Intraspecific ecomorphological variation: linear and geometric morphometrics reveal habitat-related patterns within *Podarcis bocagei* wall lizards

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Abstract

Morphological variation in relation to habitat is known to occur in several lizard groups. Comparative studies have linked morphology and habitat use, showing that locomotion is the principal mediator of this evolutionary relationship. Here, we investigate intraspecific ecomorphological variation in *Podarcis bocagei* by examining three habitat types, representing a variety between saxicolous and ground-dwelling habits. Our results indicate variation in absolute and relative limb length, but patterns are only partially concordant to biomechanical predictions. Whereas the femur and hind foot are longer in ground-dwelling lizards, confirming previous observations, the tibia and hind limb are relatively shorter, contradicting expectations. Additionally, head shape varies substantially between habitats, in line with a hypothesis of mechanical restrictions related to microhabitat and refuge use. Finally, we detect male-specific variation between habitats in total body size and head size, providing evidence for interactions between natural and sexual selection. Although performance and behaviour studies are necessary to definitely confirm the functional and evolutionary significance of the observed patterns, our study indicates that ecomorphological adaptations can arise in a very short evolutionary time in this group of lizards.

Introduction

The association between morphology and habitat is a central theme in evolutionary biology, because it reflects the way organisms adapt to their surrounding environment. The ecomorphological paradigm, establishing a causal relationship between morphology, performance and fitness (Arnold, 1983), has served as a framework for numerous studies, which have provided evidence on how whole-organism performance links morphology and ecology. Lizards have long been used as model organisms for ecomorphological studies (Pianka, 1986; Losos, 2009), the adaptive radiation of *Anolis* lizards in the Greater

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Antilles being the major cornerstone of lizard ecomorphology (Losos, 2009). Detailed studies on morphology, locomotor performance, kinematics, escape behaviour and habitat use have established a clear evolutionary link between morphology and habitat use in this radiation (Losos, 1990a,b; Irschick *et al.*, 1997; Losos *et al.*, 1998; Warheit *et al.*, 1999; Butler *et al.*, 2007), as well as in other lizard groups (Pianka, 1986; Sinervo & Losos, 1991; Warheit *et al.*, 1999; Melville & Swain, 2000; Kohlsdorf *et al.*, 2001; Bickel & Losos, 2002; Herrel *et al.*, 2002). Locomotion, being crucial for several different functions such as escape from predators, social behaviour and prey capture, is the main mediator of this link (Garland & Losos, 1994; Aerts *et al.*, 2000; Van Damme *et al.*, 2003). However, such a connection is not universal across lizard groups (see Vanhooydonck & Van Damme, 1999; Zaaf & Van Damme, 2001; Schulte *et al.*, 2004 for negative examples).

Where the morphology–performance–habitat relationship stands, main ecomorphological patterns involve total body size and limb length, which show a positive correlation with perch size and locomotor performance (Losos, 1990a; Miles, 1994; Kohlsdorf *et al.*, 2001). Although perch size is a habitat dimension relevant for arboreal species, difficult to extrapolate to other ecological situations (Melville & Swain, 2000; Zaaf & Van Damme, 2001), similar patterns of variation in body size and limb length also hold in other lizard groups (Melville & Swain, 2000; Bickel & Losos, 2002). From a different perspective, mechanical constraints related to refuge use may also be involved in shaping natural selection forces on other lizard body parts, such as the head. In some cases, no relationship seems to exist between head traits and habitat use (Tropidurinae: Kohlsdorf *et al.*, 2008). In others, such a relationship is evidenced, but the functional and evolutionary significance of observed patterns remains uncertain (Chameleons: Bickel & Losos, 2002). Finally, other studies have provided direct evidence of mechanical constraints on both body and head depth in saxicolous habitats (*Tropidurus:* Vitt *et al.*, 1997; Lacertidae: Vanhooydonck & Van Damme, 1999; populations of *Urosaurus ornatus:* Herrel *et al.*, 2001).

Notably, most ecomorphological studies have been conducted at the interspecific level. Although evolutionary trends are easier to detect at high taxonomic levels, these studies also suffer the parallel effects of phylogenetic constraints (Losos & Miles, 1994). Some of the examined traits might thus represent exaptations (sensu Gould & Vrba, 1982) rather than real adaptations (see Arnold, 1993 for other cases in lacertids). Consequently, studies of intraspecific variation might be more relevant for detecting genuine adaptive pressures, because they can provide insights to the microevolutionary mechanisms that lead to phenotypic differentiation (Rieseberg *et al.*, 2002; Kingsolver & Pfennig, 2007). Those available for different lizard groups in some cases confirm (Vitt *et al.*, 1997; Herrel *et al.*, 2001; Knox *et al.*, 2001; Irschick *et al.*, 2005b; Calsbeek & Irschick, 2007; Calsbeek *et al.*, 2007) and in others contradict (Van Damme *et al.*, 1997, 1998) the results obtained by interspecific studies. At an even finer scale, some studies have studied ontogenetic and sexual sources of ecomorphological variation at the population level (i.e. Irschick *et al.*, 2005a; Brecko *et al.*, 2008), but the interactive effects of sex/age and habitat use have scarcely been examined within lizard species (but see Butler *et al.*, 2000; Butler & Losos, 2002; Herrel *et al.*, 2002; Stuart-Fox & Moussalli, 2007).

