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The role of post-natal ontogeny in the evolution of phenotypic diversity in *Podarcis* lizards

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Abstract

Understanding the role of the developmental pathways in shaping phenotypic diversity allows appreciating in full the processes influencing and constraining morphological change. *Podarcis* lizards demonstrate extraordinary morphological variability that likely originated in short evolutionary time. Using geometric morphometrics and a broad suite of statistical tests, we explored the role of developmental mechanisms such as growth rate change, ontogenetic divergence/convergence/parallelism as well as morphological expression of heterochronic processes in mediating the formation of their phenotypic diversity during the post-natal ontogeny. We identified hypermorphosis – the prolongation of growth along the same trajectory – as the process responsible for both intersexual and interspecific morphological differentiation. Albeit the common allometric pattern observed in both sexes of any species constrains and canalizes their cephalic scales variation in a fixed portion of the phenotypic space, the extended growth experienced by males and some species allows them to achieve peramorphic morphologies. Conversely, the intrasexual phenotypic diversity is accounted for by non-allometric processes that drive the extensive morphological dispersion throughout their ontogenetic trajectories. This study suggests a model of how simple heterochronic perturbations can produce phenotypic variation, and thus potential for further evolutionary change, even within a strictly constrained developmental pathway.

Introduction

Understanding the processes producing phenotypic variation is a major goal in evolutionary studies since Darwin. Intraspecific diversity provides the raw material for the evolutionary change (Darwin, 1858) and represents one major element of biodiversity (Gaston, 1996; Takacs, 1996). Yet, we still have a limited knowledge of the processes and mechanisms through which morphological variation is produced and maintained both within and among species. In principle, morphological diversity is produced by the interplay between natural selection and developmental processes (McKinney, 1988; Gould, 2002; Futuyma, 2005). The dynamic interaction between external (selective) and internal (developmental) forces produces, selects and constrains phenotypic variation, thus representing the essence of morphological evolution (e.g. Gould, 1977; Alberch, 1982; Hall, 1992; Klingenberg, 2005). The role of the selective processes driving phenotypic diversity to adaptative changes in morphology has been elucidated in several model systems throughout

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the animal kingdom. Classical examples include studies of adaptive radiations such as Darwin's finches of Galapagos Islands, *Anolis* lizards of Caribbean islands, and cichlids of the African Great Lakes where an exceptional amount of phenotypic diversity occurs in short evolutionary times as a result of selective pressure provided by interspecific competition and ecological opportunity (Schluter, 2000; Losos, 2009). On the other hand, little investigation has been devoted to the developmental processes *producing and constraining* the phenotypic diversity on which natural selection acts. This is an exciting research theme when considering that in adaptive radiations a burst of morphologies is produced in a short evolutionary time. Although in all the above-mentioned examples, the allometric transformation represents a key process underlying the diversification in size and shape of morphological traits, the ontogenetic component of such transformations remains largely to be established. Few studies investigated whether the extensive morphological diversity associated with evolutionary radiations is explained by a less canalized – i.e. more plastic – developmental system and/or by processes of ontogenetic divergence/convergence/parallelism (but see Losos *et al.*, 2000; Klingenberg, 2010).

Among developmental processes, heterochrony – i.e. the changes in onset, offset and/or rates of growth processes – is widely regarded as one of the most important evolutionary mechanism driving morphological variation and evolution (Gould, 1977; Alberch *et al.*, 1979; McKinney & McNamara, 1991; Mabee *et al.*, 2000; see Table 1 for a synopsis of the main heterochronic processes). Heterochrony occurs at interspecific (phylogenetic) and intraspecific (tokogenetic) levels (see Reilly *et al.*, 1997). On a phylogenetic level, the study of variation among species in the timing and rate of development provided important insights into the reconstruction of evolutionary relationships (Werneburg & Sánchez-Villagra, 2009; Piras *et al.*, 2010) and the identification of developmental constraints (Gould, 1977; Maynard-Smith *et al.*, 1985) and helped to understand the evolution of morphological traits (Alberch, 1981; Cardini, 2003) and processes such as convergence and evolution of growth in related organisms (Yeh, 2002; Adams & Nistri, 2010; Wilson & Sánchez-Villagra, 2010). At the intraspecific level, variation in ontogenetic development between individuals and sexes was shown to produce phenotypic variation (Hall, 1992; Reilly, 1994; Mabee *et al.*, 2000; Badyaev *et al.*, 2001; Hollander *et al.*, 2006; Fišer *et al.*, 2008). Detailed assessment of variation in ontogenetic development across individuals, sexes, and related species is necessary for understanding the role of developmental pathways in shaping the phenotypic diversity and to reveal the processes by which phenotypic variation arises (Hall, 1992; Reilly *et al.*, 1997; Holtmeier, 2001).

Table 1 Heterochronic processes and their morphological expression. α, the age at the onset of development; β, the age at offset of development; $k\sigma$, the rate of development (i.e. the rate of change in shape); and ks, the rate of growth (i.e. the rate of change in size). Each parameter can differ in two directions, yielding the eight heterochronic perturbations. See Fig. 3 also.

Control parameter	Incremental change	Process	Morphological expression
α	$-\delta\alpha$	Predisplacement	Peramorphosis
	$+\delta\alpha$	Post-displacement	Paedomorphosis
β	$-\delta\beta$	Progenesis	Paedomorphosis
	$+\delta\beta$	Hypermorphosis	Peramorphosis
$K\sigma$	$-\delta k\sigma$	Neoteny	Paedomorphosis
	$+\delta k\sigma$	Acceleration	Peramorphosis
ks	$-\delta ks$	Proportional giantism	
	$+\delta ks$	Proportional dwarfism	

In this study, we investigated the role of post-natal ontogenetic processes in the evolution of intra- and interspecific phenotypic diversity in a diverse group of lizards that experienced an extraordinary broad and rapid phenotypic diversification.

The wall lizards genus *Podarcis* (Squamata, Lacertidae) form a homogeneous group of Palaearctic ground-dwelling species, which is the predominant reptile group in southern Europe (Arnold *et al.*, 2007). These lizards are a particularly appropriate group in which to investigate the role of the ontogenetic processes in generating phenotypic variation for several reasons. First, members of this genus exhibit a spectacular amount of intraspecific and interspecific variability in size, coloration, scalation and pholidosis patterns (Arnold, 1993, 2002; Kaliontzopoulou, 2010). Morphological variability and differentiation in these lizards are primarily influenced by sexual dimorphism and adaptation to ecological conditions. As a result of sexual constraints, males and females of different species share several common morphological features related to male–male and male–female interactions (Kaliontzopoulou *et al.*, 2007). Different *Podarcis* species and populations show convergence on a morphological body plan in peculiar environmental conditions encompassing a continuum variation in morphological traits from ground- to rock-dwelling ecomorphs as a response to the ecological opportunity provided by saxiculus – open habitats (Kaliontzopoulou *et al.*, 2010; but see also Losos, 1990; Arnold, 1998; Vanhooydonck & Van Damme, 1999; Calsbeek & Irschick, 2007; Revell *et al.*, 2007 for other examples). Second, morphological diversification in these lizards occurred in a very short evolutionary time after the group underwent a fast radiation during the latest Miocene (Poulakakis *et al.*, 2005; Brown *et al.*, 2008; Kaliontzopoulou *et al.*, 2011). In some cases, considerable phenotypic diversification in size, coloration and pholidosis can arise in *Podarcis* populations in few decades, as demonstrated by the high number of distinctive island morphs originated during recent colonizations (see, e.g. Capula & Ceccarelli, 2003 and Podnar *et al.*, 2005 for a discussion on the up to 52 subspecies of *Podarcis sicula*). Third, several studies have shown that in these lizards, allometric changes associated with size (and consequently with age) and environment account for a considerable component of both the intraspecific (intrasexual) and the interspecific morphological differentiation (Bruner & Costantini, 2007; Kaliontzopoulou *et al.*, 2007, 2008, 2010; Ljubisavljevic *et al.*, 2010). However, a detailed intra- and interspecific analyses of the developmental pathways aimed at understanding their role in shaping phenotypic diversity between sexes and species are still lacking. Moreover, the rapid and extensive morphological diversification observed in *Podarcis* lizards make this group similar in some aspects to other lizard group such as *Anolis* that experienced adaptive radiation, thus allowing the discussion of the patterns and process of phenotypic variation observed in *Podarcis* in a broader evolutionary framework.

We used landmark-based geometric morphometrics (GM) and a suite of statistical tests for multivariate allometry to analyse and compare the intra- and interspecific developmental patterns of the cephalic scales morphology during post-natal ontogeny in *Podarcis* lizards. First, we tested the hypothesis of a common multivariate allometric pattern across species and sexes for both the rate of growth (i.e. slopes) and for their morphological differences at the very early post-hatching and adult morphologies. Second, we contrasted the ontogenetic trajectories to each other at the species level in order to assess their convergence, divergence or parallelism. Third, we used residuals coming from sex/species-specific multivariate regressions of shape on size to explore how morphospace occupation in allometry-free morphospace varies between individuals, sexes and species.

The main aim of this study is to investigate whether developmental processes were a source of the phenomenal amount of morphological variation observed in *Podarcis* lizards and to discuss the role of such processes in the broader framework of evolutionary radiations.

Material and methods

Material

We took digital pictures of the head dorsal surface in 891 individuals belonging to the species *Podarcis filfolensis*, *P. muralis*, *P. sicula* and *P. tiliguerta* from the collections of the Museo di Storia Naturale (Sezione di Zoologia 'La Specola'), Universitá di Firenze (MZUF). For each species, a balanced number of males and females was included in the study. Sex determination was made on the basis of femoral pores morphology (Arnold, 2002). Young individuals with non-developed secondary sexual characters were excluded from the analyses. The sample of each species encompasses the sizes range reported for adult specimens of any species (Corti & Lo Cascio, 2002).

