Genetic and phenotypic component in head shape of common wall lizard *Podarcis muralis*

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Abstract. Head shape in lizards correlates with a wide range of environmental pressures, supporting the hypothesis that patterns of phenotypic change represent adaptive responses to selective processes. However, natural selection promotes evolutionary adaptation only if the trait under selection has enough heritable variation. In this study we used geometric morphometrics and quantitative genetics to assess the heritability patterns of the head shape and size of common wall lizards (*Podarcis muralis*). Genetic and phenotypic components were estimated using animal models, which showed that more than half of the variation in head morphology is inheritable. Furthermore, at least five independent patterns of genetically determined phenotypic change were detected. These outcomes confirm that morphological differentiation in common wall lizards may reliably be regarded as the result of adaptive processes driven by natural selection.

Keywords: evolutionary adaptation, geometric morphometrics, head shape, heritability.

Introduction

Natural selection drives adaptive processes by selecting individual phenotypes (Darwin, 1859). The base for this mechanism to work is the availability of genetic variation for the selected trait. Since the genetically determined (i.e., heritable) part of the observed phenotypic variation of any given trait under selection affects the magnitude and speed of evolutionary processes, the assessment of its value is a central point in evolutionary research. (Ridley, 2003; Collar et al., 2009). Indeed, adaptive processes cannot occur if there is not enough genetic variation in the trait experiencing selection, since changes will not be inherited (Lande, 1979). Heritability (h^2) measures the fraction of the variability in a trait that is the result of gene expression, and can be inherited by offspring. Heritability is generally used to assess if natural selection may, or may not, result in an evolutionary change in the given trait (Falconer and Mackay, 1996; Lynch and Walsh, 1998).

Morphology, including body size and shape, is among the traits that are most often cited as representative examples of natural selection and ecological adaptation (e.g., Beuttell and Losos, 1999). Adaptive relationships between morphology and ecology are mediated by performance, which is usually assessed in terms of locomotor capacities (Arnold, 1983). Adaptive variation among species from different habitats pushes species to excel in those performances that improve survival in nature (Losos and Sinervo, 1989; Sinervo and Losos, 1991; Irschick and Losos, 1999). The occurrence of evolutionary trade-offs between different performance aspects (e.g., running vs. climbing) will shape differences in morphology between animals in different habitats (Vanhooydonck and Van Damme, 2003).

In this scenario, head is a very complex system that plays a plethora of functions, including feeding, breathing, display and sensory perception, and it supplies protection to the brain and sensory systems (Zug, Vitt and Caldwell, 2001; Pianka and Vitt, 2006). The selective pressures

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acting on those different functions may be conflicting, thus driving or limiting the extent and direction of the evolution of head size and shape (Kohlsdorf et al., 2008). For example, the need of crushing large and/or hard prey leads to the evolution of large, tall and wide heads, which can accommodate large jaws and massive muscles (Herrel, De Grauw and Lemos-Espinal, 2001). By contrast, the ability to climb vertical structures conflicts with big and tall heads, which move the centre of mass away from the substrate, thus reducing locomotor performance (Vanhooydonk and Van Damme, 1999). Flat heads and bodies evolve in rock dwelling lizards for climbing, but more so for hiding in crevices and rock cavities (Vitt et al., 1997; Arnold, 1998), while contrasting environmental pressures have led to a dichotomy between short and broad heads vs. long and narrow heads in insular *Anolis* of the Greater Antilles (Harmon et al., 2005).

All above considerations suggest that head shape of reptiles is a very variable trait and changes in shape and size may represent an adaptive response to natural selection. Anyway, all the functional relationships between head morphology and environment or diet can be considered adaptive only if the observed morphological change is heritable (Lande, 1979; Adams, 2011). To date, information on the extent of heritable genetic variation in head shape of lizards is still lacking, but some studies on turtles (Myer et al., 2006) and snakes (Dohm and Garland, 1993; King, 1997; Üveges et al., 2012) suggest that the heritability of morphological traits might be high for lizards too. For example, Myer et al. (2006) estimated the maximal additive h^2 of the plastron shape in *Trachemys scripta* to be 0.52, while Üveges et al. (2012) found the h^2 of eight meristic traits of the head scales in *Vipera ursinii* to vary between 0.32 and 0.70.

