only females of *D. portschinskii* were used that reduced the total sample size of this species to 54. The summary data showing

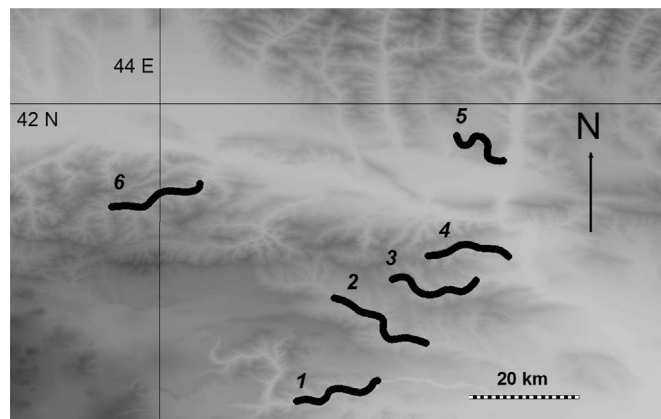


Fig. 1. Map of the Eastern Georgia, with the study areas/river valleys shown in bold lines. 1- Khrami, 2- Algeti, 3- Kojori, 4- Vere, 5- Mtskheta, 6- Tana (see also Table 1).



Fig. 2. Temporal and preanal areas of *Darevskia* lizards, with scales used in the analyses indicated.

the number of individuals from each RV studied with morphometric and genetic methods is presented in Table 1.

2.2. Scalation analysis

31 scalation characters described in Tarkhnishvili et al. (2013) were analyzed from the digital images of the lizards. After

Table 1

Numbers of individuals used for phenotypic analysis and scored microsatellite genotypes. RV – the name and the number of River Valley (see Fig. 1). PH – the number of females analyzed for phenotype; STR – the number of individuals analyzed for microsatellite profile. DD – *D. dahli*, DP – *D. portschinskii*.

RV	DD (PH)	DP (PH)	DD (STR)	DP (STR)
Khrami (1)	74	4	11	6
Algeti (2)	34	7	7	8
Kojori (3)	18	6	13	9
Vere (4)	17	6	5	4
Mtskheta (5)	30	18	11	8
Tana (6)	13	13	10	5
TOTAL	186	54	57	40

removal of invariant characters, and those showing fixed differences between *D. portschinskii* and *D. dahli*, 21 informative traits remained (Fig. 2, Table S1); all these characters vary in both taxa. No underlying information on the geographic variability was used while selecting the characters, therefore avoiding potential bias in evaluating Qst values (Whithlock 2008).

Thirteen of these traits were numeric or ordinal (from here onwards ONC): (a) the number of scales around the central temporal scale, SC-CT; (b) size of the central temporal scale, CT-SZ; (c) number of the upper labials, UL; (d) number of the scales between the central temporal and 6th upper labial (CT-UL6); (e) number of the scales between the tympanal and upper temporal, TM-UT; (f) number of the scales between tympanal and 7th upper labial, TM-UL7; (g) number of the scales between the central temporal and the third scale of the second road of postorbitals, CT-PPO3; (h) number of the scales between the central temporal and the closest upper temporal, CT-UT; (i) area of the central preanal scale (or scales, if paired), CPA; (j) number of the scales in contact with the anal scale, SC-AN; (k) number of the femoral pores on the left leg (FP); (l) length of the contact between the nasal scales, IN; (m) thickness of the interparietal scale, IN-T. The other eight characters were nominal. Prior to the further analyses, we estimated the variance of each individual ONC, and the index of qualitative variation (IQV; Reynolds 1977) of each individual nominal character, separately for 186 *D. dahli* and 54 females of *D. portschinskii*. Then, we transformed the original set of ONCs applying principal component (PCA) analysis using IBM SPSS 21.0 for Windows. The components with eigen values exceeding one were extracted. Finally, we ran categorical principal component (CPCA; Gifi 1990) analysis for the proportionally selected samples based on all 21 characters coded as numeric, ordinal, or nominal; 5 dimensions were extracted. Individual scores along the PCA and CPCA axes were saved as new variables.

2.3. Evaluating the differences and distances between RVs

We have defined each RV as the area parallel to a river bed and 100 m to both sides, where the samples were collected (Fig. 1, Table 1). We calculated the geometric center of each RV, using software ArcView GIS 3.3. We evaluated the distance among the RVs by two different methods: connecting their geometric centers with straight lines, and following lines along roadways, which in this case can be used as least-cost paths (Adriaensen et al. 2003), because the lizards can spread across the rocks positioned along the roads. Software ArcView GIS 3.3 was applied for the estimates. We downloaded 19 bioclimatic parameters (Hijmans et al. 2005) and scored all pixels within each RV for these variables; then, we calculated the geometric mean for each variable/RV, and the vectors characterizing the climate of each RV. We calculated Euclidean distances between RVs based on the bioclimatic variables using PopTools 3.2 (Hood 2010).

2.4. The analysis of microsatellite genotypes

In order to compare phenotypic variation with that scored using neutral genetic markers, we used profiles at five variable microsatellite loci (from here onwards – STR, short tandem repeats) of individuals from the same populations, published elsewhere (Table S2 from Tarkhnishvili et al. 2017). We used five primer pairs (Korchagin et al. 2007): Du215, Du281, Du418, Du47, and Du323. For detailed PCR conditions see Tarkhnishvili et al. 2017. Genotypes were scored using Genemapper v3.5 software (PerkinElmer, Waltham, MA, USA). Every locus of each individual was run two to four times to control for allelic dropout and false allele amplification.

We used software Arlequin 3.5 (Excoffier & Lischer 2010) to assess genetic variation for all genotyped individuals of *D. portschinskii* and *D. dahli*, as well as for each of the six RVs. We evaluated the total number of alleles, allele range, the observed heterozygosity, and theta H (Chakraborty & Weiss 1991) separately for *D. portschinskii* and *D. dahli*. With the same software, we evaluated pairwise Rst (the analog of the fixation index for the Stepwise Mutation model, usually applied for microsatellite genotypes (Slatkin 1995), among the RVs, in order to compare the genetic distances based on the microsatellite markers with those based on the phenotypic differences.

2.4.1. Analysis: general design

First, we inferred the overall heritability of individual scalation characters (ordinal and numeric), using the first five CPCA scores and PCA scores for the dimensions with eigenvalues exceeding one. This was based on the assumption that parthenogenetic *D. dahli* represent a single clone, and that genetic variation within this taxon, if any, does not contribute to phenotypic variation.¹ We measured overall heritability of the individual traits in *D. portschinskii* using the equation

$$c = (\text{var}_i(\text{SR}) - \text{var}_i(\text{P}))/\text{var}_i(\text{SR}) \quad (1)$$

where *i* is the character of interest, var(P) is the overall variance of the character in the parthenogen, and var (SR) is the overall variance of the character in the bisexual species. This equation assumes that the difference between the variance of the same character in bisexual and parthenogenetic species reflects the additive genetic component in the variance.

We then estimated pairwise Pst (i.e. morphological differentiation among the six river valleys, not considering heritability of a character) for 13 ONCs, scores along the five CPCA axes, and scores along the PCA axes with eigen values exceeding one as

$$\text{Pst} = \text{var}_{i,b}/(\text{var}_{i,b} + 2*(\text{var}_{i,w})) \quad (2)$$

Data were treated separately for females of *D. portschinskii* and for *D. dahli*, and calculated 'true' Qst (i.e. that considering heritability of a character) only for *D. portschinskii*,

$$\text{Qst} = (\text{var}_{i,b}\text{SR} - \text{var}_{i,b}\text{P})/((\text{var}_{i,b}\text{SR} - \text{var}_{i,b}\text{P}) + 2*(\text{var}_{i,w}\text{SR} - \text{var}_{i,w}\text{P})) \quad (3)$$

where *i* is a character, var_{i,b} and var_{i,w} are variances between RVs and within RVs respectively, var_i (SR) is a variance of the

¹ There is some genetic variation in *D. dahli* (Tarkhnishvili et al., 2017; this paper) that potentially might cause additive genetically determined phenotypic variation in this species; however, because correlation between Rst and phenotypic differentiation was present only in *D. portschinskii* and close to zero in *D. dahli*, we assumed that this potential effect can be ignored.

bisexual population, and $\text{var}_i(P)$ is variance of the parthenogenetic population. All calculations were done in Excel 2010 for Windows combining with the function Oneway ANOVA in IBM SPSS 21.0.

We used Mantel Tests (software zt; Bonnet & Van de Peer 2002) for calculating the correlation coefficients, for each of the studied taxa, between Pst and geographic distances among the geometric centers of RVs and with cost distances (along roads) among the centers of RVs (both for *D. portschinskii* and *D. dahli*); between Qst and the geographic distances (*D. portschinskii* only); between Pst and Rst based on the STR profiles (both species), and between Qst and Rst (*D. portschinskii* only). Finally, between Pst and Rst with the averaged climatic differences among the RVs (Euclidean distance), based on 19 bioclimatic variables of the respective locations. We repeated these analyses for each of thirteen ONCs, as well as for CPCA and PCA scores. Altogether, 23 characters or Principal Components X 2 (Pst and Qst) X 4 tests (straight and cost distance, Euclidean “climatic distance”, and Rst) = 184 tests for *D. portschinskii* and the same analyses but not conducted for Qst ($23 \times 4 = 92$) for *D. dahli*. Sequential Bonferroni correction (Rice 1989) was applied across the columns, to test the robustness of the most important correlations between the parameters.

Besides these analyses, we tested whether morphological Euclidean distances on the individual level, based on the same set of the numeric/ordinal characters, correlates with the exact geographic distance among the locations *within* individual river valleys, and with individual multivariate genetic distances based on the analysis of microsatellite genotypes.

As a result of this sequence of analyses, we established: (1) which of the characters have the highest heritability; (2) whether phenotypic differences among the populations correlate with geographic, climatic, or genetic distance, and whether the correlation coefficients change if additive genetic component of phenotype is used in calculations instead of simple phenotypic differences, without respect to heritability.

3. Results

3.1. Overall variation in *D. dahli* and *D. portschinskii*

All thirteen ONCs, scored for the entire sample of *D. dahli* (N = 186) and females of *Darevskia portschinskii* (N = 54) showed higher variation in *D. portschinskii* than in *D. dahli*, and for ten characters the differences were significant (F test, $P < 0.05$) (Table 2). In contrast, the index of qualitative variability (IDV) for eight nominal/categorical characters did not show higher variation in *D. portschinskii*, related to *D. dahli* (the proportion of the indices was on average close to one). The highest heritability index, estimated with equation (1) was for the characters UL (the number of upper labials), CT-PPO3 (the number of scales between the central temporal and the closest postocular), and CPA (size of preanal scale): $c = 0.60$ – 0.75 for the full dataset (Table 2).

The first two PCA axes (31% of the total variation), dominated by the contrast between the characters CT-PPO3 and SC-CT (Table 3), did not separate the species, but showed, on average, higher values and broader range of values along the 1st PCA axis in *D. portschinskii* (Fig. 3).

Individual scores along the first five CPCA and PCA axes, for both designs, varied more in *D. portschinskii* than in *D. dahli*. The heritability indices varied between 0.26 and 0.50 for the first five CPCA axes based on all 21 characters, and between 0.25 and 0.72 for the first five PCA axes based on 13 ONCs. Heritability index was highest in the first PCA axis, although the second and the third axes were more variable in both species. The 4th CPCA axis was dominated by

Table 2

Variation of 21 scalation traits and scores along the principal component axes in *D. dahli* (DD) and females of *D. portschinskii* (DP). Variation, heritability indices (c ; see the methods section) and significance of the interspecific differences (F test for equality of variance) is calculated for full samples (186 *D. dahli* and 54 *D. portschinskii*) for the original 21 characters. For eight nominal/categorical variables, the indexes of qualitative variation (IQV) shown instead of variance. The legend for abbreviations see on Fig. 2. CPCA11–CPCA15 – calculated for all 21 characters; CPCA21–CPCA25 and PCA1–PCA5 – calculated for numeric and ordinal characters (ONCs) only.

Character	VAR (DD)	VAR (DP)	C	P (F test)
SC-CT	2.791	5.195	0.463	0.0027
CT-CZ	0.399	0.639	0.376	0.0233
UI	0.070	0.271	0.741	<0.0001
CT - UL6	0.511	0.883	0.421	0.0083
TM- UT	0.413	0.602	0.315	0.0702
TM-UL7/6	0.815	1.915	0.575	<0.0001
CT-PPO3	0.446	0.976	0.543	0.0001
CT-UT	0.468	1.001	0.532	0.0002
CPA	0.460	1.550	0.703	<0.0001
CS-AN	0.551	1.264	0.564	<0.0001
FP	1.320	2.104	0.372	0.0253
IN	0.392	0.552	0.290	0.1033
IN-T	0.553	0.703	0.213	0.2521
CT-SHP	0.912	0.870	−0.055	N/A
CT-OR	0.921	0.948	0.029	N/A
TM-STR	0.642	1.000	0.358	N/A
PCT	0.756	0.912	0.171	N/A
PO2PPO3	0.990	0.814	−0.216	N/A
CPA-SH	0.942	0.618	−0.526	N/A
PRFR	0.597	0.721	0.176	N/A
IN-SD	0.893	0.907	0.015	N/A
CPCA1	0.355	0.888	0.600	<0.0001
CPCA2	0.873	1.652	0.472	0.0020
CPCA3	0.978	1.288	0.241	0.1875
CPCA4	0.684	2.214	0.691	<0.0001
CPCA5	0.937	1.367	0.314	0.0708
PCA1	0.335	1.184	0.717	<0.001
PCA2	0.542	1.487	0.635	0.001
PCA3	0.498	1.454	0.658	<0.001
PCA4	0.786	1.209	0.350	0.144
PCA5	0.867	1.147	0.245	0.340

Table 3

Component Matrix based on the CPA analysis of the 13 numeric and ordinal characters of scalation in the studied samples.

Characters	1st component	2nd component	3rd component
SC-CT	−0.714	0.392	0.121
CT-SZ	−0.539	0.132	−0.007
UI	0.200	0.425	−0.051
CT - UL6	0.624	0.037	0.464
TM- UT	0.221	0.480	−0.095
TM-UL7/6	0.496	0.392	0.343
CT-PPO3	0.680	−0.071	0.352
CT-UT	0.581	−0.480	−0.028
CPA	0.303	0.077	−0.718
CPA	−0.541	−0.076	0.617
CS-AN	0.134	0.633	−0.050
FP	0.037	0.371	0.207
IN	−0.180	−0.597	0.104

the shape of tympanal scale (Table 3), and also showed a high heritability index (Table 2), and it was most variable in *D. portschinskii*.

Genetic variation (STR genotypes) evaluated as theta H (Chakraborty & Weiss 1991) was 3.179 in *D. portschinskii* but only 1.804 in *D. dahli*. However, the two taxa did not differ significantly in average number of alleles per locus (11.4 vs 9.4) and in the observed heterozygosity (0.75 vs 0.74).

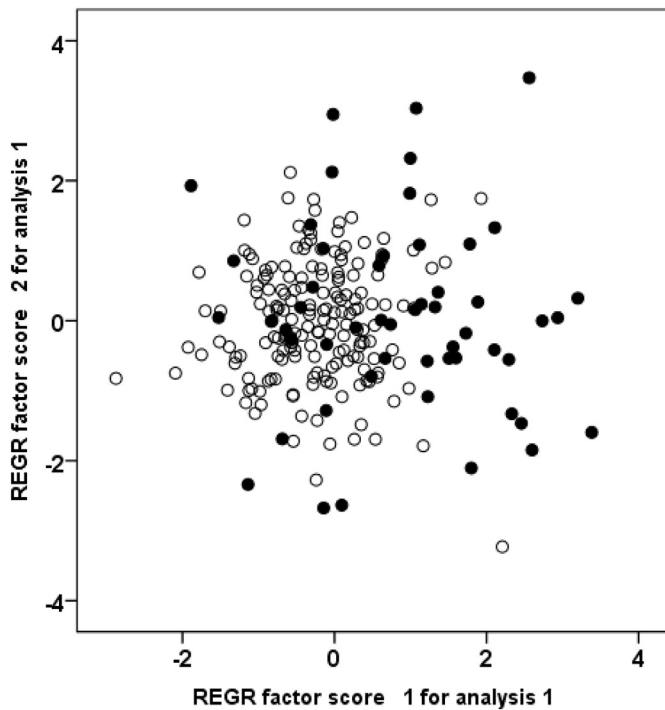


Fig. 3. Individual scores at the first two PCA axes in *D. dahli* (open circles) and *D. portschinskii* (solid circles).

In summary, bisexual *D. portschinskii* showed nearly 2X higher average phenotypic variation in numeric and ordinal characters (ONCs), and in each of the CPCA or PCA axes scores, than parthenogenetic *D. dahli*. This suggests substantial additive genetic effects in determining phenotypic variability. The size of preanal scales, the number of upper labials, and the size and the number of scales beyond the central temporal scale, have very high additive genetic components of variation, whereas characters from rostral area are quite variable within both bisexual and parthenogenetic populations, and their heritability is low. Variation in eight categorical/nominal characters was not higher in the bisexual lizards than in the parthenogen. Variation of STR genotypes, not surprisingly, was also higher in *D. portschinskii*.

3.2. Variation among the locations

Four ONCs of *D. dahli* and five of *D. portschinskii* showed significant differences between the RVs (one-way ANOVA, $P < 0.05$). From 13 studied characters, seven (size of central temporal scale, CT-SZ; number of upper labials, UL; number of scales in temporal area, specifically between CT and UL, TM and UL, CT and PPO3, CT and UT, and the number of femoral pores) varied between the river valleys in *D. portschinskii* significantly stronger than in *D. dahli* (F test, $P < 0.05$), and the same is true for the first three CPCA and the first five PCA scores; no one character varied in *D. dahli* significantly stronger (Table 4).

Hence, bisexual species showed higher morphological variation among individuals and among the sampled locations compared to the parthenogen. Especially high differences were obtained for the characters UL (more than four times higher variation in *D. portschinskii*), CTUL6, TMUL76, CTPO3; Table 4). Two of these characters are those with the highest overall heritability index.

The average pairwise fixation index (Rst, based on the STR genotypes) among the six studied river valleys (RVs) also was significantly higher in *D. portschinskii* than in *D. dahli* (0.210 vs

Table 4

Variation of morphological characters between the river valleys (mean square between the groups, F statistics, significance of among-group differences; DD - *D. dahli*, DP - *D. portschinskii*).

Trait	MSqDD	MSqDP	F DD	FDP	Sig. DD	Sig. DP
SC-CT	7.328	10.165	3.649	2.024	0.008	0.095
CT-SZ	0.706	1.390	1.428	2.397	0.234	0.053
UI	0.140	0.582	0.935	2.312	0.468	0.061
CT - UL6	0.420	2.436	0.443	3.356	0.816	0.012
TM- UT	1.109	1.813	3.843	3.473	0.006	0.010
TM-UL7/6	1.091	7.800	1.221	7.754	0.316	0.000
CT-PPO3	0.432	2.369	1.535	2.921	0.200	0.024
CT-UT	0.988	2.382	2.760	2.786	0.030	0.029
CPA	0.788	0.769	2.154	0.429	0.078	0.826
CPA	1.982	1.278	4.149	0.933	0.004	0.470
CS-AN	2.250	3.386	1.467	2.153	0.221	0.078
FP	1.019	0.779	2.249	1.279	0.068	0.291
IN	3.717	3.046	1.392	1.210	0.248	0.321
CPCA1	0.465	1.976	2.265	2.243	0.065	0.068
CPCA2	1.299	2.004	1.869	1.679	0.120	0.161
CPCA3	0.358	2.979	0.454	2.606	0.808	0.039
CPCA4	1.305	1.170	2.474	0.863	0.047	0.514
CPCA5	1.120	3.172	1.647	2.959	0.169	0.022
PCA1	0.302	2.822	0.876	2.896	0.377	0.127
PCA2	0.281	2.145	0.848	2.364	0.384	0.163
PCA3	0.168	0.335	0.311	0.271	0.592	0.617
PCA4	0.029	1.481	0.063	1.843	0.808	0.212
PCA5	0.017	0.037	0.024	0.028	0.880	0.872

Significant values ($P < 0.05$) are represented in bold.

0.013, $P < 0.001$). Out of 15 pairwise Rst values, six in *D. dahli*, and ten in *D. portschinskii* significantly exceeded zero (exact test, $P < 0.05$); hence, genetic differentiation among the populations was more than ten times higher in the sexually breeding form than in the parthenogen.

In summary, *D. portschinskii* showed stronger phenotypic as well as genotypic variation among the locations than *D. dahli*.

3.3. The influence of geographic distance and climate on variation between the locations

The phenotypic distances (Pst) based on the scores at the five CPCA axes (21 characters included) did not significantly correlate with geographic distance, neither with climatic differences between the RVs (data not shown). Phenotypic distances (Pst) based on the first three PCA scores (13 ONCs), aggregating 47% of the total variance (inferred through a broken stick analysis) showed a positive but insignificant correlation with geographic distances (Table S1). The same PCA scores averaged for the first three axes significantly (Mantel Test, $P < 0.05$) correlated with geographic distances in *D. portschinskii*, but did not correlate with geography in *D. dahli* (Table 5). The correlation with geographic distances remained significant after applying sequential Bonferroni correction across the columns. The matrix of character loading at the three PCA axes that were used for the comparison is shown in Table 3.

Replacing Pst with the corrected Qst values obtained using the equation (3), substantially increased both the correlation coefficient and the significance level when estimating correlation with geographic distance in *D. portschinskii* (Table 5, Fig. 4).

Phenotypic distances (Pst), based on the first three PCA axes, also correlated with pairwise fixation index (Rst, based the microsatellite genotypes) among the same locations in *D. portschinskii*, but not in *D. dahli*. The correlation coefficient and the significance level increased when Pst was replaced with the corrected Qst values (Table 5).

We calculated the average of the phenotypic distances based on eight numeric/ordinal characters with heritability level exceeding

Table 5
Correlation coefficients (Mantel tests) of phenotypic distances (Pst; *D. portschinskii* and *D. dahli*) and corrected phenotypic distances (Qst; *D. portschinskii* only) with (1) Fixation index (Rst) among the locations, (2) geographic distance among the locations, (3) geographic distance along the road net, and (4) climatic differences among the locations. The outputs are shown separately for *D. portschinskii* and *D. dahli* (DP, DD), and for Pst and Qst calculated for (a) average of the first three PCA axes, (b) average of eight ONCs with heritability $c > 0.4$, and (c) average of the two characters (TMUT, CPA) showing highest correlation coefficients with climate. Significance level shown in parenthesis. Correlation that remained significant ($P < 0.05$) after Bonferroni correction shown in boldface.

Mantel Test	a) 3 first PCA			
	Rst	Geodist	Geodist_corr	Ecodist
DP				
Pst	0.423 (0.029)	0.517 (0.006)	0.189 (0.229)	0.029 (0.408)
Qst	0.573 (0.021)	0.664 (0.001)	0.324 (0.175)	0.182 (0.288)
DD				
Pst	−0.315 (0.173)	−0.361 (0.144)	−0.375 (0.115)	−0.257 (0.210)
Mantel Test	b) heritability >0.4			
	Rst	Geodist	Geodist_corr	Ecodist
DP				
Pst	0.222 (0.225)	0.266 (0.179)	0.334 (0.225)	−0.159 (0.375)
Qst	0.421 (0.149)	0.246 (0.189)	0.359 (0.225)	0.001 (0.421)
DD				
Pst	0.071 (0.399)	0.108 (0.376)	0.140 (0.299)	−0.054 (0.429)
Mantel Test	c) TMUT, CPA			
	Rst	Geodist	Geodist_corr	Ecodist
DP				
Pst	0.051 (0.404)	−0.155 (0.238)	−0.219 (0.238)	0.566 (0.033)
Qst	−0.079 (0.376)	−0.106 (0.324)	−0.200 (0.246)	0.492 (0.050)
DD				
Pst	−0.144 (0.296)	−0.065 (0.433)	−0.047 (0.469)	−0.035 (0.499)

0.4 (SC-CT, UL, CT-UL6, TM-UL7/6, CT-PPO3, CT-UT, CPA, SC-AN). The distances based on these individual characters and their average did not significantly correlate with geographic and genetic distances in *D. portschinskii*, although all showed a positive correlation value.

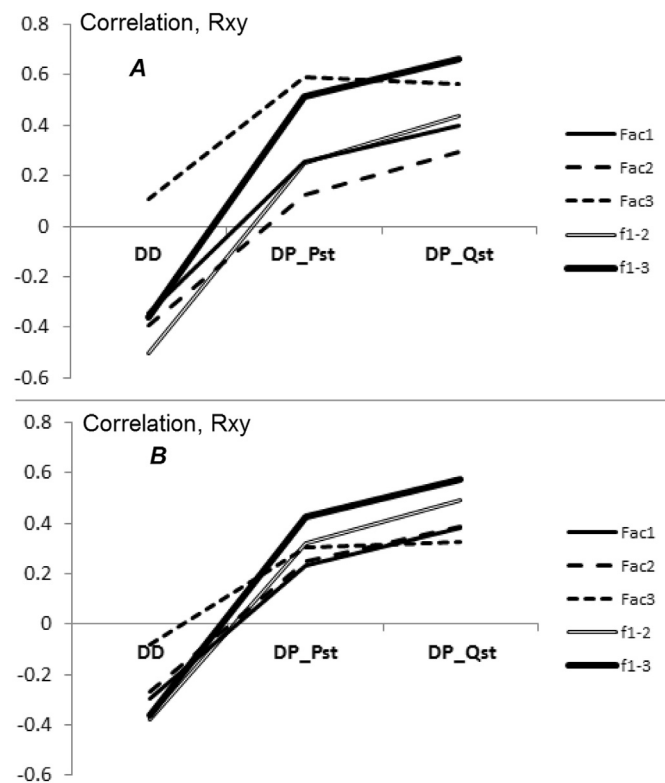


Fig. 4. Correlation coefficients between the phenotypic distances based on three first PCA scores, and linear (A) or cost (B) geographic distances. DD - *D. dahli*, Pst based on the phenotypes; DP_Pst - *D. portschinskii*, Pst; DP_Qst - *D. portschinskii*, corrected Qst value.

In conclusion, the phenotypic distances between six populations of *D. portschinskii* significantly correlated with geographic distances between the locations and with the pairwise fixation indexes based on the STR genotypes, which is not the case in *D. dahli*. The power of correlation increased when the phenotypic distances were corrected considering the heritability values (equation (3)) and Pst was replaced with 'true' Qst.

Finally, Pst based on the second PCA axis scores in *D. portschinskii*, as well as that based on the characters TMUT and CPA, showed relatively high correlation coefficient with climate (Table S1); Combination of these two characters significantly ($R_{xy} = 0.566$, $P < 0.05$) correlated with climatic differences among the RVs (but not with geography or fixation indices) in *D. portschinskii*. However, replacing Pst with Qst did not increase the correlation of the phenotypic distances with climate.

4. Discussion

Our results suggest that the parallel study of sexually reproducing organisms and their unisexual relatives may help to explore mechanisms of microevolution. Evaluating inheritance of phenotypic traits usually requires complicated and sustained experimental manipulation, which is difficult to achieve in wild animal populations. By contrast, the analysis of sympatric sexual breeders and their daughter clones provides the relevant information *in situ* without the need for controlled crosses. Corrected measures of phenotypic variation may greatly improve the robustness of the analysis of divergence mechanisms.

