

Morphological variation and sexual dimorphism of the cephalic scales in *Lacerta bilineata*

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Abstract

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The Western green lizard (*Lacerta bilineata*) is a lacertid distributed throughout Mediterranean and Central Europe. Little is known about the morphological variability and sexual shape differences in this species. In this paper, the variation of the cephalic scales in *L. bilineata* is analysed by means of a geometric morphometric approach. A main structural pattern is characterized by negative allometry of the frontal and interparietal areas, and positive allometry of the parietal and frontoparietal scales. Sexual differences are described both in size and shape. In males, the scales are generally larger, with relative shortening of the frontal area, frontoparietal enlargement, occipital lengthening and bulging of the parietal scales, which compresses and narrows the interparietal and occipital areas midsagittally. This pattern is based on a shared allometric trajectory, with males displaying a peramorphic morphotype. However, males show some shape differences in the occipital area that are not size-related, and cannot be interpreted in terms of general head enlargement. This structural trajectory can be related to the development of the skull, but the role of soft tissues (temporal, nuchal and masticatory muscles) must also be considered. The development of the jaw and nuchal muscles involved in intra- and intersexual behaviours could have played a pivotal role in the evolution of this pattern.

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Introduction

In vertebrates, most sex-related morphological differences are the result of ontogenetic scaling, heterochronic processes and allometric variations (Gould 1977; Shea 1992; Klingenberg 1998). Males are generally larger than females because of an increase in the time or rate of development, namely a prolongation or acceleration of the ontogenetic process. Depending on which of these two types of change is involved, a consequent variation of the shape will occur based on the structural relationships between the parts of a morphological system (Shea 1983). According to the principles of geometric morphometrics (e.g. Bookstein 1991; Adams *et al.* 2004), *shape* refers to the geometric properties of an object that are invariant to changes in translation, rotation and scale. Even

if a certain percentage of features can be sensitive to localized adaptations through direct sexual selection, much of the shape variation is expected to be size-related, i.e. based on a shared structural model scaled at a different magnitude.

Following a principle of subtraction (Gould 1966) we may distinguish between features that are the result of a new reorganization of the biological structure and features that represent variations of the same structural model. In the first case, these neomorphic changes probably represent direct evolutionary responses (i.e. adaptations). In the second case, variations represent responses of a whole functional system, in which causes and consequences are difficult to recognize as single and independent components. Accordingly, it can be useful to localize sexual differences that are size-free (that are likely to have a direct influence on selection) from those that

are size-dependent (that must be interpreted within the whole structural network and that may not necessarily have a direct adaptive value). Clearly, this approach represents a conceptual tool that is useful when developing evolutionary models, and the boundaries between allometric and non-allometric variations can be rather fuzzy and less discrete within the actual evolutionary processes.

This exploratory paper describes and discusses the morphological variability of the cephalic scales in *Lacerta bilineata*, a medium-sized lacertid distributed throughout Mediterranean and Central Europe (Arnold and Burton 1978; Rykena 1991; Amann *et al.* 1997). The head scales are used as useful indicators of head morphology, as they cover the entire cephalic volume and represent visible and possibly homologous anatomical references. We analyse the shape variations by means of geometric morphometric tools, to hypothesize a structural pattern representing the intra-specific and sexual variability of this taxon. The term 'structural pattern' is used here to describe the whole biomechanical relationship underlying a represented morphological variation. Assuming this inner physical environment as an important source of evolutionary changes (*sensu* Thompson 1942), the term 'structural trajectory' refers to the variants (often size-related) of such a structural model. Accordingly, we consider both the allometric structural constraints and the size-independent variables to identify further ecological or behavioural factors involved in the variability of green lizards.

The main hypothesis of this paper is that head-shape differences in male and female green lizards (if any) are only related to size variation and allometric consequences. If this hypothesis cannot be falsified, a possible sex-related selection cannot be easily differentiated from adaptations that are associated with intrinsic variations in the biological model or from other factors such as energy requirement or biomechanical constraints. In contrast, if this hypothesis is falsified, a certain percentage of morphological difference between males and females can be interpreted as a direct effect of sexual factors.

Materials and Methods

Twenty adult males and 20 adult females of *L. bilineata* were collected near Rome in summer–autumn 2003 after the reproductive season. The specimens were collected either as prey remains in nest-boxes of kestrels (*Falco tinnunculus*) or by capture–release of live individuals. Prey remains were used only when the preservation was sufficient to investigate head morphology and sexual characters. Because of the exploratory and phenetic targets of this paper, the different source of the specimens does not bias the sample. However, preliminary non-parametric tests were performed to check differences between these two groups, showing a marked phenotypic homogeneity. Each specimen was directly sexed by visual inspection of the sexual characters (e.g. presence of hemipenis).

The cephalic area was photographed in dorsal view, with the head resting on a plane and the camera orthogonal to the plane. The camera was about 20 cm from the specimen to limit the parallax distortion. A scale bar was used as metric reference to compute size and metrics. A set of repeated samplings showed a very small intraobserver error (with respect to the sample variability), which did not bias the final results. The length of the frontal scales has been measured directly on the specimens and on the scaled digital pictures using IMAGEJ (available at <http://rsb.info.nih.gov/ij/>) to check the reliability between actual diameters and two-dimensional projected photographs, showing no significant differences. This variable has been interesting in terms of sexual dimorphism, representing a useful metric reference with which to compare the absolute development of the cephalic morphology.