Lacertid lizards constitute a puzzling case within the ecomorphological paradigm. Although lacking quantitative support, detailed ecomorphological observations have long suggested evolutionary relationships between habitat use and body shape in this family (Arnold, 1987, 1998). However, formal tests of such hypotheses have given dubious results. Specifically, Vanhooydonck & Van Damme (1999) showed that lacertid species from open

habitats run faster and had long hind limbs and short fore limbs, whereas saxicolous species were dorso-ventrally flattened, with short fore and hind limbs, but correlations with habitat were not significant after controlling for phylogenetic effects. Additionally, a comparative study of morphology in relation to running and climbing capacities in lacertids suggested that long limbs typical of high-speed level running do not hinder, but rather enhance, climbing and clambering performance (Vanhooydonck & Van Damme, 2001). Further on, a detailed analysis of habitat partitioning, locomotor performance and morphology in three co-existing lacertids gave nonconclusive results and questioned the applicability of ecomorphological models to this family (Vanhooydonck *et al.*, 2000). At a finer scale, comparison between conspecific populations of *Podarcis hispanica* living in different habitats reported little morphological differentiation and contradictory results in terms of morphology–performance relationships (Van Damme *et al.*, 1997, 1998). Finally, a recent ecomorphological analysis within a population of *Podarcis melisellensis* indicated a locomotor and ecological significance of ontogenetic, but not sexual, morphological variation (Brecko *et al.*, 2008). However, a detailed intraspecific analysis of morphological variation in relation to habitat across different populations is still lacking in lacertids.

Here, we compare several populations of *Podarcis bocagei* (Seoane, 1884) to test whether morphological features are modified along with habitat structure within species. Specifically, we examine lizards from three different habitat types (HABs), representing a gradient from saxicolous to ground-dwelling ecological habits, and apply both linear and geometric morphometrics (GM) to study variation in total body size, absolute and relative size of different body parts and head shape. Based on previous predictions, we expect lizards from saxicolous habitats to have shorter limbs and flatter heads than ground-dwelling ones, although detailed predictions on other body parts and dimensions cannot be formulated based on previous observations. Additionally, we include lizards of both sexes in our analysis and examine possible interactions between sex and HAB, to gain insights into the relative force of natural and sexual selection at the intraspecific level. If interactions between natural and sexual selection exist, we predict differential responses of both sexes to HAB in those characters that are under the effect of sexual selection.

Materials and methods

Studied populations

We studied a total of 220 adult males and 191 adult females of *P. bocagei*, belonging to 11 populations from northern Portugal (Fig. 1, Table 1). Populations were sampled with an *a priori* habitat targeting to represent different habits in terms of microhabitat use, including:
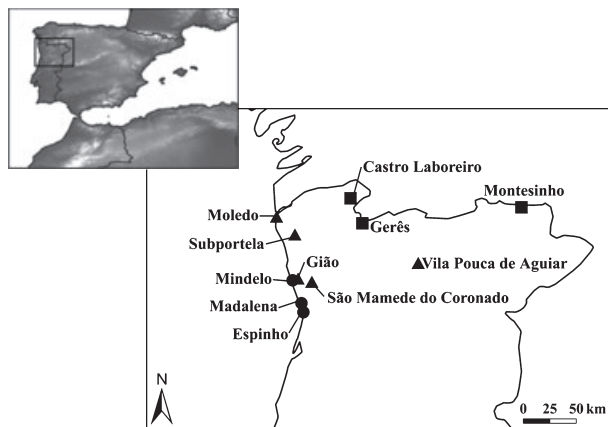
Fig. 1 Location of the study area and the sampled populations, classified by habitat type. Triangles: agricultural walls, squares: high mountain sites, circles: coastal dunes.

Table 1 Localities, geographic coordinates in WGS1984 datum, habitat type and sample sizes for males (Nm) and females (Nf) examined in this study.

Population	Geographic coordinates	Habitat	Nm	Nf
Moledo	41°50.314′N 8°52.444′W	W	20	13
Castro Laboreiro	41°59.689′N 8°14.755′W	M	20	13
Montesinho	41°58.756′N 6°47.719′W	M	22	16
Subportela	41°41.246′N 8°43.087′W	W	20	19
Gerês	41°46.940′N 8°8.708′W	M	20	20
Mindelo	41°17.909′N 8°44.221′W	D	20	18
Gião	41°18.777′N 8°41.498′W	W	21	15
S. Mamede do Coronado	41°17.118′N 8°34.471′W	W	20	20
Vila Pouca de Aguiar	41°26.750′N 7°40.331′W	W	20	20
Madalena	41°6.239′N 8°39.683′W	D	18	18
Espinho	41°1.646′N 8°38.732′W	D	19	19

W, agricultural walls; M, high mountain; D, coastal dunes.