Spatial diversification of neutral genetic markers and morphological traits commonly correlate (Merilä & Crnokrak 2001). Scientists often simply report this fact, without addressing the heritability of the components of phenotype (Leinonen et al. 2013). However, phenotypic divergence that does not explicitly consider heritability can substitute for genetically determined diversification of phenotypes (Qst) only if some robust assumptions are met (Whithlock 2008; Brommer 2011; Leinonen et al. 2006, 2013). Meanwhile, the environmentally induced and genetic variation

may be almost indistinguishable (Goldschmidt 1935; Gauze 1984; Lambert et al. 1989; Baum et al. 2010). This has several reasons. Heritability may vary among the populations, dependent on the environmental conditions (Wray & Visscher 2008). Epistatic effects (Wright 1931; Szendro et al. 2013) contribute to overall uncertainty. Some authors (Cox et al. 2006; Whitlock & Guillaume 2009; Brommer 2011; Adriaenssens et al. 2003; Ananjeva et al. 2006; Bakradze 1977; Baum et al. 2010; Bonnet and Van de Peer 2002; Leinonen et al. 2013) recommend the “common-garden” principle (crossing experiments in strictly controlled conditions) for evaluating heritability. Brommer (2011) and Holland et al. (2011) underline that the attempts of inferring “true” Qst, showing the additive genetic component of phenotype, may be strongly biased without knowledge of heritability in given environmental conditions; the proportion of within- and between-population heritability components affect the Fst-Qst ratio (Brommer 2011).

Estimating heritability experimentally is very time- and labor-expensive. Even *a priori* knowledge of the heritability of a particular character in a particular species is insufficient for calculation of “true” Qst in new environments. Parallel analysis of asexually and sexually reproducing organisms may help to overcome these complications. In this case, heritability values are measurable *in situ*, by comparing the variability of a character in syntopic populations of closely related sexual and asexual forms. This study is a test of the applicability of this methodology, and its results suggest that it could be applied to some natural systems.

4.1. Potential pitfalls

There are potential difficulties associated with the application of the method. Sexually and asexually breeding, closely related organisms may have fixed phenotypic differences—this is true for *Darevskia* taxa coexisting with their daughter clones. The clones usually are interspecific hybrids (Uzzell & Darevsky 1975; Darevsky, Kupriyanova & Uzzell 1985; Murphy et al. 2000) and they combine the characters of both parental species. *D. dahli* combines genetic markers inherited from both the paternal *D. portschinskii* and the maternal *D. mixta*; this may contribute into genetic diversity of *D. dahli*. However, this additive genetic diversity would increase rather than decrease genetic diversity of the parthenogen, and cannot explain its lower genetic variation in comparison with the paternal species; neither can this explain low genetic differentiation between the populations of *D. dahli*. Besides, some phenotypic differences between *D. dahli* and *D. portschinskii* are attributed to the mixed origin of the former, and comparing their variability may produce biased results. Indeed, *D. portschinskii* and *D. dahli* have (almost) fixed differences in the structure of the first preanal scale (paired versus singular; Darevsky 1967; Tarkhnishvili et al. 2010); they also have different sizes of dorsal scales and different coloration. For this reason, we used in our analysis only those characters, which broadly varied in both forms and, hence, their variability was comparable. Potential presence of *D. dahli* individuals that descend from more than one hybridization event (Vergun et al. 2014) may explain some phenotypic variation in this form; this possibility is considered by parallel analysis of phenotypic and genetic variation in this study. Simultaneously, Tarkhnishvili et al. (2017) provided evidence that all *D. dahli* from Georgia descend from a single hybrid individual, and even another phenotypically distinct parthenogenetic species, *Darevskia armeniaca*, may descend from the backcross of *D. dahli* with another bisexual species.

The other potential problem is that the studied populations could be exposed to slightly different habitats and microclimates within the same river valley, which could affect developmental patterns on the embryonic stage. However, the absence of correlation between the geographic distance and phenotype *within* the

same river valley suggests that this potential factor is unimportant. The third potential problem is coding. Some characters that obviously vary are difficult to code as numeric or ordinals, and they should be treated as nominal categorical variables. Our study suggests that the heritability of these categorical variations is low or undetectable.

Heritability of a trait may not be the same in two species, considering the hybrid origin of a parthenogen, i.e. non-genetic component of variation may be slightly higher or lower in a sexual breeder than in the related parthenogen. Finally, there is a certain risk that phenotypic variation in a parthenogen has a genetic component. The absence of correlation between Pst and Rst in *D. dahli*, however, suggests that genetically determined phenotypic variation in this species is minute if any.

4.2. Heritability of the characters

The patterns of variation in *D. dahli* and *D. portschinskii* are similar. Simultaneously, all thirteen ordinal and numeric characters used in this study, as well as PCA and CPCA axes varied more strongly in *D. portschinskii* than in *D. dahli*, although some variation did present in *D. dahli* as well (Table 2). For most of the characters, the differences were significant, suggesting an important additive genetic component in this variation. The size of the preanal scales and the number of upper labials showed the highest excess of variation in *D. portschinskii* and, hence, the strongest heritability index ($h^2 > 0.7$). Six characters showing the degree of metameres (the number of scales) in the temporal area and the number of scales surrounding the anal scale also showed a relatively high (>0.4 , with the average 0.52) heritability index.

Phenotypic variation in *D. dahli* suggests the presence of an important environmentally induced component of variation within the norm of reaction. Interestingly, some characters that showed a high non-genetic component of variation are commonly used in lizard taxonomy (Darevsky 1967; Bakradze 1977; Roitberg 1994; Darevsky & Tuniyev 1997; Ananjeva et al. 2006; McDiarmid et al. 2012; Gabelaia et al. 2015). Those include the number of femoral pores, shape and size of the internasal scale, presence of the contact between the nasals, size of interparietal and central temporal scales. The differences in variation of these characters between the parthenogen and the sexual breeder are insignificant, and the heritability index is low.

4.3. Genotypic vs phenotypic differentiation among the populations

The scores along the 1st and 2nd PCA axes and six out of 13 numeric and ordinal characters varied stronger between the RVs than within the RVs in *D. portschinskii*. In *D. dahli*, significant between-group variation was detected only for the 2nd PCA axis (that is dominated by the number of femoral pores and width of interparietal scale) and five characters describing the size of central temporal and anal scales. The differences among the RVs in *D. dahli* (and not only in *D. portschinskii*) suggest that the direct impact of the environment may contribute to the differences between widely separated geographic populations. Via & Lande (1985) suggested that concurrent or opposite effects of phenotypic plasticity and selection may impede the effectiveness of the latter. Conversely, the absence of correlation (as in our system) suggests a small effect of phenotypic plasticity on selection and, hence, the variation within the norm of reaction would not substantially influence the spatial distribution of variation.

Correlation between genetic differentiation and geographic distance suggests that the migration rate is a determining factor of genetic differentiation (Wright 1931; Weir 1996). Although some authors do not recommend inferring migration rates from the

fixation index (e.g. Whitlock & McCaughley 1999), the correlation between these two variables is proven by multiple empirical studies. Our relatively small set of genetic markers showed a highly distinct pattern. The presence of a significant correlation between the fixation index and geographic distance provides the evidence for long-lasting and well established spatial structure consistent with an isolation-by-distance model (Slatkin 1993). Establishing equilibrium between migration and drift requires a long time after the separation of the populations. For instance, post-glacial northern European populations of moor frog (*Rana arvalis*) do not show isolation-by-distance, different from older populations south of the Carpathian Mountains (Knopp & Merilä 2009). A similar pattern is shown for the beetle *Carabus solieri* (Garnier et al., 2004), rock cress (*Arabidopsis thaliana*, Sharbel et al., 2000), and many other terrestrial organisms. Our findings confirm that the spatial structure of the populations described in this paper is old enough for achieving drift–migration equilibrium. The individual RVs count dozens of lizard locations (Tarkhnishvili et al. 2010), and genetic drift should not act very fast, hence this time should be substantial.

Phenotypic distances in *D. portschinskii* (but not in *D. dahli*) correlate with both fixation index and geographic distances. After replacing a simple phenotype-based Pst with corrected Qst values, the correlation with geography increased. In general, the correlation between geographic distance, fixation index, and Qst suggests that migration is a major force determining the level of both genetic and phenotypic differentiation in this lizard. In other words, inherited differences in scalation pattern produce a spatial distribution pattern similar to that of neutral genetic markers. This, in turn, implies that correctly evaluated Qst value, based on the analysis of easily measurable phenotypic characters, can be used for estimating at least relative migration rates when more than two conspecific populations are analyzed.

4.4. Selection vs genetic drift: phenotypic and molecular differentiation in *D. portschinskii*

Wright (1931) showed that if population differentiation is associated solely with genetic drift, Qst should be equal to the fixation index (Leinonen et al. 2008, 2013). All pairwise Qst values calculated for *D. portschinskii* strongly exceeded fixation indices based on the microsatellite loci. On average, for individual pairwise comparisons, Qst five times exceeded the fixation index (Table S1). Even if a conservative approach (Whitlock 2008; Martin et al. 2008) is applied, one should consider the substantial role of diversifying selection in the diversification of populations of *D. portschinskii*. This pattern is in line with the observations of Merilä & Conkrak (2001) who suggested that variability of genetic and morphological traits often correlate, but Qst usually exceeds the fixation index. However, this observation is not easy to interpret. One should not *a priori* expect a stronger diversifying selection between the most geographically distant populations unless there is a correlation between climatic differences and geographic distance (which does not exist in the present study). Diversifying selection would rather decrease the correlation between geographic and genetic distances, which is not the case in this study: the correlation coefficients of geographic distance with the fixation index and with Qst are roughly equal.

Correlation of a character with environmental conditions is strong evidence of natural selection (Endler 1986). In our dataset, there were only two traits which showed a relatively high (>0.3) correlation with climatic differences. One of these characters, (CPA) has a strong additive genetic component. The other, TMUT, shows weak additive genetic component. The combination of these two characters significantly ($R_{xy} = 0.566$, $P < 0.05$) correlates with

climatic differences (but not with geography) in *D. portschinskii*. Hence, the association of phenotypes with geographic distance is not related to the climatic differences among the study areas and climate-dependent selection.

Our results suggest that the coexistence of two related forms with different modes of reproduction can be used for resolving microevolutionary questions. The estimates of phenotypic diversity, based on the analysis of numeric or ordinal characters, are higher in sexual breeders than in parthenogens, which allows researchers to infer heritability indices for individual characters. This approach may be useful for studying populations of plants, crustaceans or insects as well as many other organisms with changing modes of reproduction. It also appears that genetic drift is the leading force in phenotypic differentiation in rock lizards, and its effect is accelerated by selection driving phenotypes towards adaptive peaks.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jcz.2020.02.005>.

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Chapter 4. Head shape divergence between parthenogenetic and their paternal bisexual rock lizards in sympatry



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Head shape divergence between parthenogenetic and their paternal bisexual rock lizards in sympatry

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ABSTRACT

Head shape plays a crucial role in ecological and evolutionary processes in lizards, and scientists have studied head shape using traditional and 3D geometric morphometrics. Lizards are classic subjects for ecological studies because closely related and ecologically similar species coexist. Sympatric species tend to show higher levels of morphological divergence compared to allopatric species.

In this study, we focus on two parthenogenetic *Darevskia* species (*Darevskia dahli* and *Darevskia armeniaca*) that share a maternal species, *Darevskia mixta*, but have different paternal species (*Darevskia portschinskii* and *Darevskia valentini*). We aim to assess the relative importance of morphologically inheritable traits and environmental conditions on head shape in these parthenogenetic species. Specifically, we aim to determine whether local adaptations or intermediate phenotypes between parental species drive head shape variation in parthenogens. To accomplish this, we analyze the head shapes of samples from both parthenogens, their paternal species, and their common maternal species from 3 sites in Georgia. Our study shows that certain aspects of head shape are associated with body size and habitat climate, with high mountain species exhibiting larger and deeper heads with stronger jaws regardless of breeding mode. In addition, both parthenogens exhibit narrower and more elongated jaw areas, flatter mandibles, and thus weaker jaws compared to females of their maternal and paternal species.

1. Introduction

Head shape is an important feature in lacertid lizards. There are relationships between the morphology of lizards (head size or any other body parts) with bite force capacity, prey handling efficiency, micro-habitat use (Herrel et al., 2004; Barros et al., 2011; Verwaijen et al., 2002; Oliveira et al., 2023), even with breathing and brain protection (De Meyer et al., 2019). Simultaneously, morphological variations in lizard heads are related to environmental and ecogeographic variations. For example, the altitude and/or temperature has been shown to affect the head length (Ortega et al., 2019) and shape (Alagić et al., 2021) in some *Podarcis* lizards; geographic gradient on Tenerife island affects the head shape of Lacertid *Gallotia galloti* (Thorpe and Baez, 1987). Besides these functions, one of the largest drivers of head shape variation are phylogenetic relationships of the species (Openshaw and Keogh, 2014; De Meyer et al., 2019). Closely related lizard species resemble each

other more than distantly related ones (Openshaw and Keogh, 2014), the phenomenon generally referred to as phylogenetic signal in morphological traits (Blomberg et al., 2003; Tarkhnishvili et al., 2020a).

When closely related ecologically similar species coexist, their morphologies tend to diverge due to competition and/or reinforcement, and the level of divergence is commonly higher compared to areas of allopatry (Taylor, 1965; McDowall, 1998; Wijesundara and Freed, 2018), e.g. fish from the genus *Paragalaxias* from a lake of submontane Tasmania (McDowall, 1998), brown frogs from the Caucasus (*Rana macrocnemis* complex) (Tarkhnishvili et al., 2001), songbirds from the genus *Zosterops* in Sri Lanka (Wijesundara and Freed, 2018).

Some lizard groups include both parthenogenetic and bisexual forms (Lowe and Wright, 1966; Darevsky, 1967; Moritz, 1991; Adams et al., 2003; Tarkhnishvili et al., 2010; Petrosyan et al., 2020). Parthenogenetic species are usually of hybrid origin and share genes from both maternal and paternal ancestors. It is not clear whether the parental

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species are able to contribute their genes to the parthenogenetic vertebrate lineages after the initial hybridization (Tarkhnishvili et al., 2020b), and whether or not the hybrid origin is reflected in the morphology of the daughter species. Parthenogenetic daughter species often do not show intermediate morphological characteristics, despite their intermediate genome (Heiser Jr et al., 1965; Bemis et al., 1970; Pembleton and Baker, 1978; Parker Jr, 1979). For example, *Aspidoscelis tessellatus* (formerly–*Cnemidophorus*) resembles more its maternal species *Aspidoscelis gularis* in size than the paternal species *Aspidoscelis tigris*, although it is more widely sympatric with the latter one.

One of the groups, combining sexually breeding and parthenogenetic species, are Caucasian rock lizards (*Darevskia*) (Arribas, 1999), found mainly in the Greater and Lesser Caucasus Mountains. Parthenogenetic species (*Darevskia dahli* and *Darevskia armeniaca*) are phenotypically similar to female individuals of their paternal species, coexist in some locations and share a similar diet in rocky habitats (Darevsky, 1967; Bakradze, 1977; Tarkhnishvili, 2012), but never overlap spatially with maternal species *Darevskia mixta* (Tarkhnishvili et al., 2010).

In this paper, we focus on two parthenogenetic *Darevskia*–*D. dahli* (Darevsky, 1957) and *D. armeniaca* (Méhely, 1909), who share the maternal species *D. mixta* (Méhely, 1909), but have different paternal species, *Darevskia portschinskii* (Kessler, 1878) and *Darevskia valentini* (Boettger, 1892). *D. dahli* and its paternal species *D. portschinskii*, coexist in the mountain forest belt of central Georgia, 800–1200 m a.s.l. (Tarkhnishvili et al., 2010; Barateli et al., 2022) and the average length of adult individuals of both species varies between 5.4 and 5.8 cm. Relatively bigger-bodied, *D. armeniaca* and its paternal species *D. valentini*, coexist at elevations 1900–3100 m a.s.l. (Arakelyan et al., 2011; Galoyan et al., 2019; Barateli et al., 2022) with average body length–6.1–6.4 cm. *D. mixta*, an endemic species of Georgia (Gabelaia et al., 2017; Petrosyan et al., 2020), is distributed from 300 to 2100 m a.s.l (Ciobanu et al., 2003; Tarkhnishvili, 2012; Gabelaia et al., 2015). The average body length of *D. mixta* varies between 4.9 and 6.3 cm (Darevsky, 1967). The paternal species are closely related, but ecologically and morphologically distinct (Darevsky, 1967; Murphy et al., 2000) (Fig. 1). For example, according to Darevsky (1967) *D. valentini* has a large central temporal scale, while in *D. portschinskii* it is smaller or absent. Also, the postorbital or postfrontal bones are similar in length in most *D. valentini* and *D. portschinskii*, but unlike *D. valentini* postocular is either greater or equal to postfrontal in *D. portschinskii* (Arribas et al., 2022).

We tried to infer the relative importance of genetic inheritance and environmental conditions on the head shape of *D. armeniaca* and *D. dahli*. Specifically, if adaptations to local geographic conditions play an important role, one should expect a higher similarity of the parthenogens with females of the paternal species, with whom they commonly coexist, than with the females of the maternal species, which never share the habitat with the parthenogens. On the other hand, the phenotype of the parthenogens may be just intermediate between the two parental species, or show some specifics common for the parthenogenetic lineages but absent in the bisexual forms. For this purpose, we analyzed the head shapes of samples of both parthenogens (*D. dahli* and *D. armeniaca*), two paternal species from the same locations

(*D. portschinskii* and *D. valentini*, respectively), and their joint maternal species, *D. mixta*, from a separate location in Georgia.

2. Material and METHODS

In June–July 2018 and 2023, we collected 60 female adult individuals of five lizard species from three locations in Georgia (Table 1). Since male and female individuals of most *Darevskia* show significant sexual dimorphism in head shape, we used only female individuals from bisexual species to compare with unisexuals (Tarkhnishvili et al., 2020a). The Ilia State University Commission ethically approved all procedures with live animals for Ethical Issues and were in accordance with Article 259 of Georgian Criminal Law. General anesthesia of the lizards was used to avoid killing the animals collected in the wild. After photographing, the lizards were released to their natural habitats.

For morphological studies, we placed the individual in a plastic tube with its head pointing upwards and secured it to prevent movement. The tube was placed in the center of a cardboard circle. We then photographed 36 times from a perspective of 90° to the midline and 36 times from a perspective of 45° to the midline, moving the camera around the lizard every 10° (Fig. 2). The images were uploaded to AgiSoft PhotoScan Pro v.2.0.2 (Agisoft, 2023), which first aligned the images in 3D space and then generated 3D models of the head surface onto which 54 homological landmarks were subsequently digitized (Fig. 3). Then we exported the 3D coordinates in a “Survey points” format and imported them in R studio (R 4.2.0, 2022) for the analysis. Missing landmarks were estimated using the function “estimate.missing” and landmarks were aligned using the “gpagen” function. We extracted symmetric components using the function “bilat.symmetry” and checked it for outliers using “plotOutliers”. To illustrate the levels of differences in head shape between the individuals we conducted a principal component analysis (PCA). PCA simplifies multidimensional data by reducing dimensions to two or three, facilitating the visualization and comprehension of relationships between data points (Richardson, 2009; Paul et al., 2013). To visualize which shape patterns were reflected in these ordinations, and thus which were the most discriminating shape differences between groups, we generated landmark wireframes that reflect principal component (PC) axis variation from a PCA on the total dataset of the 54 original landmarks in R studio (R 4.2.0, 2022). The used package was “geomorph” (Adams et al., 2016; Baken et al., 2021).

In order to test for significant differences between species head shapes, we used the Procrustes aligned landmark configurations and ran Procrustes Anova with Species as terms using “lm.rpp” in the package “RRPP” (Collyer and Adams, 2018, 2023). This function uses ordinary least squares (LS) to compute the effect coefficients by performing the randomized residual permutation (Collyer et al., 2015). Afterward, we checked if the LS means of each pair of species were significantly different using the “pairwise” function in “RRPP”.

Since studied individuals substantially differ in body size, we repeated the test and included the body size in the model as a covariate to make sure the observed interspecific differences were not influenced by allometric trends. We checked in prior the model shape ~ log(SVL) + species + log(SVL):species to make sure there is no significant difference

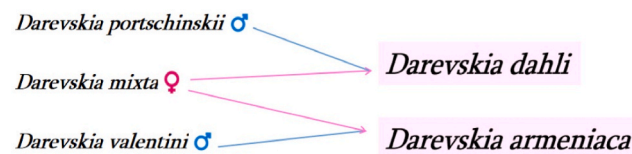


Fig. 1. Cladogram of studied species, representing the maternal parent species (*Darevskia mixta*) of both parthenogenetic species (marked in red). Based on Murphy et al., 2000; Yanchukov et al., 2022. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Sample sizes and key characteristics of three sampling locations of 5 species of *Darevskia*.

	Location	Easting	Northing	Altitude a.s.l.	Female
<i>D. dahli</i>	Kojori	44.683337°	41.648891°	1120	13
<i>D. portschinskii</i>	Kojori	44.683337°	41.648891°	1120	11
<i>D. armeniaca</i>	Sagamo Lake	43.730600°	41.293600°	2015	16
<i>D. valentini</i>	Sagamo Lake	43.730600°	41.293600°	2015	15
<i>D. mixta</i>	Rveli	43.416361°	41.871489°	824	5

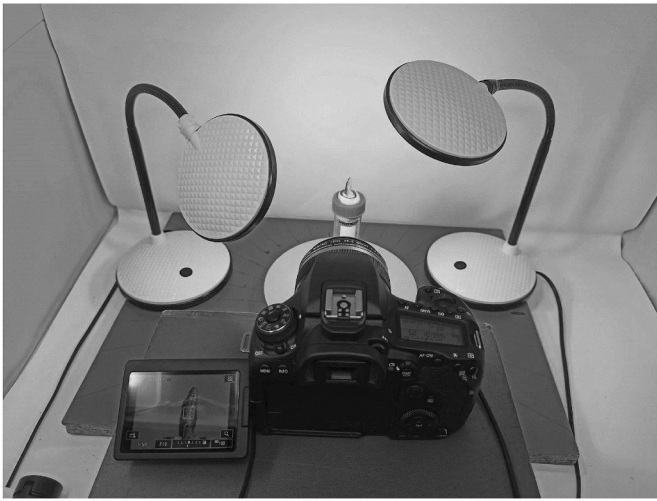


Fig. 2. Set-up for photographing the head of an immobilized lizard at different degrees and angles.

between the allometric trends of each species. After running ANCOVA, we predicted the head shape means based on the fitted values of the model and by keeping the body size associated head shape component constant. For that, we used the “predict” function in “RRPP”. All calculations were done in the R studio (R 4.2.0, 2022).

3. Results

3.1. ANOVA test on head shape

The Procrustes ANOVA and pairwise tests based on the head symmetric landmarks significantly separated all studied species pairs. According to the effect sizes (Z), females of *D. valentini* are better separated from both parthenogenetic species, *D. dahli* and *D. armeniaca* than *D. portschinskii*. *D. mixta* is in general more similar to the parthenogens than both paternal species (Table 2).

Species exhibited an allometric effect on head shape, but it was not significantly different between the studied species ($p = 0.09$), so we excluded the interaction effect ($\log\text{SVL}:\text{species}$) from the model and reran the model as $\text{shape} \sim \log(\text{SVL}) + \text{species}$. After correcting for body size, *D. mixta* and *D. armeniaca* were not significantly differentiated (Table 3). The rest of the species had a similar pattern to the previous test, e.g. *D. valentini* was most strongly separated from both parthenogenetic species, then *D. portschinskii* and *D. mixta* (Table 3).

3.2. Principle component analysis of head shape

Principal Component Analysis (PCA) resulted in five meaningful components comprising 73 % of the total variation.

Shape variation in PC1 (29 % of the total variation) was mainly concentrated in the jaw area. It varied from a narrow and elongated head shape with a flattened mandible in the jaw area (lower PC1 scores) to a wide and shortened head with a deepened mandible in the jaw area (higher PC1 scores). The PC1 axis separated *D. valentini* (higher PC scores) from *D. dahli* and *D. armeniaca* (lower PC scores), while *D. mixta* and *D. portschinskii* had intermediate scores on this axis (Fig. 4). In short,

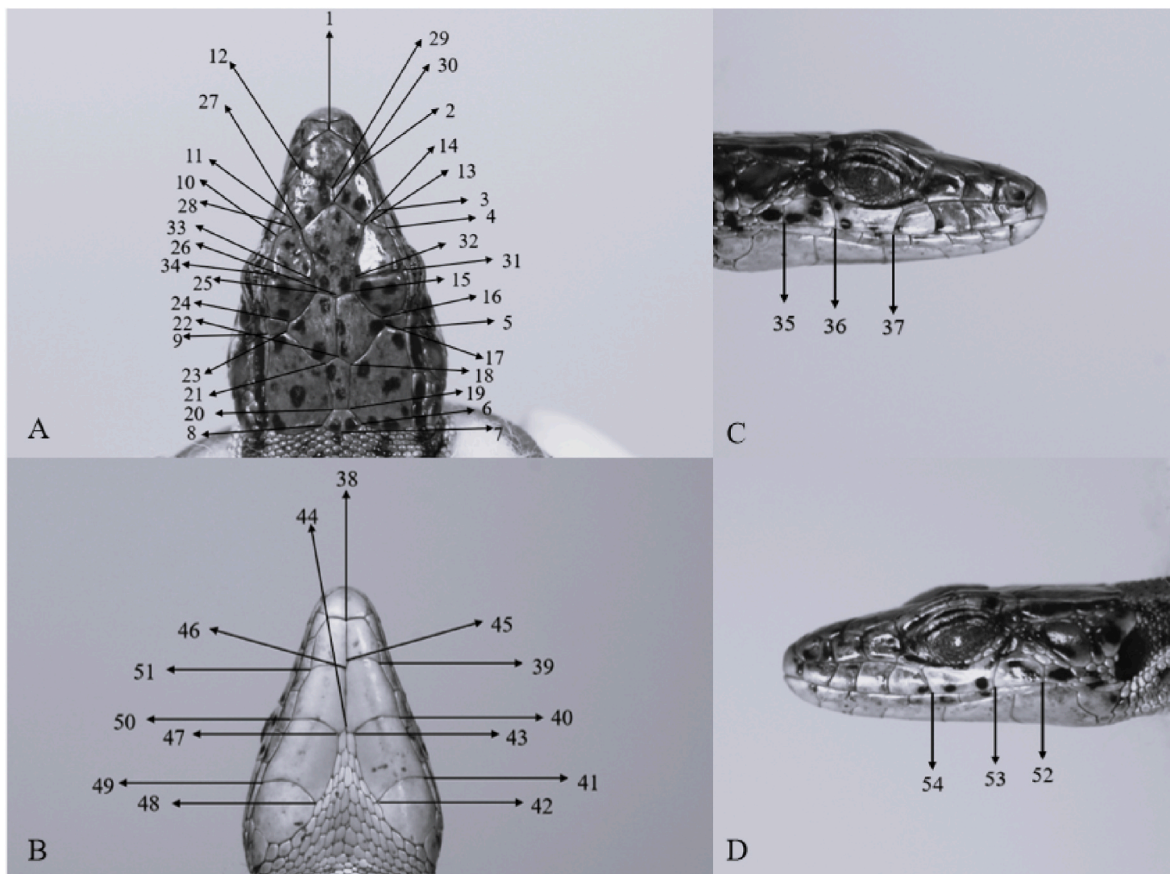


Fig. 3. Digitized three-dimensional landmarks. Landmarks are shown with black numbers. A–dorsal view of the head; B–ventral view of the head; C–view of the right profile of the head; D–view of the left profile of the head. The specimen in the photograph is *Darevskia armeniaca*.

Table 2

Results for Procrustes ANOVA for the model shape ~ species, and pairwise distances between the Least Square means on only female individuals. The statistics are performed based on randomized residual permutations. The abbreviations in the Anova table: Df–degrees of freedom, SS–Sum of Squares, MS–Mean Squares, Rsq–R-squared, F–effect size, Z–effect size which is a standard deviation of observed statistics from distributions of random outcomes, Pr(>F)–P value of the F statistics. Abbreviations in the pairwise test table: d–the difference between Species Least Square means, UCL–upper confidence limits, Pr > d–P value of the difference between Species Least Square means.