A landmark configuration was selected based on the points of junction of the frontal, frontoparietal, parietal, interparietal and occipital scales with the contiguous structures (Fig. 1). Two more landmarks were sampled at the maximum cephalic width, on the parietal outline. In general, as size increases the head becomes less flattened, with a light curvature along the midsagittal plane that may involve a certain distortion of the two-dimensional projection. The metric comparison of the frontal scales and the re-sampling of specimens show that this bias is probably limited. Nevertheless, it is recommended that future efforts should aim at improving these results through three-dimensional analyses. Two-dimensional coordinates were sampled for each photograph using TPSDIG 1.20 (Rohlf 1998a) and analysed by means of geometric morphometric procedures (Bookstein 1989; Marcus *et al.* 1993, 1996; Rohlf and Marcus 1993; Adams *et al.* 2004). The coordinate systems were superimposed through Generalized Procrustes Analysis (GPA) by translation to a common centroid, scaling to unitary size, and rotation according to a least-square procedure (see Bookstein 1991). This superimposition minimizes the differences between different configurations (Rohlf 2003). To test shape differences between sexes, a permutation test was performed

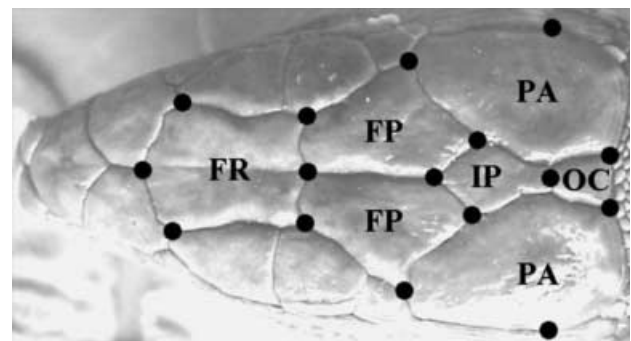


Fig. 1—Landmark configuration of the cephalic scales. FR, frontal scales; FP, frontoparietal scales; IP, interparietal scale; PA, parietal scales; OC, occipital scale.

on the Procrustes distances. Namely, after a set of randomized sampling, the observed (actual) mean difference between sexes is checked against the percentiles of the randomized distribution. This approach is useful for analyses even on a singular covariance matrix (i.e. possibly on bilateral configurations or superimposition residuals) and it is recommended when the sample size is not very large for the number of variables (D. Slice, personal communication). The GPA, permutation test and the average male/female shapes and comparison have been computed with MORPHEUS ET AL. (Slice 2000). Centroid size was used as a size index, computed as the square root of the sum of squared distances of a set of landmarks from their centroid (Marcus *et al.* 1996). Size differences between males and females were analysed by the non-parametric Mann–Whitney test ($P < 0.05$). A Principal Component Analysis (PCA) on the shape variables after superimposition was performed with TPSRELW 1.18 (Rohlf 1998b) and APS 2.3 (Penin 2000), to characterize the overall variability in head measurements. A discriminant analysis between males and females was performed with APS, to describe the sexual differences through a multivariate vector. Allometry was analysed using TPSREGR 1.20 (Rohlf 1998c), to test the correlation between centroid size and each shape components, and TPSPLS 1.11 (Rohlf 2002) to test the correlation between centroid size and a linear combination of the shape variables (Corti and Rohlf 2000). Multiple correlation between principal components and centroid size was performed with APS to localize the allometric component through the morphospace. TPS softwares and MORPHEUS ET AL. are available at <http://life.bio.sunysb.edu/morph>, while APS is available at <http://www.procruste.com>.

A major axis between centroid size and shape vector was computed using MODEL II (Legendre 2001) to describe the actual relationship between the two variables. The analysis of covariance was performed to test size-independent shape differences between males and females. This latter test requires the exclusion of the outliers, and two specimens (one female, one male) were accordingly discarded.

Geometric morphometrics allows us to analyse variability via a multivariate approach to the spatial relationships among landmarks. The result is related directly to the structural and functional network of the morphological system. Because the Procrustes superimposition minimizes the size differences, the remaining size-related part of the residual shape is the allometric component (i.e. the morphological variability resulting from size variations plus intrinsic structural constraints). In this context, geometric morphometric techniques are very useful for analyses of sexual variability and allometric patterns. Such tools, although widely applied, are sometimes discussed because of the possible biases on the statistic estimation (see Lele and McCulloch 2002; Richtsmeier *et al.* 2002; Rohlf 2003). Of course, superimposition procedures and interpolation functions produce interpretations of the actual morphology developed onto a set of *a priori* assumptions, and the results must be intended as models of

the underlying reality (Bruner 2004). That is, every superimposition approach will be influenced by its conceptual and technical framework, directly affecting the final results. For example, it is well-known that large differences associated with single landmarks will be distributed within the entire configuration through a Procrustes approach, producing artefacts in the distribution of the residuals (Rohlf and Slice 1990).