In this paper we used geometric morphometrics analyses and quantitative genetics to assess the heritability patterns of head shape of common wall lizard (*Podarcis muralis*) in order to determine genetic and not-genetic components of that trait. Quantitative genetic analyses provide a tool for studying heritability in morphological traits (Falconer and Mackay, 1996; Lynch and Walsh, 1998), as they partition the observed phenotypic variation of morphological traits into genetic (i.e., heritable) and environmental components. For our analysis we used animal models (Lynch and Walsh, 1998) that apply a linear mixed effects model in order to estimate the additive genetic variance component after controlling for the pedigree of the population.

Materials and methods

Subjects and housing

A total of 139 newborns were analysed from clutches of 35 females as a part of an experiment we carried out in the laboratory of the University of Pavia during 2010 (Galeotti et al., 2013). Females were collected by noosing in the surroundings of Pavia (Lombardy, Northern Italy), transferred to the laboratory, and maintained indoor under a natural light-dark cycle in transparent plastic jars (20 × 30 × 20 cm). Each jar was provided with a newspaper sheet as substrate, a water tank and a shelter, and fed with three mealworms (*Tenebrio molitor*) each day. Lighting was maintained with the aid of suspended 5 W incandescent lamps (from 11:00 to 17:00) and UV for calcium and vitamin D fixation was provided by UV-B lamp (18 W). Each jar was supplied with a small plastic box (10 × 10 × 5 cm) filled with wet sand to allow egg laying, and checked for eggs every morning. Eggs were removed and inserted in small plastic tubes (an egg per tube), filled with wet sand and incubated at 28°C (incubator MG316/REP) until hatching (see Galeotti et al., 2013 for details). At the end of the experiment all lizards were returned to their capture sites. For this study we selected only the clutches that produced more than one newborn (mean ± SE: 4.0 ± 0.1; range 2-7).