We selected the four species as to include (i) evolutionary extremes in the genus, from genetically uniform species (*P. sicula*, Capula & Ceccarelli, 2003; Podnar *et al.*, 2005) to possible species complex (*P. tiliguerta*, Harris *et al.*, 2005); (ii) both mainland widely distributed species (*P. sicula* and *P. muralis* that inhabit an extensive area in southern Mediterranean regions) and strictly insular species (*P. tiliguerta* and *P. filfolensis* from Corso-Sardinian and Maltese and Pelagic islands, respectively). Specimens of *P. muralis* ($N = 70$) come from two localities of Veneto region (Venice and Verona, Italy), specimens of *P. filfolensis* ($N = 65$) from Sicily (Linosa and Lampione islands) and the Maltese archipelago (Malta, Gozo, Filfa and Comino islands). In order to accurately assess intraspecific morphological diversity (see Intra- and interspecific morphological diversity paragraph), for two species, *P. tiliguerta* and *P. sicula*, we included specimens coming from a large part of their occurrence areas. As for *P. tiliguerta* ($N = 424$), we analysed specimens from Sardinia, Corsica and many micro-archipelagos. *P. sicula* specimens ($N = 331$) come from Italian Peninsula (Tuscany, Tuscan Archipelago, Calabria, and Sicily) and from Sardinia and Corsica islands. The complete list of material examined is reported in the Appendix S1.

Geometric morphometrics

We used landmark-based GM to quantify overall cephalic scales configuration shape (Bookstein, 1991; Rohlf & Marcus, 1993; Adams *et al.*, 2004; Zelditch *et al.*, 2004; Mitteroecker & Gunz, 2009). GM quantifies the shape of anatomical objects from the coordinates of repeatable landmark locations, after the effects of non-shape variation are mathematically held constant. In quantitative studies of ontogeny, GM is particularly appropriate because it allows the effective separation of size and shape components in morphological analyses, which is a basic to understand allometric changes during growth (Adams *et al.*, 2004). In addition, GM has found fruitful applications in the study of lizard head morphology because this structure in lizards is covered by relatively large scales, which facilitates the definition of landmarks, and which could be taxonomically, evolutionarily and functionally informative (Arnold, 1989; Bruner *et al.*, 2005; Kaliontzopoulou *et al.*, 2007, 2008, 2010).

The dorsal cephalic scales configuration was captured by means of 33 digitized landmarks. Landmark definitions, positions and their corresponding configurations along with cephalic scales nomenclature used in the text are shown in Fig. 1. We argue that our 2D configuration of dorsal head surface is a good approximation of the actual morphology that, although tridimensional, is rather flat. We specifically eliminated the asymmetric component of shape variation according to the methodology implemented in MorphoJ software (Klingenberg, 2011). Only the symmetric component (Klingenberg *et al.*, 2002) of shape variation was then used.

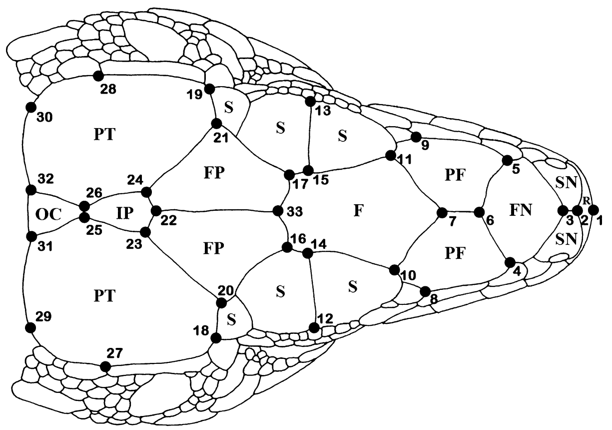

Fig. 1 Two-dimensional configuration of 33 landmarks on cephalic scales of *Podarcis* lizards used in this study. Scales nomenclature is as follows: PT, parietal; OC, occipital; IP, interparietal; FP, frontoparietal; S, subocular; F, frontal; PF, prefrontal; FN, frontonasal; SN, supranasal; R, rostral.

Successively, we aligned the set of landmark coordinates using a common generalized Procrustes analysis (GPA), which superimposes specimens to a common coordinate system after accounting for differences in position, orientation and scale (Rohlf & Slice, 1990; Bookstein, 1991; Goodall, 1991). From the aligned specimens, Procrustes shape coordinates were obtained and used as shape variables in subsequent statistical analyses (e.g. Bookstein *et al.*, 1999; Mitteroecker & Bookstein, 2008). As a measure of individual size, we used the centroid size, which is defined as the square root of the sum of squared differences between landmark coordinates and centroid coordinates for any dimension. The centroid of a configuration is the average location of all landmarks from one specimen. Centroid size was retained for subsequent allometric analyses. Then, we performed a suite of multivariate analyses for (i) comparing ontogenetic allometry of species and sexes and (ii) exploring the phenotypic variation across individuals, sexes, and species in an allometry-free empirical morphospace.

Comparing ontogenetic allometry

All subsequent analyses were performed on Procrustes coordinates of symmetric component obtained after a common GPA by developing a set of script made in R (R Development Core Team, 2011) by two of us (GF and PP), which are available from the authors upon request. To test the hypothesis that species differ in their allometric trajectories, we used a modified version of permutational procedure in Adams & Nistri (2010) and Piras *et al.* (2010). Allometric trajectories were estimated by means of multivariate regression between shape (i.e. multivariate) and size for any sex of any species separately (Monteiro, 1999). Successively, regression coefficients, predicted values and residuals are differently used in the various tests described here below.

The approach used in this study looks for differences between pairs of allometric trajectories assessed using a modification of phenotypic trajectory analysis (Adams & Collyer, 2007, 2009; Collyer & Adams, 2007). The observed allometric trajectory for each sex of each species was estimated by the set of coefficient vectors of separate regressions of shape on size. Besides the differences in slope, we tested specific hypotheses about convergence, divergence or parallelism between trajectories. This, however, does not inform about the complete overlapping of two trajectories or about truncation along a common trajectory, as pointed out by Mitteroecker *et al.* (2005). For this reason, Mitteroecker *et al.* (2005) suggest a permutation test on sums of squared residuals (SSs) calculated for two trajectories. Under the assumption of identical trajectories, the original test statistic should not be an outlier in the permutation distribution of summed squared residuals. For N permutations, the hypothesis of identical trajectories in size–shape space is rejected when $(C + 1)/(N + 1) \leq \alpha$ (the significance level set *a priori*), where C is the number of cases that result, a smaller test statistic than that for the original data. However, two distributions can still lie along an identical trajectory having different SSs; it can happen if one group is more dispersed along the trajectory than the other. Or, conversely, SSs can be identical without identical trajectories if two trajectories are parallel and the dispersion along the fitting line is the same across the two groups.

In order to test all these patterns, we suggest a suite of different multivariate tests for modelling allometric trajectories. In particular, (i) multivariate ontogenetic convergence test for testing divergence (Fig. 2a), parallelism (Fig. 2b) or convergence (Fig. 2C), (ii) permutation test for differences in mean squared error (MSE) for testing dispersion of observed data along the trajectories (Fig. 2d), (iii) a specific permutation test to check whether two distributions with equal slopes differ actually in elevation (Fig. 2e,f), (iv) a specific test for peramorphosis in order to test whether one group is peramorphic relatively to the other (Fig. 2g,h), (v) a heteroskedasticity test to assess whether non-size-dependent morphological variability significantly increases or decreases with size (Fig. 2i,j,k); heteroskedasticity is defined as the non-constant variance of dependent variable(s) – shape in our case – when regressed against a given independent variable – size in our case.

The patterns illustrated in Fig. 2 can be evaluated by applying the entire suite of specific tests presented below.

An important caveat should be made here about the estimation of multivariate slopes and intercepts in ontogenetic trajectories. In a recent paper, Huttegger & Mitteroecker (2011) highlighted that evaluating distances and angles between trajectories imply the Euclidean nature of the morphospace. This condition is rarely

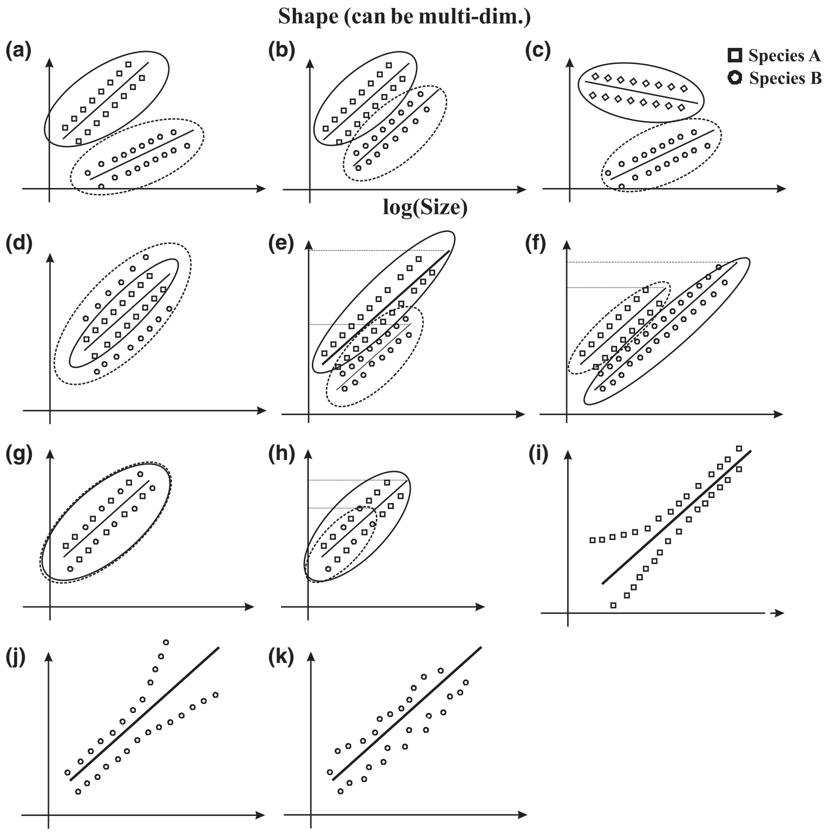

Fig. 2 Different patterns for ontogenetic trajectories in a common size–shape space. These patterns can be unambiguously identified by performing *all* the multivariate tests presented in the text. (a) divergent trajectories; (b) parallel trajectories; (c) convergent trajectories; (d) identical trajectories but unequal dispersion around fitted scores; (e) parallel trajectory where the group with juvenilized shape at equal size has a truncated growth and is paedomorph; (f) parallel trajectories where the group that is juvenilized at equal size continues to growth and is peramorphic at the end of growth; (g) identical trajectory (and equal dispersion); (h) truncation along common trajectory (equal dispersion); (i) canalized heteroskedasticity; (j) inversely canalized heteroskedasticity. (k) randomly distributed heteroskedasticity.

met in complex phenotypic spaces that often possess *affine* properties. In affine spaces, not all metrics used in Euclidean spaces are meaningful (such as Euclidean distances or angles). This is due to the fact that trajectories could be oblique for some directions relatively to size, making incommensurable the angles between them. However, we preliminarily visually checked in three dimensions – the first three Principal Components (PCs) coming from Principal Component Analysis (PCA) performed on the predicted shapes coming from *separate* multivariate regressions between shape and size against size. Usually, the first PCs of this kind of PCA explain a very high percentage of variance. If all trajectories develop in the same direction, that of course will be dependent on size, we can exclude obliquity as source of error in our calculation.