The analysis of bilateral structures requires some additional information. For example, if asymmetry is not the main target of the study (generally needing very large sample sizes to test differences that are often rather subtle) it can be a confounding factor in a multivariate approach, and requires methodological control (Klingenberg *et al.* 2002). Furthermore, multivariate approaches based on inversion of the covariance matrix are strongly biased by the redundancy of the symmetrical information (Bookstein 1996). At the same time, half configurations – used as an alternative to the entire landmarks set – do not represent the true balance of the original biological structure. The result can be an incorrect distribution of the variation among the landmarks during the superimposition procedure, with unnatural consequences such as bending of the midsagittal axis. Both the superimposition procedures and the multivariate ordination of the shape components will represent a correct consideration of the morphological variability, but the interpretation of the respective biological (structural) meaning can be misled by the artificial display of the unilateral configurations. Nevertheless, the bending of the midsagittal plane often displayed using unilateral configurations is particularly disadvantageous, both in terms of structural and visual interpretation.

In this study, a full bilateral configuration was used in the exploratory analyses of the structure (Relative Warp Analysis, Partial Least Square). A symmetrical configuration was computed for each specimen by alignment of the midsagittal profile along the x -axis, inversion of the paired landmarks, and averaging with the original data (see Klingenberg *et al.* 2002). A baseline from the anterior frontal landmark to the anterior occipital landmark was taken as the midsagittal reference. Along this baseline, asymmetrical deviations of the midsagittal profile (possibly influencing the superimposition procedure) were not noticed. This approach averages possible biases (related to the morphological variation itself or to the sampling procedure) displayed on the single sides, reducing noise. Conversely, multivariate regression onto centroid size and multivariate analysis of variance – biased by a bilateral redundant model – were computed on averaged half configurations. However, it is worth noting that there were no differences in the results (patterns or quantitative indexes) when the bilateral, unilateral or even natural (i.e. asymmetrical) configurations were used.

Another widely recognized morphometric tool is the Euclidean Distance Matrix Analysis (EDMA – see Richtsmeier *et al.* 2002 for details and references). According to this approach, all the possible inter-landmark distances between two systems of coordinates are compared as ratios.

The Form Difference Matrix (FDM) represents the whole set of compared distances, and is available for statistical tests and to form analyses. Usually, this procedure does not support a separation (either mathematical or conceptual) between size and shape, approaching ‘form’ as the actual phenotypic product. Nevertheless, although EDMA is generally used to avoid scaled metrics, a scaling factor (baseline, geometric mean, median distance, etc.) can be introduced to perform specific analyses (Cole and Richtsmeier 1998). One of the most common criticisms comparing EDMA with geometric morphometrics is the lack (in the former) of an efficient visualization tool, represented in the latter by the distortion grids, vector displacements and geometric links. Using the present case study, we introduce and propose a method to visualize the EDMA results through chromatic maps. EDMA was computed on half configuration. To compare the geometric morphometric results with the EDMA map, EDMA was computed on the Procrustes-adjusted coordinates. Results will then show similar differences, making available a comparison between the two visualization methods. Of course, a similar approach should be performed on non-superimposed data in classic EDMA studies.

The superimposed data produce a Superimposed Shape Difference Matrix (SSDM) based on the ratios of correspondent inter-landmark distances, and differences between sexes can be tested accordingly. Determinant inter-landmark distances have been considered to be those exceeding one standard deviation from the mean value. The median value of each landmark from the SSDM (i.e. the median distance of each landmark from all the others after superimposition) has been plotted onto the consensus configuration to compute the pattern of sexual variation through a Shape Difference Map (SDMAP). The values have been interpolated using a radial basis function through multiquadric interpolation (Carlson and Foley 1991). This is not an exact interpolant function, and is therefore generally available for small and scattered databases. However, interpolations with exact functions gave comparable results, and residuals from the interpolated plane are rather limited. This map is able to synthesize patterns of relative enlargement/reduction along the structure considered, through a chromatic variation. Because of the GPA superimposition, the median ratio is expected to be 1. Clearly, maps are but a visualization tool, useful to order differences in a more synthetic way, and inferences on the associated biological meaning must be developed through adequate considerations and the respective analysis of the influential landmarks (Cole and Richtsmeier 1998). EDMA has been performed using WINEDMA (Cole 2002), available at <http://oshima.anthro.psu.edu/edma.html>. SDMAP was computed using SURFER 7.0 (Golden Software, Inc.).