Data collection and head shape variables

Head shape of both mothers and newborns was quantified using geometric morphometrics (Bookstein, 1991; Rohlf and Marcus, 1993; Adams, Rohlf and Slice, 2004), which quantifies the shape of anatomical objects using the coordinates of biologically homologous landmarks, after the effects of non-shape variation have been mathematically held constant. Digital images of lizards were obtained using a Nikon D50 camera at a 1.2-million-pixel resolution, equipped with a Nikkor 60 mm AF-S Micro lens, at a fixed distance of 18 cm. Mothers were photographed the day of egg laying, whereas offspring photos were taken
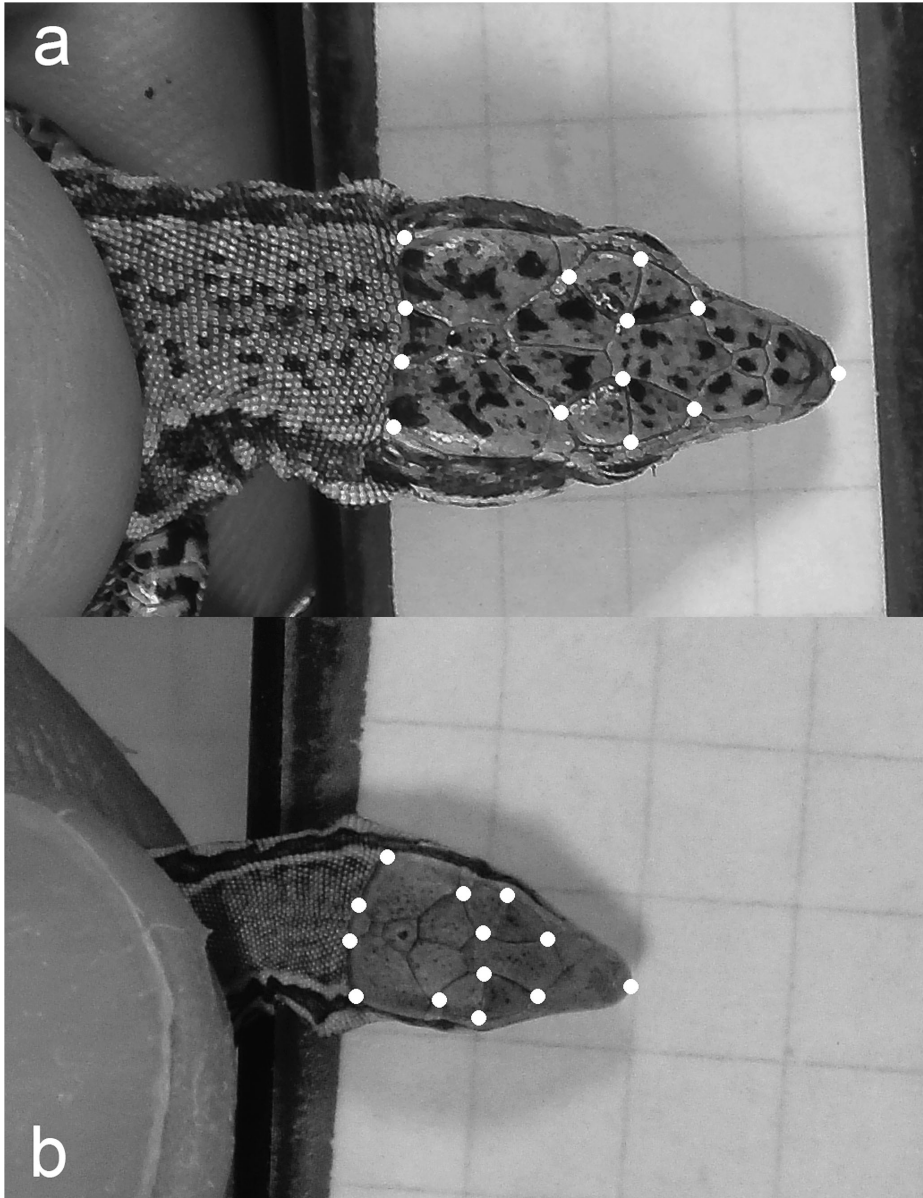
Figure 1. Location of the 13 landmarks used to quantify dorsal head shape of mothers (a) and offspring (b).

at the hatchling day. Next, the location of 13 homologous landmarks (fig. 1) was recorded on each specimens using TpsDig2 (Rohlf, 2010; available at: http://life.bio.sunysb.edu/morph/), and specimens were scaled to unit centroid size and superimposed by a generalized Procrustes analyses (GPA, Rohlf and Slice, 1990). For each specimen, a new perfectly symmetric landmark's configuration was computed in order to remove any effect of fluctuating or directional asymmetries on head shape (Klingenberg, Barluenga and Meyer, 2002; Sacchi et al., 2015). Finally, a principal component analysis (PCA) was carried out on

the variance-covariance matrix of the landmark coordinates, and the set of the first 11 PCs scores (Klingenberg, Barluenga and Meyer, 2002) was used as shape variables.

Head shape heritability using animal models

Animal model approach to estimates head shape heritability was performed using restricted maximum likelihood (REML, Lynch and Walsh, 1988; Knott et al., 1995) implemented in VCE6 (Groeneveld, Kovac and Mielenz, 2008). REML estimation is the preferred method for estimating

variance components from mixed effects models in quantitative genetics (Lynch and Walsh, 1988; de Villemereuil, Gimenez and Doligez, 2013), and it is also appropriate for methods that yield multivariate data, such as geometric morphometrics (Klingenberg and Leamy, 2001). Clutch was used as a random vector for maternal/environmental effect as in our breeding design we used a single female for each jar and thus could not disentangle maternal and environmental effects (Klingenberg and Leamy, 2001). Furthermore, we included the head size (estimated by the logarithm of the centroid size) as fixed effect to control for allometric effects. VCE6 provides a partition of the phenotypic covariance matrix P in the additive genetic G, maternal/environmental M, and residual R covariance matrices, in such a way that P = G + M + R. Given that sires were unknown in this study, we treated hatchlings as full-sibs, which provided a conservative estimate of heritability (Myers et al., 2006; Adams, 2011).