Ontogenetic convergence test

The estimation of permutation *P*-values of a multivariate slope test does not tell us whether distances between predicted shapes of two groups (in a pair comparison) at observed small sizes are different (smaller or larger) from those at observed large sizes.

To test whether developmental trajectories were convergent, parallel or divergent, we quantified in any pairs (from intraspecific–intersexual and interspecific–intrasexual comparisons) the Procrustes distances between predicted shapes at small and large sizes. To avoid distortions in predicting shapes at different size values, the predicted shapes were calculated for both size classes (small and large) at the *same* size values in any pairwise comparison. These size values to predict the shape were calculated as the cross product between the coefficient matrix of the regression model and the corresponding design matrix containing the same size values in the pair of interest. We calculated pairwise distances for each sex of the four species at both small and large sizes and contrasted them. If the morphological distance between two species is significantly larger at adult stages as compared to those at juvenile stages, there is evidence for ontogenetic divergence (Fig. 2a); if distance remains the same (i.e. the difference was not statistically significant), there is evidence for parallelism (Fig. 2b). Finally, convergence would be proven if the distance between species is significantly smaller in adults than in juveniles (Fig. 2c). To test the null hypothesis of parallelism against convergence (or divergence), we consider a random permutation procedure. A random permutation test compares the value of the test statistic (in this case the distance) for the original data to the distribution of test statistics when the data are permuted; the *P*-value of the permutation test is then obtained by counting the number of times that the statistic from permuted data exceeds the statistic for the original data and then dividing by the number of permutations performed. The reason to do a permutation test is that we do not need to depend on an assumption about the distribution of the data. In this context, given two species of interest, group affiliation was randomly reassigned via sample permutation (i.e. without replacement) across the specific pair of species of interest. Then, the difference in distances between shapes predicted at small and large sizes for each randomized sample was obtained. As previously described, the observed difference was then compared to the relevant permutation distribution of differences, consisting of 999 iterations, to obtain the corresponding *P*-value. We specify here that the pairwise permutational comparisons were obtained by permuting *any times* just across any possible pair comparison, *disregarding the other groups not in that pair*. Thus, given *n* groups, there are $(n^2 - n)/2$ possible pairwise comparisons and the same number of permutation procedures (each with 999 randomizations) that were executed (for a similar – but not identical – approach for assessing parallel evolution, see Adams, 2010; Adams & Nistri, 2010; Piras *et al.*, 2010). For this reason, from here to fore we call our approach ‘multipermutation procedure’.

The ontogenetic convergence test provides evidence for convergence, parallelism or divergence, but cannot identify identical trajectories (they would result as parallel) or shifts in elevation or along common slope. To test these hypotheses, additional tests are necessary for discerning other patterns of allometric growth.

Test for ontogenetic trajectories’ shift in elevation

Under a common slope, we tested differences in ontogenetic trajectories’ elevation similarly to the test for common slope by using a multivariate intercept test.

For any group in a pair, we first performed a multivariate regression between shape and size. We then calculated the Euclidean distances between vectors of intercept coefficients (i.e. one intercept coefficient for any dependent variable, 66 in our case, i.e. 33 landmarks in two dimensions), and we used the multipermutation procedure described above to generate a random distribution of distances. Successively, we compared (pairwise) the observed distances with the random distribution. If the observed distance between two groups falls outside the 95th percentile of the random distribution, the two groups significantly differ in elevation (as in Fig. 2e,f), otherwise their elevation is the same (as in Fig. 2d).

Test for dispersion along allometric trajectories

If two trajectories overlap in shape space and the ontogenetic convergence test is not significant, one cannot conclude that these trajectories are identical in the size–shape space. In fact, assuming two completely overlapping trajectories, one group could be more dispersed than the other around the regression line (Fig. 2d). Testing for dispersion along allometric trajectories, Mitteroecker *et al.* (2005) suggest performing a permutation test upon separate per-group multivariate regressions between shape and size. For any regression, SSs are calculated. Successively, species affiliation is randomized, and the SSs are recalculated. If the observed SSs are within the 95% of the permutation distribution, we cannot reject the null hypothesis of identical regression lines (Fig. 2g).

We argue that SSs must be corrected for the sample size because very different sample sizes do not allow direct comparison of SSs. On this basis, we used this permutation test using the MSE instead of SSs.

However, this strategy does not provide, alone, evidence of identical regression. Only if one has demonstrated that two regression lines have the same slope (i.e. they are parallel) and the same elevation (and then they coincide as in Fig. 2d,g), this test could provide their complete overlapping, thus allowing to distinguish between patterns depicted in Fig. 2d,g. For this reason, the multivariate ontogenetic convergence test, the multivariate intercept test and the MSE test should be evaluated all together.

Peramorphosis test

Following Godfrey & Sutherland (1995), it is hard to infer heterochronic processes from size and shape data without information on age. Therefore, using size as predictor of shape, the different heterochronic processes can be mainly distinguished on the basis of final morphological expression (peramorphosis or paedomorphosis) instead of actual morphogenetic processes. The different processes and their corresponding morphological expressions are reported in Fig. 3 and Table 1. In order to test this, we built a multipermutation procedure on distances between the shapes predicted at maximum size values recorded *for any group*. In order to assess significance, as described above, group affiliation is randomly reassigned and any time distances are recalculated. Then, the observed distance is compared to the randomly generated distribution. Finally, once we had assessed the significance for shape distances, we quantified the multivariate

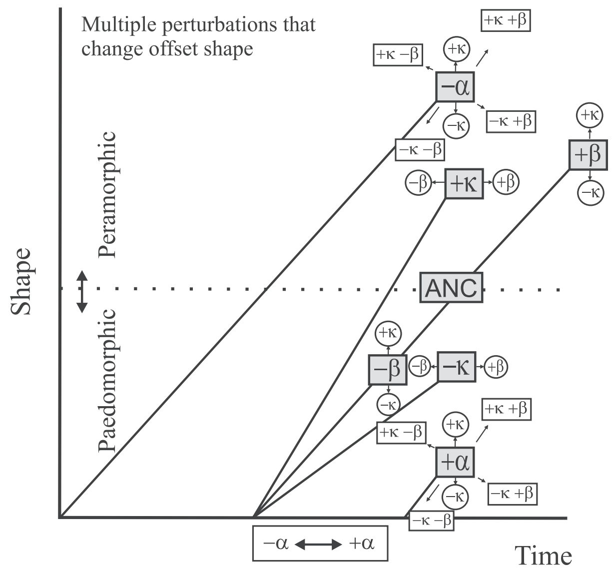

Fig. 3 Formalism for heterochronic processes and their morphological expression as proposed by Reilly *et al.* (1997) (redrawn) inspired by Alberch *et al.* (1979). Paedomorphosis and peramorphosis can result from multiple perturbations of the three developmental parameters: rate, onset and offset. Each of the simple perturbation trajectories (shaded boxes) can be shifted by one, the other, or both of the other two parameters (circles and rectangles). α, the age at the onset of development; β, the age at offset of development; $k\sigma$, the rate of development (i.e. the rate of change in shape); and ks, the rate of growth (i.e. the rate of change in size). See also Table 1.

distances between predicted shapes at maximum per-group size value and origin, i.e. the origin of coordinate system. This metric gives the information on which trajectory (in a pair) lies below the other, i.e. possesses the relatively more 'adultized' shape, as depicted in Fig. 2e,f,h.

Heteroskedasticity test

In order to assess whether non-size-dependent morphological variability significantly increases (or diminishes) with size, we performed a specific test able to detect patterns figured in Fig. 2i,l,m. Ideally, one should collect the same morphology on the same individuals during their growth. This hardly happens in experimental biological studies, and more often we deal with ontogenetic series represented by different individuals of different ages and sizes. In keeping with this, we built a procedure able to detect the course of size-independent morphological dispersion during ontogeny.

As first, we estimated linear regression models, with size as independent variable and shape (multivariate) as dependent, for each group separately. Thus, for any model, we performed the Breush–Pagan test, a test for heteroskedasticity (multivariate) in linear models available in R package 'lmtest'. If this test is significant, we are not able to recognize how and where the heteroskedasticity of residuals is concentrated along the regression curve. For this reason, we partitioned the independent variable (size in our case) in $n = 10$ intervals, each of which contained $(1/n)\%$ of individuals. Successively, for each interval, we computed the MSE on the basis of the whole linear model previously computed. Finally, we performed a linear regression between the n ordinal MSEs interval positions and their corresponding n MSEs values. If the regression is statistically significant, we obtain evidence for a positive (or negative, depending on the beta-coefficient sign) relationship between size and size-independent morphological dispersion.