Results

Considering centroid size, males have a larger cephalic structure than females ($Z = 4.84$; $P < 0.001$). Also considering

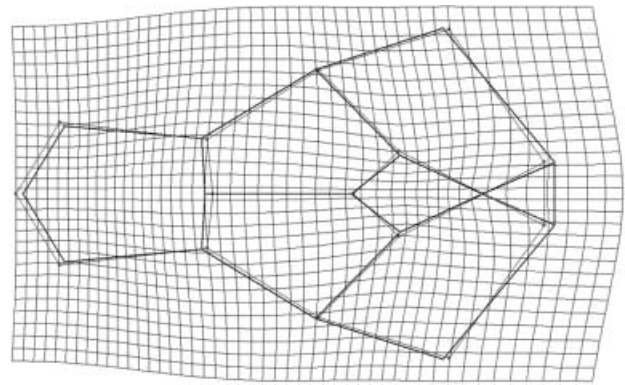


Fig. 2—Female average shape (thin line) warped onto the male average shape (bold line) after Procrustes superimposition ($\times 3$).

the length of the frontal scales, males (10.6 ± 1.4 mm) show higher values ($Z = 2.62$; $P < 0.01$) than females (9.5 ± 1.0 mm). Randomization of the male and female coordinates after Procrustes superimposition shows a significant shape difference (999 permutations; $P < 0.001$). When the female average shape is superimposed and warped onto that of the male, the main differences are the relative shortening and narrowing of the frontal scale, frontoparietal lengthening, interparietal narrowing, and a marked backward nuchal lengthening (Fig. 2).

The first two principal components account for 48% of the total variance, with the first axis separating males and females and the second axis describing a shared within-population variability (Fig. 3A). The first principal component (PC1; 30% of the total variance) is related to (from lower to higher values) frontal shortening, frontoparietal and parietal development, interparietal coronal flattening, and marked occipital enlargement (Fig. 3B). PC1 is normally distributed, with males showing higher values ($P < 0.01$). PC2 (18% of the total variance) mainly involves (from lower to higher values) forward development of the interparietal scale, associated with occipital stretching and minor frontal shortening (Fig. 3C). This component mainly separates individuals with midsagittally developed interparietal scales and narrow occipital area from individuals with shorter interparietal scales and widened occipital scales. PC2 is not normally distributed because of a tail shifted to lower values in some males. However, no significant sex differences were found. The subsequent components show variance of less than 10%.

A partial least-square correlation between the shape vector and centroid size is highly significant ($R = 0.84$; $P < 0.001$). Larger cephalic structures show development of the parietal, frontoparietal and occipital scales, relative shortening of the frontal areas, and coronal flattening of the interparietal scale (Fig. 4). The correlations between centroid size and the whole shape variables are also significant (Wilk's lambda = 0.13, $P < 0.001$; Generalized Goodall F -test: $F_{16,6} = 9.9$, $P < 0.001$), and display the same morphological pattern.

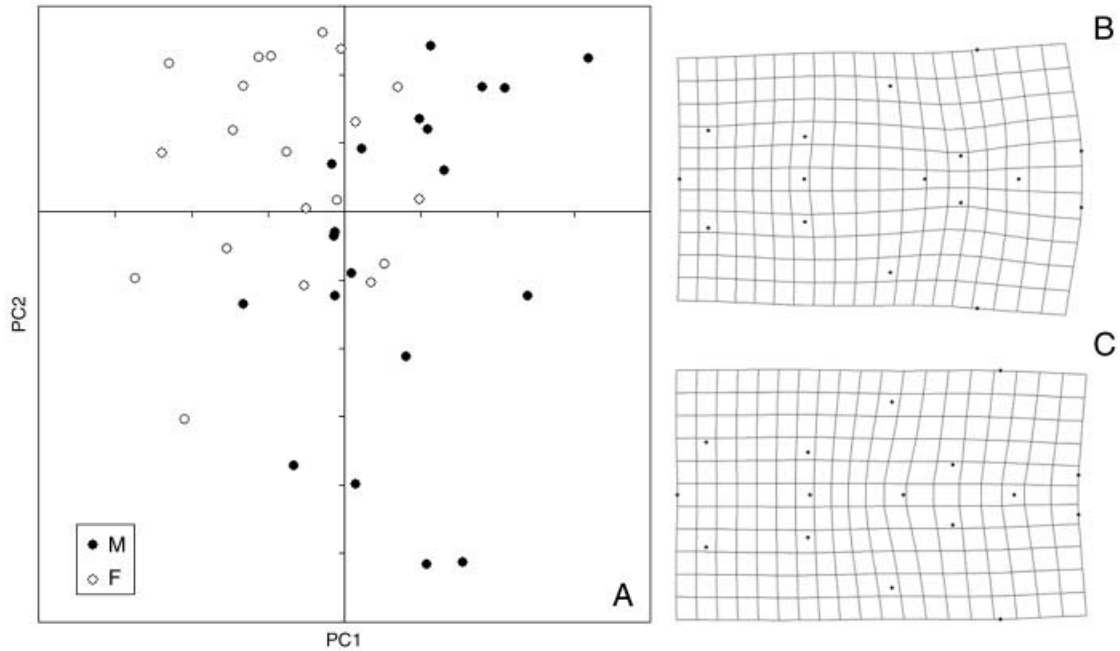


Fig. 3—Principal Component Analysis: —**A.** Plot of the first two principal components with the respective patterns described by distortion grids at the positive extremes: —**B.** PC1, 30% of the total variance; —**C.** PC2, 18% of the total variance. M, males; F, females.