The amount of phenotypic, genetic, and maternal/environmental variation in head shape was assessed by examining the eigenvalues of the phenotypic and additive covariance matrices, which allows evaluation of the amount of variation associated with the different axes in the shape space (Klingenberg and Leamy, 2001). Then, the matrix GP^{-1} was estimated (where P^{-1} is the generalized inverse of P) and used as the multivariate analogue of heritability (Roff, 1997; Klingenberg and Leamy, 2001). The eigenvectors of the GP^{-1} matrix represent the directions of the response to selection in phenotype space, and the eigenvalue corresponding to the first eigenvector is the maximum additive heritability, h^2_{max} (Klingenberg and Leamy, 2001; Meyers et al., 2006; Adams, 2011). Nonetheless, the eigenvalues of all other eigenvectors (h^2_i) were considered in order to assess the extent of the response to selection. We then assessed the statistical significance of the h^2_{max} as well as of the other h^2_i estimates using a permutation procedure, in which individuals were randomly assigned to clutches, the G and P matrices recalculated in VCE6, and eigenvalues obtained. Because the calculations of the variance components using REML were computationally intensive, 999 permutations were used to generate a theoretical distribution of possible h^2_{max} and h^2_i for comparison to the observed h^2_{max} and h^2_i estimates. The MP^{-1} matrix was then estimated to examine the contribute of the not-genetic (environmental/maternal) component on phenotypic variation of head shape, and eigenvalues and statistical tests were obtained following the same procedures as for the GP^{-1} matrix.

Although our primary focus was on head shape, we also performed a separate analysis on the head size, since both shape and size contribute to characterize the head as a whole. The analysis of head size was performed using the same model as for head shape, and h^2 was obtained by dividing the genetic by the phenotypic variance.

Results

Phenotypic and genotypic variation of head shape

The total variance for the phenotypic covariance matrix as estimated through its trace was 18.86×10^{-4}, whereas the total variance associated with the additive covariance matrices were 9.51×10^{-4} for the genetic component, and 3.83×10^{-4} for the maternal/litter component (in dimensionless units of squared Procrustes distance). Consequently, the additive genetic component accounted for near half of the total phenotypic variation and was three times of the maternal/environmental component, even if these values cannot be interpreted as heritability of head shape, as shape is a multivariate object (Klingenberg and Leamy, 2001). The eigen decomposition of phenotypic and additive covariance matrices showed that much of variation was concentrated in the first few PCs. In the genetic and particularly in maternal/environmental matrices emerged a tendency for the last eigenvalues to become slender and drop to zero (fig. 2). Indeed, the first three dimensions in the genetic and maternal/environmental covariance matrices captured 75.4% and 83.8% of the total variation respectively, whereas the same dimension in the phenotypic covariance matrix explained only 63.0% of head shape variation. The axes' orientation in the additive genetic and phenotypic covariance matrices was similar (table 1), since the angle between the first major axes of the two matrices was just 28°, the angle between the second axis of the genetic matrix and the third axis in the phenotypic matrix was 40°, while the third axis in the genetic matrix diverged from the second axis in the phenotypic matrix for only 31°. The axes' orientation in the additive maternal/environmental and phenotypic covariance matrices was much less similar, as axes diverged from one another for more than 45° (table 1).
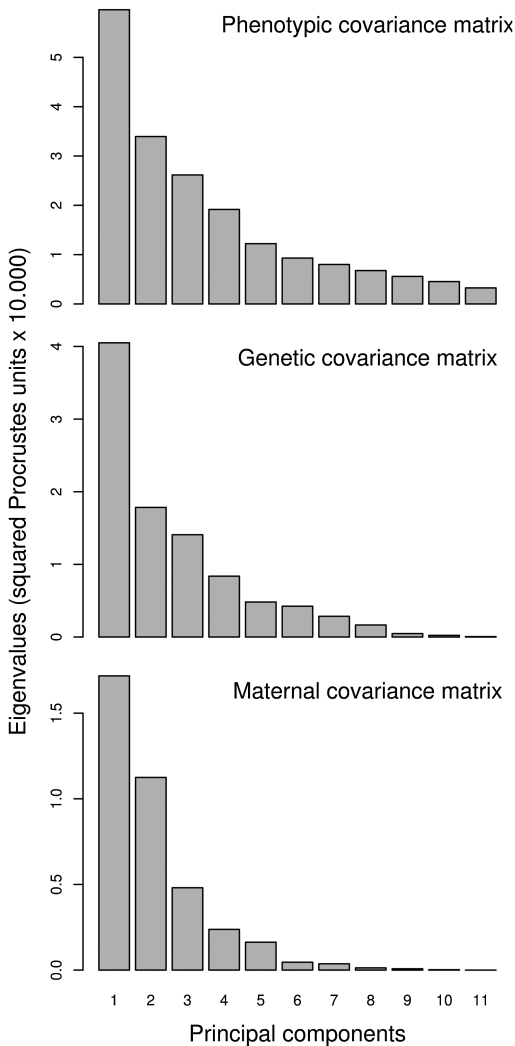
Figure 2. Eigenvalues of the P, G and M variance covariance matrices.