ANOVA table	Df	SS	MS	Rsq	F	Z	Pr(>F)
species	4	0.085	0.021	0.482	12.8	9.4	0.0001
Residuals	55	0.091	0.002	0.518			
Total	59	0.176					
Pairwise tests		d	UCL (95 %)	Z-effect Size	Pr > d		
<i>armeniaca:dahli</i>		0.048	0.028	3.7	0.0001		
<i>armeniaca:mixta</i>		0.054	0.039	3	0.0011		
<i>armeniaca:portschinskii</i>		0.061	0.03	4.5	0.0001		
<i>armeniaca:valentini</i>		0.067	0.028	5.1	0.0001		
<i>dahli:mixta</i>		0.051	0.04	2.7	0.0029		
<i>dahli:portschinskii</i>		0.052	0.031	3.6	0.0001		
<i>dahli:valentini</i>		0.07	0.029	5	0.0001		
<i>mixta:portschinskii</i>		0.059	0.041	3.1	0.0007		
<i>mixta:valentini</i>		0.066	0.039	3.9	0.0001		
<i>portschinskii:valentini</i>		0.062	0.03	4.4	0.0001		

Table 3

Results for the linear model shape ~ log(SVL) + species, and pairwise distances between the means on only female individuals with its statistics. The abbreviations in the linear model table: Df–degrees of freedom, SS–Sum of Squares, MS–Mean Squares, Rsq–R-squared, F–effect size, Z–effect size, Pr(>F)–P value of the F statistics. Abbreviations in the pairwise test table: d–the difference between Species Least Square means, UCL–upper confidence limits, Pr > d–P value of the difference between Species Least Square means.

Anova table	Df	SS	MS	Rsq	F	Z	Pr(>F)
logsvl	1	0.022	0.022	0.126	13.4	5.2	0.0001
species	4	0.065	0.016	0.369	9.9	7.4	0.0001
Residuals	54	0.089	0.002	0.505			
Total	59	0.176					
Pairwise tests		d	UCL (95 %)	Z-effect Size	Pr > d		
armeniaca:dahli		0.044	0.032	2.9	0.001		
armeniaca:mixta		0.043	0.045	1.4	0.0758		
armeniaca:portschinskii		0.058	0.042	2.9	0.0011		
armeniaca:valentini		0.07	0.029	4.7	0.0001		
dahli:mixta		0.049	0.039	2.6	0.0035		
dahli:portschinskii		0.053	0.032	3.4	0.0001		
dahli:valentini		0.072	0.04	3.8	0.0001		
mixta:portschinskii		0.06	0.039	3.2	0.0001		
mixta:valentini		0.064	0.054	2.3	0.0091		

both parthenogens had narrower and more elongated jaws and flatter mandibles than the females of any of their ancestral species.

PC2 (16 % of the total variation) varied from a long dorsal head with a shorter and flatter mandible and in general flat head (low PC2 scores) to a short dorsal head with a longer and deeper mandible and overall deeper head shape (high PC2 scores). It separated *D. portschinskii* and partially *D. dahli* (lower PC2 scores) from *D. armeniaca* and *D. valentini* (higher PC2 scores). *D. mixta* was grouped with *D. valentini* and *D. armeniaca*, but leaned toward intermediate scores on the PC2 axis (Fig. 4). PC2 positively correlated with the log-transformed snout–vent–length (Pearson’s R = 0.62) (Fig. 5).

PC3 (15 % of the total variation) varied from the deep head with rounded dorsal and deep mandible (low PC3 scores) to the flatter head and wide and flatter mandible (high PC3 scores). PC4 (8 % of the total variation) varied from a shorter and laterally more curved (outwards) mandible and narrow dorsal head (low PC4 scores) to a longer and inward-curved mandible and wider dorsal head (high PC4 scores).

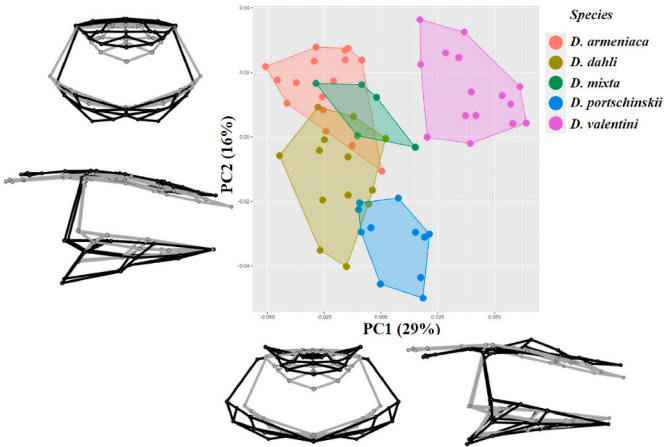


Fig. 4. Principal component analysis (PCA) score plot. Black lines represent maximum PC values and gray–minimum PC values. PC1–shows that *D. valentini* has a wider head, while according to PC2–*D. dahli* and *D. portschinskii* have longer and flatter heads, with slightly wider jaw areas. The numbers in the parentheses represent the percentage from the total variation for each axis.

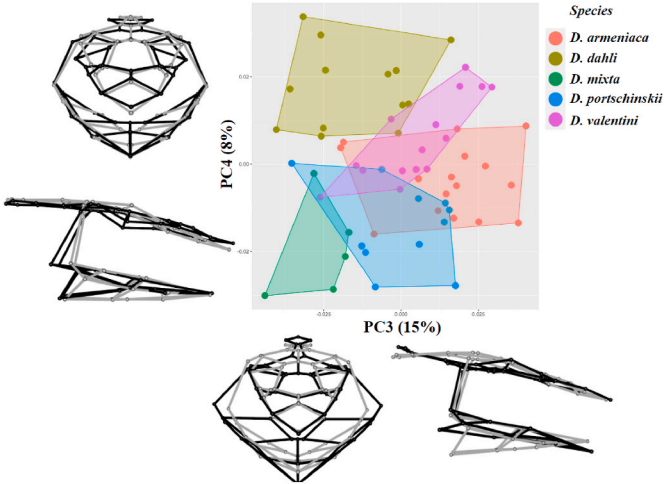


Fig. 5. Principal component analysis (PCA) score plot. PC3 overlaps in all species. PC4 shows that *D. dahli* has a longer and flatter mandible and a larger/broader interparietal scale compared to the other four species. The numbers in the parentheses represent the percentage from the total variation for each axis.

Combined, PC3 and PC4 axes separated *D. mixta* from both parthenogens and *D. valentini*, with *D. portschinskii* keeping the intermediate position (Fig. 5).

To visualize the morphospace after excluding the effect of body length on head shape, we used the fitted values from the model shape ~ log(SVL) + species and predicted the 95 % confidence intervals for the mean shape of each species, by keeping the body length effect on the head shape constant (Fig. 6). The 73 % of the total allometry–free shape variation showed that maternal *D. mixta* is much closer based on the head shape to the parthenogenetic species, than paternal *D. valentini* and *D. portschinskii*. Also, *D. valentini* and *D. armeniaca* are less similar to each other than *D. portschinskii* and *D. dahli* (Fig. 6).

4. Discussion

To summarize our results, there is no clear reflection of mixed genetic ancestry in the head shape of the parthenogens, although they have more affinity to females of the maternal, than of the paternal species. Simultaneously, there are certain specifics of head shape associated with

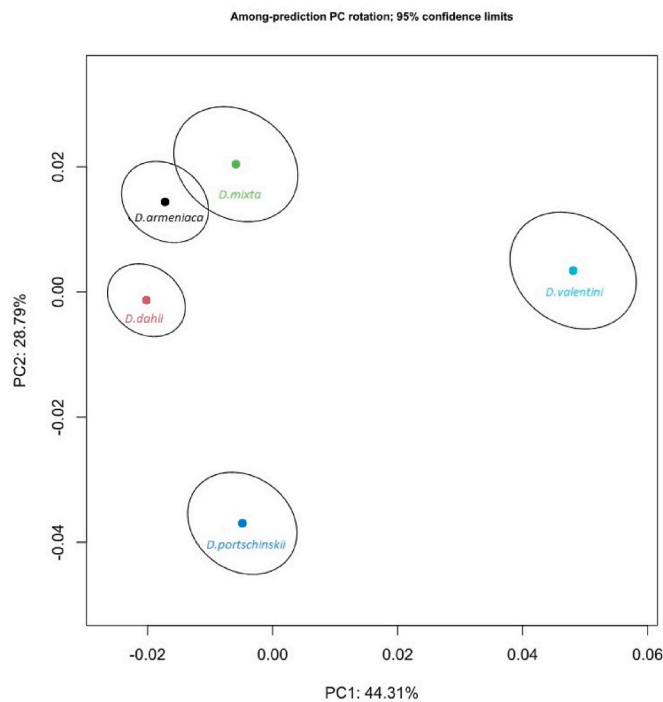


Fig. 6. 95 % confidence ellipses of the predicted values for species means generated by bootstrapped residuals. Residuals are derived from the model fit with the formula: shape $\sim$ log(SVL) + species.

body size and/or climate of the habitat (high mountain species are bigger irrespective of the breeding mode and have deeper heads with stronger jaws). Besides, both parthenogens have narrower and more elongated jaw areas with flatter mandibles, hence weaker jaws than females of their respective paternal and maternal species. For example, *D. armeniaca* from Sagamo Lake have stronger jaws and deeper heads than low elevation inhabiting species, but weaker (narrower) jaws compared to females of paternal *D. valentini*, which also live at Sagamo Lake.

The morphological variation of lizard species is influenced by genetic (e.g. gene drift, mutations) as well as environmental factors (e.g. temperature, humidity, food availability, etc.) (Runemark et al., 2010; Oliveira et al., 2023). According to Adams (2011), despite shared selection pressures, different genetic patterns could drive unique head–shape evolution in *Plethodon* salamanders. If the environment has a substantial impact on head shape in *Darevskia*, we should expect higher similarity of the parthenogens with females of the paternal species, with whom they commonly coexist, than with the females of the maternal species, with whom they never share the habitat. Simultaneously, the previous morphological studies on parthenogenetic hybrids show that unisexual species tend to exhibit an intermediate morphological resemblance to either both parental species or a closer resemblance to one of them (Espeche et al., 2023). According to studies on parthenogenetic *Teius suquiensis*, it demonstrated intermediate morphological traits compared to bisexual *T. teyou* and *T. oculatus* (Espeche et al., 2023), whereas parthenogenetic *A. tessellatus* is morphologically more similar to its maternal than to the paternal species (Parker Jr, 1979).

Our study suggested a complex interaction of inheritance and environment in the determining head shape of the parthenogens. The general shape, relative size and depth of the head, and size of jaws are correlated with the body size of the individuals, which in turn is under the strong influence of climate and elevation (Darevsky, 1967). Indeed, body size growth with the elevation and related temperature and precipitation is shown for many *Darevskia* species, including the studied *D. valentini*, and other *Darevskia*, such as *Darevskia caucasica*, *Darevskia*

parvula (Darevsky, 1967; Volynchik, 2014; Bülbül et al., 2016) and some other lizard species, such as *Podarcis liolepis* (Ortega et al., 2019), *Lio-laemus* spp. (Pincheira-Donoso et al., 2008). This is most likely a simple pattern following Bergmann's rule (1847), suggesting that a decrease in surface/volume ratio makes body temperature better controlled because heat loss is slower. However, this is not the sole trend in lizards (Ashton and Feldman, 2003), e.g. lacertid *Eremias argus* shows the reverse of Bergmann's rule (Deme et al., 2023). Many squamates at higher altitudes have larger individuals of the same species than at lower altitudes. Obviously, head shape is linked with body size with allometric dependencies, which causes the inevitable shape change following the size change.

4.1. Differences between bisexuals and parthenogens

Even though there were slight differences in the sizes of *D. dahli* (SD=0.35) with *D. portschinskii* (SD=0.40) and *D. armeniaca* (SD=0.32) with *D. valentini* (SD=0.36), in addition to the latter two being much larger than the former two, the differences between the parthenogens and paternal species' females were clearly expressed in the degree of head and mandible width. The parthenogens have much narrower and elongated heads with flatter mandibles, whereas the paternal species have a stronger head shape, especially *D. valentini*. Moreover, these differences account for nearly one-third of the total variation in head shape, whereas body size-associated changes explain only 16 % of the variation. One potential reason for that could be behavioral differences between the parthenogens and their bisexual ancestors. Specifically, it was repeatedly shown that both males and females of bisexual rock lizards perform territorial behavior, forcing conspecific females to avoid occupied individual plots (Galoyan, 2011; Galoyan, 2013; Tselariou et al., 2016; Barateli et al., 2021) and, wider jaws lead to stronger bites (Kohlsdorf et al., 2008; De Meyer et al., 2019), which is obviously important for successful defense of the territory. In contrast, parthenogens do not display territorial behavior, and having broad and strong jaws is way less important. Although the strength of the jaws may also be related to the size and morphology of most commonly consumed food organisms (Verwajen et al., 2002), this is an unlikely explanation of the differences between coexisting parthenogenetic and paternal lizards, since the lizards are not specialized feeders and just consume invertebrates that are present in a specific habitat (Darevsky, 1967).

The impact of shared ancestry on the morphology of the parthenogens is way less clear. The body size of *D. armeniaca* and *D. dahli* is closer to their respective parental species than to the body size of the parental species of the other parthenogen; however, this can equally be a result of the concurrent adaptation of a parthenogen and its parental species to a similar environment. Procrustes ANOVA analysis showed a higher similarity of size-removed head shape of the parthenogens, especially that of *D. armeniaca*, to their maternal *D. mixta* than to the females of the patrilineal ancestors. A stronger maternal effect on the head shape is recorded for some other squamates, including parthenogenetic lizard *A. tessellatus* (Parker Jr, 1979) or meadow viper (*Vipera ursini rakosiensis*) (Oliveira et al., 2023). Noble et al. (2014) had shown experimentally that maternal effect rather than simple Mendelian inheritance determines body size and some behavioral characteristics in skinks. Maternal effect can explain the higher similarity of the parthenogens to their matrilineal than to the patrilineal ancestors. On the other hand, females of some of the parental species may be less territorial than the others, dependent on their habitat type (e.g. *D. mixta* is more broadly using various microhabitat types than *D. valentini* and especially *D. portschinskii*, which are strict rock dwellers (Darevsky, 1967; Tarkh-nishvili, 2012)), and parallel behavioral adaptations could explain different degree of similarity between the parthenogens and their ancestors rather than direct ancestral influence. Detailed behavioral observations on these species may shed light on this question in the future.

In summary, some characteristics differentiate the parthenogens from their bisexual ancestral species, and others are associated with

body size and elevation of typical habitats. Although the effect of maternal species is also likely present, the impact of intermediate genetics or habitat type on head shape is shaded by the differences between the reproductive biology, which is not associated with aggressive territorial behavior in parthenogens, in contrast with their bisexual ancestors.

CRedit authorship contribution statement

Natia Barateli: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Mariam Gabelaia:** Writing – review & editing, Visualization, Supervision, Software, Methodology, Formal analysis, Conceptualization. **Giorgi Iankoshvili:** Writing – review & editing, Investigation, Data curation. **David Tarkhnishvili:** Writing – review & editing, Methodology, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Natia Barateli reports administrative support and equipment, drugs, or supplies were provided by Ilia State University. Natia Barateli reports a relationship with Ilia State University that includes: employment. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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RESEARCH ARTICLE

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Genotypic similarities among the parthenogenetic *Darevskia* rock lizards with different hybrid origins

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Abstract

Background: The majority of parthenogenetic vertebrates derive from hybridization between sexually reproducing species, but the exact number of hybridization events ancestral to currently extant clonal lineages is difficult to determine. Usually, we do not know whether the parental species are able to contribute their genes to the parthenogenetic vertebrate lineages after the initial hybridization. In this paper, we address the hypothesis, whether some genotypes of seven phenotypically distinct parthenogenetic rock lizards (genus *Darevskia*) could have resulted from back-crosses of parthenogens with their presumed parental species. We also tried to identify, as precise as possible, the ancestral populations of all seven parthenogens.

Results: We analysed partial mtDNA sequences and microsatellite genotypes of all seven parthenogens and their presumed ancestral species, sampled across the entire geographic range of parthenogenesis in this group. Our results confirm the previous designation of the parental species, but further specify the maternal populations that are likely ancestral to different parthenogenetic lineages. Contrary to the expectation of independent hybrid origins of the unisexual taxa, we found that genotypes at multiple loci were shared frequently between different parthenogenetic species. The highest proportions of shared genotypes were detected between (i) *D. sapphirina* and *D. bendimahiensis* and (ii) *D. dahli* and *D. armeniaca*, and less often between other parthenogens. In case (ii), genotypes at the remaining loci were notably distinct.

Conclusions: We suggest that both observations (i-ii) can be explained by two parthenogenetic forms tracing their origin to a single initial hybridization event. In case (ii), however, occasional gene exchange between the unisexual and the parental bisexual species could have taken place *after* the onset of parthenogenetic reproduction. Indeed, backcrossed polyploid hybrids are relatively frequent in *Darevskia*, although no direct evidence of recent gene flow has been previously documented. Our results further suggest that parthenogens are losing heterozygosity as a result of allelic conversion, hence their fitness is expected to decline over time as genetic diversity declines. Backcrosses with the parental species could be a rescue mechanism which might prevent this decline, and therefore increase the persistence of unisexual forms.

Keywords: *Darevskia*, Parthenogenesis, Microsatellites, Mitochondrial DNA, Backcrosses, Allele conversion, Caucasian rock lizards

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Background

Parthenogenesis has both advantages and shortcomings compared to sexual reproduction [1–10]. One significant advantage of parthenogenesis relative to sexual reproduction is the absence of males, which allows the allocation of more resources into the production of offspring [5, 6]. On the other hand, the lack of genetic recombination leads to an accumulation of deleterious mutations in the genome of parthenogens, whereas in sexual breeders, recombination and natural selection can eliminate recessive deleterious mutations [8, 11, 12]. All this, coupled with the lack of novel recombinant genotypes, dramatically reduces the ability of the parthenogens to adapt to changes in the environment [5, 7, 10]. Occasional true sex, even if it occurs only once in many thousands of generations, solves these problems; therefore, it is an effective reproduction strategy [8]. The fact that parthenogenesis with occasional true sex is found throughout the tree of life, being pervasive in plants, prokaryotes, protists, insects, and multiple other phyla [1, 13–18], supports the advantage of this form of reproduction.

Unlike plants and invertebrates, parthenogenesis in vertebrates is rare, and parthenogenetic vertebrates are usually unable to enrich their genomes by occasional sex. There are only a few exceptions: 1) A gynogenetic Amazon molly fish (*Poecilia formosa*) exhibits recombination, which has occurred after the initial hybridization [19, 20]. 2) The North American salamanders, *Ambystoma*, are also gynogenetic and can incorporate genetic elements received horizontally from closely related sexual species [21]. Both systems appear to be among the oldest vertebrate parthenogenetic lineages, with an estimated age of ~ 5 Mye [20, 22].

In all known cases of unisexual species of reptiles, parthenogenesis appears to be obligate [23], and no gene exchange with sexually reproducing species has been documented (although hypothesized [24]). Parthenogenetic forms are most common among the squamates (19 genera [25]). For all but one of these genera, parthenogenesis results from interspecific hybridization [25, 26]. Consequently, such unisexual species initially possess highly heterozygous genotypes, and “freezing” this high initial diversity enables remarkable evolutionary persistence of many parthenogenetic vertebrates [23, 27, 28]. In some groups, hybrid parthenogens are diverse and occupy large geographic areas: the North American whip-tail lizards (*Aspidoscelis*) and Caucasian Rock Lizards (*Darevskia*) have produced multiple unisexual lineages in parallel [29–37].

Caucasian rock lizards are the first group of vertebrates where parthenogenesis was discovered. Lantz & Cyren [38] were among the first who noted the absence of males in some populations of rock lizard *Lacerta saxicola armeniaca* (later designated *Darevskia armeniaca*),

from Armenia. Darevsky [39] documented locations where only females were present and suggested they were obligate parthenogens. In his 1967 treatise, he described four parthenogenetic forms (subsequently given species status) from Armenia and Georgia [40]: *D. armeniaca*, *D. dahli*, *D. unisexualis*, and *D. rostombekowi*. Later, another species from Turkey was discovered, *D. uzzelli* [41]. Finally, two new parthenogenetic forms, *D. sapphirina* and *D. bendimahiensis* were described from the vicinity of Lake Van in Turkey [42]. All these parthenogens live in the area between the Lesser Caucasus Mountains and Lake Van (Fig. 1).

All parthenogenetic *Darevskia* descend from hybridization between bisexual representatives of two clades within the genus: the maternal ancestry comes exclusively from the *caucasica* clade (*D. mixta*, *D. raddei*) and the paternal ancestors from the *rudis* clade (*D. valentini*, *D. portschinskii*). These two clades separated between ten [35] and 25 million years ago [43, 44]. *D. armeniaca* and *D. dahli* share mitochondrial DNA descended from the Western Caucasian *D. mixta*. Five other unisexuals: *D. unisexualis*, *D. rostombekowi*, *D. uzzelli*, *D. bendimahiensis*, and *D. sapphirina* descended matrilineally from at least two distinct populations of *D. raddei*, currently found south of the Lesser Caucasus Mountains [36, 45]. *D. mixta* and *D. raddei*, although they belong to the same clade within *Darevskia*, are genetically distinct, and they are not even sister species; hence the parthenogens matrilineally associated with *D. raddei* and *D. mixta* are also distinctive, and their mitochondrial haplogroups are reciprocally monophyletic [45].

The details of patrilineal ancestry are less clear. The bisexual species *D. portschinskii* shares the highest proportion of allozyme alleles with *D. dahli* and *D. rostombekowi*, and is partially sympatric with both of them in the eastern Lesser Caucasus. *D. valentini* from the higher altitudes south and west of the Lesser Caucasus has the highest number of allozyme alleles shared with five other parthenogens [45]. The use of small sample sizes genotyped for allozyme markers with low allelic diversity was a significant limitation in the latter study, and did not allow detailed resolution of a reticulate speciation scheme (Fig. 1 in [45]). Disentangling the paternal ancestral contribution is further complicated because, unlike *D. mixta* and *D. raddei* on the maternal side, *D. portschinskii* and *D. valentini* exchange genes via introgression between themselves and with other species within the *rudis* clade [46].

For decades, the number of hybridization events that led to a series of parthenogenic forms in *Darevskia* has been subject to debate. Parker et al. [47] did not exclude the occasional presence of more than one clonal hybrid lineage within the same taxon. Later publications also indirectly suggest polyclonality: Fu et al. [48] discovered

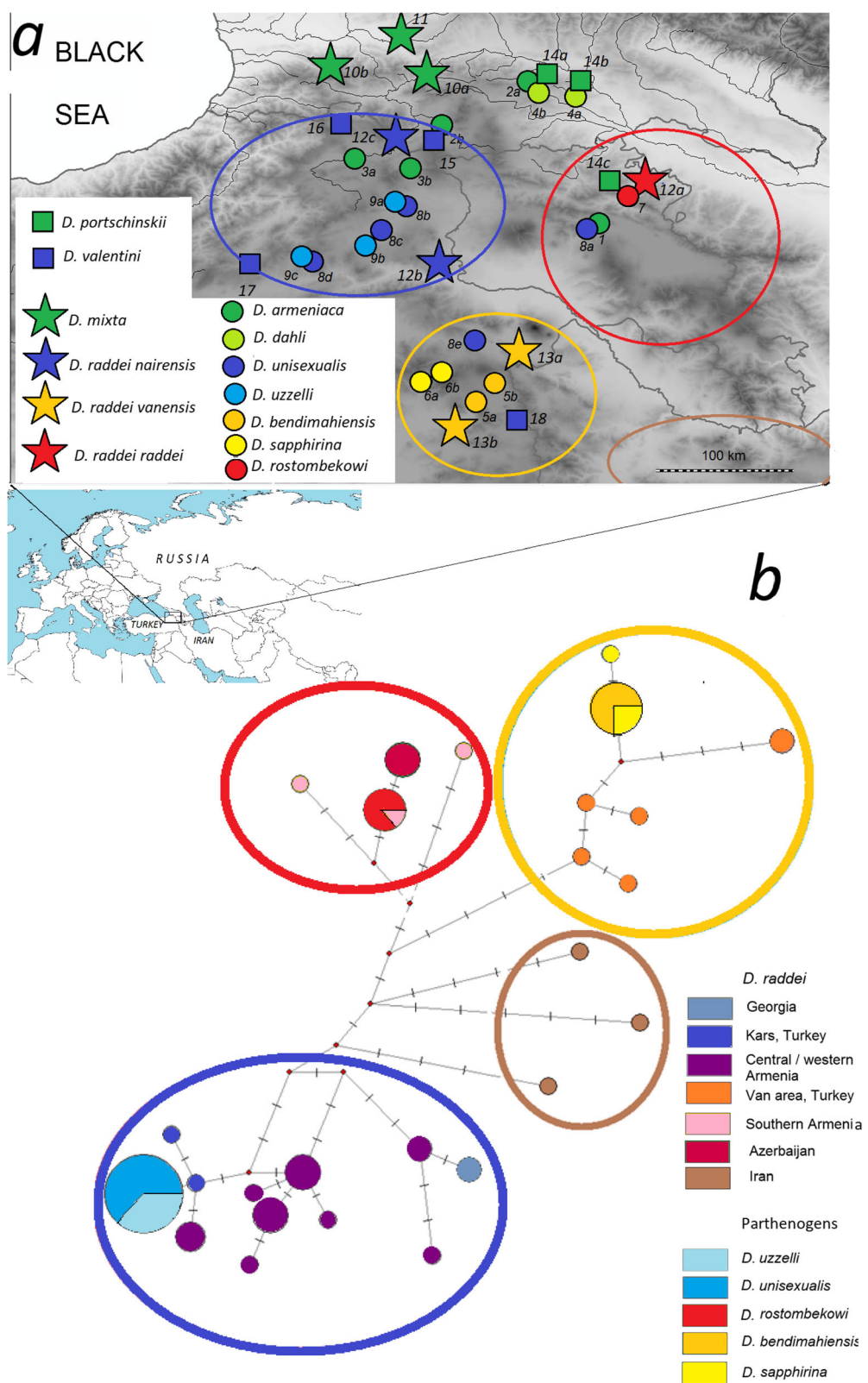


Fig. 1 (See legend on next page.)

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Fig. 1 Map of sampling locations (a) and Median Joining network linking *D. raddei* and its daughter parthenogenetic forms (b). Location numbers (same as in Table 3) are shown on the map. Parental species in boldface. *D. armeniaca*: 1 - Hrazdan, Armenia; 2a - Didgori, Georgia; 2b - Khosplio, Georgia; 3a - Ardahan, Turkey; 3b - Çıldır, Turkey; *D. dahli*: 4a - Kojori, Georgia; 4b - Didgori, Georgia; *D. bendimahiensis*: 5a - Muradiye, Turkey; 5b - Çaldıran, Turkey; *D. sapphirina*: 6a - Patnos, Turkey; 6b - Pınarlı, Turkey; *D. rostombekowi*: 7 - Dilijan, Armenia; *D. unisexualis*: 8a - Hrazdan, Armenia; 8b - Hanak, Turkey; 8c - Horasan, Turkey; 8d - River Ağrı, Turkey; *D. uzzelli*: 9a - Kars, Turkey; 9b - Sarıkamış, Turkey; 9c - Horasan, Turkey; *D. mixta*: 10a - Akhaldaba, Georgia; 10b - Abastumani, Georgia; 11 - Ambrolauri, Georgia; *D. raddei raddei*: 12a - Sotk Village, Armenia; *D. raddei nairensis*: 12b - Digor, Turkey; 12c - Vardzia, Georgia; *D. raddei vanensis*: 13a - Doğubeyazıt, Turkey; 13b - Muradiye, Turkey. *D. portschinskii*: 14a - River Khrami, Georgia; 14b - Kojori, Georgia; 15 - Mets Sepasar, Armenia; *D. valentini*: 15 - Akhalkalaki, Georgia; 16 - Ardahan, Turkey; 17 - Erzurum, Turkey; 18 - Çaldıran, Turkey. Circles of the same color are delimiting sampling areas in (a) and related haplogroups (b). Brown circle delimits haplogroups of *D. raddei* from Iran downloaded from GenBank, unrelated to any known parthenogenetic form, and hence not included in further analyses. The Caucasus Map was prepared in software QGIS (<http://qgis.osgeo.org>). Eurasia's map at the bottom of the figure is modified from World Map Blank.svg (Public domain)

allozyme variation within *D. unisexualis* (three genotypes), *D. uzzelli*, and *D. bendimahiensis* (two genotypes each). Polymorphisms were also recorded for *D. armeniaca* [36, 49, 50], *D. dahli* [51, 52], *D. unisexualis* [37, 48], *D. rostombekowi* [53], *D. uzzelli* and *D. bendimahiensis* [48]. Vergun et al. [54], Ryskov et al. [53], and Girnyk et al. [55] examined the nucleotide sequence variation in amplicons containing the microsatellite repeats, in *D. dahli*, *D. rostombekowi*, and *D. armeniaca*. The variation in the repeat lengths at individual loci was attributed to mutations within the clonal parthenogenetic lineages, while the single-nucleotide variants in the regions flanking the repeats were assumed to be due to independent hybridization events. However, no formal model-based criteria were applied to distinguish between multiple hybridizations vs mutation accumulation scenarios, which cannot be excluded *a priori* [54, 56, 57].