(i) agricultural walls, (ii) high mountain sites and (iii) coastal dunes. Although *P. bocagei* is considered to be a ground-dwelling species (Sá-Sousa, 2001), especially when compared to most other forms of the *P. hispanica* species complex (Harris & Sá-Sousa, 2002), it shows considerable variation in terms of microhabitat use. Here, lizards coming from habitats characterized as *agricultural walls* are primarily saxicolous, living and taking shelter in

piled rocks. Those from *coastal dunes* show primarily ground-dwelling habits, moving on the sand and taking shelter in low dune vegetation. Finally, lizards from *high mountain* sites represent an intermediate between the previous two situations, moving both on the ground and on big rocks or on scattered constructions. We selected the sampled populations to minimize the potential effects of geographic and genetic variation, at least as far as mtDNA and nuclear markers are concerned (Pinho *et al.*, 2007a,b). All lizards were captured by noose or by hand, measured and photographed in the field (see below) and then released back to their capture locations.

Morphological data

We used linear biometry to describe total body size and absolute and relative size of body parts and GM to describe dorsal and lateral head shape. To quantify biometric variation, we measured nine linear characters to the closest 0.01 mm: head length (HL), head width (HW), head height (HH), trunk length (TRL), fore limb length (FLL), femur length (FL), tibia length (TBL), length of the hind foot including the toe and nail (4TL) and total hind limb length (HLL). To examine head shape variation, we took high-resolution photographs of the dorsal and lateral view of the head. To capture head shape by GM, we digitised 30 dorsal and 16 lateral landmarks (see Fig. 3 and Table 1 in Kaliontzopoulou *et al.*, 2007 for a detailed description) using tpsDig software (Rohlf, 2008a). All landmarks corresponded to anatomically homologous points and therefore constituted 'real' (type 1) landmarks. To avoid asymmetry effects for the dorsal view (Corti & Rohlf, 2001), we averaged symmetric landmarks along the mid-line and performed all statistical analyses based on this half-configuration. However, for pattern visualization, we reflected landmarks back along the mid-line to obtain a symmetric, full configuration which facilitates the observation of shape patterns (Kaliontzopoulou *et al.*, 2007).

Statistical analyses

All biometric variables were log-transformed before analyses to ensure normality (Lilliefors test, $P > 0.1$) and homoscedasticity (Levene's test, $P > 0.05$). To obtain a general estimate of total body size and size-corrected estimates of biometric variables (body shape), we first projected the log-transformed raw measurements on an isometric vector to obtain a multivariate representation of the isometric size of each specimen (mSIZE). Then, we regressed the initial variables on this vector and used the obtained residuals as size-corrected variables. To analyse the effect of HAB and sex (SEX) on total body size, size-constrained (raw, i.e. SIZE) and relative (residual, i.e. SHAPE) biometric characters, taking into account our sampling scheme, we applied a (M)ANOVA design

examining the effects of HAB, capture locality (SITE) as a factor nested in HAB, SEX and the interaction term SEX*HAB on each of the above datasets. Then, we performed canonical variates analyses (CVA) in each sex separately to detect which traits contributed the most in ecomorphological variation, for size-constrained (SIZE) and size-corrected (SHAPE) datasets.

Concerning GM analyses, we first performed a Generalized Procrustes Analysis (GPA or GLS, Rohlf & Slice, 1990) using tpsRelW (Rohlf, 2008b) to standardize the size, translate and rotate the landmark configurations. Using the same software, we extracted partial warp scores (including the uniform component), which were used as shape variables. We applied the MANOVA scheme described above on shape variables to examine head shape variation. To visualize head shape differences between habitat groups in each sex separately, we used tpsSplin software (Rohlf, 2004) to superimpose the mean shape configuration for each HAB group on the global mean shape for the corresponding sex. All statistical analyses were performed using NTSYSpc v. 2.21c (Rohlf, 2009), except for (M)ANOVAs which

were performed using STATISTICA v. 7.1 (Stat Soft Inc., 2005). Because the same data were used for multiple comparisons (Curran-Everett, 2000), we used the false discovery rate procedure to adjust *P*-values (Benjamini & Hochberg, 1995).

Results

Total body size differed by sex, HAB and their interaction (mSIZE, Table 2), with males larger than females (Unequal N Tukey's Honestly Significant Difference (HSD), $P < 0.001$ in all cases). No habitat-related size variation was detected for females (Unequal N Tukey's HSD, $P > 0.1$ in all cases), but males from dunes were larger than those from walls and mountain habitats (Unequal N Tukey's HSD, $P < 0.05$ in all cases) (Fig. 2), also resulting to a more marked sexual dimorphism in this habitat. Significant sexual and among-habitats variation was observed for both raw and size-corrected biometric variables. Among-sites variation (factor SITE) was also significant in several cases, but explained less variation than HAB (results not presented).

Table 2 Results of the (M)ANOVAs conducted on isometric body size (mSIZE), absolute values (SIZE) and residuals of biometric variables after isometric-size correction (SHAPE) and dorsal and lateral head shape, treating SITE as nested in habitat type (HAB) and testing the interaction SEX*HAB.