Visualizing ontogenetic allometry

We visualized allometric trajectories using predicted shapes along the regression curve (Mitteroecker *et al.*, 2005). Here, we used the allometric vector computed in MorphoJ software (Klingenberg, 2011) to perform separate linear regressions of shape on size ($\log_{10}$CS) for each species. Predicted values are obtained and plotted against size. As with patterns in Procrustes shape variables, each point in morphospace was scaled by its centroid size, allowing a visual assessment of allometry.

The allometric patterns of growth from juvenile to adult stages were visualized by means of thin-plate spline deformation grids. In the thin-plate spline analyses, the configuration of each specimen is integrated in a grid, which deformation illustrates as shape changes relative to a reference (average) configuration. Technical details about this procedure can be found in Bookstein (1989, 1991).

Intra- and interspecific morphological diversity

Evaluating differences in morphological disparity in an allometry-free empirical morphospace, we used for the following analyses the allometry-free data represented by residuals from group-specific regressions between shape and size (males and females separately for the four species, thus eight regressions were performed). To test multivariate dispersion of allometry-free shape data around their centroid, we computed per-group morphological disparity as the average Euclidean distance from group centroid and then we performed a permutation test using 'betadisper' and 'permutest' functions available in R package 'vegan' (Oksanen *et al.*, 2011). We pairwise contrasted the multivariate morphological dispersions of each group around group centroid and by means of our multipermutation procedure. We assessed the significances in disparity differences via pairwise comparisons. We argue that our results are meaningful mainly for *P. sicula* and *P. tiliguerta* for which we have a very large sample in comparison with other species, which allows us testing whether intraspecific–intersexual differences in morphological disparity occur.

Results

Ontogenetic changes

In the four studied *Podarcis* species, an evident dorso-rostral morphological variation in dorsal view is clearly visible during the ontogenetic change and in female–male comparison, with a marked sexual dimorphism. These ontogenetic changes are depicted in the deformation grids in Fig. 4 and are described following scales nomenclature reported in Fig. 1.

During the post-natal ontogeny in both sexes of each species, lengthening of the rostral portion of the cephalic scales configuration is observed as well as a lateral expansion of occipital portion. Moreover, a marked lengthening of frontoparietal scales with, consequently, an anterior shortening of frontal scale is evident. The inter-parietal scale shows a strong reduction in size, from juvenile to adult stages, with a development of occipital scale anteriorly. This is due to the posterior growth of head, with enlargement of parietal scales and the lengthening of frontoparietals. In the rostral portion, a slight lengthening of frontonasal and rostral scale anteriorly and a posterior lengthening of prefrontal scales are present. The subocular scales show a slight posterior lengthening with a lateral compression due to the lateral development of frontoparietal scales and frontal one.

However, local morphological change differed between sexes. In males, the frontoparietal scales become longer than frontal scales and the posterior contour of parietal scales become large, acquiring a convex lateral profile at the level of parietal–subocular contact. The occipital scale becomes longer than interparietal scale due to lengthening of the former. In females, lengthening of the frontoparietal scales is less pronounced than in males so that at adult stage the extension of these scales is comparable to that of the frontal scale. During growth, the enlargement of parietal scales in females is smaller with respect to males and evenly distributed along the length of this scale, resulting in an overall tapered profile of the whole cephalic scales configuration.

Notwithstanding common patterns of ontogenetic change between and within sexes, some species-specific pattern of scales shape change during growth can also be pointed out. In males of *P. filfolensis* and *P. muralis*, a marked posterior lengthening of the occipital scale (and also of the intraparietal scale in the former) during growth produces a slightly convex profile at the posterior

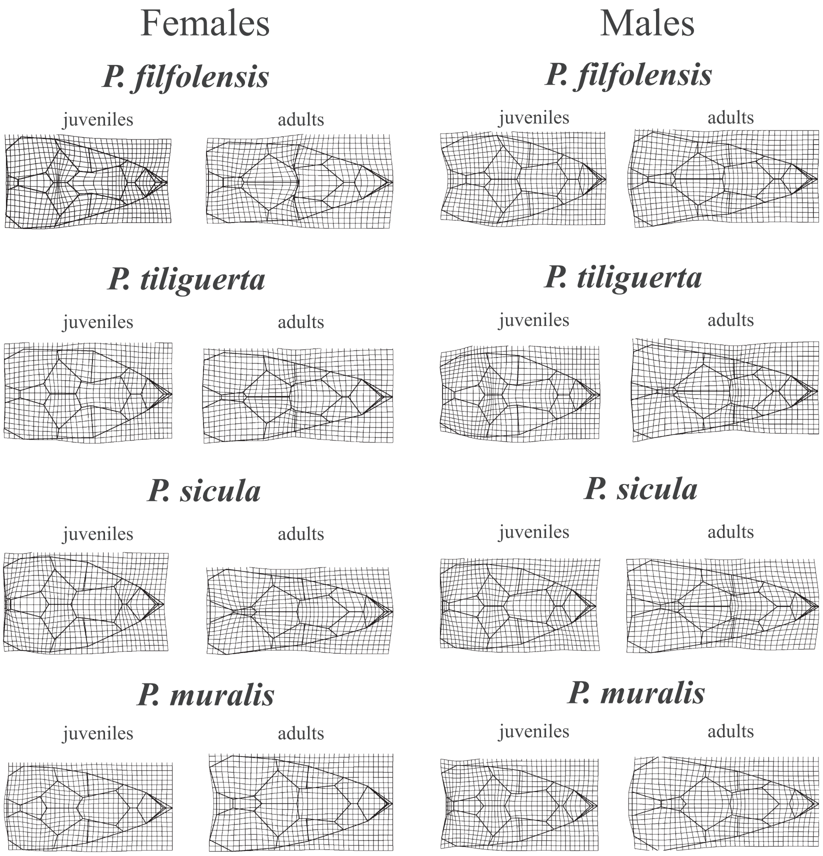

Fig. 4 Thin-plate spline deformation grids showing shape changes associated with growth for the eight groups separately (sexes and species).

side of encephalic shield. Whereas in *P. filfolensis* and *P. tiliguerta*, the frontonasal scale undergoes a posterior lengthening with a consequent length reduction of the prefrontal scales, in *P. sicula* and *P. muralis* a reverse pattern occur with an anterior lengthening of the prefrontal scales and a consequent posterior shortening of the frontonasal scale. In adult females of *P. filfolensis*, a marked lengthening of frontoparietal scales is evident, and in *P. sicula* females, a strong reduction in size of interparietal scale is associated with lengthening of occipital one. Finally, some species show peculiarities in cephalic scales shape at juvenile and adult stages. In juveniles of *P. muralis*, the frontoparietal scales are longer than in other species, and in *P. tiliguerta* and *P. sicula* juveniles, subocular scales are laterally expanded. The adult males of *P. tiliguerta* have a frontal scale posteriorly shortened.

Comparison of ontogenetic trajectories

The *Podarcis* lizards studied show a common allometric pattern in cephalic scales morphology during post-natal ontogeny. Species and sexes clearly exhibit a overlap in the morphospace occupation and show a shared direction of the ontogenetic change relative to first three PCs (explaining about 70% of total variance) (Fig. 5a). Overall, the ontogenetic trajectories of males and females of any species are extremely similar in their course (Fig. 5b,c).

Visual inspection of first three PCs computed on predicted shapes coming from separate multivariate regressions between shape and size does not reveal evident obliquities between trajectories, thus allowing testing differences in slope and intercept.

The ontogenetic convergence test does not show any divergence or convergence between predicted shapes at common small and large sizes (ontogenetic convergence test, $P > 0.05$; see Table 2). In the observed common size range, the distances between predicted shapes (at common small and large sizes) remain constant in all pairwise comparisons, thus suggesting that ontogenetic trajectories of both sexes of any species are parallel.

The test for shift in elevation among ontogenetic trajectories found differences in elevation for three pair comparisons (*P.til.*♂–*P.til.*♀, *P.til.*♂–*P.sic.*♂, and *P.til.*♂–*P.mur.*♂; multivariate intercept test, $P < 0.05$; see Table 2). The comparison between males of *P. muralis* and *P. tiliguerta* is at the limit of significance level (multivariate intercept test, $P = 0.048$). On the basis of these results, we can assert that the majority of the trajectories lie along the same line and have the same intercept.

The dispersion along ontogenetic trajectories was unequal in four pair comparisons, three of which concern males of *P. tiliguerta* that were more dispersed than conspecific females and than males of *P. sicula* and *P. filfolensis* (MSE test, $P < 0.05$; see Table 2). The other