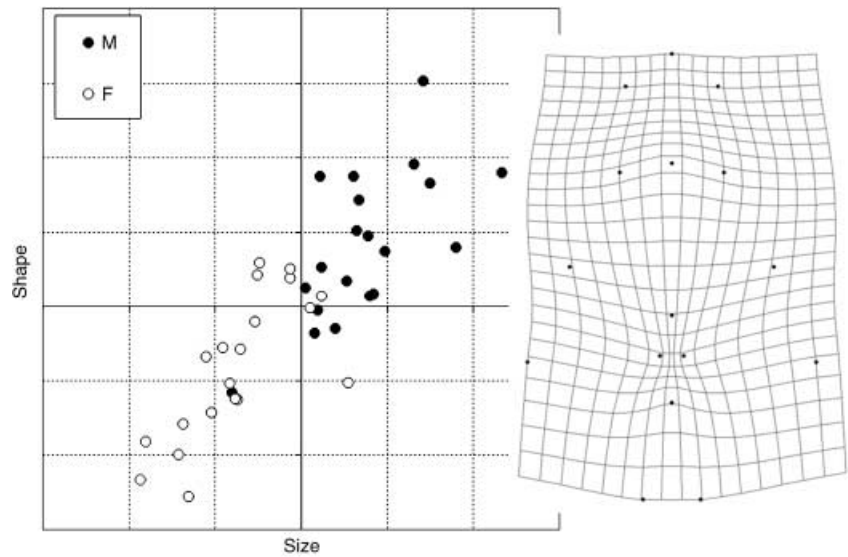


Fig. 4—The plot shows the relationship between centroid size and shape vector (M, males; F, females). The distortion grid shows the warp along the allometric vector, from smaller to larger heads (frontal scale, top; occipital scale, bottom).

A multiple regression between the principal components and the centroid size shows that the allometric variation is almost entirely related to PC1 ($R = 0.76$; $P < 0.001$). When the relationship between size and shape is considered by means of a major axis regression in males and females separately, the confidence for the estimation of the respective slopes and intercepts fails to separate the male and female trajectories. Similarly, the analysis of covariance between size

and shape in males and females fails to show significant differences after size has been removed ($F_{1,36} = 0.18$; $P = 0.67$).

Discriminant analysis of males and females shows a pattern similar to the one described for the allometric variation. The only difference is occipital narrowing associated with occipital stretching in males. However, the difference is not statistically significant because of the small sample size and the number of variables. To check this pattern, we selected a

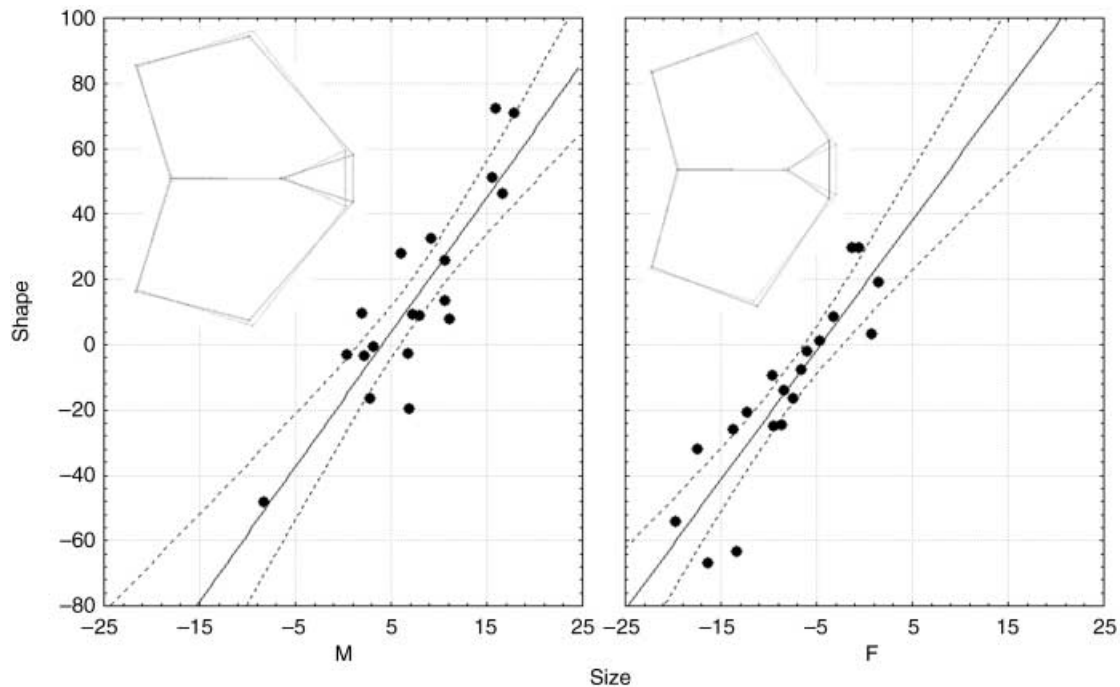


Fig. 5—Relationship between size and shape in males (M) and females (F) for the parieto-occipital subset of landmarks (dashed outline: 95% confidence intervals); the linked configurations show the pattern along the discriminant vector superimposed on the consensus, for each group.

subset of landmarks to characterize the parietal and occipital morphology. The allometric regression for this subconfiguration confirms the pattern based on the entire data set, with size-related occipital stretching. The discrimination is significant ($R = 0.63$; $P < 0.001$) and related to stretching plus narrowing of the occipital scale in males (Fig. 5). Similarly, the analysis of covariance between size and shape for males and females is significant when the parieto-occipital subset is used ($F_{1,36} = 9.27$; $P < 0.005$); a major axis regression shows two trajectories with differences in intercepts (95% confidence interval) but not in slopes (Fig. 5). A certain degree of occipital narrowing in males is also shown by the pairwise comparison between mean shapes but is minimized and obscured by major changes in the other scales. However, when the male and female average shapes are compared, the occipital scale does not show any enlargement associated with the parietal development.