Although the possibility of ongoing gene flow from a parental species into the parthenogenetic forms of reptiles is usually not considered, it can not be excluded completely [24]. Darevsky and Kulikova [58] mentioned the presence of hybrids between parthenogenetic *D. armeniaca* and sexually reproducing *D. valentini*; all of them were triploid, and females were sterile [58–60], although later some triploids with fully developed gonads were found [61–63]. Freitas et al. [37] genotyped multiple triploid individuals in sympatric populations of *D. valentini*, *D. armeniaca*, and *D. unisexualis*, but ruled out gene introgression from the presumed paternal species into parthenogenetic lineages. Triploid individuals were also found where parthenogenetic *D. dahli* and *D. armeniaca* are sympatric with *D. raddei* in Armenia [60, 64, 65].

Tarkhnishvili et al. [24] studied multiple genotypes at five microsatellite loci in *D. armeniaca*, *D. dahli*, their maternal ancestor *D. mixta*, and anticipated paternal ancestors *D. portschinskii* and *D. valentini*. The majority of individuals of both parthenogens had coincident genotypes at two loci and different most-frequent genotypes at the three other loci. The authors concluded that the most plausible explanation of this pattern is rare backcrossing that leads to the integration of parts of a different paternal

genome into the genome of existing parthenogenetic form; hence, *D. armeniaca* is a result of backcross between parthenogenetic *D. dahli* with *D. valentini*, and not a hybrid between *D. valentini* and *D. mixta*.

In summary, unexpectedly high levels of genetic variation, and apparent post-hybridization reorganization and redistribution of alleles in *Darevskia* contradict the standard theory, which predicts relative genomic stasis. Coinciding microsatellite genotypes in *D. dahli* and *D. armeniaca* and high genetic diversity within these and other unisexual forms raise questions on the possible role of interbreeding between parthenogenetic and bisexual lizards in the diversification of parthenogenetic *Darevskia* (and, perhaps, other groups of parthenogenetic lizards).

In the present study, we aimed to clarify some important questions about the origin and population genetic structure of parthenogenetic *Darevskia*. We analyze the microsatellite genotypes and mitochondrial haplotypes in all seven parthenogenetic species of rock lizards from Turkey, Georgia, and Armenia, as well as in their presumed parental populations. We attempt to identify, as precisely as possible, maternal and paternal source populations of each parthenogen. While several studies with similar goals were conducted earlier [36, 45], including a recent one [37], none of them included all parthenogenetic *Darevskia* species, nor did they sample the ranges of the ancestral species on the scale presented here. Importantly, we examined the evidence for (i) a single initial hybridization event in the origin of each parthenogenetic *Darevskia*, a hypothesis shared by most authors [35, 37, 45] and (ii) an alternative hypothesis, that posits new parthenogenetic forms may have resulted from the backcross of a hybrid parthenogen with a paternal bisexual species [24]. Our results suggest that the origin and evolution of the parthenogenetic forms of *Darevskia* are far more complicated than considered earlier. The genetic similarities between some parthenogenetic lineages can hardly be explained solely by coincidence. Simultaneously, we exclude the multiclonal origin of any of the described parthenogenetic species of *Darevskia*.

Results

Matrilineal ancestry of the parthenogens

The best-fit substitution model (that with the lowest BIC score) for all samples of the parthenogens and their presumed ancestral species was HKY + G, assuming variable base frequencies, stable transition rates, and stable transversion rates. The Maximum Likelihood tree (Fig. 2) based on the analysis of 683 bp of mitochondrial cytochrome b gene, both of the novel samples and those downloaded from GenBank showed the presence of three well-supported clades within *D. raddei* and its daughter parthenogens: 1) *D. raddei* from southern Georgia and the Kars

area in Turkey (nominal subspecies *D. r. nairensis* and *D. r. raddei*), which also includes all individuals of *D. unisexualis* and *D. uezelli*. One sequence from GenBank marked as “*D. rostombekovi*” (GenBank #MH247113) was also clustered with this clade. However, this might be a result of an error in species identification, since all other *D. rostombekovi* belong to a different clade. This sequence was excluded from the rest of the analyses.) 2) a clade including *D. raddei vanensis*, *D. bendimahiensis*, and *D. sapphirina*; and 3) a clade including individuals from southern Armenia and Azerbaijan, which also includes *D. rostombekovi* (colored boxes in Fig. 2).

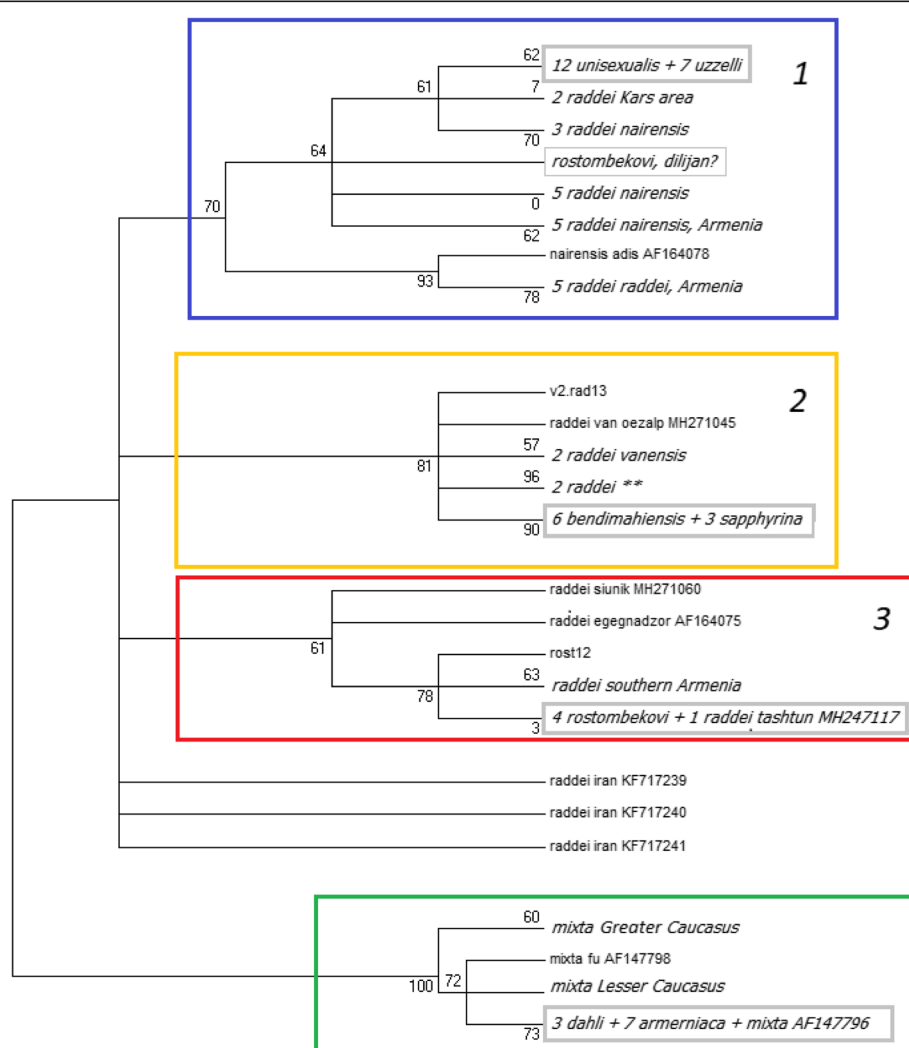


Fig. 2 Maximum Likelihood 50% consensus rule tree of haplotypes associated with different populations *D. raddei* and five its parthenogenetic daughter species, based on the HKY + G model. The 683 bp fragment of the mitochondrial *Cytochrome b* gene is used. The tree with the highest log likelihood (−799.7117) is shown. The tree is based on 42 novel sequences and 62 sequences downloaded from GenBank. Bootstrap values exceeding 50% are shown on the nodes and branches. Individual clades are shown in color boxes. Clade 1 (blue box) – *D. r. nairensis* and *D. r. raddei* from the northernmost part of the species range and their daughter parthenogenetic species; clade 2 (yellow box) – *D. r. vanensis* and its daughter parthenogenetic species. Clade 3 (red box) – *D. r. raddei* from southern Armenia and Azerbaijan and their daughter parthenogenetic species; *D. mixta* and its daughter parthenogens (green box) are used as an outgroup for *D. raddei* and its daughter parthenogens. For the relative position of *D. raddei* and *D. mixta* in the phylogenetic tree of *Darevskia*, see [45, 66]

The published sequences of *D. raddei* from Iran belonged to three additional lineages in a polytomy. The individuals of *D. mixta* were from both the Greater and the Lesser Caucasus lineages [67] and all *D. armeniaca* and *D. dahli* are clustered with the Lesser Caucasus clade.

The median-joining network of all individuals (Fig. 1b) revealed 24 haplotypes of *D. raddei*, seven of *D. mixta*, and seven of their daughter species. Six for those descending from the maternal lineages of *D. raddei* and one of *D. mixta* (a previous study that analyzed shorter sequences of many more *D. dahli* and *D. armeniaca* revealed the presence of multiple haplotypes of these species, although the majority were identical to that described in [24]). There is little variation among individuals of the same parthenogenetic form. Simultaneously, (1) most of the individuals of *D. armeniaca* and *D. dahli* share the same haplotype, which is also present in a single individual of *D. mixta* from Borjomi Gorge (see also [24]; (2) all individuals of *D. uzzelli* and *D. unisexualis* share the same haplotype, which is close to the haplotypes of *D. raddei* from Digor near Kars, Turkey (subspecies *D. r. nairensis*); (3) all *D. rostombekowi* share the same haplotype, which is also recorded in *D. raddei* from Tashtun in Southern Armenia (subspecies *D. r. raddei*); (4) all *D. bendimahiensis* samples, and all but one *D. sapphirina* share a common haplotype closest to the haplotype of *D. raddei* from Doğubeyazit and Özalp in the Lake Van area, Turkey (subspecies *D. r. vanensis*, Fig. 1b). In summary, parthenogens that matrilineally descend from *D. raddei* stem from at least three geographically distinct lineages of this species.

Genetic diversity of parthenogens and their putative ancestors

The entire data set of microsatellite genotypes is presented in Table S1. As expected, genetic diversity of all parthenogenetic forms is lower than in their putative paternal and maternal species; the average allelic richness [68] in the parthenogens is 1.9 times lower than in their presumed ancestors (Fig. 3a); this difference was significant (t-test, $P = 0.0018$). Among the parthenogens, allelic richness was the lowest in *D. rostombekowi* (16.34) and the highest in *D. uzzelli* (24.33). In all parthenogenic lineages, observed heterozygosity was higher than expected under Hardy-Weinberg equilibrium in a sexually breeding population with similar allele frequencies. These results are consistent with the assumption of their hybrid origins: *D. uzzelli* had the smallest excess of observed heterozygosity, and *D. unisexualis* had the highest.

In sexually breeding species, some deficit of heterozygotes was observed, consistent with the expectation of the Wahlund effect [69] among geographically isolated populations (Fig. 3b). The unexpected result was that all parthenogenetic forms had relatively high proportions of

homozygous loci. The highest proportion of homozygous loci was found in parthenogenetic *D. rostombekowi* (60%); while in *D. bendimahiensis* and *D. sapphirina*, the proportion of homozygous loci exceeded 50% (Fig. 3c); Table S1.

Bayesian inference: clustering of the parthenogens with sexually breeding species

For sexually reproducing species, the highest ΔK was observed at $K = 3$, while the BSRK method showed the highest support for $K = 7$ (Fig. 4). Clustering sexually breeding individuals at $K = 3$ separated *D. mixta*, *D. raddei*, and a third group comprised of *D. portschinskii* and *D. valentini* individuals from different geographic populations. At $K = 7$, the clustering procedure separated (1, 2) *D. mixta*; *D. raddei* from (3) Armenia and Kars area, and (4) the Lake Van area; (5) *D. portschinskii*; *D. valentini* from (6) southern Georgia and Ardahan area and (7) from the Lake Van area. The separation of the clusters was imperfect, probably due to the presence of ancestral polymorphisms and / or convergent STR alleles (Fig. 5).

At $K = 3$, we estimated different proportions of each of the three clusters in each parthenogenetic form. The two most common clusters in *D. armeniaca* and *D. dahli* were associated with *D. valentini* + *D. portschinskii* and *D. mixta*. A small proportion of *D. armeniaca* ancestry was attributed to *D. raddei* (Fig. 5).

At $K = 7$, *D. dahli* and *D. rostombekowi* shared 30–40% of ancestry with *D. portschinskii*, but the latter also had a substantial proportion of ancestry shared with *D. valentini* from the Lake Van region. The latter geographic population also contributed to *D. sapphirina*, *D. bendimahiensis*, and *D. unisexualis*, whereas *D. armeniaca* and *D. uzzelli* had more shared ancestry with *D. valentini* from southern Georgia and neighboring parts of Turkey (Ardahan). *D. sapphirina*, *D. bendimahiensis*, and *D. unisexualis* shared most of their ancestry with *D. raddei vanensis*, whereas *D. armeniaca* and *D. uzzelli* appeared to be more associated with *D. raddei nairensis* and *D. r. raddei* (the two latter groups were not distinguishable at $K = 7$).

All parthenogenetic forms had the most considerable ancestry fraction shared with their presumed parental species, but also possessed a significant contribution from genetic variation associated with other *Darevskia* from the same geographic area (Fig. 5).

Non-model-based analysis of microsatellite genotypes in parthenogens

Genotype diversity

As would be expected in the absence of sexual reproduction in parthenogenetic populations, in each unisexual species we identified 1–3 common genotypes, and a larger number of rare genotypes, that only differed by a few alleles at the individual microsatellite loci (Table 1). The highest diversity of genotypes was

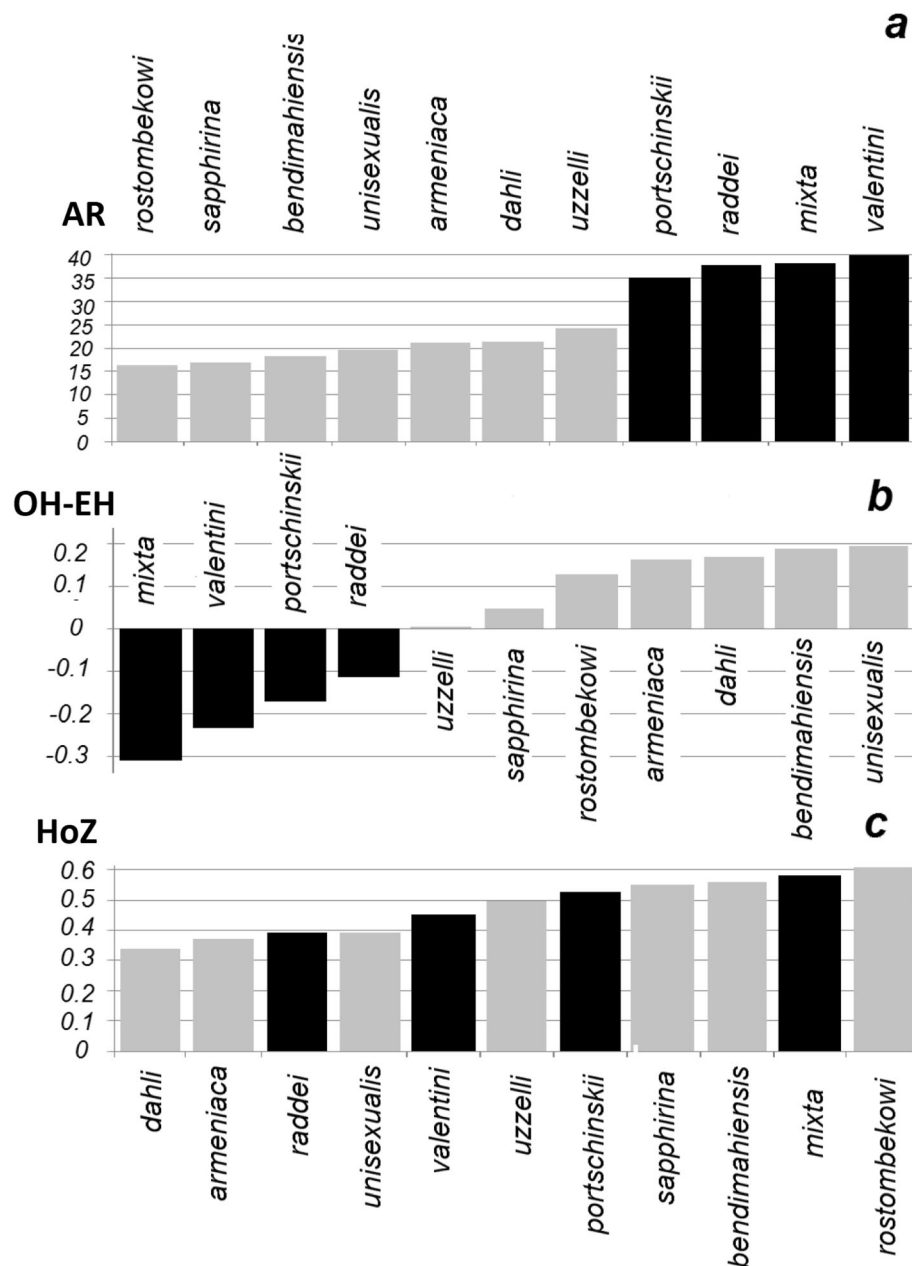


Fig. 3 **a** Allelic richness (the value of the index of Petit et al. [67]) (AR); **b** the difference between the observed and expected heterozygosity (OH – EH); **c** and the overall proportion of homozygous alleles (HoZ). Parthenogenetic taxa (grey) and their presumed ancestral species (black)

observed in *D. armeniaca* and the lowest in *D. sapphirina*. The frequency of the single most frequent genotype varied between 0.207 in *D. dahli* and 0.533 in *D. sapphirina*; the cumulative proportion of the two most common genotypes varied between 0.414 in *D. dahli* and 0.800 in *D. sapphirina*.

The minimum spanning networks, based on averaged pairwise Bruvo distances between individual microsatellite genotypes, are shown on the NJ cladogram in Fig. 6a and b. There are two main clusters, corresponding to the groups

matrilineally descended from *D. raddei* and from *D. mixta*. Within the *D. raddei*-derived cluster, the species with common matrilinear ancestry from *D. r. vanensis* (*D. bendimahiensis* and *D. sapphirina*) formed a tight group with the individual distances between the two species comparable to those within *D. unisexualis*. At the same time, the two parthenogenetic species with mitochondrial ancestry stemming from *D. r. nairensis* (*D. unisexualis* and *D. uzzelli*) did not form a single cluster. Instead, *D. unisexualis* appeared to be much closer to *D. rostombekowi* than to *D. uzzelli* (Fig. 6c).

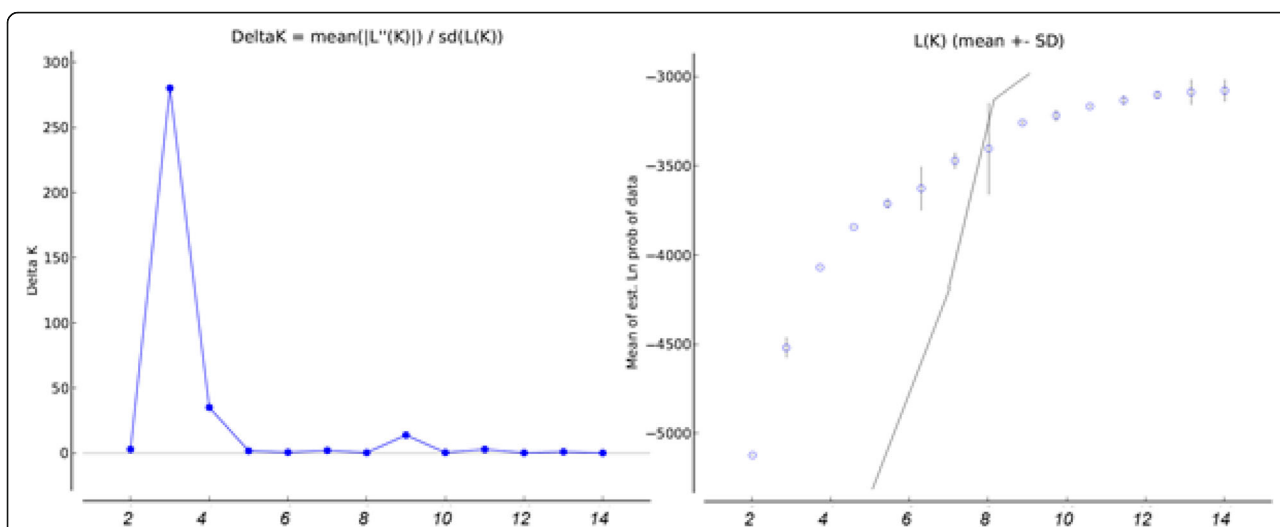


Fig. 4 ΔK and BSRK (the number of K selected with broken stick method) based on the clustering of bisexual species only (*D. mixta*, *D. raddei*, *D. portschinskii* + *D. valentini*) with admixture model, $1 \leq K \leq 15$, 10 runs for each analysis

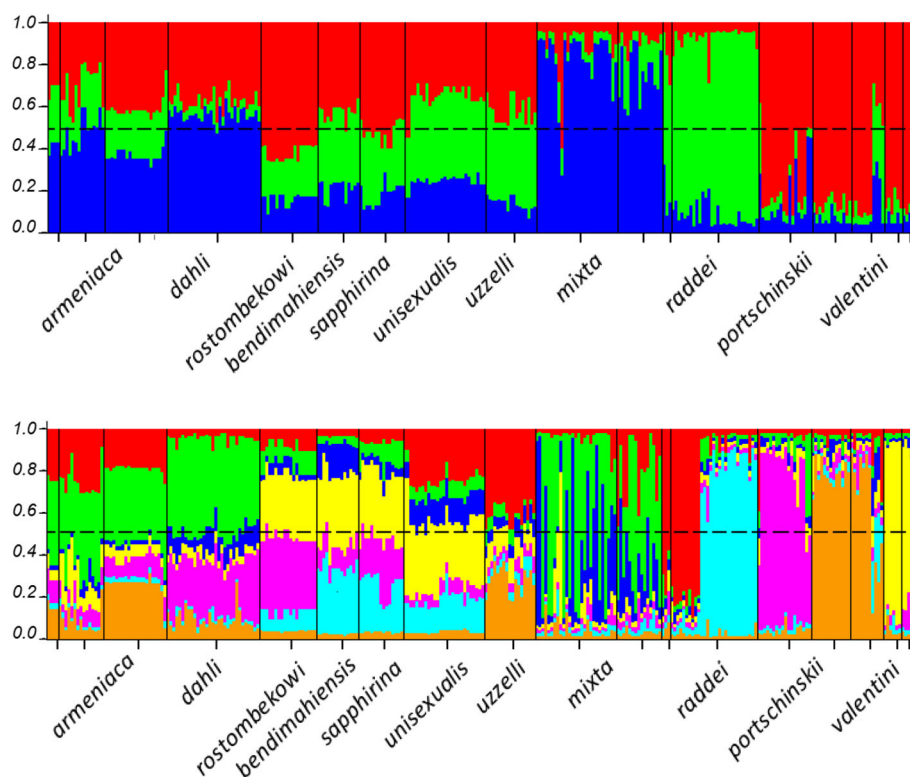


Fig. 5 STRUCTURE clustering outcome with K = 3 (upper panel) and K = 7 (lower panel). Admixture model applied, no LOCPRIOR used, POPFLAF = 1 for sexually breeding species (*D. mixta*, *D. raddei*, *D. portschinskii*, *D. valentini*). With K = 7, two geographic populations of *D. mixta* are from the Lesser and the Greater Caucasus respectively; the red cluster marks populations of *D. raddei* from Armenia and northern Turkey, blue cluster - *D. raddei* *vanensis*, brown cluster - *D. valentini* from Georgia and northeastern Turkey, yellow cluster - *D. valentini* from the Lake Van area

Table 1 The number of the microsatellite genotypes and their distribution per individuals in seven parthenogenetic species of *Darevskia*. N - Sample size, GT - the number of distinct microsatellite genotypes (all differences at least at one locus considered (individuals with missing data at some loci are treated as distinct genotypes), DR - the Bruvo distance between the two most distant genotypes, MD - the Bruvo distance between the two most common genotypes, 1st, 2nd, 3rd, 4th - proportion of the first, second, third, and fourth most common genotypes in the sample

	N	GT	DR	MD	1st	2nd	3rd	4th
<i>D. armeniaca</i>	44	22	0.266	0.097	0.227	0.091	0.068	0.068
<i>D. dahli</i>	29	26	0.594	0.075	0.103	0.069	0.069	0.069
<i>D. unisexualis</i>	27	12	0.321	0.153	0.185	0.148	0.148	0.111
<i>D. uzzelli</i>	17	12	0.409	0.055	0.176	0.118	0.118	0.118
<i>D. rostombekowi</i>	19	5	0.113	0.113	0.474	0.263	0.158	0.053
<i>D. bendimahiensis</i>	14	9	0.181	0.028	0.357	0.143	0.071	0.071
<i>D. sapphirina</i>	15	7	0.278	0.266	0.267	0.200	0.200	0.133

Shared alleles and genotypes between the parthenogens and their presumed ancestral species

Each parthenogenetic species shared at least a few alleles with each bisexual species. *D. dahli* and *D. armeniaca* shared more alleles with their matrilineal ancestor, *D. mixta*, while the other parthenogens had closer links to *D. raddei*. The shared allele pattern on the patrilineal side was less specific, combining alleles found in both presumed paternal populations (*D. portschinskii* and *D. valentini*). When single and two-locus shared diploid genotypes were considered, the similarities between (i) *D. portschinskii* and *D. rostombekowi* (ii) *D. valentini* and *D. bendimahiensis* and (iii) *D. valentini* and *D. rostombekowi* became more prominent relative to other bisexual-parthenogen pairs.