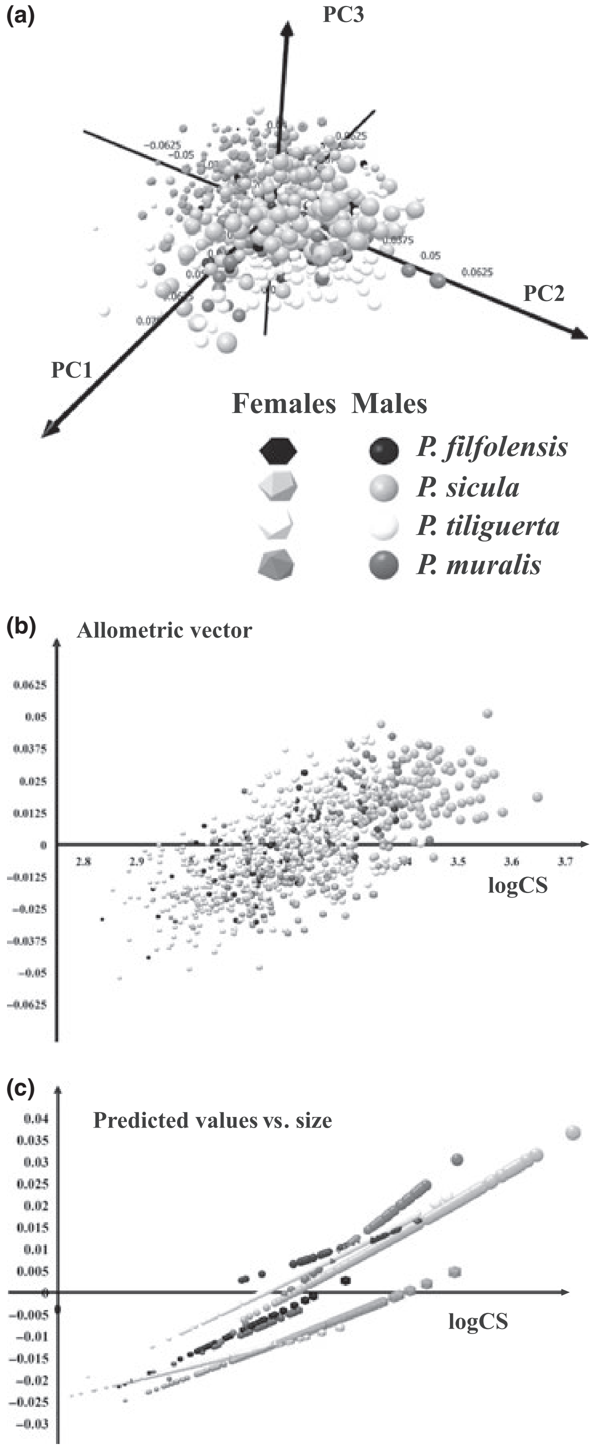

Fig. 5 Allometric scatterplots of shape variation in sexes and species. In all graphics points, dimensions are proportional to size. (a) visualization of allometric trajectories by means of PCA on original aligned shapes. (b) visualization of allometric trajectories as visualized by MorphoJ allometric vector; (c) visualization of allometric trajectories as linear regressions of per-group predicted shape and size ($\log_{10}$CS).

Table 2 Results of pairwise comparison of ontogenetic trajectories. Significant *P*-values are given in bold. *P. fil., Podarcis filfolensis*; *P .mur., P. muralis*; *P. sic., P. sicula*; *P. til., P. tiliguerta*; ♂: males; ♀: females.

Pairwise comparison	Multivariate ontogenetic convergence test	Multivariate intercept test	Mean squared error test	Peramorphosis test
P.fil.♀–*P.fil.*♂	0.052	0.116	0.613	0.146
P.fil.♀–*P.mur.*♀	0.825	0.578	0.681	**0.007**
P.fil.♀–*P.sic.*♀	0.274	0.071	0.087	**0.010**
P.fil.♀–*P.til.*♀	0.438	0.177	0.244	**0.023**
P.mur.♀–*P.mur.*♂	0.838	0.550	0.201	**0.001**
P.mur.♀–*P.sic.*♀	0.068	0.439	**0.032**	**0.013**
P.mur.♀–*P.til.*♀	0.982	0.542	0.098	**0.001**
P.sic.♀–*P.sic.*♂	0.993	0.370	0.979	**0.001**
P.sic.♀–*P.til.*♀	0.689	0.209	0.964	**0.002**
P.til.♀–*P.til.*♂	0.914	**0.013**	**0.049**	**0.001**
P.fil.♂–*P.mur.*♂	0.727	0.153	0.221	**0.002**
P.fil.♂–*P.sic.*♂	0.790	0.283	0.063	**0.001**
P.fil.♂–*P.til.*♂	0.724	0.357	**0.016**	0.071
P.mur.♂–*P.sic.*♂	0.998	0.202	0.353	**0.001**
P.mur.♂–*P.til.*♂	0.865	**0.048**	0.149	**0.002**
P.sic.♂–*P.til.*♂	0.723	**0.006**	**0.020**	**0.001**

Table 3 Multivariate distance (Euclidean) from origin for shape predicted at maximum observed size values for any group. Abbreviations as in caption of Table 2.

Group	Multivariate distance
P.fil.♀	1.000352
P.fil.♂	1.000355
P.mur.♀	1.000184
P.mur.♂	1.000736
P.sic.♀	1.000151
P.sic.♂	1.000716
P.til.♀	1.000295
P.til.♂	1.000542

Table 4 Results of heteroskedasticity test. Significant *p*-values are given in bold. Abbreviations as in captions of Table 2.

Group	Breusch–Pagan test	*P*-value	R^2
P.fil.♀	**< 0.0001**	0.948	−0.124
P.fil.♂	**< 0.0001**	**0.037**	0.368
P.mur.♀	**< 0.0001**	0.693	−0.102
P.mur.♂	**< 0.0001**	0.272	0.042
P.sic.♀	**< 0.0001**	0.182	0.112
P.sic.♂	**< 0.0001**	0.484	−0.054
P.til.♀	**< 0.0001**	0.659	−0.096
P.til.♂	**< 0.0001**	**0.012**	0.514

significant pair comparison returned *P. sicula* females as having a larger MSE than of *P. muralis* females.

The differences between shapes predicted at maximum per-group size values were highly significant in all comparisons (peramorphosis test, $P < 0.05$, see Table 2) except for the comparisons between *P. filfolensis* females and males and between the latter and *P. tiliguerta* males (peramorphosis test, $P > 0.05$). Thus, a wide occurrence of paedo-peramorphosis is evident in the contrasted trajectories. In all intraspecific comparison between the two sexes (except in *P. filfolensis*), males were significantly peramorphic in comparison with females because peramorphosis tests were highly significant and male shapes showed a higher multivariate distance (Euclidean) from origin (see Table 3).

Results from tests concerning non-size-dependent morphological variability around ontogenetic trajectories are provided in Table 4. In all the females and in the males of *P. sicula* and *P. muralis*, the non-size-dependent morphological variability does not change during the ontogeny. In males of only two species, *P. filfolensis* and *P. tiliguerta*, the morphological variability increases (see the pattern in Fig. 2j) with size (heteroskedasticity test, $P < 0.05$, see Table 4), although the former case is at the limit of significance level (heteroskedasticity test, $P = 0.04$).

Intra- and interspecific morphological diversity and differentiation

Evaluating differences in morphological disparity, we found an extensive intraspecific morphological variation that is not evenly distributed across sexes. Indeed, in most of the species, males show higher morphological disparity than females, and this difference is statistically significant in *P. muralis*, while in *P. filfolensis*, females are significantly more dispersed than males (Disparity tests, $P < 0.05$; see Table 5).

Discussion

Ontogenetic patterns in Podarcis

Several studies show that variation in ontogenetic patterns may contribute to morphological variation both at intra- and interspecific levels (see Hollander *et al.*, 2006). Although developmental pathways may canalize the phenotypic variation of species and sexes in definite portions of the morphospace, thus representing an evolutionary constrain, recent studies demonstrate that ontogenetic plasticity lets allometries themselves to evolve, allowing the phenotypic variation to explore further portions of the morphospace (Adams & Nistri,

Table 5 Results of disparity test between groups based on residuals between shape and size. Abbreviations as in captions of Table 2.

Pairwise comparison	Disparity test *P*-value
P.fil.♀–*P.fil.*♂	**0.043**
P.mur.♀–*P.mur.*♂	**0.011**
P.sic.♀–*P.sic.*♂	0.114
P.til.♀–*P.til.*♂	0.090

2010; Wilson & Sánchez-Villagra, 2010; for an exhaustive discussion, see Klingenberg, 2010). What is the contribution of the ontogenetic trajectories variation in shaping intraspecific (intersexual) and interspecific patterns of morphological differentiation in *Podarcis* lizards?

The results of this study provided straightforward evidence for a common allometric pattern in the *Podarcis* lizards cephalic scales morphology during post-natal ontogeny. In all *Podarcis* lizards studied here, the rate of ontogenetic growth per unit *size* (as we have no indications about *ages* of individuals) was the same across sexes and species. The hypotheses of morphological ontogenetic convergence or divergence between species or between sexes are rejected because the shape differences between sexes and species remain unvaried during growth. Differences in the ontogenetic trajectories' intercept and dispersion were found only in *P. tiliguerta* males relative to females and to *P. sicula* and *P. filfolensis* males. Also in two Iberian *Podarcis* species (*P. carbonelli* and *P. bocagei*), Kaliontzopoulou *et al.* (2008) pointed out that sexes of both species follow ontogenetic trajectories with similar slope but different intercept. However, in the case of *P. tiliguerta*, a caution is required because this species likely represents a species complex (Harris *et al.*, 2005), and as such, ontogenetic assessments may conflate allometry with species or locality differences. Unfortunately, as the number and the geographical distribution of the evolutionary entities embodied in this species complex are still unknown, it is not possible to incorporate locality in our analyses and determine whether this greatly affects the resulting ontogenetic pattern and its interpretation.

Taking into account all these results, we argue that the pattern of allometric growth in *Podarcis* lizards affects and constrains the morphological change of all species and sexes in the same way. During post-natal ontogeny, allometry channels cephalic scales morphology variation of these lizards in occupying a fixed portion of phenotypic space. In view of the fact that some straightforward ecological (including dietary) differences exist between the studied species (Capula *et al.*, 1993; Pérez-Mellado & Corti, 1993; Vanhooydonck *et al.*, 2000; Arnold, 2002; Corti & Lo Cascio, 2002), the common allometric growth pattern of *Podarcis* lizards would reflect their shared evolutionary history rather than a common morphology corresponding to a functional optimum. However, selection cannot be ruled out, as it can act directly in maintaining the shared developmental trajectory itself. Moreover, in these lizards, sexual selection constrains could drive the output of the heterochronic processes acting along the trajectories itself as discussed in the next section.

Intraspecific and interspecific heterochrony

The importance of integrating intraspecific and interspecific comparisons when studying heterochronic processes has been invoked by Reilly *et al.* (1997) and then followed by few studies concerning fishes (Mabee *et al.*, 2000; Holtmeier, 2001). These studies, where ontogenetic variation is addressed through both intraspecific and interspecific comparisons, reported a weak relationship between intraspecific and interspecific heterochronic processes. Notwithstanding the common allometric growth pattern we observed in *Podarcis* lizards, what is the role of the intraspecific and interspecific heterochrony in shaping the morphological variation within and among species?