		SIZE				SHAPE			
		HAB	SITE	SEX	SEX*HAB	HAB	SITE	SEX	SEX*HAB
	d.f.	2	8	1	2	2	8	1	2
mSIZE	F	5.23	1.73	559.97	4.03				
	p	**0.010**	0.106	**<0.001**	**0.027**				
HL	F	4.67	2.31	515.41	1.65	2.36	2.07	3.60	2.15
	p	**0.015**	**0.029**	**<0.001**	0.213	0.110	0.052	0.074	0.132
HW	F	17.38	3.21	692.81	2.21	36.93	3.77	17.31	3.16
	p	**<0.001**	0.004	**<0.001**	0.126	**<0.001**	**<0.001**	**<0.001**	0.058
HH	F	0.67	5.78	493.59	10.13	5.22	8.74	5.31	6.03
	p	0.535	**<0.001**	**<0.001**	**<0.001**	**0.010**	**<0.001**	**0.031**	**0.005**
TRL	F	8.05	1.49	20.77	3.09	10.81	1.90	123.06	2.53
	p	**<0.001**	0.177	**<0.001**	0.061	**<0.001**	0.074	**<0.001**	0.097
FLL	F	6.75	1.87	550.61	2.68	2.56	5.78	6.08	0.29
	p	**0.002**	0.078	**<0.001**	0.086	0.096	**<0.001**	**0.021**	0.772
FL	F	24.41	3.99	412.59	3.94	39.97	10.66	0.02	0.24
	p	**<0.001**	**<0.001**	**<0.001**	**0.029**	**<0.001**	**<0.001**	0.900	0.805
TBL	F	6.94	5.20	506.27	2.52	40.92	11.34	8.05	0.08
	p	**0.002**	**<0.001**	**<0.001**	0.097	**<0.001**	**<0.001**	**0.008**	0.921
4TL	F	24.31	7.72	717.63	0.96	16.84	8.96	21.11	2.95
	p	**<0.001**	**<0.001**	**<0.001**	0.412	**<0.001**	**<0.001**	**<0.001**	0.069
HLL	F	9.66	2.16	776.08	0.81	26.50	14.03	41.65	8.09
	p	**<0.001**	**0.042**	**<0.001**	0.471	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Head dorsal	F					6.31	3.16	18.63	1.12
	p					**<0.001**	**<0.001**	**<0.001**	0.268
Head lateral	F					13.06	6.63	13.63	1.62
	p					**<0.001**	**<0.001**	**<0.001**	**0.007**

Significant *P*-values (at $\alpha = 0.05$) are marked in bold letter. See Materials and methods for variables' abbreviations.

d.f., degrees of freedom for each effect, with additional 1 d.f. for intercept and 397 d.f. for error terms; F, value of the statistic; *P*, corresponding FDR adjusted *P*-value.
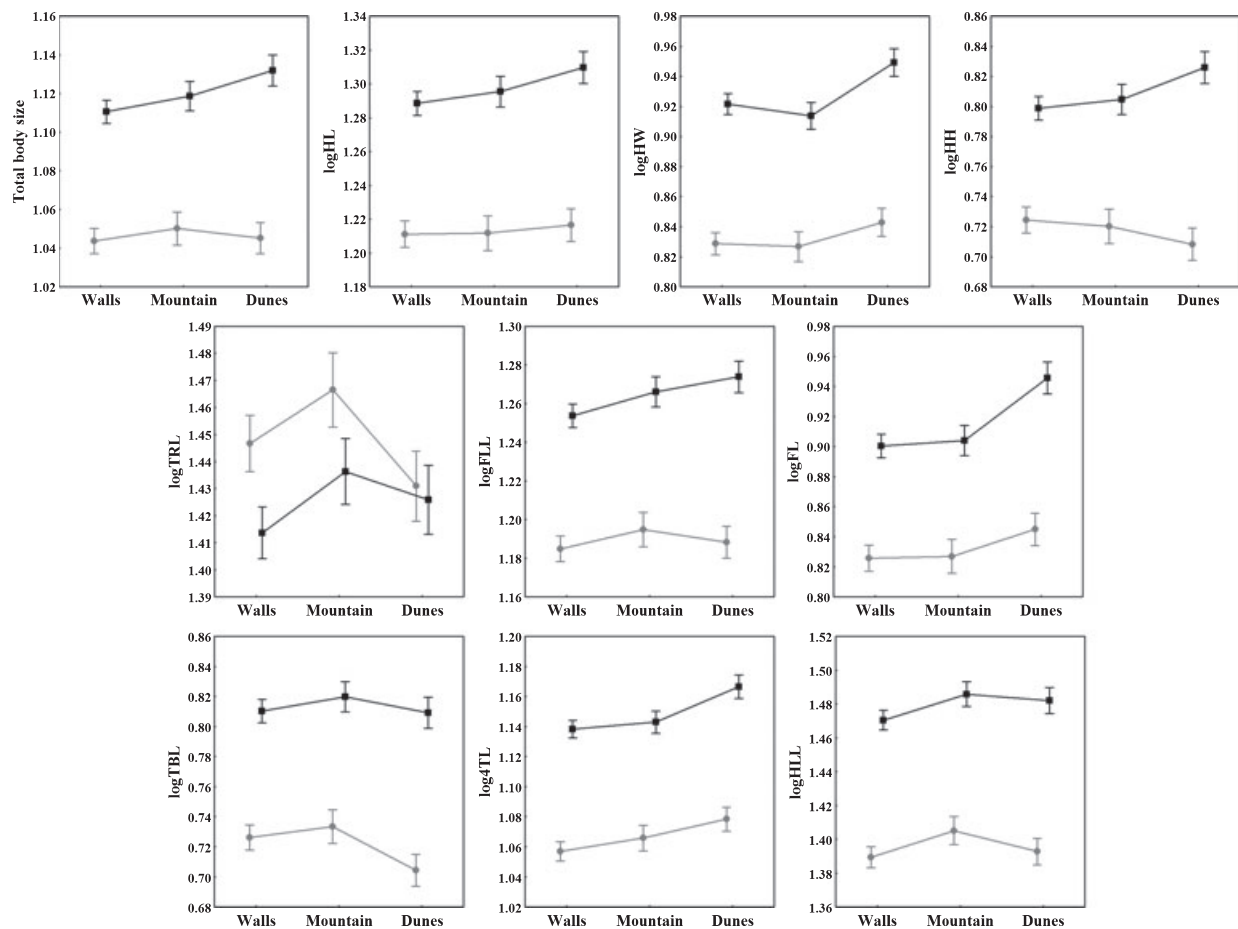
Fig. 2 Means and 95% confidence intervals of multivariate body size (see text for definition) and raw biometric variables in the three examined habitat types, for males (black) and females (grey). See Materials & Methods for variables' abbreviations. See Table 2 and Results for significance of observed differences.

