

Evolution of extreme ontogenetic allometric diversity and heterochrony in pythons, a clade of giant and dwarf snakes

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Ontogenetic allometry, how species change with size through their lives, and heterochrony, a decoupling between shape, size, and age, are major contributors to biological diversity. However, macroevolutionary allometric and heterochronic trends remain poorly understood because previous studies have focused on small groups of closely related species. Here, we focus on testing hypotheses about the evolution of allometry and how allometry and heterochrony drive morphological diversification at the level of an entire species-rich and diverse clade. Pythons are a useful system due to their remarkably diverse and well-adapted phenotypes and extreme size disparity. We collected detailed phenotype data on 40 of the 44 species of python from 1191 specimens. We used a suite of analyses to test for shifts in allometric trajectories that modify morphological diversity. Heterochrony is the main driver of initial divergence within python clades, and shifts in the slopes of allometric trajectories make exploration of novel phenotypes possible later in divergence history. We found that allometric coefficients are highly evolvable and there is an association between ontogenetic allometry and ecology, suggesting that allometry is both labile and adaptive rather than a constraint on possible phenotypes.

KEY WORDS: Geometric morphometrics, heterochrony, ontogenetic allometry, Pythonidae.

Allometry, the relationship between biological traits and size (Huxley and Teissier 1936), and its role as both a contributor and constrainer of phenotypic diversity, has been a central focus in evolutionary biology for the last century (Huxley and Teissier 1936; Gould 1966; Mosimann 1970; Klingenberg 1998; 2016). Ontogenetic allometry, the relationship between biological traits and size through ontogeny (Alberch et al. 1979; Voje et al. 2013), is likely also a major contributor to biological diversity, but it is comparatively less well understood. The few studies that have evaluated it among closely related species have concluded that it has played a major role in that clade's phenotypic diversity through ontogeny (Zelditch et al. 2003; Adams and Nistri 2010; Piras et al. 2011). The evolutionary mechanisms that cause ontogenetic allometric trajectories to shift during evolution (see below), thus impacting phenotypic diversity at different developmental stages, remain unclear (Zelditch et al. 2003; Klingenberg 2010; Pélabon et al. 2014). However, extrinsic forces like selective pressures or phenotypic plasticity imposed by ecological

factors can affect the intrinsic forces of development (Frankino et al. 2005). Allometry and size–shape relationships have been considered constraints that size and growth impose to the morphologies that organisms can adopt (Simpson 1944; Gould and Lewontin 1979; Maynard Smith et al. 1985). Yet, allometric trajectories themselves can be biological traits under selection and not just constraints (Weber 1990; Frankino et al. 2005; Adams and Nistri 2010; Wilson and Sanchez-Villagra 2010; Klingenberg 2010; Urošević et al. 2013; Porto et al. 2013; Giannini 2014).

There are several properties of ontogenetic allometric trajectories that can shift the array of phenotypes observed in a clade at any given ontogenetic stage (Zelditch et al. 2012; Sheets and Zelditch 2013). When examined in a statistical regression framework, modifications in the slopes of the allometric regressions, which define the magnitude and direction of shape change with size, can lead to patterns like ontogenetic convergence or divergence. In some European plethodontid salamanders, juveniles start off with very different shaped feet but converge onto

