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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.

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

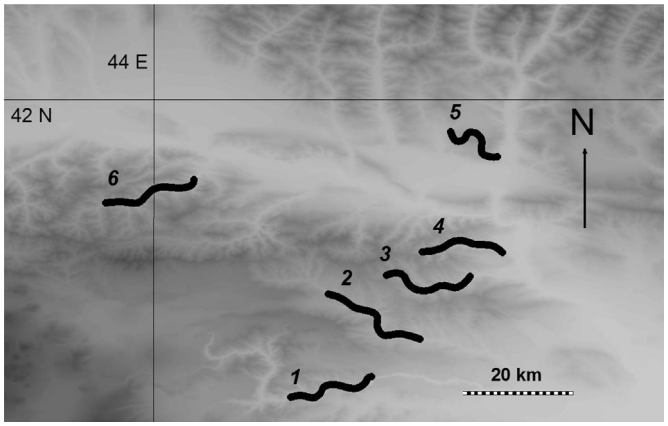

Fig. 1. Map of the Eastern Georgia, with the study areas/river valleys shown in **bold** lines. 1- Khrami, 2- Algeti, 3- Kojori, 4- Vere, 5- Mtskheta, 6- Tana (see also Table 1).

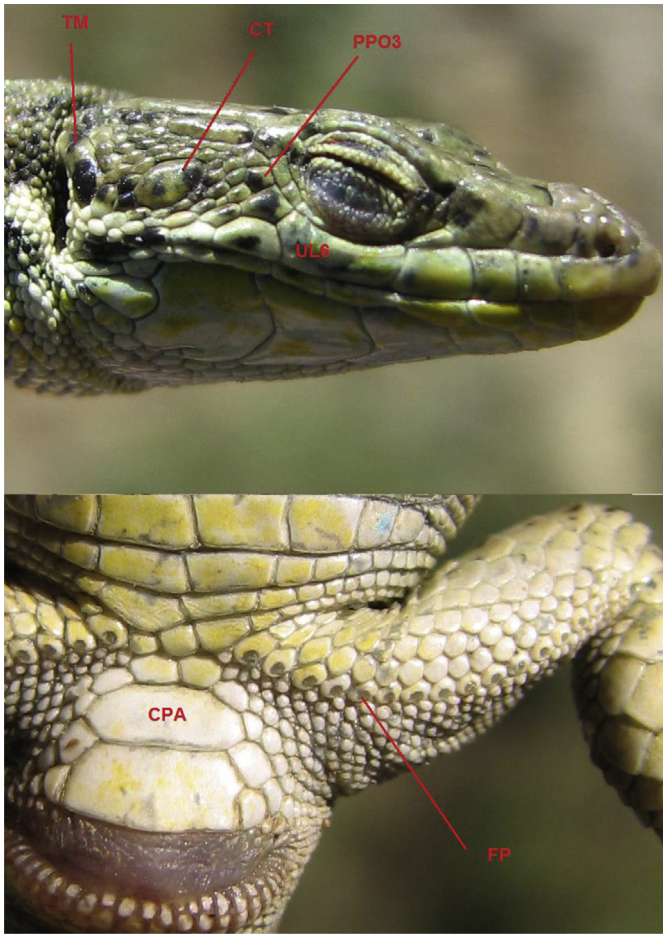

Fig. 2. Temporal and preanal areas of *Darevskia* lizards, with scales used in the analyses indicated.

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

$$c = (var_i(SR) - var_i(P))/var_i(SR) \quad (1)$$

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

$$Pst = var_{i,b}/(var_{i,b} + 2^*(var_{i,w})) \quad (2)$$

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*,

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

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 $var_i(P)$ 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*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 portschinsklii* (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 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).

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
Ul	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
Ul	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

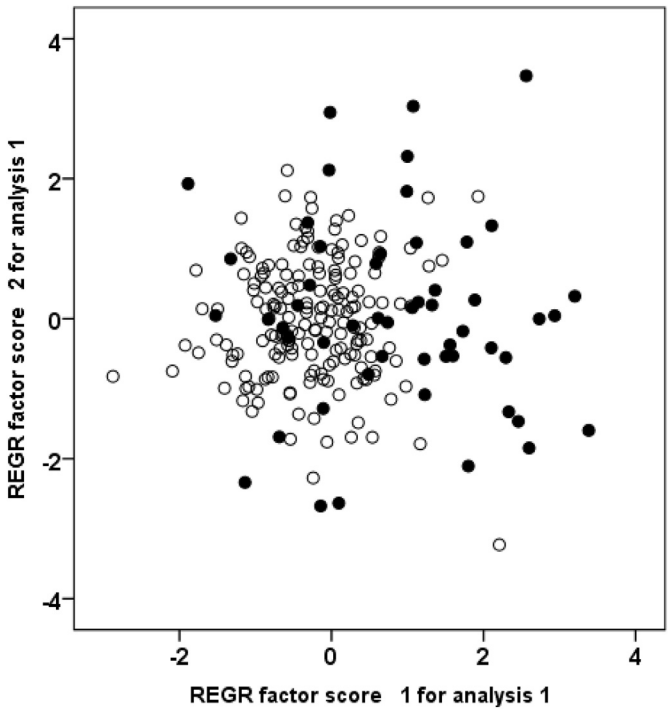

Fig. 3. Individual scores at the first two PCA axes in *D. dahli* (open circles) and *D. portschinskii* (solid circles).

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, CTPPO3; 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 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*.

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.

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.

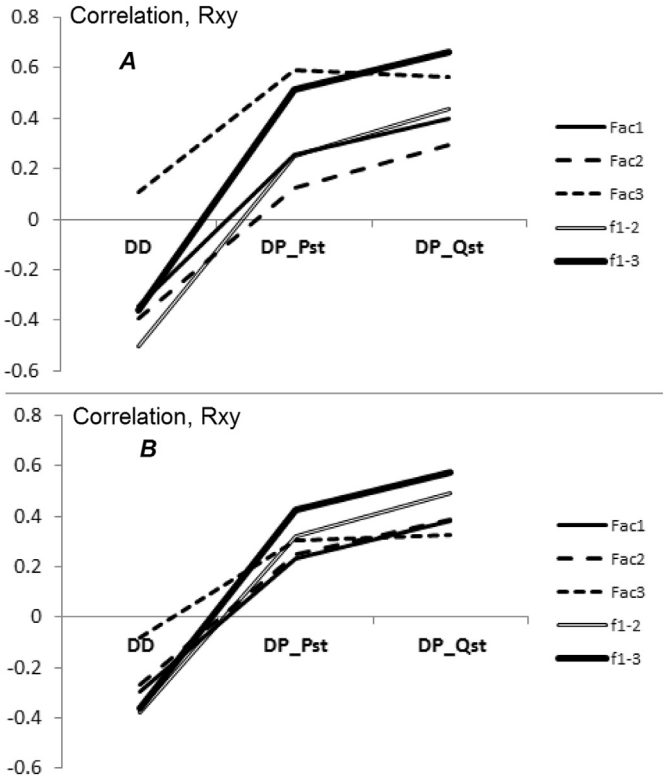

Fig. 4. Correlation coefficients between the phenotypic distances based on three first PCA scores, and linear (A) or cost (B) geographic distances. DD - *D. dahli*, Pst based on the phenotypes; DP_Pst - *D. portschinskii*, Pst; DP_Qst - *D. portschinskii*, corrected Qst value.

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 ($Rxy = 0.566$, $P < 0.05$) 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; Adriaensen 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 metamerisation (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 (Whithlock 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 ($Rxy = 0.566$, $P < 0.05$) 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

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