Size of body parts

The effect of SEX was always significant for raw biometric variables, and the effect of HAB was significant in all cases except for HH (Table 2). For head characters (i.e. HL, HW, HH), there were no significant differences between females (Unequal N Tukey's HSD, $P > 0.1$ in all cases), but there was a tendency towards larger head size in males coming from dunes, as compared to those from walls and high mountain sites (Fig. 2; Unequal N Tukey's HSD, $P < 0.05$ always between walls and dunes, and for HW also between mountain and dunes). Concerning limb variation, FL was higher in males from dunes (but not significantly so in females), and 4TL was higher in lizards of both sexes in dunes when compared to the other two groups (Fig. 2; Unequal N Tukey's HSD, $P < 0.01$ in all cases). HAB*SEX significant interactions were observed for HH and FL for which sexual dimorphism was more pronounced in dunes (Fig. 2).

Shape of body parts

For size-corrected variables, the effects of SEX and HAB were significant in most cases, but the interaction term SEX*HAB was only significant for HH and HLL (Table 2). Examination of the traits that are most relevant for habitat-related variation (see further on) revealed that lizards living in dunes had relatively longer femurs (FL), shorter tibias (TBL) and longer hind feet (4TL), (Unequal N Tukey's HSD, $P < 0.01$; Fig. 3). Additionally, females living in dunes had relatively wider heads (HW, Unequal N Tukey's HSD, $P < 0.01$; Fig. 3) and males living in dunes had relatively shorter hind limbs (HLL, Unequal N Tukey's HSD, $P < 0.001$; Fig. 3).