The comparison between male and female mean forms based on the Euclidean distance matrix after superimposition also supports significant shape differences in the cephalic scales ($T = 1.251$; $P = 0.002$). The mean and median ratio between males and females is 0.99, in agreement with the size normalization of the superimposition procedure. Figure 6(A) shows the influential inter-landmark distances. Females show a relative lengthening of those diameters involved in the development of the frontal scale, plus a relatively wider interparietal scale. In contrast, in males the occipital area increases the relative distance from the parietal structures,

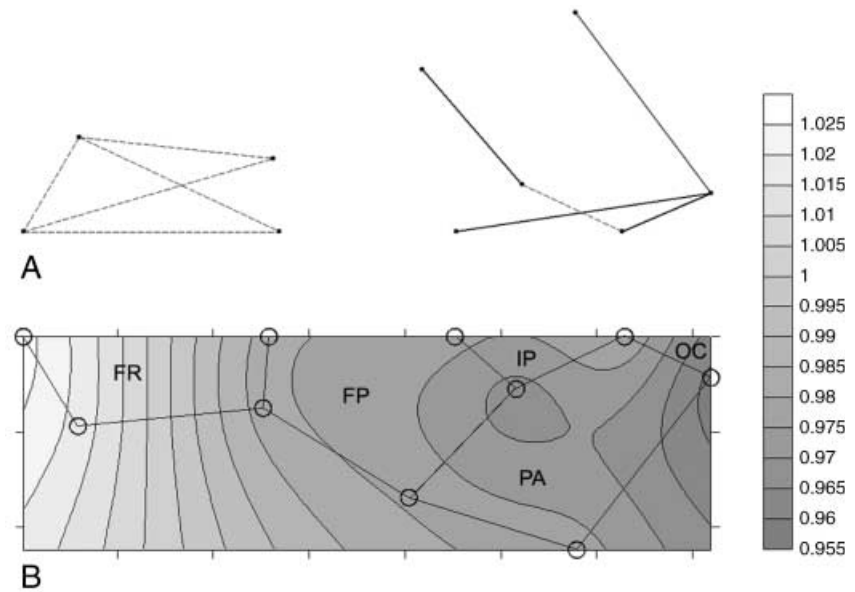
with widening of the fronto-parietal areas associated with the interparietal backward shortening and relative narrowing. The shape-difference map (Fig. 6B) synthesizes this pattern, with males showing a relative shortening of the frontal scale, parietal enlargement and occipital lengthening. In males, peaks (i.e. maximum increased distances) are represented by the lateral point of the interparietal scale, and by the occipital posterior landmark, while the function slopes down (relative length reduction) at the frontal scale toward the anteriormost point. This figure is easily comparable with Fig. 2.

Discussion

Although direct sexual selection can have a major role in the evolution of neomorphic structures (i.e. associated with reorganization of the structural networks, and changes of the biological model), sexual differences are often related to allometric patterns and heterochronic processes (e.g. Shea 1992; Fairbairn 1997). Many features of the skull are similar in small lacertids, and heterochronic processes are believed to play a major role in the within-taxa variability (Barahona and Barbadillo 1998).

In this paper, we investigated how size and sex influence the head shape in *Lacerta bilineata*, using two-dimensional coordinates of the cephalic scale pattern. Sexual dimorphism of the cephalic scales in the green lizard is expressed in both size and shape. As size increases there is relative shortening of the frontal area and relative development of the fronto-

Fig. 6—Sexual differences by Euclidean Distance Matrix Analysis computed on the Procrustes superimposed configurations. —**A.** Right-half configuration showing the influential inter-landmark distances, above (dotted line, male negative allometry) or below (solid line, male positive allometry) one standard deviation. —**B.** Shape Difference Map (left-half configuration). The median values from the Shape Difference Matrix of each landmark are interpolated using a multiquadric radial basis function. The grey-scale refers to the ratio between female and male median values after superimposition (white, female positive allometry, ratio > 1; black, male positive allometry, ratio < 1). Males show a relative frontal shortening, parietal development and nuchal lengthening.



parietal, parietal and occipital scales. Thus, the lengthening of the frontal scale does not keep pace with the general head enlargement, displaying a negative allometry of this area. This enlargement is more pronounced in the nuchal (parieto-occipital) areas. The interparietal scale is compressed laterally by the development of the parietal scales. Therefore, the interparietal narrowing is considered a secondary result of the bulging of the parietal scales as size increases. It is worth noting that, in general, lizards keep on growing after their acquisition of reproductive status. Therefore, a clear separation between static and ontogenetic allometry (biologically determined in other taxa such as mammals) is not feasible, lacking detailed species-specific information on the timing and rate of development.