One of the central findings of our work is that the heterochronic processes responsible for the intraspecific (intersexual) differentiation also account for the interspecific differentiation, and result in paedo-peratypic/paedo-peramorphic shapes (sensu Reilly *et al.*, 1997). To our knowledge, this is the first study that explores the variation in ontogeny within and between species and where a consistent pattern of heterochrony is found across the intraspecific and interspecific levels.

At the intraspecific level, we found in all the intersexual comparisons males to be peramorphic (*peratypic* sensu Reilly *et al.*, 1997) relative to females, except in *P. filfolensis* (discussed below). The sexual dimorphism in cephalic scales configuration in these lizards is entirely due to the allometric shape change associated with larger size achieved by males. It is hard to infer heterochronic processes from size and shape data without information on age (Godfrey & Sutherland, 1995). Yet, we suggest that the morphogenetic process underlying the peramorphic male condition (i.e. just a morphological expression) is the hypermorphosis due to the truncation of female growth in comparison with males [see the pattern in Fig. 2h and the heterochronic process classification proposed by Alberch *et al.* (1979) in Table 1]. We can reasonably exclude that the larger size observed in males is due to an accidental bias in the maximum age between males and females included in our sample (which consists of around 900 individuals). Indeed, it was demonstrated that *Podarcis* males reach larger size than females with the same age (Raia *et al.*, 2010). Thus, whereas in females the offset of growth occurs earlier, in males the investment of resources in growth remains significant so that they achieve larger head sizes and the associated morphological peramorphic condition. However, a contribution of developmental acceleration in the ontogenetic hypermorphosis of *Podarcis* males head shape cannot be ruled out with our data. In other species, for which the timing of the ontogenetic events was better known, the peramorphic males condition is due to developmental acceleration in males as compared to females (Kelly & Adams, 2010).

The evolutionary significance of larger head male size in lizards has been deeply investigated. As pointed out also in previous studies in *Podarcis*, the increase in head size is associated with a general head enlargement due to a positive allometric pattern of the parietal area (Bruner

& Costantini, 2007; Kaliontzopoulou *et al.*, 2007, 2008; Ljubisavljevic *et al.*, 2010). A wider and larger head allows the development of enlarged nuchal and jaw adductor muscles and thus an increased bite force performance of males, which is implied in competitive behaviour and copulation (Mouton & van Wyk, 1993; Herrel *et al.*, 1996, 2001; Verwaijen *et al.*, 2002; Husack *et al.*, 2006). Thus, head size in lizards is a sexually selected trait (Braña, 1996; Olsson *et al.*, 2002; Kaliontzopoulou *et al.*, 2007). In this study, we identify the ontogenetic process of hypermorphosis as the proximate mechanism through which sexual selection demand on head shape and size are optimized within a strictly constrained developmental pathway.

Interestingly, in the insular lizard *P. filfolensis*, we did not find a relationship of paedo-peramorphosis between females and males, and in general any trace of sexual dimorphism in cephalic scales configuration. Moreover, we observed a trend of increasing sexual size dimorphism from insular species (*P. filfolensis* and *P. tiliguerta*) to mainland species (*P. sicula* and *P. muralis*) as highlighted in Table 6. These results are consistent with the expectation of the 'island syndrome' hypothesis, which predicts that island population of small vertebrates are less sexually dimorphic (including rodents, birds, and lizards, see Raia *et al.*, 2010 for an overview but also for the 'reversed island syndrome' hypothesis). Nevertheless, an explicit test and an appropriate experimental design are needed to confirm this preliminary hypothesis.

Likewise, also at the interspecific level, the phenotypic differentiation implies a paedo-peramorphic patterning. Due to their larger maximum sizes, the cephalic scale morphology of some species appears peramorphic relative to other species. A reverse pattern was observed between males and females. In male–male comparisons, mainland species (*P. sicula* and *P. muralis*) are peramorphic relative to island species (*P. filfolensis* and *P. tiliguerta*), whereas female island species are peramorphic relative to female mainland species. This pattern is a consequence of the higher size achieved by male mainland species and female island species as discussed above.

In the studied *Podarcis* lizards, both the intraspecific (intersexual) and the interspecific phenotypic differentiation is exclusively driven by allometric (heterochronic) factors related to post-natal ontogeny as demonstrated by the fact that both slope and elevation, and dispersion along trajectories are shared by the most of pairwise intersexual and interspecific comparisons.

Table 6 Size (logCS) range and mean for each group. Abbreviations as in captions of Table 2.

Group	logCS min	logCS max	logCS mean
P.fil.♀	2.836	3.264	3.076
P.fil.♂	3.102	3.382	3.255
P.mur.♀	2.971	3.189	3.115
P.mur.♂	2.994	3.447	3.276
P.sic.♀	2.931	3.442	3.163
P.sic.♂	3.105	3.648	3.362
P.til.♀	2.855	3.254	3.037
P.til.♂	2.877	3.430	3.209

In addition, Bruner & Costantini (2007) found that the morphological differentiation in cephalic scale morphology between *P. sicula* and *P. muralis* is mostly explained by allometric variation, although in their study only adult specimens were considered and sexes were pooled, thus preventing the identification of intersexual and ontogenetic patterns. On the other hand, for the two Iberian species *P. bocagei* and *P. carbonelli*, some morphological sexual dimorphism independent from size was found both at juvenile at and adult stages (Kaliontzopoulou *et al.*, 2008).

Patterns of intra- and interspecific morphological variation and differentiation

A shared allometric patterning does not imply a cutback of the phenotypic diversity in these species. We have showed how the ontogenetic mechanism of trajectories truncation/elongation enlightens the phenotypic differentiation between sexes and species. However, an additional source of variation accounts for the phenomenal amount of morphological diversity observed in *Podarcis* lizards. Indeed, the high degree of individual (intrasexual) morphological dispersion along common allometric trajectories accounts for a large occupation of the allometric space in both sexes of any species allowing for the high morphological diversity observed at any size. The morphological dispersion along the ontogenetic trajectories does not vary with size, with the only exception of *P. tiliguerta* and *P. filfolensis* males, which showed increased morphological variability at large sizes. Thus, there is a considerable amount of phenotypic diversity that is not accounted for by ontogenetic allometry and is present at any age stage in these lizards. This result, in combination with the rejection of the hypotheses of ontogenetic convergence or divergence between the ontogenetic trajectories, suggests that the cephalic scales variation is equally developmentally constrained during the growth from juveniles to adults but also that this constrain allows a large degree of morphological variation. Understanding which evolutionary process engendered and shaped this non-allometric component of phenotypic diversity requires further investigation. For example, investigating the relationship between genetic divergence and size variation across the entire *Podarcis* clade could clarify whether this group experienced a sort of least evolutionary resistance trajectory (Marroig & Cheverud, 2005) during its evolution. However, this is behind the scope of the present study.

To explore how the non-allometric component of phenotypic diversity is distributed within and among species, we analysed each sex of any species in an allometry-free morphospace. Intraspecific morphological variation is not evenly distributed across sexes. In most of the species, males were always more variable than females as evidenced by their higher average morphological disparity. The contribution of the ontogenetic processes in the unequal morphological diversity between sexes could stem from the additional portion of the morphospace experienced by males, but not by females, during growth. Because of hypermorphosis, males keep growing longer than females attaining extended shape. This additional subset of morphological states related to large sizes of males, together with the non-size variation around them, could account for (or at least contributes to) their higher morphological diversity.

Main implications and conclusion

This study outlines the importance of integrating intra- and interspecific comparisons in order to properly appreciate the role of developmental processes in shaping the phenotypic diversity across sexes and species. The few previous studies that explored the ontogenetic variation within and between species (Mabee *et al.*, 2000; Holtmeier, 2001) found a weak relationship between heterochronic processes at the intra and interspecific level. By contrast, we found the heterochronic process of hypermorphosis as the mechanism responsible for both the intraspecific (intersexual) and interspecific morphological differentiation in *Podarcis* lizards.

A more plastic developmental system is a general expectation for groups that experienced extensive morphological diversification, so that either phenotypic variation can arise through the alteration in developmental constraints or from environmentally induced components of developmental variation (Hall, 1992; Fusco & Minelli, 2010; see also Klingenberg, 2011). In *Podarcis* lizards, we found that a shared allometric pattern between sexes and species constrains and canalizes their morphological variation in a fixed portion of the phenotypic space. Neither growth rate changes nor ontogenetic convergence/divergence are the mechanisms mediating the generation of phenotypic diversity in these lizards. Rather, heterochronic processes of truncation/elongation of the ontogenetic trajectories account for the extensive phenotypic differentiation between sexes and species. Interspecific and intersexual variation arises from a simple hypermorphic transformation that allows males and some species to occupy an extended portion of the morphospace marked by wider and larger head. Given the obvious evolutionary implications of this trait from intra and intersexual competition to interspecific and intersexual differentiation in microhabitat use, diet and behaviour (see e.g. Butler & Losos, 2002; Verwaijen *et al.*, 2002; Husack *et al.*, 2006), it is clear that hypermorphosis could contribute substantially to the ecomorphological diversity of these lizards. The observed pattern of post-natal ontogeny in *Podarcis* suggests that heterochronic processes such as those concerning changes in onset or offset of morphogenetic growth processes could produce remarkable morphological variation. Moreover, a broad diversification of morphological traits that are functionally and ecologically relevant can be generated as quickly as it does not imply any change in the overall ontogenetic trajectory. Therefore, heterochronic processes are candidate mechanisms for rapid (and extensive) phenotypic diversification, which is one central prediction of adaptive radiations. These findings suggest a model of how, even within a strictly constrained developmental pathway, simple heterochronic perturbations can produce phenotypic variation that is functionally and ecologically significant, and thus potential for adaptive evolutionary change. Several common features of the *Podarcis* system with the classic and well-known *Anolis* model suggest the relevance of these ontogenetic processes in the framework of adaptive evolutionary radiations. Likewise in *Podarcis, Anolis* interspecific and intersexual variation concern the same morphological traits, so that the sexes differ for the same morphological characters (e.g. size, body length, limb length), which discriminate species adapted to distinctive habitats (ecomorphs) (Butler *et al.*, 2007). Given the allometric basis of the morphological differentiation and the mosaic nature of morphological evolution (de Beer, 1954; Frankino *et al.*, 2005; Clarke & Middleton, 2008), it becomes of interest testing whether heterochronic processes played a role in the processes of ecomorphological evolution during adaptive radiation of anoles and other groups. Interestingly, these insights come in times when the recent availability of full genomes of some *Anolis* lizards and progresses in understanding their developmental biology (Sanger *et al.*, 2008) allow for more thorough analyses of developmental and evolutionary basis of morphological variation.