Table 1. Relative orientations of the first three axes of the phenotypic covariance matrix with respect to the genetic and maternal/environmental covariance matrices.

Phenotypic covariance matrix	Genetic covariance matrix			Maternal/ environmental covariance matrix		
	PC1	PC2	PC3	PC1	PC2	PC3
PC1	28°	63°	85°	56°	81°	65°
PC2	87°	89°	31°	62°	45°	68°
PC3	71°	40°	82°	78°	56°	61°

The patterns of variation we found in the first three PCs of the phenotypic covariance matrix were diverse and involved the simultaneous

changes of landmark positions in different portions of the head (fig. 3). PC1 (31.03% of the total variation) included longitudinal shifting of landmark positions, mainly loaded on the tip of the snout and the occipital region (fig. 3). PC2 (17.69% of the total variation) principally accounted for displacements in the positions of the landmarks associated with the ocular regions and the outer margins of the occipital portion of the head (fig. 3), while PC3 (14.3% of the total variation) included changes in position of near all landmarks and accounted for a longitudinal compression with an enlargement in the lateral plan (fig. 3).

Shape changes associated with the first three PCs in the additive genetic covariance matrix (fig. 3) were similar to those observed in the phenotypic covariance matrix, given the orientation pattern of the axes of the two matrices (table 1). Indeed, landmark displacements included in PC1 (41.42% of the total variation) were similar to PC1 of the phenotypic covariance matrix, and the same occurred for PC2 (17.51% of the total variation) with respect to PC3 of the phenotypic covariance matrix (fig. 3). Similarities occurred also between the pattern of landmarks' displacement observed in PC2 and PC3 of the phenotypic and additive genetic covariance matrices respectively (fig. 3), even if to a lesser extent than in the other two previous cases. Therefore, the genetic and phenotypic patterns of variation were similar, even not identical.