In the teiid *Tupinambis merianae*, a similar geometric morphometric analysis of the skull showed an allometric vector characterized by rostrum enlargement, midface narrowing and elongation, brain-case narrowing, orbital reduction, and increase of the lateral brain-case surface (Monteiro and Abe 1997). Although the allometric pattern described in the present analysis shows many localized differences from the process in *T. merianae*, they both converge to two common results. First, there is a shared anteroposterior growth gradient, increasing from the frontal to the occipital districts. Second, the parietal area shows the greatest positive allometry, with longitudinal stretching and general enlargement. The same sequence has been described for the ossification pattern of the lacertid skull (Barahona and Barbadillo 1998): premaxilla, maxilla and nasal bones, followed by the posterior areas of the frontal bone, and finally the parietal bones with the neurocranial elements.

The allometric relationships in the green lizard represent both a major pattern of variability and the principal source of

sexual dimorphism. Males show allometric relative reduction of the frontal area, widening of the frontoparietal and parietal scales, and interparietal narrowing. In contrast, the occipital scale lengthens without widening, while in the allometric pattern the occipital scale undergoes a general enlargement. The consequence is a relative narrowing of the occipital area. Therefore, in males the parietals appear to widen not only at the expense of the interparietal surface but also by reduction of the occipital one. This result can falsify the null hypothesis which explained male morphology in terms of scaled shape variation of the female pattern. Thus, this character may represent a sexual feature independent of the size-related shared morphogenetic trajectory. Occipital scale widening in males could be limited by further non-allometric midsagittal parietal development. Hence, the male morphotype could represent a scaled (peramorphic – *sensu* Shea 1983) variation of the female model, with some sexual (non-size-related) characters in the occipital area.

It is assumed that during development the scales either grow or do not grow but that no absolute reduction takes place. Concerning the length of the frontal scales, males show an absolute enlargement but a relative reduction when compared to females (i.e. negative allometry). For this reason, the frontal scales can be used to fix a minimum pace between the male and female shapes. By superimposing all the specimens according to this baseline (frontal scale length), we can visualize a useful sexual comparison of the cephalic scales (Fig. 7A). Showing the frontal scale length the smallest increment along the female–male trajectory, and assuming that no actual reduction occurs in males, this superimposition is useful to compare the sexual variations under the same minimum difference, appreciating the remaining variation. It is worth noting that this baseline

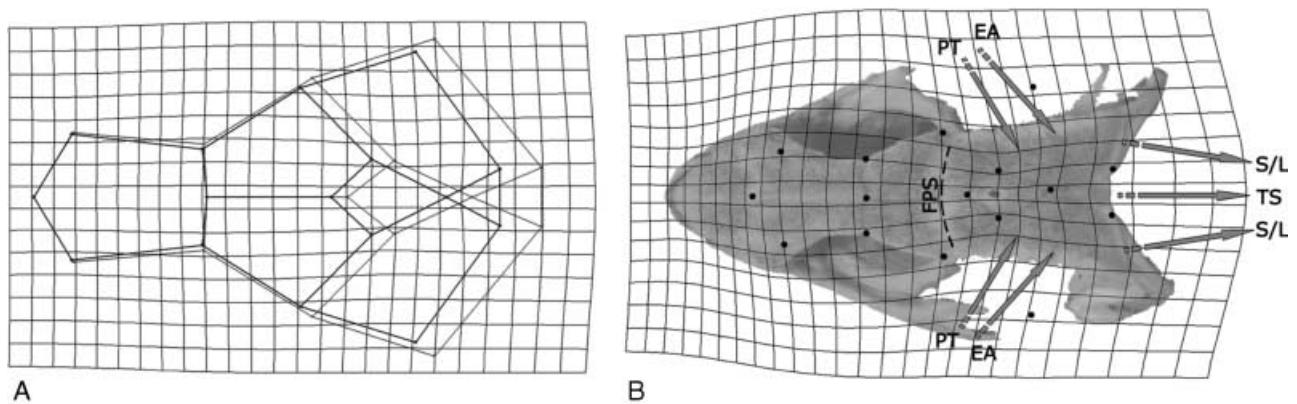


Fig. 7—Structural relationships between the cephalic scales. —**A**. Female (bold links) shape superimposed and warped onto the male (thin links) shape by baseline alignment (Bookstein superimposition) using the most anterior and most posterior midsagittal landmarks of the frontal scale; —**B**. Diagram showing the relationship between this pattern, the vault bones and the occipito-parietal muscular system (arrows). FPS, frontoparietal suture; PT, pseudotemporal muscle (deep); EA, external adductor (superficial); TS, *transverso-spinalis capitis*; S/L, *suboccipitalis* (deep) and *longissimus* (superficial) *capitis*.

superimposition represents just a descriptive comparison aimed at showing differences between males and females at the same frontal length, and is not intended as a conventional analytical approach. Except for minor frontal widening, the growth pattern is largely related to the development of the parietal and frontoparietal scales and to minor occipital and interparietal longitudinal lengthening. In this superimposition, there is the clear absence of occipital widening associated with the parietal development (i.e. there is relative occipital narrowing). Part of this growth pattern is probably related to the skull development. In the four-step scheme described for the lizard's skull (Barahona and Barbadillo 1998), the parietals are the last bones to ossify, from the lateral to midsagittal areas, and they cover the supraoccipital elements as size increases. According to this model, the ontogenetic stages are reached in a species-specific range of body size.