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References

Adams, D.C. 2010. Parallel evolution of character displacement driven by competitive selection in terrestrial salamanders. *BMC Evol. Biol.* **10**: 72.

Adams, D.C. & Collyer, M.L. 2007. Analysis of character divergence along environmental gradients and other covariates. *Evolution* **61**: 510–515.

Adams, D.C. & Collyer, M.L. 2009. A general framework for the analysis of phenotypic trajectories in evolutionary studies. *Evolution* **63**: 1143–1154.

Adams, D.C. & Nistri, A. 2010. Ontogenetic convergence and evolution of foot morphology in European cave salamanders (Family: Plethodontidae). *BMC Evol. Biol.* **10**: 216.

Adams, D.C., Rohlf, F.J. & Slice, D.E. 2004. Geometric morphometrics: ten years of progress following the ''revolution''. *Ital. J. Zool.* **71**: 5–16.

Alberch, P. 1981. Convergence and parallelism in foot morphology in the neotropical salamander genus *Bolitoglossa.* I. Function. *Evolution* **35**: 84–100.

Alberch, P. 1982. Developmental constraints in evolutionary processes. In: *Evolution and Development* (J.T. Bonner, ed.), pp. 313–332. Springer, New York.

Alberch, P., Gould, S.J., Oster, G.F. & Wake, D.B. 1979. Size and shape in ontogeny and phylogeny. *Paleobiology* **5**: 296–317.

Arnold, E.N. 1989. Towards a phylogeny and biogeography of the Lacertidae: relationships within an old-world family of lizards derived from morphology. *Bull. Brit. Mus. Nat. Hist.* **55**: 209–257.

Arnold, E.N. 1993. Phylogeny and the Lacertidae. In: *Lacertids of the Mediterranean Region* (E.D. Valakos, W. Bohme, V. Perez-Mellado & P. Maragou, eds), pp. 1–16. Hellenic Zoological Society, Athens.

Arnold, E.N. 1998. Structural niche, limb morphology and locomotion in lacertid lizards (Squamata, Lacertidae); a preliminary survey. *Bull. Nat Hist. Mus. Lond.* **6**: 63–89.

Arnold, E.N. 2002. *A Field Guide to the Reptiles and Amphibians of Britain and Europe*. Collins, London.

Arnold, E.N., Arribas, O. & Carranza, S. 2007. Systematics of the palaearctic and oriental lizard tribe Lacertini (Squamata: Lacertidae: Lacertinae), with descriptions of eight new genera. *Zootaxa* **1430**: 1–86.

Badyaev, A.V., Hill, G.E. & Whittingham, L.A. 2001. The evolution of sexul size dimorphism in the house finch. IV. Population divergence in ontogeny. *Evolution* **55**: 2534–2549.

de Beer, G.R. 1954. *Archaeopteryx lithographica.* A Study Based Upon the British Museum Specimen. British Museum (Natural History), London.

Bookstein, F.L. 1989. Principal warps: thin-plate splines and the decomposition of deformations. *IEEE Trans. Pattern Anal. Mach. Intell.* **11**: 567–585.

Bookstein, F.L. 1991. *Morphometric Tools for Landmark Data: Geometry and Biology*. Cambridge University Press, Cambridge.

Bookstein, F.L., Schäfer, K., Prossinger, H., Seidler, H., Fieder, M., Stringer, C. *et al.* 1999. Comparing frontal cranial profiles in archaic and modern *Homo* by morphometric analysis. *Anat. Rec.* **257**: 217–224.

Braña, F. 1996. Sexual dimorphism in lacertid lizards: male head increase vs female abdomen increase? *Oikos* **75**: 511–523.

Brown, R.P., Terrasa, B., Pérez-Mellado, V., Castro, J.A., Hoskisson, P.A., Picornell, A. *et al.* 2008. Bayesian estimation of post-Messinian divergence times in Balearic Island lizards. *Mol. Phyl. Evol.* **48**: 350–358.

Bruner, E. & Costantini, D. 2007. Head morphological variation in *Podarcis muralis* and *Podarcis sicula*: a landmark-based approach. *Amphibia-Reptilia* **28**: 566–573.

Bruner, E., Costantini, D., Fanfani, A. & Dell'Omo, G. 2005. Morphological variation and sexual dimorphism of the cephalic scales in *Lacerta bilineata*. *Acta Zool.* **86**: 245–254.

Butler, M.A. & Losos, J.B. 2002. Multivariate sexual dimorphism, sexual selection, and adaptation in Greater Antillean *Anolis* lizards. *Ecol. Mon.* **72**: 541–559.

Butler, M.A., Sawyer, S.A. & Losos, J.B. 2007. Sexual dimorphism and adaptive radiation in *Anolis* lizards. *Nature* **447**: 202–205.

Calsbeek, R. & Irschick, D.J. 2007. The quick and the dead: correlational selection on morphology, performance, and habitat use in island lizards. *Evolution* **61**: 2493–2503.

Capula, M. & Ceccarelli, A. 2003. Distribution of genetic variation and taxonomy of insular and mainland populations of the Italian wall lizard, *Podarcis sicula*. *Amphibia-Reptilia* **24**: 483–495.

Capula, M., Luiselli, L. & Rugiero, L. 1993. Comparative ecology in sympatric *Podarcis muralis* and *P. sicula* (Reptilia: Lacertidae) from the historical centre of Rome: what about competition and niche segregation in an urban habitat? *Boll. Zool.* **60**: 287–291.

Cardini, A. 2003. The Geometry of the Marmot (Rodentia: Sciuridae). Mandible: phylogeny and patterns of morphological evolution. *Syst. Biol.* **52**: 186–205.

Clarke, J.A. & Middleton, K.M. 2008. Mosaicism, modules, and the evolution of birds: results from a Bayesian approach to the study of morphological evolution using discrete character data. *Syst. Biol.* **57**: 185–201.

Collyer, M.L. & Adams, D.C. 2007. Analysis of two-state multivariate phenotypic change in ecological studies. *Ecology* **88**: 683–692.

Corti, C. & Lo Cascio, P. 2002. *The Lizards of Italy and Adjacent Areas*. Chimaira Verlag, Frankfurt–am–Main.

Darwin, C. 1858. On the tendency of species to form varieties, and on the perpetuation of varieties and species by natural means of selection. I. Extract from an unpublished work on species, II. Abstract of a letter from C. Darwin, Esq., to Prof. Asa Gray. *J. Proc. Linn. Soc. Zoology* **3**: 45–53.

Fisěr, C., Bininda-Emonds, O.R.P., Blejec, A. & Sket, B. 2008. Can heterochrony help explain the high morphological diversity within the genus *Niphargus* (Crustacea: Amphipoda)? *Org. Div. Evol.* **8**: 146–162.

Frankino, W.A., Zwaan, B.J., Stern, D.L. & Brakefield, P.M. 2005. Natural selection and developmental constrains in the evolution of allometries. *Science* **307**: 718–720.

Fusco, G. & Minelli, A. 2010. Phenotypic plasticity in development and evolution: facts and concepts. *Phil. Trans. R. Soc. B.* **365**: 547–556.

Futuyma, D.J. 2005. *Evolution*. Sinauer Associates, Sunderland, MA, USA.

Gaston, K.J. 1996. What is biodiversity. In: *Biodiversity: A Biology of Numbers and Difference* (K.J. Gaston, ed.), pp. 1–9. Blackwell Science Ltd, Oxford, UK.

Godfrey, L.R. & Sutherland, M. 1995. Flawed inference: why size-based tests of heterochronic processes do not work. *J. Theor. Biol.* **172**: 43–61.

Goodall, C. 1991. Procrustes methods in the statistical analysis of shape. *J. R. Statist. Soc. B* **53**: 285–339.

Gould, S.J. 1977. *Ontogeny and Phylogeny*. Harvard University Press, Cambridge, MA, USA.

Gould, S.J. 2002. *The Structure of Evolutionary Theory*. Harvard University Press, Cambridge.

Hall, B.K. 1992. *Evolutionary Developmental Biology*. Chapman and Hall, London.

Harris, D.J., Pinho, C., Carretero, M.A., Corti, C. & Böhme, W. 2005. Determination of genetic diversity within *Podarcis tiliguerta* using mtDNA sequence data, with a reassessment of the phylogeny of *Podarcis*. *Amphibia-Reptilia* **26**: 401–407.

Herrel, A., Van Damme, R. & De Vree, F. 1996. Sexual dimorphism of head size in *Podarcis hispanica atrata*: testing the dietary divergence hypothesis by bite force analysis. *Neth. J. Zool.* **46**: 253–262.

Herrel, A., de Grauw, E. & Lemos-Espinal, J.A. 2001. Head shape and bite performance in xenosaurid lizards. *J. Exp. Zool.* **290**: 101–107.

Hollander, J., Adams, D.C. & Johannesson, K.K. 2006. Evolution of adaptation through allometric shifts in a marine snail. *Evolution* **60**: 2490–2497.

Holtmeier, C.L. 2001. Heterochrony, maternal effects, and phenotypic variation among sympatric pupfishes. *Evolution* **55**: 330–338.