The changes associated with the first three PCs in the additive maternal/environmental covariance matrix differed in general from those associated with the phenotypic covariance matrix (fig. 3), although some landmarks had analogous displacements (e.g., the snout tip). For example, changes in the landmarks of the snout involved in PC1 were the same as in PC1 of the phenotypic covariance matrix, but landmark displacements accounted for the same PCs in the occipital region were entirely different (fig. 3). These comparisons suggested that the
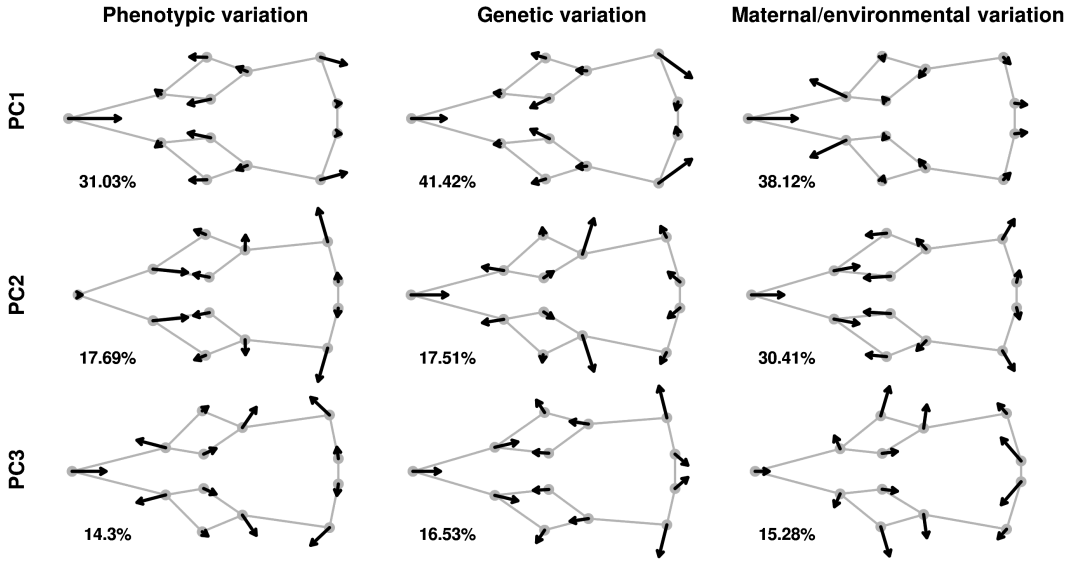
Phenotypic variation   **Genetic variation**   **Maternal/environmental variation**

Figure 3. Comparison of phenotypic change of head shape according to the first three PC for the P, G, and M variance covariance matrices. The arrows illustrate the shape evolution from positive to negative values along PCs.

maternal/environmental pattern of shape variation was quite dissimilar with respect to the phenotypic pattern of variation.

The analysis of the GP^{-1} matrix revealed that the first eigenvalue (h^2_{max}) was 0.95 and the second (h^2_2) was 0.89, whereas the first five exceeded 0.69 (fig. 4). From these results we can draw two main conclusions: first, a considerable proportion of head shape variation seems to be genetically inherited, and second, selection seems to act in multiple (at least five) directions in which driving evolutionary changes. Shape changes characterized by each of these five eigenvectors were distinct and depicted different deformation patterns as represented by a different subset of landmarks (fig. 5). The pattern included in the first eigenvector represents a shortening of the snout with a general longitudinal stretching of the occipital region. The second eigenvector included the position displacement of the four landmarks at the posterior margin of the head, controlling the position of the posterior margin of the occipital region. The third eigenvector included particularly the landmarks that define the position of the eyes
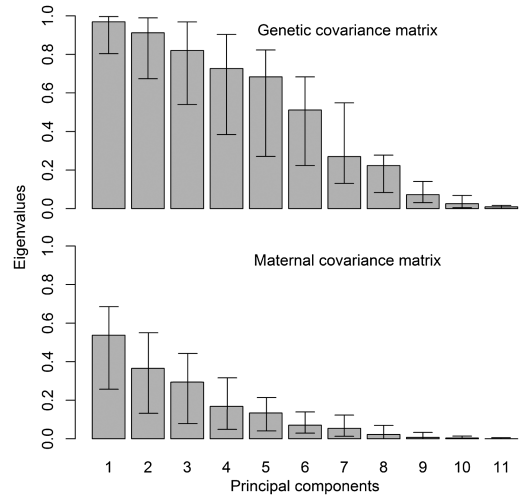

Figure 4. Eigenvalues of the GP^{-1} and GM^{-1} matrices with the 95% confidence interval after 999 bootstraps.

and their rotation with respect to the longitudinal axis of the head. The fourth eigenvector involved the head sides and described a general enlargement and expansion of the head, while the fifth eigenvector depicted a slendering of the head shape, particularly in the occipital region.