The CVAs conducted on raw and size-corrected biometric data gave consistent patterns across sexes when contributing variables were considered (Table 3). Limb variation was the main source of among-habitat
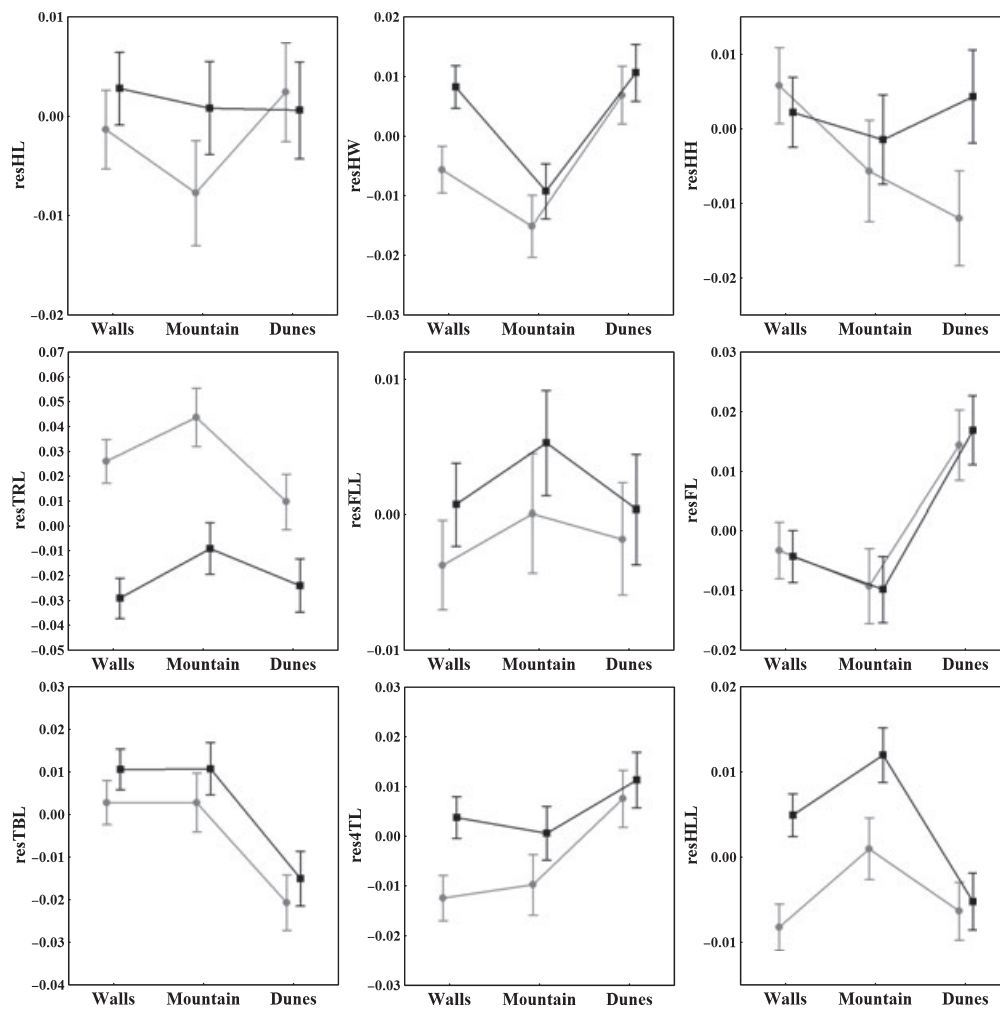
Fig. 3 Means and 95% confidence intervals of residuals of biometric variables in the three examined habitat types, for males (black) and females (grey). See Materials and methods for variables' abbreviations. See Table 2 and Results for significance of observed differences.

differentiation, showing high loadings on both canonical axes, considering both raw and size-corrected datasets (Table 3).

Geometric head shape

manova comparisons on dorsal and lateral head shape indicated a significant effect of SEX and HAB for both landmark configurations; additionally, for lateral head shape, the interaction SEX*HAB was also significant (Table 2). Head shape differentiation between habitat groups as visualized through the examination of deformation grids of group means revealed common patterns in both sexes. For the dorsal view, the main emerging shape difference was a local enlargement of the parietal area of the head and a reduction of the interparietal scale in lizards living in dunes when compared to the other two HABs (Fig. 4). Patterns were more evident for lateral

head shape (Fig. 5); lizards from dunes had a relatively higher posterior head area. In turn, lizards from walls and mountain habitats showed a flattening of the posterior area of the head which resulted in more protruding eyes. Surprisingly, lizards from walls seemed to have a relatively more robust head when compared to those from mountain habitats.

Discussion

Evolutionary relationships between morphological and ecological variation have long been established at the interspecific level for numerous lizard groups. Our study on 11 populations of *P. bocagei* occupying three different HABs indicates important ecomorphological variation at the intraspecific level, and some of the emerging patterns fit previous observations. Lizards from agricultural walls (saxicolous) are smaller in total body size, with smaller

Table 3 Eigenvalues (EV), percentage of among-group variability explained (% exp.), correlations with variables for the canonical variates (CV) extracted from the among-groups relative to within-group variance covariance matrix and percentage of correct classification (% cor.) for absolute values (SIZE) and residuals of biometric variables after isometric-size correction (SHAPE) for each sex separately.

| | SIZE | | | | SHAPE | | | |
| | Males* | | Females† | | Males‡ | | Females§ | |
CR	CV1	CV2	CV1	CV2	CV1	CV2	CV1	CV2
EV	0.719	0.140	0.583	0.207	0.609	0.128	0.573	0.205
% exp.	83.75	16.25	73.83	26.17	82.60	17.40	73.59	26.41
HL	0.246	**0.445**	−0.119	0.010	0.015	−0.166	−0.293	−0.273
HW	**0.517**	0.201	**−0.346**	0.039	**−0.577**	**−0.641**	**−0.622**	−0.228
HH	0.295	**0.436**	0.236	−0.268	−0.119	−0.056	0.304	**−0.613**
TRL	−0.094	**0.578**	0.353	0.228	0.219	**0.522**	0.400	0.236
FLL	0.187	**0.631**	0.050	**0.310**	0.164	0.207	0.018	0.278
FL	**0.570**	**0.599**	−0.338	0.131	**−0.640**	0.348	**−0.591**	−0.014
TBL	−0.134	0.166	**0.458**	0.013	**0.566**	**−0.564**	**0.594**	−0.174
4TL	**0.456**	**0.567**	−0.368	0.382	−0.303	0.099	**−0.538**	0.300
HLL	−0.019	**0.604**	0.158	**0.485**	**0.638**	−0.049	0.225	**0.620**
% cor.	64.08		68.83		63.12		67.54	

The most highly contributing variables are marked in bold letter. See Material & Methods for variables' abbreviations.