Many higher numbers of shared microsatellite alleles and genotypes were observed among the parthenogenetic species. The highest proportion of shared alleles (0.62) was between *D. sapphirina* and *D. bendimahiensis*; followed by *D. dahli* and *D. armeniaca* (0.33), *D. unisexualis* and *D. rostombekowi* (0.34), *D. unisexualis* and *D. uzzelli* (0.29), *D. unisexualis* and *D. bendimahiensis* (0.28), and finally, *D. armeniaca* and *D. uzzelli* (0.24). For single- and two-locus genotypes, the strongest overlap was between (i) *D. bendimahiensis* and *D. sapphirina* (0.49 at single loci and 0.23 at two loci) and (ii) *D. armeniaca* and *D. dahli* (0.30 and 0.07). Substantial overlap was also observed in the following species pairs: *D. rostombekowi* - *D. bendimahiensis* (0.19 and 0.02), *D. rostombekowi* - *D. sapphirina* (0.18 and 0.017), *D. rostombekowi* - *D. unisexualis* (0.18 and 0.019), *D. unisexualis* - *D. bendimahiensis* (0.12 and 0), *D. uzzelli* - *D. armeniaca* (0.11 and 0.004). The proportion of alleles shared between parthenogens, and their presumed ancestors is shown in Fig. 7.

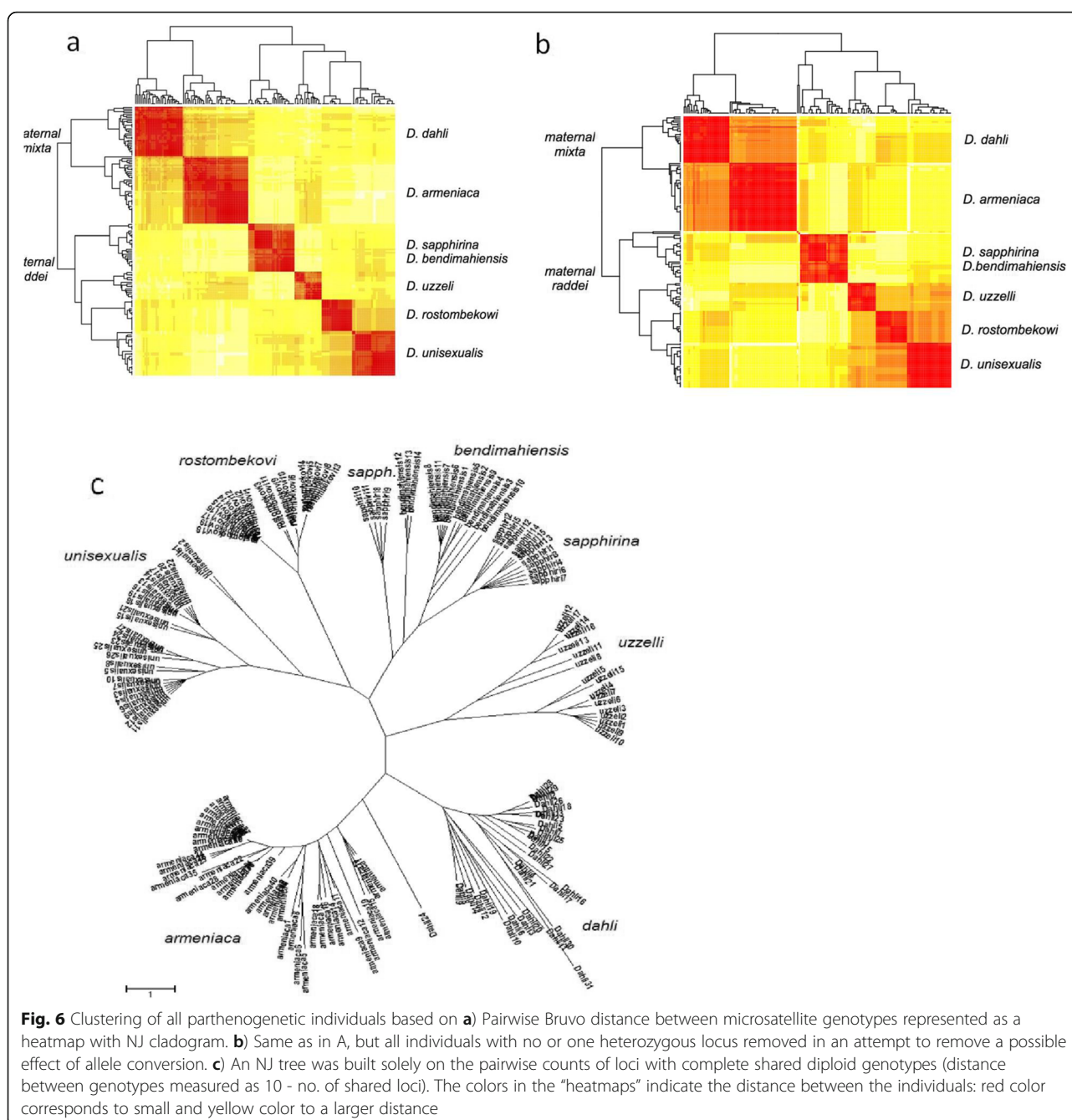
Shared single and multilocus genotypes among the parthenogenetic populations: more details

We found that a few specific genotypes were overrepresented in certain pairs of species (Table 2, for complete information on individuals with shared genotypes, see Table S1). The majority of individuals in the *D. armeniaca* - *D. dahli* comparison shared a three-locus genotype, and two individuals also shared a five-locus genotype (Table S1). In *D. bendimahiensis* - *D. sapphirina* pair, the five-locus genotype was shared by most individuals; at the same time, 7 and 3 individuals, respectively, possessed an identical genotype at six loci. Fewer loci (up to 3 in *D. rostombekowi* and *D. unisexualis*) were shared in the same genotype among other pairs of species (Table 2).

To illustrate this pattern in more detail, we overlaid the number of shared loci on the minimum spanning networks based on Bruvo distances between the full multilocus genotypes, in four pairs of the parthenogenetic species with the highest proportion of identical genotypes (Fig. 8). In all pairs, the closest individuals that belong to two different parthenogenetic species also have the maximum number of shared loci (six in *D. armeniaca* - *D. dahli* and *D. bendimahiensis* - *D. sapphirina* pair, 3 in *D. rostombekowi* - *D. unisexualis*, and 2 in *D. armeniaca* - *D. uzzelli* pair).

The distribution of homozygotes versus heterozygotes among the shared genotypes was also non-random concerning species pairs. That is, *D. armeniaca* and *D. dahli* shared exclusively heterozygous genotypes (Table 2), and a substantial proportion of identical heterozygous genotypes was also observed between *D. bendimahiensis* and *D. sapphirina*. In all other species pairs (and trios), only homozygous genotypes were shared.

To verify the null hypothesis that the identical genotypes shared between different parthenogenetic forms may have resulted from n independent coincidence of parental alleles during hybridization of their respective bisexual ancestors, we calculated the theoretical probabilities of independently repeated formation of the individual multilocus genotypes (Table 2, see the Methods for the quantitative approach chosen). A more conservative approach was used to compute homozygous genotypes' probabilities, assuming they are the result of heterozygosity loss or other forms of gene conversion [28, 70, 71]. Overall, the calculated probabilities were tiny, e.g., in case of the most frequent three-locus genotype shared by *D. armeniaca* and *D. dahli*, $Pad2 = 4.6 \times 10^{-6}$. Similarly, the chance of coincidence of a five-locus genotype most commonly shared between *D. sapphirina* and *D. bendimahiensis* is $Psb2 = 1.83 \times 10^{-7}$. The probabilities of independent formation of two coinciding two-locus genotypes clustering together most *D. armeniaca* and some of *D. uzzelli* (Table 2), even considering the conservative approach based on the homozygosity of



the shared genotype, are 1.71×10^{-3} and 5.73×10^{-4} , respectively. In other words, if the first species emerged independently as a result of hybridization between a male of *rudis* clade with a female of *D. mixta*, and the second species resulted from hybridization between a male of *rudis* clade with a female of *D. raddei*, in only 1/100 of successful hybridizations would one expect coincidence at the locus Du255, which, coincidentally, is present in over 75% of the individuals of either species. As a purely theoretical exercise, we illustrate the dynamics of coincidence probabilities with respect to the number of loci in

a genotype, for hetero- and homozygotes (Fig. 9). Note that all parental allele frequencies in this figure are set to 0.5, i.e., substantially higher than the observed frequency of any allele in any presumed parental species, according to our data.

Finally, we show in Fig. 6c that the overall distribution of shared genotypes among parthenogenetic species, as an NJ cladogram based solely on the number of shared genotypes, is almost identical to a direct measure of genetic distance (such as Bruvo distance on full genotypes, Fig. 6a). Both cladograms differ from the mtDNA tree in

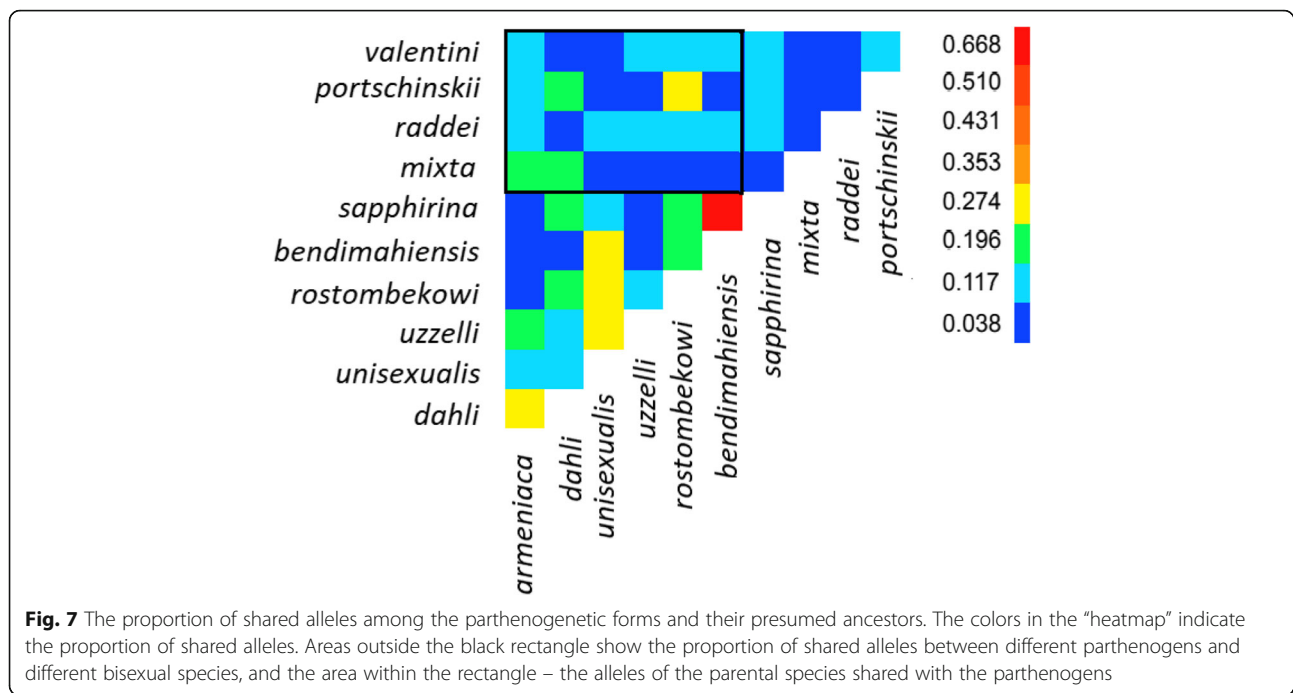


Fig. 2, showing a closer affinity of *D. unisexualis* to *D. rostombekowi*, rather than to *D. uzzelli*. Note that *D. unisexualis* and *D. rostombekowi* share a high proportion of homozygous genotypes, which could have been caused by the loss of heterozygosity in the clonal lineages. Such a process can inflate genotypic similarities between species and, hence, contribute to the discrepancies between the mtDNA and microsatellite data in this particular case. However, when we excluded all homozygotes from the entire parthenogen dataset and redraw the heatmap, the NJ cladogram's topology does change (Fig. 6c). The heterozygotes-only heatmap in Fig. 6b demonstrates the presence of three major groups with distinct matrilineal ancestry: (i) *D. mixta*, (ii) *D. raddei* *vanensis*, and (iii) *D. r. raddei* + *D. r. nairensis*.

Discussion

Discussion of the origins of parthenogenetic *Darevskia* usually posits several background premises: (1) each of seven parthenogenetic forms of *Darevskia* derives from independent hybridization events [32, 33, 54, 55]; (2) the parental species of the extant parthenogens belong to different clades within *Darevskia* [36, 37, 45]; (3) high heterozygosity of the parthenogens derives from their hybrid origin; (4) all extant parthenogenetic lineages originated in relatively recent geological past, i.e. within the last 200,000 years [32, 36]; (5) genetic exchange between parthenogenetic lineages and sexually reproducing *Darevskia* is rare or nonexistent [32, 37, 61, 72]. Hence, all parthenogenetic lineages of *Darevskia* are expected to be evolutionary dead ends, which, due to high heterozygosity levels ab

initio, nevertheless may be successful for short periods [33, 60, 73, 74], and thus provide good examples of geographic parthenogenesis [27, 75].

The analysis presented here suggests that the origin of the parthenogens and their evolutionary perspective is more complicated than previously thought. Parthenogens descend from at least four maternal lineages, and thus four hybridization events. Even more intriguing is that some single- and multilocus microsatellite genotypes are shared between different parthenogenetic forms. Finally, multiple homozygous loci are present in some parthenogens, which is unexpected given their hybrid origin.

The putative parental lineages of the parthenogens: mitochondrial lineages and recombinant genotypes

Our results confirm and further elaborate previous suggestions [37, 45] on the parental lineages of parthenogenetic *Darevskia*. Mitochondrial DNA sequencing shows that seven parthenogenetic forms of *Darevskia* matrilineally descend from at least four geographic populations. *D. armeniaca* and *D. dahli* descend from *D. mixta* from Borjomi Gorge (see also [24, 67], *D. unisexualis* and *D. uzzelli* descend from *D. raddei* *nairensis* (northwestern Armenia and the vicinity of Kars in Turkey; see also [36]), *D. rostombekowi* from *D. raddei* *raddei* (east and south of Armenia), and *D. bendimahiensis* + *D. sapphirina* from *D. raddei* *vanensis*. This finding is consistent with the distribution of the presumed maternal cluster component among the same species in our STRUCTURE analysis.

Table 2 Pairwise comparisons of the most frequent multi- and single-locus genotypes between different parthenogenetic forms of *Darevskia*. Heterozygous genotypes are highlighted in bold

species pair / trio	loci in genotype	alleles in genotype	frequency first species	frequency second species	Prob. of random coincidence ^a
<i>armeniaca</i> - <i>dahli</i>	Du183	189–193	0.677	0.875	4.6×10 ^{−6}
	Du47	278–286			
	Du323	185–213			
<i>bendimahiensis-sapphirina</i>	Du161	391–391	0.5	0.733	1.83×10 ^{−7}
	Du183	193–201			
	Du47	282–282			
	Du323	217–217			
	Du215	204–220			
<i>armeniaca-uzzelli</i> (1)	Du255	204–204	0.5	0.176	1.71×10 ^{−3}
	Du215	192–192			
<i>armeniaca-uzzelli</i> (2)	Du255	204–204	0.55	0.118	5.73×10 ^{−4}
	Du418	140–140			
<i>unisexualis-rostombekowi</i>	Du161	395–395	0.074	0.263	4×10 ^{−7}
	Du418	136–136			
	Du47	282–282			
<i>unisexualis-rostombekowi</i>	Du161	391–391	0.815	0.737	3.03×10 ^{−4}
	Du418	136–136			
frequent genotypes shared between three species					
<i>sapphirina-rostombekowi-bendimahiensis</i>	Du47	282–282	fixed in all three species		4.14 × 10 ^{−3}
<i>armeniaca-unisexualis-rostombekowi</i>	Du418	136–136	0.138; 1; 1		3.28 × 10 ^{−4}
<i>dahli-uzzelli-sapphirina</i>	Du161	399–399	0.680; 0.353; 0.267		1.17 × 10 ^{−3}
<i>dahli-uzzelli-bendimahiensis</i>	Du418	144–144	0.267; 0.667; 0.625		1.79 × 10 ^{−2}

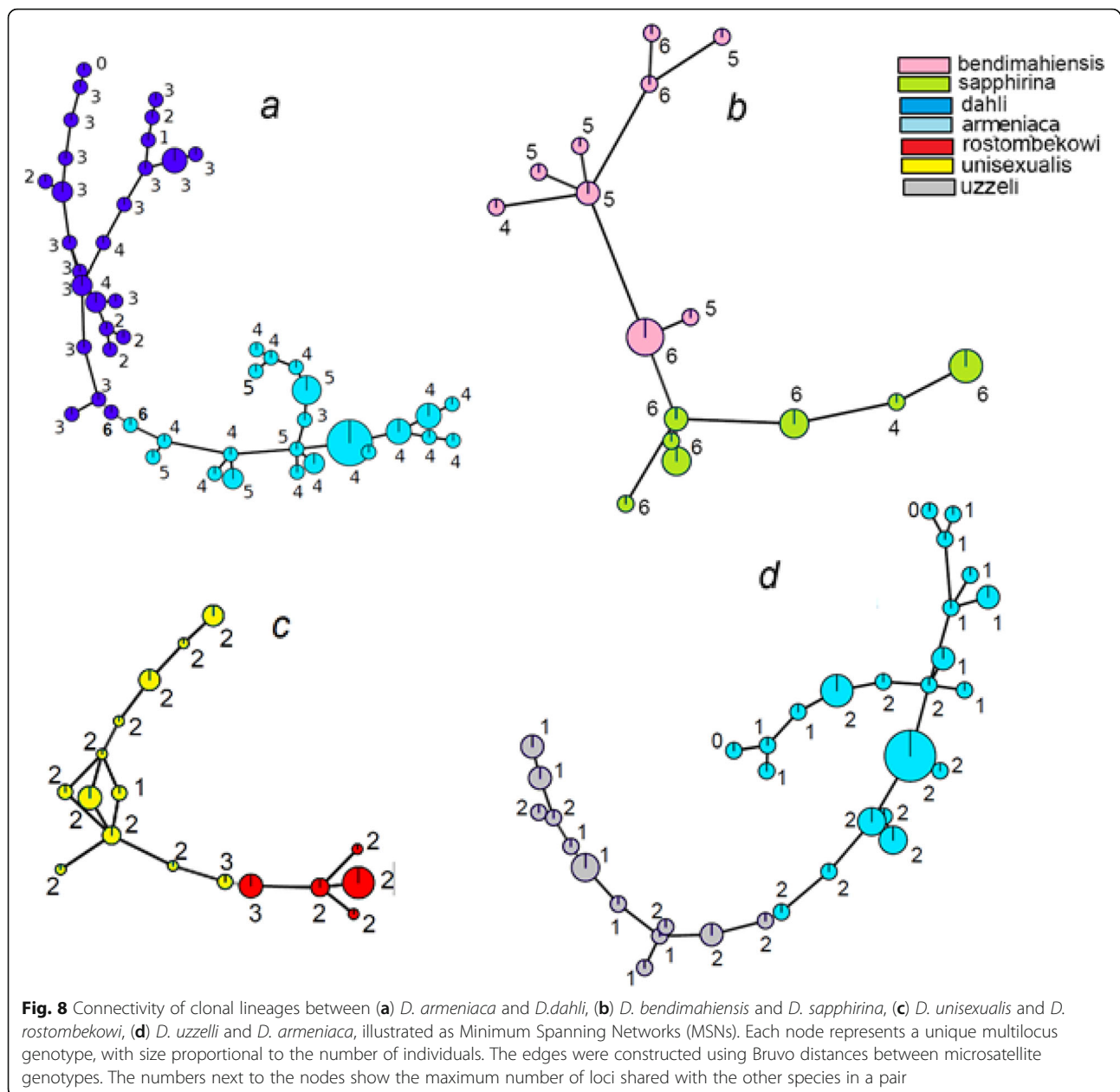
^a Probability of random coincidence between two or three parthenogens is calculated on the assumption of random union of the respective parental genotypes during hybridization using current allele frequencies from presumed parental spp

However, patrilineal ancestry remains unclear. *D. portschinskii* and *D. valentini* show a broad introgressive pattern between each other and the neighboring populations of closely related *D. rudis* [46]; hence genetic distances between geographically distant conspecific populations may be greater than between nominally different species from neighboring locations. Therefore, the STRUCTURE results of this group should be interpreted carefully. Our analysis separates (i) *D. portschinskii*, (ii) *D. valentini* from southern Georgia, Armenia, and Kars area in Turkey, and (iii) *D. valentini* from the Lake Van area; the latter is more different from the former two taxa (i-ii) than they were from each other. *D. dahli* and *D. rostombekowi* showed the highest proportion of *D. portschinskii* ancestry; *D. armeniaca* and *D. uzzelli* were most related to the northern *D. valentini* (ii), whereas *D. unisexualis*, *D. bendimahiensis*, and *D. sapphirina* – are more similar to *D. valentini* from the Lake Van area (iii). Finally, *D. unisexualis* shares ancestry between *D. raddei nairensis* and *D. raddei vanensis* in our analysis,

consistent with the conclusions of Freitas et al. [37], who used a different set of microsatellite markers; see Fig. 6 in their paper). Figure 10a shows the presumed matrilineal and patrilineal origin of the parthenogenetic *Darevskia*, summarizing the information reported in our paper and previous publications. These findings are only partly consistent with previous analyses that explored the origins of parthenogenetic *Darevskia* [36, 37, 45] and which left open the question, whether more than two ancestral species have contributed to the ancestry of particular parthenogenetic taxa. We also note that occasional similarity in the composition of microsatellite alleles between different presumed ancestral populations might be homoplasy typical in the evolution of microsatellite loci [76].

Coincidence of microsatellite genotypes between *D. armeniaca* and *D. dahli*

The case of these two parthenogenetic species is especially puzzling. Murphy et al. [45] hypothesized that *D. armeniaca* and *D. dahli* might have different patrilineal



ancestors, based on analysis of allozyme alleles. Matrilinically, both forms undoubtedly derive from *D. mixta*, which could have hybridized with both *D. portschinskii* and *D. valentini* in the past. *D. armeniaca* and *D. dahli* are phenotypically distinct and easy to identify. Adults have different body sizes, coloration, and scalation patterns in the temporal and anal regions [24, 35, 40]. These phenotypic differences are comparable with those between sexually reproducing *Darevskia* species, e.g., *D. portschinskii* and *D. valentini* (the present-day ranges of these two presumed paternal species overlap with *D. dahli* and *D. armeniaca*). More importantly, *D. armeniaca* and *D. dahli* occupy distinct geographic areas and

altitudinal ranges (1500–3000 and 800–2000 m a.s.l., respectively), although sometimes individual *D. dahli* are found in marginal locations of *D. armeniaca*. Could these two species emerge from completely different hybridization events?

Tarkhnishvili et al. [24] found two heterozygous single-locus genotypes (at Du47 and Du323) shared at high frequencies in both *D. armeniaca* and *D. dahli*. Vergun et al. [54] and Girnyk et al. [55] genotyped 111 specimens of *D. dahli* and 111 *D. armeniaca* from Armenia using four out of the ten loci included in our analysis (Du215, Du281, Du323, and Du47). Comparing the data between these two studies, the heterozygous

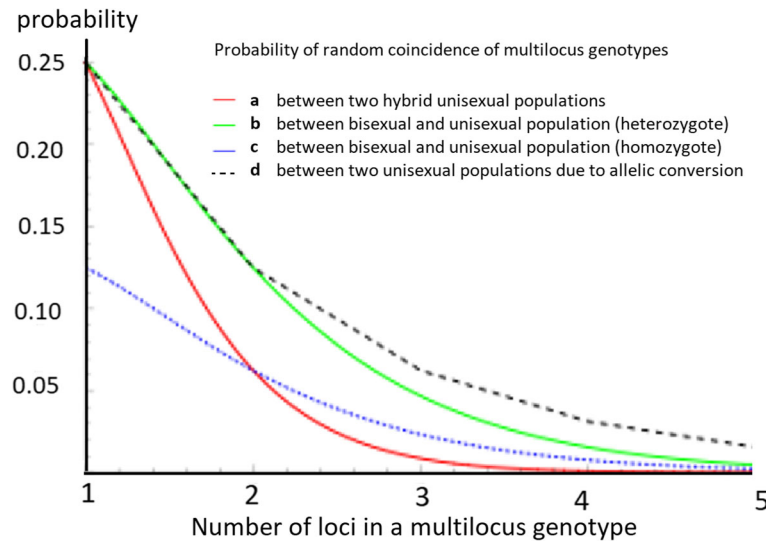


Fig. 9 Probability of independent coincidence of a multilocus genotype between (a) random coincidence in two unisexual populations (heterozygous or homozygous genotypes); (b) random coincidence in a parthenogenetic and a bisexual population: all-heterozygous genotype; (c) random coincidence in a parthenogenetic and a bisexual population: all-homozygous genotype; (d) probability of coincidence in two parthenogenetic populations due to due to allelic conversion (only possible for homozygous genotypes) (see text). X-axis: the number of loci in a multilocus genotype; Y-axis: the respective probability values. All allele frequencies in all populations are set to $\frac{1}{2}$

genotype comprised of alleles 184 and 211 at the locus Du323 was shared by nearly all specimens of *D. armeniaca* (0.94) and one-third of *D. dahli* (0.27). We have now determined that the vast majority of both species share an identical genotype at three loci, a few also share four- and five-locus genotypes and two individuals even share a six-locus genotype. Calculated from the present-day frequencies of the respective alleles in the parental bisexual species, the probability of accidental fixation of a three-locus *heterozygous* genotype in two individuals descending from different hybrid ancestors is close to zero (Table 2). Of course, if the same combinations of alleles were present at (much) higher frequencies in the actual ancestral populations at the time of initial hybridization, this would increase the chance of coincidence. Although we will never have access to the “true” ancestral populations, the concerted sweep of the same alleles at multiple independent loci from near fixation in the past to very low frequencies at present is just as unlikely. The latter drives us to conclude that a single common hybrid ancestor must have existed for both *D. dahli* and *D. armeniaca*, with a subsequent divergence of the two forms, leading to pronounced phenotypic, ecological, and genetic differences. We further suggest that a backcrossing hypothesis could explain the current genetic differentiation of these parthenogenetic species: i.e., enrichment of gene pools of at least one of the two parthenogenetic forms as a result of backcrossing with one of the parental species [24].

Additional evidence contradicting the hypothetical origin of *D. armeniaca* from direct hybridization between

D. mixta and *D. valentini* is the structure of ranges of these two presumed parental species, which are separated by a distance of over 50 km (in contrast with *D. mixta* and *D. portschinskii*, the presumed ancestors of *D. dahli*, whose ranges are parapatric).

Hence, the earlier hypothesis of Tarkhnishvili et al. [24] is supported by evidence from additional microsatellite loci and larger samples of the scored loci and individuals. If *D. dahli* is descended from a single hybridization event between female *D. mixta* and a *D. portschinskii* male, *D. armeniaca* could emerge as a result of enrichment of the original parthenogenetic genome by backcrossing with bisexual males. This conclusion is consistent with the results of Freitas et al. [37], which showed an admixture of alleles associated with at least three different presumed ancestral populations, similar to the results published here, but based using different loci. Freitas et al. [37] use the closeness of the proportion of maternal ancestry to 0.5 as an argument for the absence of backcrosses. Our results, however, suggest that the proportion of presumed patrilineal ancestry is significantly different from 0.5. Conclusive validation of this hypothesis will require the analysis of genomic data from the parthenogens and their ancestors.