At the opposite, the first two eigenvalues of the MP^{-1} matrix were 0.53 and 0.37 respectively, while the others became slender (fig. 5).
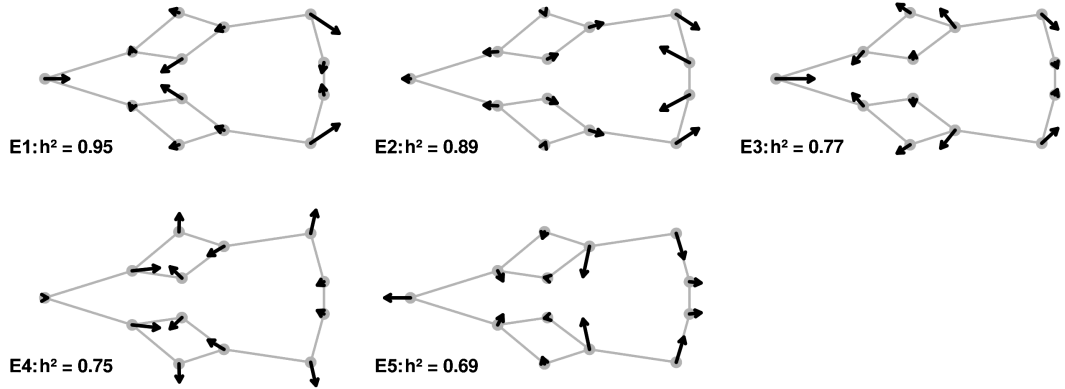
Figure 5. Phenotypic change associated to the first five eigenvectors of the GP^{-1} matrix (E1 to E5), displaying the heritable pattern of variation in head shape of common wall lizard. The values reported below the images correspond to the eigenvalues, which estimate the h^2 coefficient for the corresponding pattern of phenotypic change. The arrows visualize shape evolution from positive to negative values along each eigenvector.

Phenotypic and genotypic variation of head size

The mean logarithm of the head's centroid size was 5.84, with a standard error of 0.01. The additive variances estimated by VCE6 were 41.2 for the genetic component and 36.0 for the maternal/environmental effect, while the residual variance was nearly null (3.5×10^{-7}). Consequently, h^2 of head size was 0.53, with a 95% confidence interval of 0.51-0.56 (after 999 bootstraps). In order to assess why the residual variance was virtually absent, we plotted the density distribution of the logarithm of the head's centroid size and we found a clear bimodal pattern with the peaks at 4.35-4.40 and 4.50-4.55 mm respectively (fig. 6). The coefficient of variation for the logarithm of the head's centroid size within family was on average $1.5 \pm 0.2\%$ SE, suggesting that offspring were very similar in head size within clutch. Consequently, the two peaks in the density distribution corresponded to mothers laying clutches with small vs. large headed offspring respectively.

Discussion

Head shape exhibits an amazing variability in lizards, which has been shown to correlate
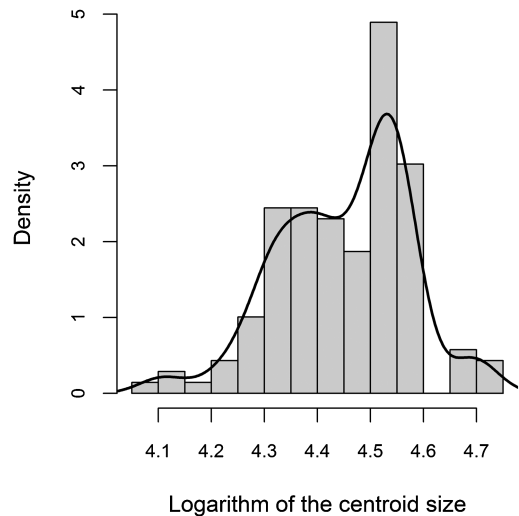

Figure 6. Density distribution of the logarithm of the centroid size for the common wall lizard offspring. The line represents a density kernel.

with environmental pressures (Vitt et al., 1997; Arnold, 1998; Vanhooydonk and Van Damme, 1999; Herrel, De Grauw and Lemos-Espinal, 2001; Harmon et al., 2005), supporting the hypothesis that patterns of phenotypic change in head shape should be the result of adaptive responses to selective processes. However, natural selection promotes evolutionary adaptation only if the trait under selection has enough genetic