Husack, J.F., Lappin, A.K., Fox, S.F. & Lemos-Espinal, J.A. 2006. Bite–force performance predicts dominance in male venerable collared lizards (*Crotaphytus antiquus*). *Copeia* **2006**: 301–306.

Huttegger, S. & Mitteroecker, P. 2011. Invariance and meaningfulness in phenotype spaces. *Evol. Biol.* **38**: 335–352.

Kaliontzopoulou, A. 2010. Proximate and evolutionary causes of phenotypic diversification: morphological variation in Iberian and North African *Podarcis* wall lizards. PhD Thesis, University of Barcelona, Barcelona.

Kaliontzopoulou, A., Carretero, M.A. & Llorente, G.A. 2007. Multivariate and geometric morphometrics in the analysis of sexual dimorphism variation in *Podarcis* lizards. *J. Morphol.* **268**: 152–165.

Kaliontzopoulou, A., Carretero, M.A. & Llorente, G.A. 2008. Interspecific and intersexual variation in presacral vertebrae number in *Podarcis bocagei* and *P. carbonelli*. *Amphibia-Reptilia* **29**: 288–292.

Kaliontzopoulou, A., Carretero, M.A. & Llorente, G.A. 2010. Intraspecific ecomorphological variation: linear and geometric morphometrics reveal habitat-related patterns within *Podarcis bocagei* wall lizards. *J. Evol. Biol.* **23**: 1234–1244.

Kaliontzopoulou, A., Pinho, C., Harris, D.J. & Carretero, M.A. 2011. When cryptic diversity blurs the picture: a cautionary tale from Iberian and North African *Podarcis* wall lizards. *Biol. J. Linn. Soc.* **103**: 779–800.

Kelly, C.D. & Adams, D.C. 2010. Sexual selection, ontogenetic acceleration, and hypermorphosis generates male trimorphism in Wellington tree weta. *Evol. Biol.* **37**: 200–209.

Klingenberg, C.P. 2005. Developmental constraints, modules, and evolvability. In: *Variation: A Central Concept in Biology* (B. Hallgrimsson & B.K. Hall, eds), pp. 219–247. Elsevier Academic Press, Burlington, MA, USA.

Klingenberg, C.P. 2010. There's something afoot in the evolution of ontogenies. *BMC Evol. Biol.* **10**: 221.

Klingenberg, C.P. 2011. MorphoJ: an integrated software package for geometric morphometrics. *Mol. Ecol. Res.* **11**: 353–357.

Klingenberg, C.P., Barluenga, M. & Meyer, A. 2002. Shape analysis of symmetric structures: quantifying variation among individuals and asymmetry. *Evolution* **56**: 1909–1920.

Ljubisavljevic, K., Urosevic, A., Aleksic, I. & Ivanovic, A. 2010. Sexual dimorphism of skull shape in a lacertid lizard species (*Podarcis* spp., *Dalmatolacerta* sp., *Dinarolacerta* sp.) revealed by geometric morphometrics. *Zoology (Jena)* **113**: 168–174.

Losos, J.B. 1990. The evolution of form and function: morphology and locomotor performance in West Indian *Anolis* lizards. *Evolution* **44**: 1189–1203.

Losos, J.B. 2009. *Lizards in an Evolutionary Tree*. University of California Press, Berkeley.

Losos, J.B., Douglas, A.C., Glossip, D., Goellner, R., Hampton, A., Roberts, G. *et al.* 2000. Evolutionary implications of phenotypic plasticity in the hindlimb of the lizard *Anolis sagrei*. *Evolution* **54**: 301–305.

Mabee, P.M., Olmstead, K.L. & Cubbage, C.C. 2000. An experimental study of intraspecific variation, developmental timing, and heterochrony in fishes. *Evolution* **54**: 2091–2106.

Marroig, G. & Cheverud, J. 2005. Size as a line of least evolutionary resistance: diet and adaptive morphological radiation in New World Monkeys. *Evolution* **59**: 1128–1142.

Maynard-Smith, J., Burian, R., Kauffman, S., Alberch, P., Campbell, J., Goodwin, B. *et al.* 1985. Developmental constraints and evolution. *Q. Rev. Biol.* **60**: 265–287.

McKinney, M.L. 1988. *Heterochrony in Evolution*. Plenum Press, New York.

McKinney, M.L. & McNamara, K.J. 1991. *Heterochrony: The Evolution of Ontogeny*. Plenum Press, New York.

Mitteroecker, P. & Bookstein, F. 2008. The evolutionary role of modularity and integration in the hominoid cranium. *Evolution* **62**: 943–958.

Mitteroecker, P. & Gunz, P. 2009. Advances in geometric morphometrics. *Evol. Biol.* **36**: 235–247.

Mitteroecker, M., Gunz, P. & Bookstein, F.L. 2005. Heterochrony and geometric morphometrics: a comparison of cranial growth in *Pan paniscus* versus *Pan troglodytes*. *Evol. Dev.* **7**: 244–258.

Monteiro, L.R. 1999. Multivariate regression models and geometric morphometrics: the search for causal factors in the analysis of shape. *Syst. Biol.* **48**: 192–199.

Mouton, N. & van Wyk, J.H. 1993. Sexual dimorphism in cordylid lizards: a case study of the Drakensberg crag lizard, *Pseudocordylus melanotus*. *Can. J. Zool.* **71**: 1715–1723.

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., O'Hara, R.B., Simpson, G.L. *et al.* 2011. Vegan: community ecology package. R package version 1.17-7. http://vegan.r-forge.r-project.org.

Olsson, M., Shine, R., Wapstra, E., Ujvari, B. & Madsen, T. 2002. Sexual dimorphism in lizard body shape: the roles of sexual selection and fecundity selection. *Evolution* **56**: 1538–1542.

Pérez-Mellado, V. & Corti, C. 1993. Dietary adaptations and herbivory in lacertid lizards of the genus *Podarcis* from western Mediterranean islands (Reptilia: Sauria). *Bonn. Zool. Beitr.* **44**: 193–220.

Piras, P., Colangelo, P., Adams, D.C., Buscalioni, A., Cubo, J. & Kotsakis, T. *et al.* 2010. The *Gavialis-Tomistoma* debate: the contribution of skull ontogenetic allometry and growth trajectories to the study of crocodilian relationships. *Evol. Dev.* **12**: 568–579.

Podnar, M., Mayer, W. & Tvrtković, N. 2005. Phylogeography of the Italian wall lizard, *Podarcis sicula*, as revealed by mitochondrial DNA sequences. *Mol. Ecol.* **14**: 575–588.

Poulakakis, N., Lymberakisa, P., Valakos, E., Pafilis, P., Zouros, E. & Mylonas, M. 2005. Phylogeography of Balkan wall lizard (*Podarcis taurica*) and its relatives inferred from mitochondrial DNA sequences. *Mol. Ecol.* **14**: 2433–2443.

R Development Core Team 2011. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0. URL http://www.R-project.org.

Raia, P., Guarino, F.M., Turano, M., Polese, G., Rippa, D., Carotenuto, F. *et al.* 2010. The blue lizard spandrel and the island syndrome. *BMC Evol. Biol.* **10**: 289.

Reilly, S.M. 1994. The ecological morphology of metamorphosis: heterochrony and the evolution of feeding mechanisms in salamanders. In: *Ecological Morphology: Integrative Approaches in Organismal Biology* (P.C. Wainwright & S.M. Reilly, eds), pp. 319–338. University of Chicago Press, Chicago.

Reilly, S.M., Wiley, E.O. & Meinhardt, D.J. 1997. An integrative approach to heterochrony: the distinction between interspecific and intraspecific phenomena. *Biol. J. Linn. Soc.* **60**: 119–143.

Revell, L.J., Johnson, M.A., Schulte, J.A., Kolbe, J.J. & Losos, J.B. 2007. A phylogenetic test for adaptive convergence in rock-dwelling lizards. *Evolution* **61**: 2898–2912.

Rohlf, F.J. & Marcus, L.F. 1993. A revolution in morphometries. *Trends Ecol. Evol.* **8**: 129–132.

Rohlf, F.J. & Slice, D. 1990. Extensions of the procrustes method for the optimal superimposition of landmarks. *Syst. Biol.* **39**: 40–59.

Sanger, T.J., Losos, J.B. & Gibson-Brown, J.J. 2008. A developmental staging series for the lizard genus *Anolis*: A new system for the integration of evolution, development, and ecology. *J. Morph.* **269**: 129–137.

Schluter, D. 2000. *The Ecology of Adaptive Radiation*. Oxford University Press, Oxford.

Takacs, D. 1996. *The Idea of Biodiversity*. Philosophies of Paradise. Johns Hopkins University Press, Baltimore, MD, USA.

Vanhooydonck, B. & Van Damme, R. 1999. Evolutionary relationships between body shape and habitat use in lacertid lizards. *Evol. Ecol. Res.* **1**: 785–805.

Vanhooydonck, B., Van Damme, R. & Aerts, P. 2000. Ecomorphological correlates of habitat partitioning in Corsican lacertid lizards. *Funct. Ecol.* **14**: 358–368.

Verwaijen, D., Van Damme, R. & Herrel, A. 2002. Relationships between head size, bite force, prey handling efficiency and diet in two sympatric lacertid lizards. *Funct. Ecol.* **16**: 842–850.

Werneburg, I. & Sánchez-Villagra, M.R. 2009. Timing of organogenesis support basal position of turtles in the amniote tree of life. *BMC Evol. Biol.* **9**: 82.

Wilson, L.A.B. & Sánchez-Villagra, M.R. 2010. Diversity trends and their ontogenetic basis: an exploration of allometric disparity in rodents. *Proc. R. Soc. B* **277**: 1227–1234.

Yeh, J. 2002. The evolution of development: two portraits of skull ossification in pipoid frogs. *Evolution* **56**: 2484–2498.

Zelditch, M.L., Swiderski, D.L., Sheets, H.D. & Fink, W.L. 2004. *Geometric Morphometrics for Biologists: A Primer*. Elsevier/Academic Press, Amsterdam.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Museum label information for all *Podarcis* specimens analyzed.

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