Shared genotypes in *D. bendimahiensis* and *D. sapphirina*

Two other parthenogenetic forms, *D. bendimahiensis* and *D. sapphirina*, share the highest proportion of identical genotypes (5-locus genotype in most individuals) among all other pairs. Such a coincidence is virtually impossible if the two forms descend from two different hybridization events. Our results show there is very little genetic difference between *D. bendimahiensis* and *D.*

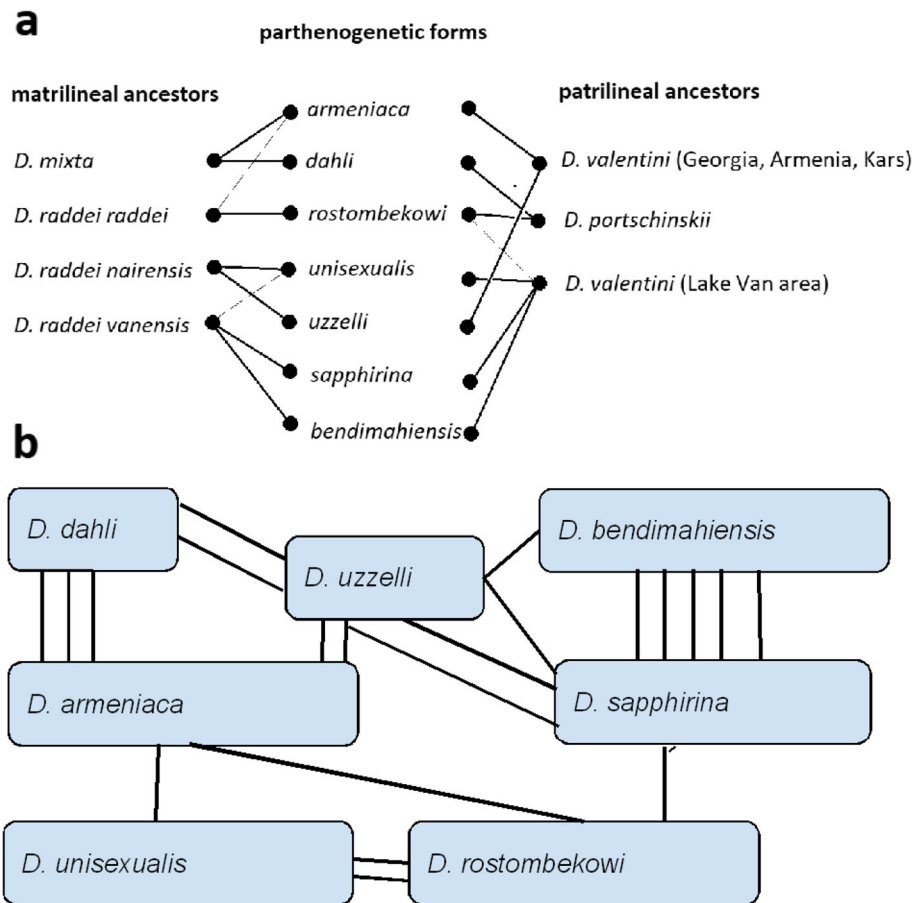


Fig. 10 Origin and genotypic overlap in parthenogenetic *Darevskia*. **a** Matrilineal and patrilineal ancestry of seven parthenogenetic forms of *Darevskia*, based on mitochondrial DNA sequences and STRUCTURE analysis at $K = 7$. Solid lines show matrilineal (left) and patrilineal (right) ancestors of the parthenogens, according to [45] and our data (this paper), specifying geographic populations of the presumed ancestors. Some parthenogens, however, were associated with more than two populations, and dashed lines show the links with these “third” populations (see the text below). **b** The scheme of the most frequent shared genotypes linking the parthenogenetic species of *Darevskia* to each other. Multiple lines demonstrate the numbers of loci with the shared genotypes of different parthenogenetic species

sapphirina, those that exist are best explained by the accumulation of mutations in geographically isolated populations that belong to the same hybrid lineage.

Shared genotypes in other parthenogenetic forms

Figure 10b indicates the presence of shared genotypes in other pairs of the parthenogenetic species, based on the total number of shared genotypes: *D. armeniaca* - *D. uzzelli* (genotypes shared at three loci), *D. rostombekovi* - *D. sapphirina* (2 loci), *D. rostombekovi* - *D. unisexualis* (1 locus), *D. dahli* - *D. uzzelli* - *D. sapphirina* (1 locus), *armeniaca* - *unisexualis* - *rostombekovi* (1 locus), etc. All parthenogenetic forms share genotypes at at least one microsatellite locus (Fig. 10b).

Shared genotypes are found between species pairs descending from both the same and the different maternal lineages, such as *D. armeniaca* and *D. uzzelli* or *D. rostombekovi* and *D. sapphirina*. In such genotypes, no

more than two loci are shared between two individuals (except for a single 3-locus genotype between *D. unisexualis* and *D. rostombekovi*). However, in such cases, the shared genotypes are rare, and they are always homozygous. Below we consider the possibility of homozygous genotypes being the product of allelic conversion, requiring only one allele to be shared originally at a given locus.

Backcrosses as possible way for diversification of parthenogenetic forms

Descent from a common ancestral hybrid individual explains the presence of shared genotypes at multiple loci between the parthenogenetic species, in particular *D. dahli* and *D. armeniaca*. However, even those pairs of parthenogens with the highest number of shared genotypes also have some loci with fixed or nearly fixed differences. How can these differences be explained?

Microsatellites are highly mutable [77], and the estimated per-locus mutation rate in *Darevskia* reaches 0.1428 per generation [57]. Potentially, one could explain polymorphism within the parthenogens by the aggregation of de novo mutations. However, additional evidence caused multiple authors to interpret genetic variation within the parthenogens as polyclonality, i.e., descent from different F1 hybrids [48, 49, 51, 52, 54, 55]; but see [53]. In our study, the presence of multiple and fixed differences between *D. armeniaca* and *D. dahli* is probably not solely the result of accumulated mutation; at least such extent of divergent phenotypic evolution is unknown in vertebrate parthenogens. We suggest that these genotypic differences (as well as genotypic differences within the forms previously interpreted as polyclonality) could derive from post-hybridization backcrosses with bisexual species of *Darevskia*.

Directional selection in parthenogens is less effective than in sexually reproducing species because clones cannot generate variant genotypes [8]. Moreover, because deleterious mutations are more common than beneficial mutations and difficult to eliminate (Muller's Ratchet [12]), obligatory parthenogens are thought to be evolutionary "dead ends." The process of fitness decline in parthenogenetic lineages is accelerated if crossing over between homologous chromosomes of hybrid parthenogens occurs during gonadal maturation: it causes gradual loss of heterozygosity described in detail in [70]. In parthenogenetic *Aspidoscelis* and *Ambystoma*, the lack of recombination between homologous chromosomes prevents loss of heterozygosity (crossing-over between identical sister chromatids still goes on but has no effect) [70, 78] (however meiosis pattern in *Darevskia* may be different [79]).

The possibility of occasional provision of genes from sexually reproducing individuals into the genomes of parthenogenetic forms alters the evolutionary prospects for asexual lineages. We argue that this is indeed likely in *D. armeniaca* and *D. dahli*. In some populations of *D. armeniaca* and *D. unisexualis*, coexisting with *D. valentini*, the proportion of phenotypically-identified backcross individuals exceeds 36% of the entire parthenogenic population [78]. These authors describe eleven karyotyped backcrossed *D. unisexualis* × *D. valentini* specimens; 5 were triploid females, three triploid males, two intersexual triploid hybrids, and one tetraploid male [62]; another two tetraploid individuals were reported in Freitas et al. [37]. Difficulty in segregating the triploid set of chromosomes during normal meiosis would present an obvious fertility problem to such hybrids [33, 45, 80–82]. Consequently, early authors hypothesized that all hybrid triploids are sterile [58, 61, 72]. On the other hand, successful all-triploid hybrid unisexual lineages exist among parthenogenetic *Aspidoscelis* sp. [29, 70, 83]. In *Darevskia*, no all-triploid parthenogenetic populations have ever been

found. However, Arakelyan et al. [62] showed the presence of mature gonads in some triploid females and males, similar to earlier described for *Cnemidophorus* [84]. Spangenberg et al. [63] showed that defects in chromosome synapsis of triploid hybrid male *D. unisexualis* × *D. valentini* did not block meiosis, although the 281 spermatozoa studied in their paper all showed some developmental defects. In conclusion, backcrossing of parthenogenetic *Darevskia* with their presumed paternal species is documented, although definitive evidence of fertile F1 is still absent.

In vertebrates, perhaps the best-documented evidence of incorporating parental genomes into asexual lineages can be found in North American salamanders *Ambystoma* [78, 85, 86]. In *Ambystoma*, backcrosses between the diploid bisexual species and triploid parthenogens can result in either (i) elevation of ploidy in the parthenogens, or (ii) replacement of one of their three haploid genomes with a new and different one. The offspring ploidy (3n) in the second scenario is not altered, but their genetic diversity is substantially increased [78]. Similarly, strong evidence exists for backcross hybridization and introgression of parental genetic elements into the genomes of gynogenetic Amazon molly fish (*Poecilia formosa*), although the details of the actual cytogenetic mechanism are less clear [19, 20].

In some fish, triploid females can produce haploid eggs [87]. The chance that a gamete containing a copy of each chromosome is produced following triploid meiosis decreases exponentially with the number of chromosomes. The probability of meiosis in which all univalents pass to the same pole is equal to $0.5^{(x-1)}$, where x is the number of haploid chromosomes in gene pool [88]. *Darevskia* have $n = 19$ chromosomes in a haploid complement [32, 63, 89]; hence, the probability of producing haploid or diploid gametes by a triploid individual is 0.0000038147 (one out of 262,144 gametes). This is certainly a rare event; however, considering the relatively high proportion of triploid individuals in some parthenogenetic populations and the high number of gametes produced by males, viable gametes may appear occasionally.

Homozygous loci in parthenogens

We assume that two allelic copies at each locus are inherited from the maternal and the paternal bisexual parents. This assumption is consistent with higher observed overall levels of heterozygosity in the parthenogens compared to bisexual taxa. However, homozygous loci are common in *D. rostombekowi* (six out of ten scored loci in most individuals), *D. bendimahiensis*, and *D. sapphirina* (up to five loci) and *D. unisexualis* (four loci).

The frequencies of the respective alleles differ strongly between the putative maternal and paternal ancestors, at least in their respective present-day populations. Unless genetic drift in the bisexual populations has been particularly strong since hybridization, the chance of the

random generation of a homozygous individual, one who is homozygous at so many loci, is meager. The fact that we observe multilocus homozygous genotypes shared between different parthenogenetic species, (e.g., 3-locus homozygous genotype is shared between *D. unisexualis* and *D. rostombekowi*) is even more puzzling, and requires considering alternative processes that increase the frequency of homozygotes post-hybridization.

Indeed, the loss of heterozygosity in hybrid parthenogenetic lineages is a frequently observed phenomenon [6] and can be caused by pairing and crossing over homologous non-sister chromosomes. The segregation of recombinant gametes is expected to result in a random dropout/meiotic conversion of one or the other allele from the clonal lineage and increased homozygosity of the parthenogenetic population. On the other hand, Lutes et al. [70] described an efficient mechanism of maintaining heterozygosity in hybrid parthenogenetic lizards (*Aspidoscelis*). According to this paper, pairing and crossing-over occur only between the identical sister chromatids, which effectively rescues the unisexual lineage from the rapid loss of heterozygosity. The same process has been observed in mole salamanders [21]. Although unisexual forms of both *Aspidoscelis* and *Darevskia* are seemingly able to maintain heterozygosity, a general mechanism described in Lutes et al. [70] might not always work to perfection: rare occasional recombination between homologs would inflate the frequencies of homozygotes over time. The meiotic mechanism in the diploid parthenogenetic *Darevskia* has not yet been described in sufficient detail; however, Spangenberg et al. [63] observed crossing-over in trivalent of non-sister homologous chromosomes in a triploid male from a cross between *D. unisexualis* × *D. valentini*. This possibility suggests that homologous recombination is possible, at least under certain conditions.

Alternative mechanisms of gene conversion are known, but not for vertebrate parthenogens. For instance, in bdelloid rotifers, oocytes are formed through mitotic divisions, with no evidence of chromosomal pairing [90]. In this case, gene conversion can be biased towards one or the other allele, thereby driving the proportions of the parental genomes from 1:1 ratio. Finally, elements of one parental genome may be directly favoured by natural selection in the parthenogens. Interestingly, the proportions of homozygous loci shared between *D. rostombekowi*, *D. dahli*, and *D. bendimahiensis* and, respectively, either of their bisexual parents is visibly biased towards the paternal alleles (*D. portschinskii* and *D. valentini*).

As we mentioned before, the proportion of homozygous loci differs between the parthenogenetic forms. The loss of heterozygosity is a slow process, a product of genetic drift, hence taking thousands of generations to have an effect. Different proportion of homozygous loci in different parthenogenetic lineages is explained either

with different age, or different population size. The parthenogens with the lowest proportion of homozygous loci have broader ranges and consequently probably have larger populations than those with a higher proportion of homozygous loci. Therefore, the time it takes for an allele to reach fixation is directly proportional to the population size of a given parthenogenetic form. The high proportion of homozygous loci in *D. rostombekowi* could reflect its small range and small population size. The loss of heterozygosity inevitably leads to the decline of fitness, and eventual extinction of unisexual lineages [8, 11, 12, 91], unless the parthenogenetic genotype is enriched as a result of an extraspecific augmentation of genetic diversity.

Hypotheses related to parthenogenesis in rock lizards: a synthesis

Here we approach a critical question raised by previous authors [32, 34, 37, 45, 72]: how often does hybridization between different bisexual lineages lead to parthenogenesis? Theory predicts that the number of genetic incompatibilities increases exponentially with the number of nucleotide differences between diverging populations, amounting to ever more substantial barriers to gene exchange [92]. Chromosome pairing and segregation of gametes during meiosis is a complex process, where incompatibilities may cause hybrid infertility or inviability [93]. However, a switch to parthenogenetic reproduction may not require compatibility between homologous parts of the genome. In spiny loaches (*Cobitis*), hybrid parthenogenesis occurs only at an advanced stage of divergence [80]. Darevsky [40] suggested that parthenogens may result from hybridization between *any* species that are diverged insufficiently to prevent hybridization completely, but sufficiently different to undermine the “normal” mechanisms of gametogenesis. By contrast, Moritz et al. [33] argued that only *specific* evolutionary lineages can produce parthenogens upon hybridization, irrespective of the evolutionary distance between them. Neither of these hypotheses is completely validated or disproved so far; and, as we will see, this discussion remains important *only if* all parthenogenetic lineages of rock lizards indeed derived from F1 hybrids between different sexually reproducing individuals.

The results reported here question this assumption. At least in the case of *D. dahli* - *D. armeniaca*, development of one of the two parthenogenetic lineages could have included backcrosses with the male of the paternal species, whose offspring then switch to exclusively parthenogenetic reproduction.

Interestingly, the ranges of all parthenogenetic *Darevskia* either overlap or are parapatric [36, 40]. They form a single geographic area between the mountains of the Central Lesser Caucasus and Lake Van. Even though representatives of the *rudis* and *caucasica* clades coexist throughout the Greater Caucasus and the Western

Lesser Caucasus (Doğu Karadeniz in Turkey), no parthenogenetic forms have ever been recorded there. This fact, together with the presence of overlapping microsatellite genotypes, forces us to challenge the dominant hypothesis about the independent origin of seven parthenogenetic *Darevskia* and search for additional potential explanations.

North American whiptail lizards (*Aspidoscelis*), similar to *Darevskia*, include up to 15 hybridogenous parthenogenetic forms (<http://reptile-database.reptarium.cz/>). The origin of these parthenogens remains a subject of extensive discussion, comparable with that on *Darevskia*. Five out of seven parthenogenetic *Aspidoscelis* from New Mexico are triploid [94, 95]. The presence of triploid forms is clear evidence of successful backcrosses between the diploid parthenogens and their sexually breeding relatives [96]. However, these backcrosses giving rise to new parthenogenetic lineages have never been observed in nature, suggesting that the phenomenon is unusual. The latter is not surprising for the reasons discussed above for *Darevskia*: the chance to find an instantly reproductively successful triploid hybrid is meager. Cole et al. [97] and Lutes et al. [96] managed to produce a hybrid tetraploid parthenogenetic lineage of *Aspidoscelis* in laboratory conditions, which can be taken as evidence of such a possibility in nature.

Conclusions

We argue that enrichment of parthenogenetic genetic variation by occasional backcrosses might exist in Caucasian rock lizards, and potentially in other parthenogenetic reptiles. Backcrossed polyploid hybrids are relatively frequent in *Darevskia*, although no direct evidence of recent gene flow has been previously documented; here we showed coincidence of multilocus genotypes in different species of parthenogenetic rock lizards, a fact difficult to explain if gene flow between the parthenogens and their sexually breeding ancestors is excluded. Our results suggest that the mechanism of maintaining heterozygosity described earlier [70] may be imperfect, and the parthenogens are gradually losing heterozygosity as a result of the allelic conversion. Hence, their fitness is expected to decline over time as genetic diversity declines. Backcrossing with the parental species could be a rescue mechanism that might prevent this decline and increase the persistence of unisexual forms.

The apparition of a new hybrid parthenogenetic lineage from sexually reproducing parents is an exceptionally rare event in reptiles—simple coexistence of species from specific lineages or lineages separated at a specific evolutionary distance is an insufficient precondition for such evolutionary novelty. However, a parthenogenetic lineage may survive for a long time and adapt to changing environments if backcrosses occasionally enrich its genome.

Methods

Sampling

Samples for DNA analysis were collected between 2010 and 2018 in Georgia, Turkey, and Armenia (Fig. 1). All individuals were hand-caught, temporal, dorsal, and anal areas photographed for verification of species (available upon request, also see Tarkhnishvili et al., 2010, [46] for details), and tail-tips removed from live animals for DNA extraction. Procedures for live-animal handling were approved by the Animal Research Ethics committees of Ilia State and Bülent Ecevit universities. In all instances, the species could be identified unambiguously in the field: in the case of morphologically similar *D. bendimahiensis* and *D. sapphirina*, the samples were collected from the respective *terrae typica* [42]. We collected samples from 19 populations of seven parthenogenetic *Darevskia*, and 14 populations of four presumably ancestral bisexual species (Table 3, Fig. 1). Samples of the parthenogens included 44 *D. armeniaca*, 29 *D. dahli*, 28 *D. unisexualis*, 17 *D. uzzelli*, 14 *D. bendimahiensis*, 15 *D. sapphirina*, and 19 *D. rostombekowi* altogether. Samples of the presumed bisexual ancestors included 42 *D. mixta*, 6 *D. raddei raddei*, 10 *D. raddei nairensis*, 19 *D. raddei vanensis*, 33 *D. valentini*, and 15 *D. portschinskii*.

DNA extraction, sequencing, and analysis of mtDNA haplotypes

DNA was extracted from tissue samples using a Qiagen tissue kit (QIAamp DNA Mini Kit, Qiagen, Germany) according to the manufacturer's instructions [98]. To check for contamination and pipetting error, a negative control (reagents only) was used for each extraction procedure and PCR. Two different primer pairs (H15915 – L15369 and H15488 – L15153, [45, 48]) were used for amplification of a 683 bp fragment of the cytochrome b mitochondrial gene [24, 45, 46]. The PCR conditions were as follows: 20 µl total volume, with 2–4 µl template DNA, 1 U of GoTaq Flexi DNA Polymerase (Promega), 1X GoTaq Green Flexi DNA Polymerase buffer, 1 mM of MgCl₂, 0.2 mM of each dNTP and primer concentrations at 0.1 µM. The PCR profile included initial denaturation at 93 °C for 3 min, followed by 30 cycles at 93 °C for 1 min, 53 °C for 1 min, and 69 °C for 2 min and 70 °C for 10 min for the final extension. The amplicons were sequenced on an ABI 3130 sequencer in both directions to ensure sequence accuracy. Sequences were edited using SEQSCAPE 2.5 (Applied Biosystems Inc. Foster City, CA, USA), and unique sequences (haplotypes) were deposited in GenBank (accession no KM496573–82).

We used mitochondrial sequences of *D. mixta* and its daughter parthenogens *D. armeniaca* and *D. dahli*, as well as the sequences of *D. raddei* and its daughter parthenogens *D. unisexualis*, *D. uzzelli*, *D. bendimahiensis*, *D. sapphirina*, and *D. rostombekowi* to identify the most likely maternal population of each parthenogenetic form. Mitochondrial DNA analysis included 46 samples

Table 3 The number of samples and the localities of the seven parthenogenetic *Darevskia* species and their presumed ancestors

Species/ population	Location as shown in Fig. 1	MtDNA (our samples) ^a	MtDNA (GenBank) _b	GenBank Accession #	STR profiles ^c
<i>D. armeniaca</i>	1 - Hrazdan	2	Armenia - 2	MH247120, AF147799	4
	2a,b – Didgori, Khospio	3	Georgia- 46		17
	3a,b - Ardahan, Çıldır	0	Turkey-0		21
<i>D. dahli</i>	4a,b – Kojori, Didgori	2	Georgia – 0 Armenia-1	AF147800	29
<i>D. bendimahiensis</i>	5a,b – Muradiye, Çaldıran	2	Turkey - 2	MH271047, MH271049, MH271050, AF164084,	14
<i>D. sapphirina</i>	6a,b – Patnos, Pınarlı	2	Turkey - 1	AF164083	15
<i>D. rostombekowi</i>	7 - Dilijan	2	Armenia - 3	AF164089-AF164091; MH247116	19
<i>D. unisexualis</i>	8a – Hrazdan	0	Armenia - 9	AF164085-AF164088 MH271034-MH271035 MH271053-MH271054 MN015183	2
	8b,c,d – Hanak, Horasan, Ağrı	3	Turkey - 0		20
<i>D. uzzelli</i>	9a,b,c- Kars, Sarıkamış, Horasan	2	Turkey - 5	MH271037-MH271039 MH271041, AF164082	17
<i>D. mixta</i>	10a,b – Akhdaldaba, Abastumani	0	Georgia - 10	KM496573-KM496579 AF147796 - AF147798	25
	11 - Ambrolauri	0	Georgia - 3	KM496580- KM496582	17
<i>D. raddei</i> <i>raddei</i>	12a - Sotk	2	5 (Armenia)	MH247117, MH271052, MH271060, MH271063, AF164075	3
<i>D. raddei</i> <i>nairensis</i>	12b	2	Armenia-16	AF164074, AF164077-AF164081; U88606, U88607, U88613; MH247032, MH247051, MH247055, MH247057, MH247113-MH247116	10
	12c - Vardzia	3	Georgia-0		3
<i>D. raddei</i> <i>vanensis</i>	13a,b – Doğubeyazıt, Muradiye	4	2 (Turkey)	MH271045, AF164076	19
<i>D. raddei</i>	Northern Iran (undefined)		3 (Iran)	KF717239, KF717241	0
<i>D. portschinskii</i>	14a,b – Khrami, Kojori	N/A	Georgia	N/A	10
	14c – Mets Sapasar	N/A	Armenia	N/A	3
<i>D. valentini</i>	15 - Akhalkalaki	N/A	Georgia	N/A	13
	16 - Ardahan	N/A	Turkey	N/A	7
	17 - Erzurum	N/A		N/A	5
	18 - Çaldıran	N/A		N/A	4

^a the number of MtDNA sequences generated and analysed within current research^b MtDNA sequences downloaded from GenBank, used in the present paper; the area of origin reported in GenBank nearest to the respective sampling location in our data is shown^cThe number of individual microsatellite genotypes generated and analysed in the current paper

of *D. dahli*, 20 of *D. armeniaca*, and 14 of their presumed matrilineal ancestor *D. mixta* obtained in an earlier study [24], some of these samples were re-analyzed for a longer fragment of the *Cyt-b* gene (683 bp)

(Table 3). We included all homologous cytochrome b sequences of parthenogenetic *Darevskia* and their presumed matrilineal ancestors, *D. raddei* and *D. mixta*, available from GenBank, along with the novel sequences

obtained for this study. The total number of individuals of each species, the GenBank accession numbers, and their source populations are provided in Table 3.

To infer the most likely local matrilineal ancestor populations of the parthenogenetic species descended from *D. raddei*, we reconstructed the haplotypes tree as well as the haplotype network—Maximum Likelihood (ML) tree of seven parthenogenetic taxa and their presumed ancestors was reconstructed using MEGA 7.0 [99]. The best-fit substitution model and prior specifications for ML analysis were inferred with the same software (Maximum Likelihood method was applied); the Model with the lowest BIC scores (Bayesian Information Criterion) was considered to describe the substitution pattern the best. The support for branching patterns was estimated by 500 bootstrap replications. Considering reciprocal monophyly of *D. mixta* and *D. raddei* [45, 66], we used the former species and its daughter parthenogens as outgroups with respect to *D. raddei* and its daughter parthenogens.

The Median-Joining network [100] was used for illustrating the degree of differences between individual haplotypes of *D. raddei* and haplotypes of parthenogens matrilineally descending from this species (*D. unisexuallis*, *D. uzzelli*, *D. bendimahiensis*, *D. sapphirina*, *D. rostombekowi*). The software NETWORK 5.0 [100] was used. A similar network showing the differences between individual haplotypes of *D. mixta* and its daughter parthenogens *D. armeniaca* and *D. dahli* was published earlier [24].

Microsatellite genotyping

We inferred genotypes of 289 individual lizards at ten microsatellite loci: Du161, Du183, Du231, Du255, Du365 [101], Du418, Du47, Du281, Du323, and Du215 [102]. However, in 142 individuals, the locus Du418 could not be reliably scored, hence approximately half of the individuals were studied for nine and not ten loci (maximum number of missing loci per individual = 3, the overall proportion of missing data = 12.39%). Microsatellite PCR reactions were performed using the Qiagen Multiplex PCR kit with primer concentrations of 0.11–0.14 μ M (ca 0.1 mM). Thermal cycling was performed at 95 °C for 15 min, followed by touchdown of 15 cycles of 94 °C for 30 s, 56 °C for 45 s incorporating a stepwise decrease of 0.5 °C at each cycle, 72 °C for 1 min, then 22 cycles of 94 °C for 1 min, 48 °C for 90 s, and 72 °C for 1 min, followed by a final extension at 60 °C for 30 min. PCR multiplexes were developed to reduce the time and cost of genetic analyses. We amplified the ten loci in three multiplex PCR reactions with following primer combinations: multiplex I - Du161, Du231, Du255, Du183, Du365; multiplex II: Du47, Du281, Du418 and multiplex III: Du323 and Du215. Amplicons were run on a 3130xl Genetic Analyser (Applied Biosystems), using

deionized formamide and Genescan size standard LIZ 500 (Applied Biosystems Inc. Foster City, CA, USA). We amplified and scored all loci at least three times, and calculated genotyping errors (allelic dropouts, false alleles, and genotype reliability), following the guidelines described in [103, 104]. We set the reliable change index (RCI) threshold value as 95% and verified every individual twice for heterozygosity, and three times for homozygosity, since allelic dropouts are less likely to be detected at homozygous loci [105].

Since we frequently observed identical genotypes shared among the different parthenogens (see the Results section), we first validated the trivial hypothesis that these coincidences resulted from misidentification of genotypes. We examined the cases when alleles in the high-frequency genotypes of the parthenogens were also shared with one or more bisexual species, and tested their frequencies for HW proportions in the respective bisexual populations.

Analysis of allele and genotype frequencies and differentiation between taxa

We used the R packages *adeigenet* [106], *hierfstat* [107], and *PopGenReport* [108] to infer allele and genotype diversity estimates within and between conspecific populations of all parthenogenetic species and their presumed ancestors.

The proportions of shared alleles were calculated per individual pairs of parthenogenetic species using the function *propShared* in *adeigenet* and averaged across the respective groups. The same approach was used when calculating the proportions of shared multilocus genotypes, i.e., by treating each combination of a given number of loci as one haploid locus and each corresponding genotype as an allele. The minimum spanning networks [109], based on averaged pairwise Bruvo distances between individual microsatellite genotypes, were built using the R packages *poppr* [110, 111] and *igraph* [112]. The Bruvo distance [113] between two microsatellite alleles is calculated as:

$$d = 1 - (2^{-|x|}) \quad (1)$$

where x is a number of repeat units that set the two alleles apart.