*Wilk's lambda = 0.511, $F_{18,418} = 9.279$, $P < 10^{-20}$.

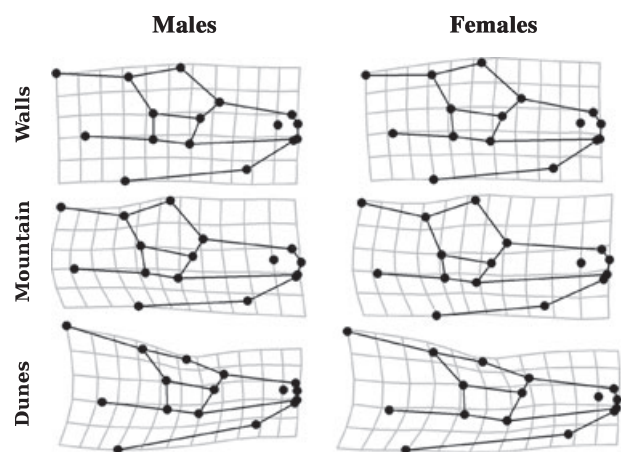†Wilk's lambda = 0.523, $F_{18,360} = 7.644$, $P = 3.33 * 10^{-16}$.

‡Wilk's lambda = 0.551, $F_{16,420} = 9.121$, $P < 10^{-20}$.

§Wilk's lambda = 0.528, $F_{16,362} = 8.526$, $P < 10^{-20}$.
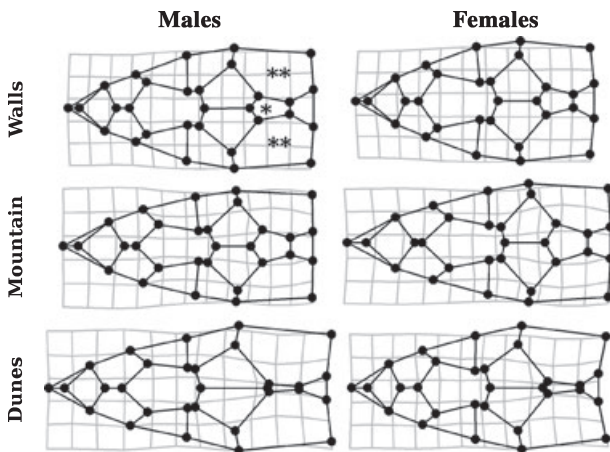
Fig. 4 Mean dorsal head shape for the three habitat types examined, in each sex separately. Patterns are exaggerated five-fold to enhance visualization. *Interparietal scale, **parietal scales. Notice that deformation grids were produced for comparison of shape patterns within each sex and therefore comparisons across sexes do not make sense here.

Fig. 5 Mean lateral head shape for each of the habitat types examined in males (left) and females (right). Patterns are exaggerated five-fold to enhance visualization. Notice that deformation grids were produced for comparison of shape patterns within each sex and therefore comparisons across sexes do not make sense here.

heads, shorter femurs, longer tibias and shorter hind feet than those living in coastal dunes (ground-dwelling). Lizards from high mountain sites (mixed ecological habits) lie between these extremes, being more similar to saxicolous or ground-dwelling ones depending on the character examined. GM give a detailed image of head shape modifications, consistent with the hypothesis of mechanical constraints related to microhabitat and refuge use. Finally, habitat-related differentiation is more striking in males than in females, pointing towards interactions between sexual and natural selection.

Size patterns: interactions between ecomorphological and sexual variation

While ecomorphological analyses in the past mainly examined relative trait variation, based on the impor-

tance of relative limb length for locomotion (Losos, 1990a,b), variation in the absolute size of certain traits, such as total body size and head dimensions, might be more relevant under the hypothesis of habitat constraints on morphology. Male *P. bocagei* with saxicolous habits are smaller in total body size and present lower absolute values for all three dimensions of the head than ground-dwelling ones, suggesting natural selection constraints involving refuge use (Fig. 2). This pattern is interesting, because no such differentiation was detected in a large-scale study including several lacertid species (Vanhooydonck & Van Damme, 1999), confirming that within-species and between-species patterns might vary substantially. On the contrary, no such differences are observed for female lizards (Fig. 2).