(i.e., heritable) variation on which the selective processes can actually work (Lande, 1979). Otherwise, the observed variability is not adaptive because natural selection will not yield evolution on the given trait. Our results showed that head shape holds a large component of heritable genetic variation. Indeed, the animal model analysis gave an estimate for the first five dominant eigenvectors of the GP^{-1} matrix to be higher than 0.69 (the maximum additive heritability, h^2_{max} being 0.95). These values are consistent with the findings of previous studies that analysed heritability of morphological traits in other species of reptiles (e.g., plastron in *Trachemys scripta*, Myer et al., 2006; head scales in *Vipera ursinii*, Üveges et al., 2012), or used geometric morphometrics (head shape in *Plethodon* ssp., Adams, 2001). Our high estimates of heritability indicate that the head shape of common wall lizards has a substantial genetic base; therefore observed patterns of phenotypic variation can be reliably regarded being the result of adaptive processes shaped by natural selection.

Using the animal model approach we were able to find at least five main patterns of genetically inherited phenotypic change in head shape, revealing that head shape in lizards harbours a wide array of adaptive responses to natural selection. The genetic patterns of morphological change include longitudinal and lateral stretching, dilatation or shrinkage in a well determined portion of the head (e.g., the nape rather than the face or the ocular region), and are wholly consistent with the phenotypic changes that have been regarded as adaptive or ontogenetic responses in lizards. For example, the widening of the occipital region with the simultaneous outward rotation of eyes matches the pattern of variation of sexual dimorphism found in island populations (Sacchi et al., 2015), as well as the developmental process leading to sexual dimorphism characterized by the prominent jaw muscles of males (Sacchi et al., 2015). Recently, Lazic et al. (2015) used geometric

morphometrics to analyse the patterns of phenotypic variation in head shape of adult common wall lizards, and found a relationship between morphology and habitat: individuals living in urban environment were more variable than those of rural areas. Interestingly, they found most of the variability to be contained in the distal part of the parietal region, and the phenotypic patterns of the first PCs (see fig. 2 in Lazic et al., 2015) match those we found for the genetic component. Therefore, the morphological variability observed between rural and urban populations may have a consistent genetic base, and could have been arisen and maintained by selection. Furthermore, the variation in cranium shape in five species of morphologically similar *Podarcis* lizards was shown not to be phylogenetically originated, but to be largely influenced by species ecology, and particularly by species having a terrestrial vs. saxicolous habit (Urosevic et al., 2012). In particular, saxicolous species (including *P. muralis*) had elongated parietals, elongated cranium bases, shortened anterior parts of the dorsal cranium, and reduced chambers of the jaw adductor muscles, leading to a flattened head, well adapted to dwell on vertical surfaces and to seek refuge in crevices. Terrestrial species on the other hand exhibited more variation in head shape, which appeared more elongate and robust with enlarged chambers of the jaw adductor muscle, reduced skull bases and shortened parietals. These patterns of phenotypic change are concordant with the genetic pattern we found in our study, supporting that when species are subjected to different environmental pressures, their evolutionary responses to selection result in distinct head shapes because each species evolves along a diverging heritability trajectory.

A second relevant result of our study was the low residual variance in the analysis of head size heritability, as the animal model assigned the entire variability of the head size to the genetic and maternal components. This result

likely depended on the response to the analysis to mixture data, i.e. data with bimodal (polymodal) distribution resulting from the presence of sub-populations within an overall population. Depending on ventral colouration (see Sacchi et al., 2013 for details), common wall lizard females adopt an r or K strategy, thus producing large clutches of small offspring or small clutches of large offspring (Galeotti et al., 2013). In our study, females were randomly selected for morph, as the only constraint we imposed concerned the minimum number of individuals per clutch (i.e., two). Our sample included females from all morphs, and the r/K dichotomy was clearly evident in the density distribution of offspring size. Furthermore, morph strategies are genetically determined (Calsbeek et al., 2010), and the head size variance within family in our sample was minimal. All above things considered, the animal model supplied an estimate of h^2 by detecting high similarity of individuals within family resulting from both mother strategy (genetic effect) and condition (maternal effect). Mixture data are not expected to affect the estimation of variance component by the model since the mother size was not considered and genetic relationships among offspring are derived only from the pedigree.

In conclusion, our study supports the hypothesis that head shape variability in lizards has a substantial genetic component, and morphological differentiation within and among species may actually be explained as the result of adaptive processes driven by selection.

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