Bayesian analysis of the microsatellite genotypes

STRUCTURE v2.2 [114] separated the dataset into groups with the least within-locus and between-locus disequilibria. Bayesian clustering is a standard method of population analysis, especially in cases where hybridization is involved [114]. However, populations of clonally reproducing organisms do not satisfy the main assumption of STRUCTURE, i.e. that genotype

frequencies are independent and tend towards linkage equilibrium. For this reason, we first ran the analysis for bisexual species only, to infer the number of clusters with the highest log-likelihood. The procedure was repeated ten times for each a priori delimited number of clusters (K), ranging from 2 to 15, for each of three settings: (1) admixture model without a priori information on the location (no LOCPRIOR option); (2) no admixture model without LOCPRIOR option applied; (3) no admixture model,

with LOCPRIOR option applied. MCMC parameters were set with a burn-in period of 100,000 and 100,000 post-burn-in replicates. The admixture model without a priori information on the location showed fully consistent clustering among different runs; hence we inferred the population structure based on the setting (1). We calculated ΔK statistics based on the difference between two consequential K values [115, 116] using Structure Harvester website [117]. Since the log likelihood monotonically increased with K, we considered K corresponding to the highest ΔK as the most likely number of ancestral population clusters before the interspecific hybridization/first appearance of the parthenogenetic hybrids. Additionally, we inferred the number of clusters satisfying the broken-stick rule (BSRK [118]). The resulting value of K with the highest support was then used in the subsequent analysis involving both sexual and parthenogenetic populations, following the procedure described in [119].

We next estimated the ancestry proportions gained by each parthenogenetic individual from the anticipated bisexual parental clusters.

We assigned POPFLAG = 1 to sexual species and POPFLAG = 0 to asexuals, which treats parthenogens as admixed individuals of unknown origin. In this analysis, MCMC parameters settings were as in the clustering bisexual species; both for $K=K_{\max}$ (ΔK) and $K=BSRK$, the procedure was repeated ten times, and the run with the highest $\ln P(D)$ [115] was chosen and discussed.

Probability of independent coincidence of multilocus genotypes

In cases where the same multilocus genotypes were identified in two or more parthenogenetic species, we tested the null hypothesis of their independent and random coincidence. Based on the assumption that a specific parthenogenetic genotype had resulted from hybridization between the maternal (*D. mixta* or *D. raddei*) and paternal (*D. valentini* and *D. portschinskii* combined) bisexual taxa, and given the frequencies of the respective alleles in the putative ancestors:

$$P_{(L,n)} = \sum_i^{2n} \prod_{i \neq j}^n p_{li} q_{lj} \quad (2)$$

where $P_{(L,n)}$ is the probability of a hybrid multilocus genotype L at n loci; p_{li} - frequency of allele i at locus l in the paternal bisexual population; q_{lj} - frequency of allele j at locus l in the maternal bisexual population.

For example, for two loci A and B :

$$P_{A_1A_2B_1B_2} = p_{A_1}p_{B_1}q_{A_2}q_{B_2} + p_{A_2}p_{B_1}q_{A_1}q_{B_2} + p_{A_2}p_{B_2}q_{A_1}q_{B_1} + p_{A_1}p_{B_2}q_{A_2}q_{B_1}$$

where $P_{A_1A_2B_1B_2}$ is the probability of a hybrid genotype with the alleles A_1A_2 and B_1B_2 ; p_{A_1} - frequency of A_1 in the paternal population, q_{A_1} - frequency of A_1 in the maternal population, etc. If the genotype in question was homozygous, we applied a conservative approach, considering that the homozygosity could be a result of the allelic conversion, leading to loss of heterozygosity when only one parental allele is retained at a locus (see Results and Discussion). In this case, the probability of a parthenogenetic, homozygous locus with allele i was considered equal to a sum of frequencies of this allele in both presumed parental taxa divided by two (assuming that the probability of loss of each of two alleles in a genotype is the same). Hence, we considered

$$P_{(L,n)} = \prod_{l=1}^n \left(\frac{p_{li}}{2} + \frac{q_{li}}{2} \right) \quad (3)$$

where p_{li} and q_{li} are the frequencies of the allele i at locus l in the presumed parental populations. These frequencies are multiplied with each other and (if applicable) with those calculated using eq. (2). To calculate the probability of random coincidence, i.e. the chance of occurrence of the same hybrid genotype twice, the respective values in (2) or (3) were squared. In case of single locus genotypes that were shared between three different parthenogenetic species, the probability of chance coincidence of the respective genotypes was calculated by raising the respective values of (3) to the third degree.

Supplementary information

Supplementary information accompanies this paper at <https://doi.org/10.1186/s12862-020-01690-9>.

Additional file 1 : Table S1. Excel file with microsatellite genotypes of all studied parthenogenetic and sexually reproducing individuals, with the indication of species name.

Abbreviations

BIC: Bayesian information criterion; BSRK: The optimal number of clusters K, based on Bayesian STRUCTURE analysis, selected with broken stick method; K: The number of clusters, studied with Bayesian STRUCTURE analysis; MCMC: Markov Chain Monte Carlo; ML: Maximum likelihood; RCI: Reliable change index; STR: Short tandem repeats

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Authors' contributions

DT and AY initiated the study and obtained funding, AY, MKŞ, KC, FM, ME, YK, ÇI, EG, MA, NB and GI performed fieldwork and/or provided samples, MM, MG, SK, AY and FÇ performed labwork, DT, AY together with MG analysed the data, MA and EG made important contribution to the revision of literature, CLA critically reviewed the consequential versions of the manuscript and corrected English for grammar and style. DT and AY wrote the paper and all authors contributed substantially to the final version of the manuscript. All authors have read and approved the manuscript.

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Availability of data and materials

All data generated or analysed during this study are included in this published article and its supplementary file. All individual STR profiles are presented in supplementary Table S1; GenBank accession numbers of additional unique sequences produced in the framework of current study are given in the text of the paper.

Ethics approval and consent to participate

Ethical Commission for Research Projects of Ilia State University reviewed methodology, study protocols and approved this research. Permit #1018. No anesthesia, euthanasia or any kind of animal sacrifices have been carried out during the study. Field Research: tissue samples (tail tips) were obtained with negligible harm to live animals. Fieldwork and collection of samples in Turkey was done under the permits no. E.1284045 from the Ministry of Agriculture and Forestry of Turkey and no. 91330202 from the Animal Research Ethics Committee of Bülent Ecevit University. No permits are required for field study of the *Darevskia* species in Georgia.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Precise paternal ancestry of hybrid unisexual ZW lizards (genus *Darevskia*: Lacertidae: Squamata) revealed by Z-linked genomic markers

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Precise paternal ancestry of hybrid unisexual ZW lizards (genus *Darevskia*: Lacertidae: Squamata) revealed by Z-linked genomic markers

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We genotyped multiple populations of all seven parthenogenetic species of rock lizards, genus *Darevskia*, as well as their putative sexual parental populations, using double digest RAD-sequencing genomic markers. Taking advantage of the conserved homology of the ZW/ZZ sex chromosomes among lacertid lizards, we aligned our short sequence reads to a reference Z chromosome assembly of the common wall lizard *Podarcis muralis*. This provided unique insight into the origin of all-female hybrid populations, which inherited their single Z chromosome exclusively from a paternal ancestor. The mapped Z-linked loci were used to construct a robust time-calibrated phylogeny. In each parent–offspring species pair, the geographically nearest population of the paternal species was identified as the most likely ancestor of the respective parthenogen, mirroring the trend observed previously on the maternal side in mitochondrial DNA. The estimated splits between the sampled paternal sexual ancestors and their daughter species occurred much earlier than suggested previously and during two narrow time periods: (1) the parthenogenetic *Darevskia armeniaca*, *D. dahli*, *D. uzzelli* and *D. rostombekowi* dated back to ~0.5 or ~0.9 Mya, depending on the calibration point used, while (2) *D. bendimahiensis*, *D. sapphirina* and *D. unisexualis* appear to have diverged ~1 or ~2 Mya.

ADDITIONAL KEYWORDS: hybridization – parthenogenesis – RAD-sequencing – rock lizards – ZZ/ZW species.

INTRODUCTION

Obligate parthenogenetic reproduction is widely documented in the order Squamata (lizards and snakes), and in the majority of cases is immediately preceded by hybridization between sexual representatives of

phylogenetically distant lineages (Neaves & Baumann, 2011; Dedukh *et al.*, 2020). The majority of extant obligate parthenogenetic hybrid species investigated to date appear to be relatively young, suggesting that parthenogenesis is unlikely to be maintained for very long on the evolutionary timescale (Moreira *et al.*, 2021), despite demonstrated ecological advantages in the short term (Tarkhnishvili *et al.*, 2010).

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At the same time, to correctly estimate the time of origin of the parthenogenetic populations, one needs to (1) identify those populations of the sexual species that are most closely related to the true parents of the hybrid parthenogens, and (2) genotype both hybrid and parental populations with sufficient resolution in order to obtain robust age estimates (i.e. based on molecular clock or demographic simulation approaches). The difficulty in fulfilling these requirements leads to wide confidence intervals of age estimates in most studies on parthenogenetic reptiles (Tarkhnishvili *et al.*, 2010).

The rock lizards of the genus *Darevskia* Arribas, 1999 were the first example of hybrid parthenogenesis discovered in vertebrates (Darevsky, 1958, 1966), and remain one of the most well-studied systems (Murphy *et al.*, 2000; Badaeva *et al.*, 2008; Tarkhnishvili, 2012; Tarkhnishvili *et al.*, 2010, 2020; Freitas *et al.*, 2016; Galoyan *et al.*, 2019; Spangenberg *et al.*, 2020). In *Darevskia*, two maternal (*D. mixta* and *D. raddei*) and two paternal (*D. valentini* and *D. portschinskii*) sexual species have produced seven nominal parthenogenetic species (*D. armeniaca*, *D. dahli*, *D. unisexualis*,

D. rostombekowi, *D. uzzelli*, *D. bendimahiensis* and *D. sapphirina*; Fig. 1). Their distribution is centred south of the Lesser Caucasus mountains in Georgia, Armenia and Turkey, as well as the Lake Van basin in eastern Turkey (Fig. 2). The hybrid origin of the parthenogenetic *Darevskia* and the identity of their parents at the species level was determined using only a few allozyme and mitochondrial DNA (mtDNA) markers (reviewed in Murphy *et al.*, 2000), and was broadly confirmed in all later genetic studies (Freitas *et al.*, 2016, 2019; Tarkhnishvili *et al.*, 2020). Notably, the parental sexual species themselves were later shown to harbour high levels of genetic diversity and complicated geographical structure (Tarkhnishvili *et al.*, 2020), and at least on the paternal side, they are characterized by widespread interspecific gene flow today as well as in the past (Tarkhnishvili *et al.*, 2013; Freitas *et al.*, 2019). Regarding the origin of the parthenogenetic species, these recent findings imply that the general view of reticulate evolution within *Darevskia* such as summarized two decades ago by Murphy *et al.* (2000) lacks sufficient detail, in terms

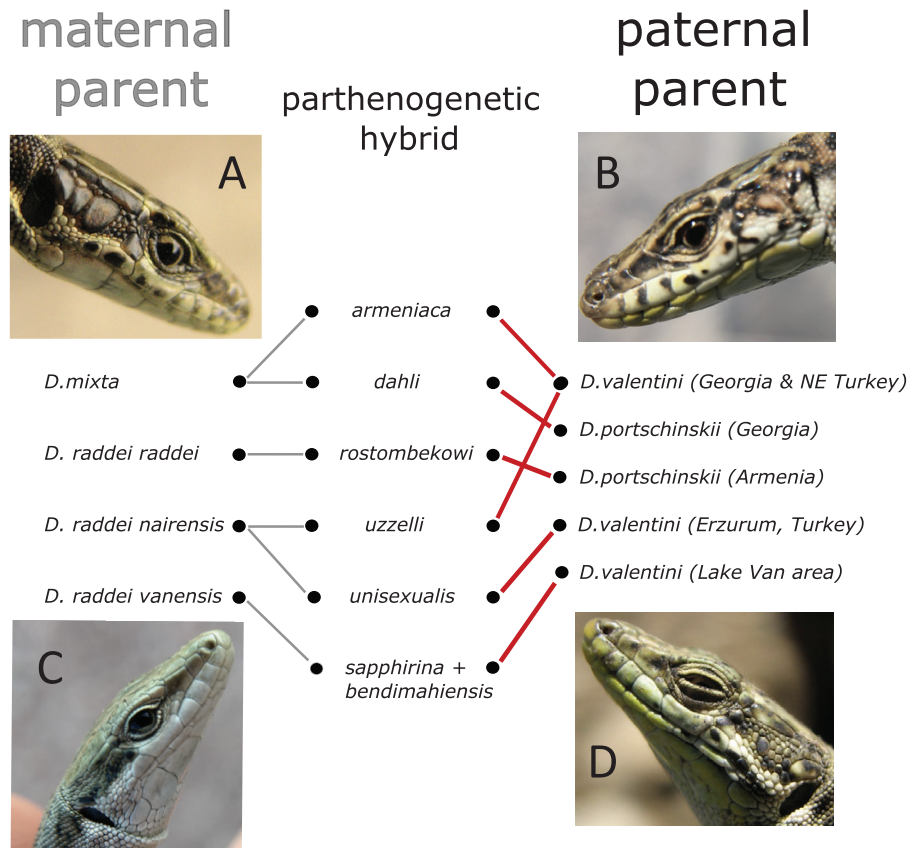


Figure 1. Schematic origin of parthenogenetic taxa in the genus *Darevskia*. The list of matrilineal ancestors on the left is taken unmodified from figure 10 in Tarkhnishvili *et al.* (2020), while details of the patrilineal ancestry on the right were updated using the results of the present study. The insert photographs indicate the sexual ancestral species: maternal *D. mixta* (A) and *D. raddei* (C), and paternal *D. valentini* (B) and *D. portschinskii* (D).

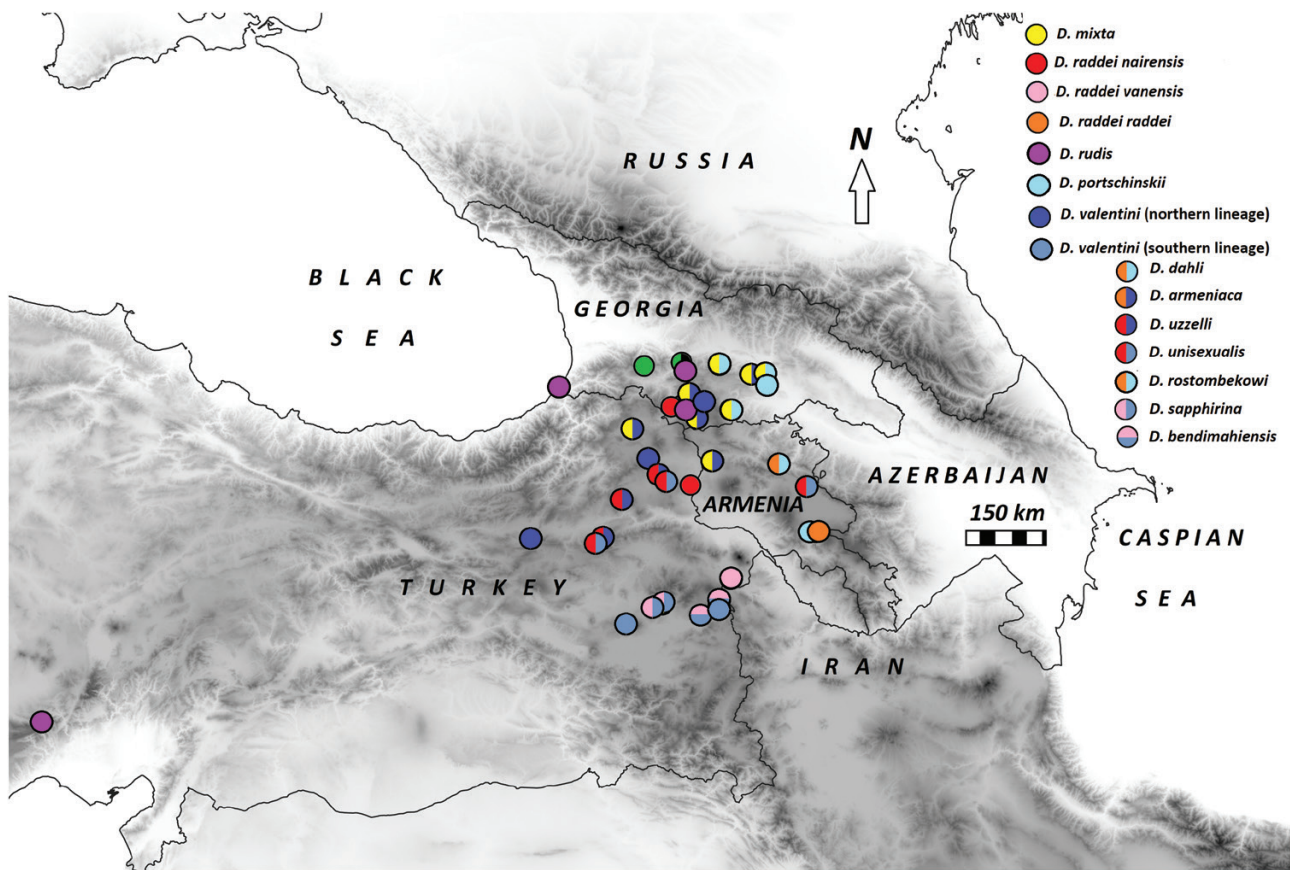


Figure 2. Sampling locations of seven parthenogenetic taxa, their presumed ancestral sexual species and their close relatives.

of both geographical sampling and genome resolution. While a number of recent studies have compared the genetic distances of various paternal and hybrid parthenogenetic populations using a handful of mitochondrial and nuclear DNA (nDNA) markers, their results lacked genomic resolution (Tarkhnishvili *et al.*, 2020), and only a few species were studied locally (Freitas *et al.*, 2016; Ryskov *et al.*, 2017; Girnyk *et al.*, 2018; Vergun *et al.*, 2020). Estimates of the time of the origin of some parthenogenetic species have been made (Murphy *et al.*, 2000; Freitas *et al.*, 2016; Murtskhvaladze *et al.*, 2020), and in all cases they were based on mtDNA sequences. This effectively measures the time of divergence of the parthenogenetic lineage from its nearest maternal parent, but lacks validation based on analysis of the paternal genomes. Considering broad variation of rates of molecular evolution throughout the tree of life (Ho, 2020), this complicates true estimation of the time of origin of the parthenogenetic species.

For the first time in *Darevskia*, we use high-throughput genotyping [double-digest restriction site associated sequencing (ddRAD-seq)] to obtain

genetic data in all parthenogenetic and their putative parental species over the entire area where parthenogenesis occurs. As far as our data allow, we attempt to resolve the exact paternal ancestry of all known parthenogenetic species, and estimate the respective divergence times between the extant parthenogenetic and paternal populations. To this end, we perform phylogenetic analysis using multiple short sequences from one region of the genome, which in parthenogenetic *Darevskia* has been inherited exclusively from the paternal side: the Z chromosome.

Homologous and well-differentiated ZZ/ZW sex chromosomes with shared origin dating back as far as 85 Mya are characteristic for all lacertid lizards (Rovatsos *et al.*, 2016, 2019; Stöck *et al.*, 2021). The ZZ/ZW system possesses several attractive properties compared to XX/XY. The Z chromosome can show a signature of polymorphism similar to the faster evolving X relative to the autosomes (Mank *et al.*, 2007, 2010; but see Axelsson *et al.*, 2004). Assuming an even sex ratio and similar variance in reproductive success between the sexes, the effective population size on Z is expected to be three-quarters that of the autosomes.

The Z chromosome spends two-thirds of its time in the males, and therefore should experience a slightly higher mutation rate than autosomes. Hence, neutral substitutions on the Z should proceed slightly faster than on the autosomes and on the W (Wilson Sayres & Makova, 2011). At the same time, sex chromosomes in general are expected to be less prone to introgression following interspecific hybridization (i.e. due to reduced fitness of the heterogametic sex), which has been demonstrated empirically for X (Macholán *et al.*, 2011; Maroja *et al.*, 2015) as well as for Z (Storchová *et al.*, 2010). In short, the sex chromosomes and the Z chromosome in particular can be expected to more adequately reflect the history of divergence even in cases where incomplete reproductive isolation and secondary hybridization complicate phylogenetic analyses at other parts of the genome.

In *Darevskia*, all-female parthenogenetic populations are diploid and heterogametic (ZW). The presence of female heterogametic chromosomes has been directly confirmed in *Darevskia portschinskii*, *D. raddei* (Spangenberg *et al.*, 2019; Rovatsos *et al.*, 2019), *D. valentini*, *D. unisexualis* (Spangenberg *et al.*, 2017, 2020a), *D. armeniaca* (Kupriyanova, 2009) and *D. rostombekowi* (Spangenberg *et al.*, 2020b). Assuming that the hybrid species resulted instantly from the union between the paternal (Z) and maternal (W) gametes, the Z chromosome in parthenogens is then inherited directly and exclusively from the paternal parent. This eliminates the need to phase and correct the assignment of haplotypes to the respective parental genomes: a non-trivial task because many alleles will be shared by both parental populations due to ancestral polymorphism (Tarkhnishvili *et al.*, 2020). In contrast, the Z chromosome is expected to be completely free of maternally derived alleles and can be used in phylogenetic analysis as a single sequence unit – in contrast to the seemingly ‘private’ alleles on the autosomal sequences, which could still be inherited from the mother. The only differences from the ancestral paternal variant are therefore due to accumulation of novel mutations, which offers unique insight into the evolutionary history of the parthenogenetic populations after hybridization has occurred.

MATERIALS AND METHODS

Live lizards were collected in Turkey, Georgia and Armenia, and tissue samples (tail tips) were taken with negligible harm to the animals, as described previously (Tarkhnishvili *et al.*, 2020). The animals were released immediately following the procedure. Sex was identified based on external morphological characters and only adult females were included in

the present study to avoid possible bias due to unequal dosage of Z chromosomes between sexes. In total, 99 individuals were analysed using ddRAD-seq and these represented all seven parthenogenetic species as well as their respective sexual parents from multiple localities (Fig. 2; Supporting Information Table S1). In particular, our sampling covered all currently identified, genetically distinct subspecies/geographical variants of the parental taxa *D. mixta*, *D. raddei*, *D. portschinskii* and *D. valentini* found near the parthenogens, as well as *D. rudis rudis* and *D. rudis obscura* from Georgia that are known to exchange genes with both *D. portschinskii* and *D. valentini* (Tarkhnishvili *et al.*, 2013), and a distant *D. rudis bolkardaghica* from the Taurus mountains in southern Turkey, representative of the large distribution range of *D. rudis* (Arribas *et al.*, 2013; Candan *et al.*, 2021). Eleven individuals of *D. raddei*, three of *D. mixta* and one of *D. derjugini*, all representing the maternal clade of parthenogenetic *Darevskia* (Murphy *et al.*, 2000), were used as outgroups in the phylogenetic analysis.

GENOMIC LIBRARY PREPARATION AND BIOINFORMATIC PROCESSING

Genomic DNA was extracted from alcohol-preserved tissues as described by Tarkhnishvili *et al.* (2020), and genomic ddRAD library preparation and sequencing was outsourced to a commercial facility (Floragenex Inc., OR, USA). Briefly, DNA samples were digested with restriction enzymes *Pst*I and *Mse*I (New England Biolabs), and genomic libraries were constructed and multiplexed using a standard protocol (see Supporting Information), and sequenced on two lanes of an Illumina HiSeq instrument (100-bp single-end reads). No differences were observed in the FASTA read quality between the two batches of samples. Reads with quality score < 30 were discarded. Raw reads were de-multiplexed and adaptors and indexes removed using Stacks 2.57 software (Rochette *et al.*, 2019), and quality checked in FastQC. We then aligned the reads to the reference genome of *Podarcis muralis* (Andrade *et al.*, 2019), NCBI assembly GCF_004329235.1, using the default mismatch settings in BowTie 2 (Langmead & Salzberg, 2012) and selecting the conservative end-to-end flag to ensure that the entire read matches the reference. Only those reads successfully mapped to the *Podarcis* Z chromosome (NCBI assembly CM014761.1) were retained for downstream analyses (Supporting Information Table S1). The remaining autosomal and unplaced sequences are outside of the scope of the present study but will be used in future analyses. The reference *P. muralis* genome does not include any annotations specific to the W chromosome. After alignment and converting of the individual sequences into BAM format with SAMtools (Li *et al.*, 2009) we

performed reference-based assembly in Stacks 2.57 using the software default settings for variant calling. To maximize the number of useful RAD loci per sample, we assumed that each individual comes from its own separate population with size = 1.

PHYLOGENETIC ANALYSIS

All Z-linked ddRAD loci were concatenated and used in the phylogenetic analysis. Large genome-wide datasets with a high proportion of missing loci typically perform better relative to small datasets where the number of loci has been sacrificed to increase cross-individual coverage (Wiens & Morrill, 2011; Roure *et al.*, 2013; Talavera *et al.*, 2021). Principal component analysis (PCA) was performed using the adegenet (Jombart 2008) R package, and the co-ancestry matrix was constructed in fineRADstructure (Malinsky *et al.*, 2018). A phylogeny of the Z chromosome was calculated using a Bayesian approach in BEAST v.2.6.3 (Bouckaert *et al.*, 2014) on the CIPRES Science gateway (Miller *et al.*, 2010). Prior to building the trees, the most plausible substitution models were selected using MEGA-X software (Kumar *et al.*, 2018). BEAST was run using the Yule process, and with a random distribution of the offspring number between individuals; Markov chain Monte Carlo iterations = 100 000 000. The strict as well as the relaxed exponential clock models were used to obtain a range of divergence time estimates.

CORRELATION ANALYSIS OF THE DISTANCES

We analysed the association between the geographical and genetic distances for all pairs of parthenogenetic and sexual individuals from the paternal clade. Geographical distances were measured as the Euclidean distance based on the exact geographical coordinates, between the locations of 60 studied parthenogenetic individuals and each of 21 individual-based locations of *D. portschinskii*, *D. valentini* and *D. rudis*. Genetic distances were measured as the proportion of nucleotide substitutions within each pair of parthenogenetic and sexual individuals. Since these only represented a subset of all possible individual pairs, the Mantel test could not be applied and Pearson's correlation coefficients, both direct and ln-transformed, were inferred between the geographical and genetic distances.

RESULTS

After alignment to the *Podarcis* genome with an average mapping success rate of 53%, and the reference-based assembly of the ddRAD loci in Stacks 2.57, a total of

14 588 loci were mapped to the Z chromosome in all 99 female individuals, of which 3659 loci contained 6806 informative single nucleotide polymorphisms (SNPs). Only 56 loci were genotyped in all individuals, but at least 47 (48%) of the studied individuals were genotyped at 12 143 (83%) loci, while at most two individuals were missing at 5302 (36%) loci (details in Supporting Information Table S2). Missing data per se do not constitute a major source of bias in phylogenetic analyses, especially when Bayesian methods are employed (Wiens & Morrill, 2011; Roure *et al.*, 2013), and thus our dataset contained sufficient information for further phylogenetic analysis.

The Bayesian tree topology (Fig. 3) clearly differentiated two major phylogenetic clades in *Darevskia*, previously designated by Murphy *et al.* (2000) as 'caucasica' (i.e. including the species *mixta*, *raddei* and *derjugini*) and 'rudis' (including nominal *rudis*, *portschinskii* and *valentini*). The sexual species *mixta* and *raddei* from the 'caucasica' clade, although known to be maternal parents of the parthenogenetic taxa, appeared just as distant from them on the Z chromosome as they were from the paternal sexual species of the 'rudis' clade. In contrast, the Z chromosomes of parthenogens clustered closely with their respective paternal species, down to the level of local geographical populations. In particular, *armeniaca* appeared in the same sub-clade with *valentini* samples from Georgia; *dahli* with *portschinskii* collected in Georgia; *rostombekowi* with *portschinskii* south of Lake Sevan in Armenia; *uzzelli* with *valentini* from Ardahan, Turkey; *unisexualis* with *valentini* from Erzurum, Turkey; and two parthenogenetic taxa, *bendimahiensis* and *sapphirina*, with *valentini* populations from Çaldıran (NE of Lake Van, Turkey). The same pairs of paternal sexual and daughter parthenogenetic populations separated clearly on the individual-based PCA and fineRADstructure plots (Supporting Information). In all analyses, *D. sapphirina* and *D. bendimahiensis* appear as a single undifferentiated clade, thus questioning their current status as two separate species (Schmidtler *et al.*, 1994; Tarkhnishvili *et al.*, 2020). The choice of the molecular clock substitution model (strict or relaxed exponential) had no effect on the tree topology.