It should be noted that the suture between the frontal and parietal bones lies under the frontoparietal scale, and the areas posterior to this suture show a marked generalized lengthening (Fig. 7B). However, the role of soft tissues (e.g. the masticatory and nuchal muscles) cannot be excluded. In lacertids, there seem to be no sex differences in the presence or morphology of the cranial bony elements (Barahona and Barbadillo 1998). Furthermore, the endochondral elements are related to the negative allometry of the brain development, while the dermal structures are sensitive to muscle tensions, such as those of the jaw adductors and the cervical muscle (Monteiro and Abe 1997). In particular, the parietal area is subjected to stress from the external superficial adductor (including the *levator anguli oris*) inserted on the rictal plate and on the lateral surface of the mandible, and the superficial pseudotemporal, acting on the coronoid process

(Haas 1973). The principal nuchal muscles are the superficial *longissimus capitis* and the deeper *suboccipitalis*, lying under the posterior borders of the parietal scales, and the *transverso-spinalis capitis* acting midsagittally under the occipital scale (Guibé 1970). These structures are responsible for the head movement and suspension, and therefore are particularly sensitive to variation in size and weight of the head component (mostly bones and masticatory muscles). The intimate relationship between dermal bones and scales suggests a tight contact and a structural interaction between these two systems, which must be investigated further.

The growth process is related to the increase of the frontoparietal interdigitation and adhesion of the osteoderms on the bone surface and sutures, involving an increase of cranial robustness and loss of mobility in the mesokinetic axis (Barahona and Barbadillo 1998). The anteroposterior growth gradient leads to anterior displacement of the frontoparietal suture, with further reduction of cranial kinesis (Monteiro and Abe 1997). This increased orofacial robustness may be related to dietary changes, i.e. more omnivorous or even durophagous habits, and food habits are (after phylogeny) the most important determinant in shaping the lizard skull (Stayton 2005). At larger sizes, there is increased bite force, improved prey handling efficiency and a consequent shift to larger and harder foods (Verwajen *et al.* 2002). The female to male trajectory involves further parietal development with respect to the basic allometric parietal enlargement, compressing the interposing interparietal and occipital scales. Thus, the parietal bulging can be considered a consequence of the allometric anteroposterior growth gradient plus a further sex-related hypertrophy. Functionally, these processes are related to the development of the jaw adductors. In green lizards, the food habits change from the juvenile to adult

stages, but there are no differences between males and females (Angelici *et al.* 1997). The male positive allometric relationship in *Lacerta* and *Podarcis* has also been related to the female positive allometric development of the abdomen (Braña 1996). Because of the lack of dietary differences between male and female green lizards, other behavioural and biological components are thought to be involved in their sexual dimorphism. For example, the hypertrophy of the jaw adductors could be necessary during male–male territorial tournaments and during male–female mating behaviour. Thus, if male parietal development is not involved in adult sex-related food selection, it may be related to other factors such as behavioural variables via genetic and/or physiological responses.

It should be noted that in this study we refer essentially to the relative development/reduction of structures comparing sexes, with no direct references to or assumptions on the developmental processes. Even if such descriptions of sexual shape differences in terms of static allometry may be useful to suggest evolutionary hypotheses, considering the problems related to the relationship between the evolutionary and static variations (e.g. Lande 1979; Cheverud 1982; Lande and Arnold 1983) these hypotheses need to be tested and supported on a more dynamic background, namely promoting ontogenetic and interspecific comparisons. Furthermore, a detailed morphological analysis of the nuchal anatomy of this species is necessary to verify and quantify the differences in the male and female allometric trajectories, as well as sex-specific morphometrics on larger samples.

The chromatic map from the EDMA results largely synthesizes the sexual differences already pointed out using the thin-plate spline distortion grids. Both visualizations are based on interpolant functions and Procrustes-adjusted data, so the similar result is of course expected. We think that this procedure can be successfully applied (with or without scaling adjustments) to the EDMA studies, improving and promoting the methodological and conceptual application of this approach. Considering the difficulty of displaying EDMA results through graphics (Cole and Richtsmeier 1998), the interpolation maps may be useful tools to better visualize the morphological patterns and to synthesize morphological information. Clearly, a correct use of such synthetic tools cannot replace a detailed analysis of the local variations, including the presence of single influential landmarks biasing the mapping resolution, or in contrast the elimination of biologically relevant differences because of the interpolation procedure. The analysis (mathematical as well as visual) of the residuals as departures from each landmark value to the interpolated plane can be very useful in this sense.

As a final methodological comment, we wish and suggest that geometric morphometrics and EDMA should be used as complementary tools, and should be considered together to strengthen and improve the resolution of the morphological analyses.

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