This difference in habitat-related variation between both sexes points to interactions between natural and sexual selection (Table 2, Fig. 2). Lacertids in general (Braña, 1996) and *P. bocagei* specifically (Kaliontzopoulou *et al.*, 2007, 2008) are known to be highly dimorphic in both total body size and head size and shape, because of sexual selection on males driven by male antagonistic behaviour and mating (Kaliontzopoulou *et al.*, 2008). How this system comes in balance with other evolutionary pressures, such as the mechanical limitations posed by a saxicolous way of life, has never been considered in lacertids. Our results indicate that such an interaction may in fact be important at the intraspecific level, size sexual dimorphism being amplified in ground-dwelling populations, where no restrictions because of habitat apply. Possibly, habitat openness may provide the conditions for increased male home ranges, which would in turn increase the evolutionary pressures on characters associated to their maintenance, such as body and head size (Stamps, 1983). Although taxonomically limited across lizards (*Anolis*: Butler *et al.*, 2000; Butler & Losos, 2002; Losos *et al.*, 2003; Butler, 2007; Butler *et al.*, 2007; *Bradypodion*: Stuart-Fox & Moussalli, 2007), other studies have also found significant modifications of sexual dimorphism in different habitats, suggesting that the interaction between sexual and natural selection may give variable morphological outcomes.

Head shape: evidence from GM

Head variation in lacertids can be very effectively investigated using geometric morphometric methods (Kaliontzopoulou *et al.*, 2008). This study reinforces such a view; our results reveal significant habitat-related variation in head shape, which confirms biomechanical predictions. Although the results obtained for dorsal head shape are more difficult to interpret in functional terms, patterns are clearer for lateral head shape (Fig. 5). For both sexes, lizards coming from coastal dunes (ground-dwelling) present an enlargement of the posterior area of the head, whereas lizards from high mountain sites and agricultural walls (more saxicolous) have flatter heads,

with protruding eyes. At the interspecific level, this last pattern is known to be characteristic of saxicolous lacertid species and is related to the use of crevices as refuges (Arnold, 1973).

Limb variation: potential ecomorphological significance

Contrary to what is observed for total body size and head characters, variation of absolute and relative limb length is generally concordant between sexes. As previously observed in other lizard species (Vitt *et al.*, 1997; Herrel *et al.*, 2001; Irschick *et al.*, 2005a,b; Calsbeek *et al.*, 2007), limb characters are the main source of within-species ecomorphological variation in *P. bocagei* (Table 3). Such variation is to some extent in line with previous observations and biomechanical predictions; the femur and the hind foot (i.e. FL and 4TL) are longer, both in absolute (Fig. 2) and in relative (Fig. 3) terms, in populations living in coastal dunes. This is also the case for absolute total HLL, at least in males (Table 2, Fig. 2). Longer hind limbs and distal hind limb parts could be beneficial for ground-dwelling lizards, because they increase the thrust provided during running and enhance sprint speed (Garland & Losos, 1994; Aerts *et al.*, 2000; Van Damme *et al.*, 2003). In fact, although information on lacertids is lacking, foot length is known to be the only hind limb element actually correlating to stride length in *Anolis* lizards (Irschick, 2002).

However, the patterns observed for relative tibia (TBL) and HLL contradict our initial hypothesis, because both characters are relatively shorter in ground-dwelling lizards (Fig. 3). According to biomechanical predictions, reduced TBL could enhance stability during climbing by lowering the centre of balance (Arnold, 1998), and we therefore expected saxicolous populations to have shorter limbs and/or tibias. The same is true for total HLL. A detailed study of the kinematics of running and sprint performance in *P. bocagei* from different HABs would provide answers on the exact functional significance and evolutionary basis of the observed morphological patterns, because behavioural modifications may also be involved.

An interesting pattern emerging from the observation of size-constrained vs. size-corrected limb characters (Fig. 2 vs. Fig. 3) is the importance of body size in character variation. Although both sets are involved in ecomorphological differentiation, trait variation is evidently under the influence of total body size: whereas habitat-related differentiation is amplified for some characters when size effects are removed (i.e. femur, tibia, hind foot), for others it is eliminated (FLL in males) or even inverted (HLL in males). This fact, in combination with the complex ontogenetic patterns observed for limb traits in this species (i.e. Kaliontzopoulou *et al.*, 2010), reinforces the view that limb characters are highly plastic. Examination of the ontogeny of limb traits in

different habitats may help to understand the exact mechanisms underlying limb ecomorphological variation. Information on this is extremely scarce for lacertids (but see Sorci *et al.*, 1996), but evidence from other lizard groups indicates that phenotypic plasticity during ontogeny may be playing a crucial role in shaping the patterns observed (Losos *et al.*, 2000; Kolbe & Losos, 2005).

Conclusions

We have shown that both absolute and relative morphological traits, as well as head shape quantified by GM, show significant variation in relation to HAB among populations of *P. bocagei*. Following biomechanical predictions, ground-dwelling lizards have longer hind limb distal elements, but relative limb patterns in some cases confirm and in others contradict previous observations. Total body size and head variation point to interactions between natural and sexual selection, because habitat-related patterns are only observed in males and are concordant with a hypothesis of ecologically constrained sexual variation. However, a detailed study quantifying microhabitat use and locomotion performance in lizards of both sexes in different habitats would provide definite answers on the microevolutionary mechanisms modifying morphology in this species. In any case, our data provide evidence of very fast-evolving ecomorphological adaptation in this group, because *P. bocagei* has expanded its range after a major, recent bottleneck and presents very low genetic variation (Pinho *et al.*, 2007a).

Acknowledgments

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