We found a strong correspondence of the geographical and genetic distances between the parthenogenetic population and its closest paternal relative. For example, one population of *D. bendimahiensis* was found just 3 km from its closest paternal parent *D. valentini* in Çaldıran (Lake Van area), and the two geographically distant populations of *D. portschinskii* in Georgia and in Armenia clustered locally with their respective daughter species *D. dahli* and *D. rostombekowi* (Fig. 2). Overall, the correlation

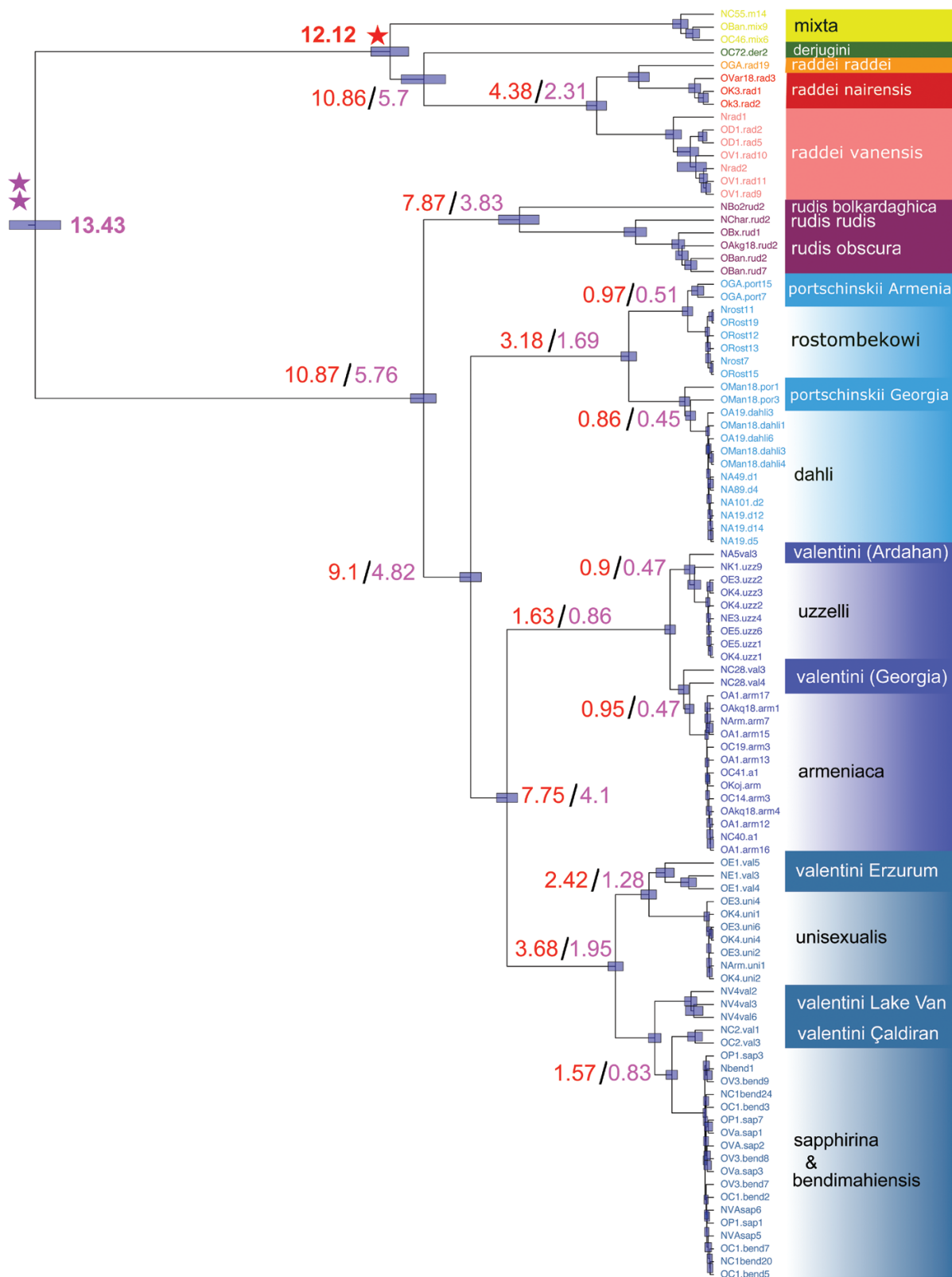


Figure 3. Bayesian phylogeny of parthenogenetic taxa and their parental sexual species constructed from Z-linked ddRAD loci. BEAST v.2.6.3 tree, calibrated by (i) the *raddei-mixta* split, indicated by the single red star symbol, at 12.12 Mya

coefficient between the geographical and genetic distance was 0.265 (the total number of distances compared was 1260). After log-transformation of both geographical and genetic distances, the correlation coefficient increased to 0.477, suggesting that genetic distance does not greatly increase for those sexual populations which are very remote from the target parthenogenetic locations (Fig. 4).

To estimate the node ages on our Z chromosome tree, we calibrated the molecular clock using (1) the previous divergence time estimates between *D. raddei* and *D. mixta* as reported in three different studies (14.4, 12.12 or 8.76 Mya: respectively Kumar *et al.*, 2017, Murtskhvaladze *et al.*, 2020 and Garcia-Porta *et al.*, 2019), or (2) divergence between *D. valentini* and *D. raddei* (20.6, 18.53 or 13.43 Mya), as reported in the same studies. The resulting divergence time estimates of the parthenogenetic species from their closest extant paternal relative are given in Table 1. The timings of four such events (i.e. the divergence of *armeniaca*, *dahli*, *rostombekowi* and *uzzelli*) grouped within roughly the same period, which we dated to as early as 1.16–1.84 Mya (median times) under the relaxed exponential clock model calibrated according to the TimeTree online resource (Kumar *et al.*, 2017), or to as recent as 0.45–0.51 Mya under the strict clock model calibrated according to Garcia-Porta *et al.* (2019). The divergence of *unisexualis* and *bendimahensis-sapphirina* took place within a broader and earlier time frame, i.e. from 2.18–3.43 to 0.83–1.28 Mya, respectively. The 95% probability intervals between these two major groups overlapped when the relaxed exponential clock model was used, but remained separate when the strict clock model was chosen (Table 1; Fig. 3).

DISCUSSION

The molecular phylogeny built on the basis of Z chromosome sequences provides insight into paternal ancestry, as well as the place and time of origin of parthenogenesis in *Darevskia* lizards. The first remarkable observation is that the splits of four parthenogenetic species (*armeniaca*, *dahli*, *uzzelli* and *rostombekowi*) from their respective paternal ancestor (local populations of *valentini* and *portschinskii*) all occurred within a short period of time. All species in this group are today found in a compact area centred

in the Lesser Caucasus (Figs 2, 5), and could indeed point to a rapid series of hybridizations with the maternal species *mixta* and *raddei*, probably preceded by simultaneous range expansions due to a climatic event. While our estimates of the split times depend heavily on the choice of clock model and calibration points (Table 1), we argue that the strict clock model might be a more reasonable assumption for closely related species (Langley & Fitch, 1974; Tiley *et al.*, 2020). In this case, the middle Pleistocene Climatic Transition, i.e. glacial cycles increasing in amplitude and duration (Clark *et al.*, 2006), could have acted as a trigger for the origin of parthenogenesis around ~1 or ~0.5 Mya in the Lesser Caucasus. The geographical model of the last time (Last Glacial Maximum) when conditions were unsuitable for rock lizards over most of their current range clearly indicates the areas just north of the Lesser Caucasus as possible refugia for this group (Fig. 5), and the same refugia were probably used by *Darevskia* lizards during the earlier glacial maxima as well.

Shared geography (both occur on the south-western side of the Kars–Erzurum Plateau) coupled with the change in climate could also have been responsible for the origin of parthenogenetic *bendimahensis-sapphirina* and *unisexualis* species. However, their divergence times from the closest extant paternal populations of *valentini* are considerably older compared to those in the Lesser Caucasus and not simultaneous (from 2.18–3.43 to 0.83–1.28 Mya; Table 1, Fig. 3). If the strict clock model is chosen, the divergence times overlap roughly with the earliest Pleistocene glacial cycles Gibbard *et al.* 2010. In fact, our 2.42 Mya estimate of the split of *D. unisexualis* from its paternal ancestor *D. valentini*, under the strict clock model and 12.12 Mya calibration at the *mixta-raddei* node according to Murtskhvaladze *et al.* (2020), corresponds very closely to its estimated divergence from the maternal ancestor *D. raddei* reported in the same study (2.59 Mya; Murtskhvaladze *et al.*, 2020). In summary, it seems plausible that the earlier origin of *unisexualis* and *bendimahensis-sapphirina* was triggered by a different climatic event(s), and could have followed the range expansion of the parental species from the refugial areas south of the Lesser Caucasus (Fig. 5).

While we have high confidence in the derived topology of our Z chromosome tree, our ability to

(Murtskhvaladze *et al.*, 2020) or (ii) by the *valentini-raddei* split, indicated by the double purple star, at 13.43 Mya (Garcia-Porta *et al.*, 2019). The names of two major clades in the genus *Darevskia* (maternal ‘caucasica’ and paternal ‘rudis’) that participated in hybridization and produced parthenogenetic taxa are given according to Murphy *et al.* (2000). Alternative divergence time estimates (Mya) that followed from the two different calibration points (i–ii) are shown next to the selected nodes in red (i) and purple (ii) font. Bars at the nodes represent 95% highest posterior density intervals. All nodes with ages shown had very high Bayesian support values (>0.99).

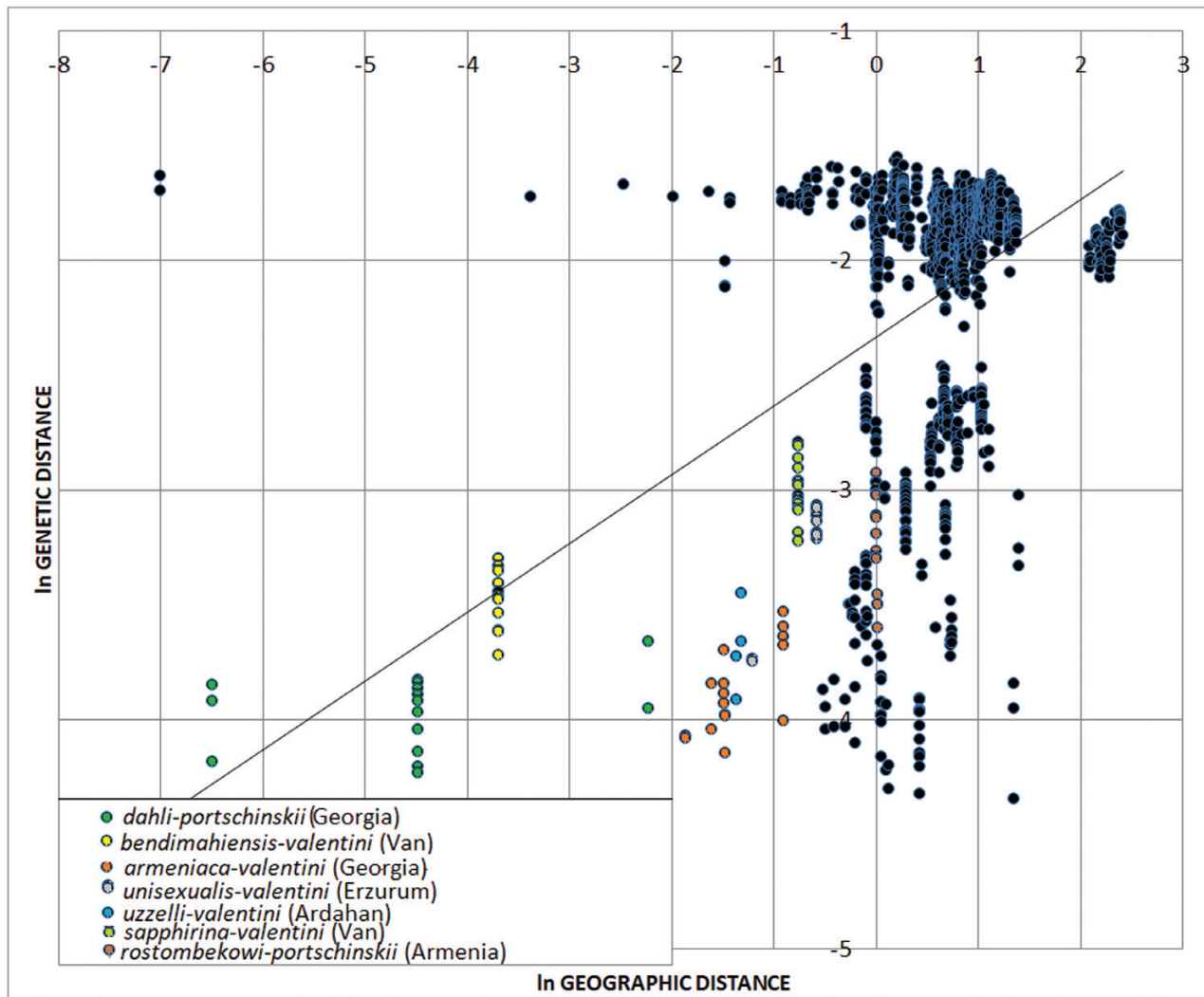


Figure 4. Genetic distances between the parthenogenetic and sexual individuals plotted against straight geographical distances between their locations. Numbers are ln-transformed. Distances between the individual parthenogens and their presumed patrilineal ancestral populations are shown with different symbols (see key).

estimate the true divergence times could be limited by a number of factors. First, the sexual populations examined might not necessarily include the ‘true’ paternal ancestral lineages, due to them either being extinct or not sampled. This is less of a concern in the nominal subspecies *D. v. valentini* (Georgia and Ardahan in Turkey) and all *portschinskii* samples, which have been studied phylogeographically (Tarkhishvili *et al.*, 2013; Rato *et al.*, 2021), but is more relevant to *D. valentini* from Erzurum and the Lake Van basin, where more ancient phylogenetic lineages have recently been discovered (Candan *et al.*, 2021). Second, while using the molecular clock, it is hard to avoid relative bias in the estimation of the divergence time between the old and recently diverged lineages. Our calibration is based on different timescales, with

the earliest datings of the most basal split between the ‘rudis’ and ‘caucasica’ clades (Murphy *et al.*, 2000) around 20 Mya (Kumar *et al.*, 2017; Murtskhvalandze *et al.*, 2020), which corresponds to those from a number of earlier publications (Pyron & Burbrink, 2014; Roquet *et al.*, 2014; Zheng and Wiens, 2016), although we also used the third calibration based on a more recent divergence (13.43 Mya) suggested by Garcia-Porta *et al.* (2019). If calibrations are placed on the ancient nodes, the ages of young nodes are likely to be overestimated (Tiley *et al.*, 2020). In addition, the largest posterior density intervals for the inferred time tend to show positive skewness for tip branches taxa and negative skewness for basal branches (Beavan *et al.*, 2021). This also explains the unexpectedly long inferred average divergence time of the Z

Table 1. Estimated divergence times (Mya) of parthenogenetic species from their closest extant paternal population. Two combinations of the calibration point + model used to construct the tree in [Figure 3](#) are highlighted in bold.

	Calibrated with <i>mixta</i> — <i>raddei</i> split		Calibrated with <i>valentini</i> — <i>raddei</i> split	
	T strict clock	Relaxed exponential	T strict clock	Relaxed exponential
Kumar et al. (2017)	<i>14.4 ± 0.55</i>	<i>14.4 (6.0–30.1)</i>	<i>20.6 ± 0.8</i>	<i>20.6 (9.7–41.0)</i>
<i>dahli-portsch</i> (Georgia)	1.02 ± 0.02	1.45 (0.24–1.7)	0.69 ± 0.01	1.03 (0.21–1.43)
<i>rostop-portsch</i> (Ardahan)	1.16 ± 0.02	1.16 (0.22–1.6)	0.79 ± 0.01	0.82 (0.2–1.5)
<i>armen-val</i> (Georgia)	1.06 ± 0.02	1.60 (0.25–2.0)	0.72 ± 0.01	0.96 (0.2–1.68)
<i>uzzelli-val</i> (Ardahan)	1.05 ± 0.02	1.84 (0.21–2.49)	0.72 ± 0.01	1.30 (0.2–2.46)
<i>unisex-val</i> (Lake Van)	2.88 ± 0.06	3.43 (0.8–4.0)	1.96 ± 0.04	2.42 (0.6–3.2)
<i>bendi-val</i> (Lake Van)	1.87 ± 0.02	2.18 (0.5–2.8)	1.27 ± 0.02	1.53 (0.4–2.5)
<i>sapph-val</i> (Lake Van)	1.87 ± 0.02	2.18 (0.5–2.8)	1.27 ± 0.02	1.53 (0.4–2.5)
Murtskhvaladze et al. (2020)	12.12 ± 0.55	<i>12.12 (5.1–25.0)</i>	<i>18.53 ± 0.7</i>	<i>18.53 (8.0–36.9)</i>
<i>dahli-portsch</i> (Georgia)	0.86 ± 0.02	1.22 (0.23–1.5)	0.62 ± 0.02	0.92 (0.21–1.43)
<i>rostop-portsch</i> (Ardahan)	0.97 ± 0.02	0.98 (0.2–1.5)	0.71 ± 0.02	0.74 (0.2–1.5)
<i>armen-val</i> (Georgia)	0.89 ± 0.02	1.14 (0.23–1.8)	0.65 ± 0.02	0.86 (0.2–1.67)
<i>uzzelli-val</i> (Ardahan)	0.89 ± 0.02	1.55 (0.2–2.47)	0.64 ± 0.02	1.17 (0.19–2.45)
<i>unisex-val</i> (Lake Van)	2.43 ± 0.06	2.88 (0.6–3.3)	1.77 ± 0.04	2.17 (0.59–3.1)
<i>bendi-val</i> (Lake Van)	1.57 ± 0.02	1.83 (0.41–2.5)	1.14 ± 0.02	1.38 (0.39–2.4)
<i>sapph-val</i> (Lake Van)	1.57 ± 0.02	1.83 (0.41–2.5)	1.14 ± 0.02	1.38 (0.39–2.4)
Garcia-Porta et al. (2019)	<i>8.76 ± 0.47</i>	<i>8.76 (4.8–21.0)</i>	13.43 ± 0.53	<i>13.43 (5.6–26.9)</i>
<i>dahli-portsch</i> (Georgia)	0.62 ± 0.02	0.88 (0.21–1.42)	0.45 ± 0.01	0.67 (0.2–1.4)
<i>rostop-portsch</i> (Ardahan)	0.70 ± 0.02	0.71 (0.2–1.5)	0.51 ± 0.01	0.53 (0.19–1.62)
<i>armen-val</i> (Georgia)	0.64 ± 0.02	0.72 (0.2–1.66)	0.47 ± 0.01	0.62 (0.2–1.65)
<i>uzzelli-val</i> (Ardahan)	0.64 ± 0.02	1.12 (0.19–2.45)	0.47 ± 0.01	0.85 (0.18–2.43)
<i>unisex-val</i> (Lake Van)	1.75 ± 0.06	2.08 (0.59–3.1)	1.28 ± 0.03	1.58 (0.56–2.8)
<i>bendi-val</i> (Lake Van)	1.14 ± 0.02	1.32 (0.39–2.4)	0.83 ± 0.01	1.00 (0.37–2.1)
<i>sapph-val</i> (Lake Van)	1.14 ± 0.02	1.32 (0.39–2.4)	0.83 ± 0.01	1.00 (0.37–2.1)

chromosome of some conspecific individuals from the same population, but does not undermine conclusions on the divergence time between the parthenogens and their closest patrilineal ancestor populations. While our results raise the possibility of much older continuous existence of all obligate parthenogenetic forms in *Darevskia* than previously suggested ([Moritz et al., 1992](#); [Freitas et al., 2016](#)), achieving more robust conclusions is possible by combining the molecular clock analysis with other approaches. In particular, demographic modelling based on genomic data with large sample sizes in parthenogenetic populations, recently performed on another parthenogenetic lizard species, *Aspidoscellis laredoensis*, produced a maximum age estimate of ~500 kya ([Barley et al., 2022](#)), which coincides with our most recent estimates (0.45–0.51 Mya).

As far as the same sexual species are concerned, the topology of our Z chromosome tree shows two differences compared to the previously suggested phylogenies of *Darevskia*. In particular, *D. derjugini*, which is a sister taxon to *D. mixta* on the full mitogenome tree in [Murtskhvaladze et al. \(2020\)](#), as well as on the transcriptomic data-based tree in [Garcia-Porta et al.](#)

(2019), is instead found on the same branch with *D. raddei* on our Z chromosome tree ([Fig. 3](#)). In the second case, the discrepancy includes our results and [Murtskhvaladze et al. \(2020\)](#), on the one side, and [Garcia-Porta et al. \(2019\)](#), on the other, regarding the positions of *D. rudis*, *D. valentini* and *D. portschinskii* (i.e. the ‘rudis’ group; [Murphy et al., 2000](#)). Our Z chromosome results agree with the mitogenome tree, placing *D. rudis* as a sister taxon to both *D. valentini* and *D. portschinskii*, while the transcriptome data, as well as some earlier mtDNA-based studies, suggest that *D. portschinskii* is an outgroup ([Tarkhnishvili et al., 2013](#); [Garcia-Porta et al., 2019](#); [Candan et al., 2021](#)). Further complicating the picture, the node datings within this subclade are drastically different between the mitochondrial and nuclear/genomic DNA-based phylogenies. In particular, while the entire ‘rudis’ group appears to have diverged from the common ancestor <1 Mya on the mtDNA tree, the most basal of the three species is separated by at least 4–5 Myr (our most recent median time estimates) according to our results ([Fig. 3](#); [Table 1](#)) and to those of [Garcia-Porta et al. \(2019\)](#). We suggest that these differences could be explained by hybridization among the three species,

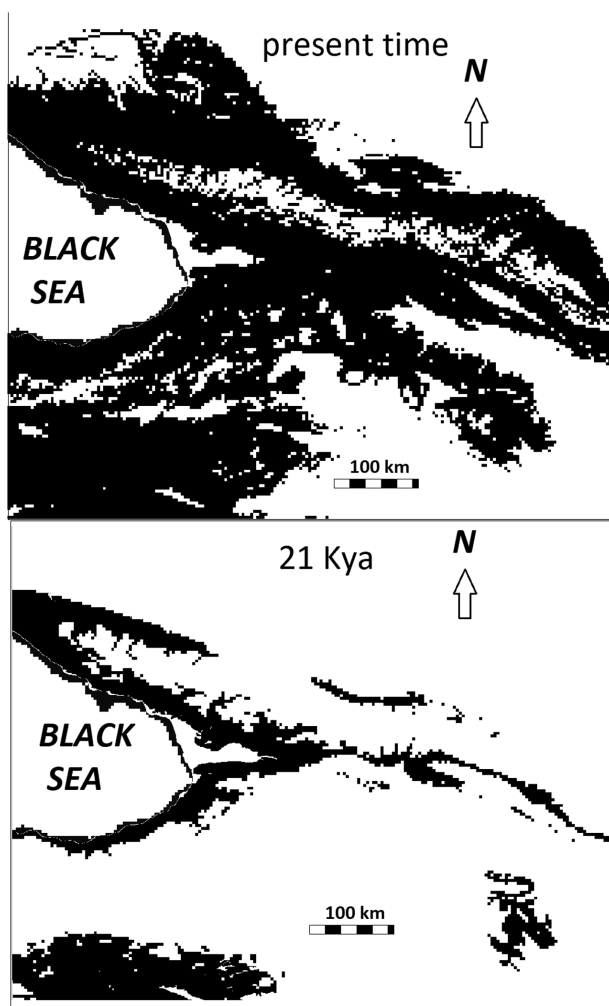


Figure 5. Geographical area with annual mean temperature $> 0\text{ }^{\circ}\text{C}$ and annual precipitation $> 500\text{ mm}$ at present (upper panel) and 21 kya (Last Glacial Maximum), according to Hijmans *et al.* (2005) and the MIROC (Braconnot *et al.*, 2007) palaeoclimatic model.

as well as by differences in introgression among different parts of the genome. Introgression of mtDNA (Tarkhnishvili *et al.*, 2013) as well as microsatellite genotypes (Freitas *et al.*, 2019) has been demonstrated for all three species of the ‘rudis’ group. Moreover, the populations which were sampled in different studies could have possessed introgressed vs. non-introgressed haplotypes. In particular, *D. portschinskii* from some Georgian populations, used in the study of Murtskhvaladze *et al.* (2020), has mitochondrial haplotypes more closely related to *D. rudis obscura* and *D. valentini* from southern Georgia than to the populations from Armenia and south-eastern Georgia. Both mtDNA and autosomal elements may introgress more readily compared to genes located on sex chromosomes (Babik *et al.*, 2005; Macholan *et al.*,

2011; Zieliński *et al.*, 2013; Hassanin, 2015). One could speculate that the introgression of Z chromosomal sequences occurs much more rarely, and populations of *D. portschinskii* and *D. valentini* that are paraphyletic at some mtDNA and nuclear sequences (Tarkhnishvili *et al.*, 2013; Candan *et al.*, 2021, Garcia-Porta *et al.*, 2019) are monophyletic when the Z chromosome is considered.

In conclusion, our approach to constructing the phylogeny using Z-linked genomic markers proved to be very useful in identifying the genetically closest paternal sexual populations of each parthenogenetic species in *Darevskia* rock lizards. We have also pinpointed at least one major period when the hybridization and switch to parthenogenesis occurred almost simultaneously in four different instances, all seemingly confined to the same geographical area in the Lesser Caucasus, while the origin of two other parthenogenetic taxa must have taken place earlier in geological time. In addition, we found that the Z chromosome-based phylogeny of the sexual paternal species themselves also presents different divergence time estimates compared to previous studies based on mtDNA. Further analysis of autosomal genomic markers will provide more resolution into the very complex history of the origin and evolution of parthenogenesis in this group.

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and F.M. took part in the fieldwork; M.A., E.G., Ç.I. and Y.K. provided additional samples; M.M., M.G. and F.Ç. performed DNA extraction; A.Y., M.E. and D.T. analysed the data. A.Y. and D.T. wrote the paper and all authors read and approved the final version of the manuscript.

CONFLICT OF INTEREST

All authors declare no conflicts of interest. Following manuscript acceptance, the last co-author asked about disavowing his affiliation, but this request was not granted given where the research was conducted, in keeping with industry standards.

DATA AVAILABILITY

All individual sequences aligned to the Z chromosome of *Podarcis muralis* are available in BAM format from NCBI Sequence Read Archive accessible at <https://www.ncbi.nlm.nih.gov/sra/PRJNA769575>.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Methods. ddRAD library preparation protocol (performed by Floragenex, Inc., OR, USA).

Figure S1. Principal component analysis on individual Z-linked genotypes. Ellipses indicate pairs of parthenogenetic species + their inferred paternal bisexual population.

Figure S2. fineRADstructure co-ancestry plot constructed from the individual Z-linked genotypes. Species/population names are indicated on the left and individual samples names are listed along the x-axis. The scale bar on the right represents the absolute co-ancestry values inferred by fineRADstructure.

Table S1. Individual lizard samples used in the study.

Table S2. Distributions of ddRAD loci across samples and SNPs across loci (from populations.log.distributions file following the Stacks 2 assembly).