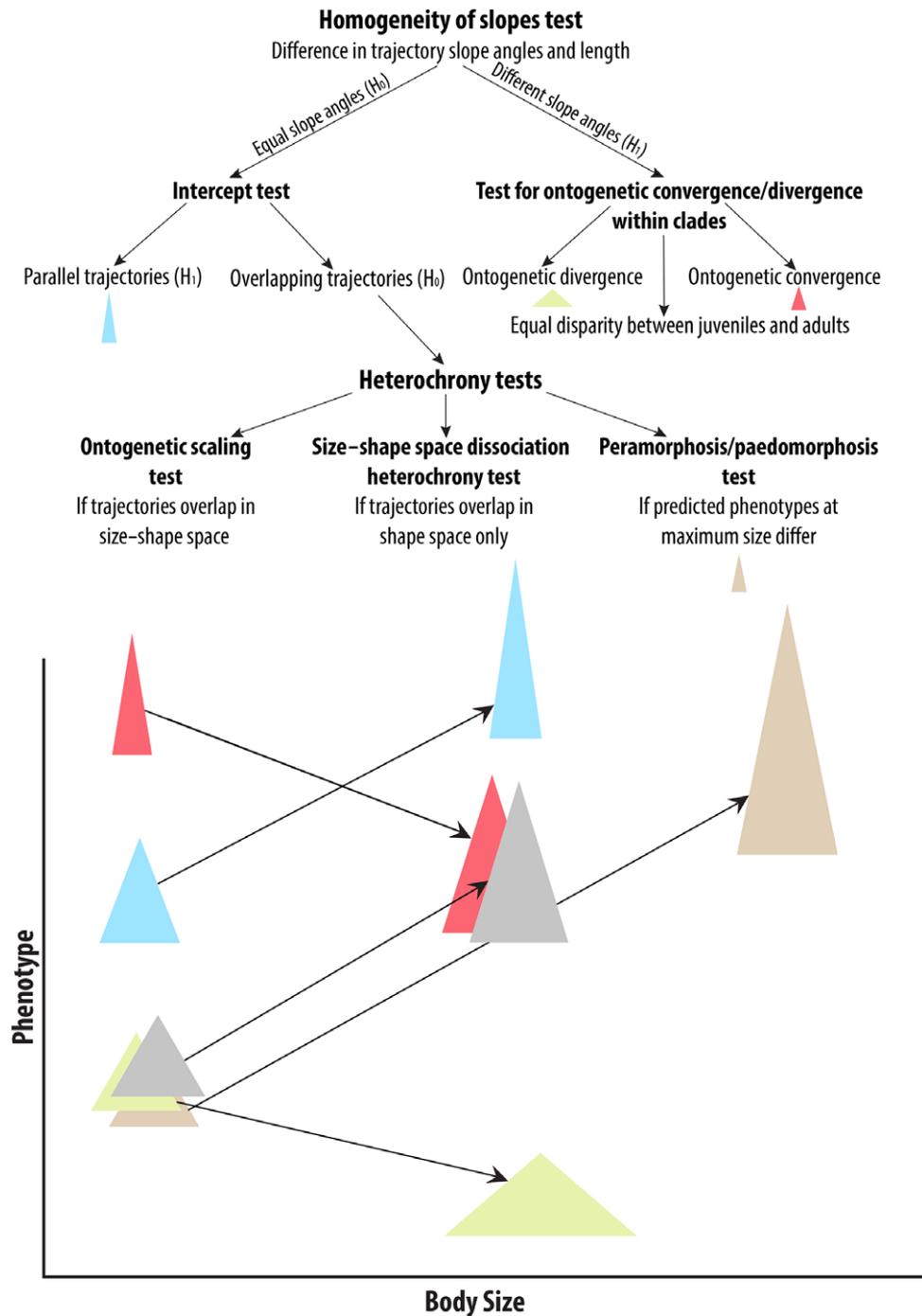


Figure 1. Schematic of the workflow to identify the different processes and patterns of ontogenetic allometric and heterochronic changes. See Section “Materials and Methods” for details on each step and the analyses. Below, a phenotype versus body size illustration of a hypothetical example of patterns our study seeks to uncover. The gray triangles are a reference trajectory; the green illustrate ontogenetic divergence; the red ontogenetic convergence; the blue parallel ontogenetic trajectories or shifts in the intercept; the other heterochrony. The other triangles are peramorphic or the gray triangles are paedomorphic, in respect to each other.

a similar foot morphology as they grow, which is thought to be an adaptation to climbing (Adams and Nistri 2010). Conversely, damselfishes show a conserved larval skeletal morphology while they inhabit the homogeneous oceanic environment, but diverge into a disparate array of adult morphologies to use the more

complex and diverse microhabitat of the coral reef (Frédérich and Vandewalle 2011). Trajectories can shift in their regression intercept, producing parallel trajectories that have the same direction of shape change but never share the same phenotype (Frédérich and Vandewalle 2011; Hipsley and Müller 2017). If trajectories

overlap (share an intercept and slope), heterochronic changes, where a decoupling between shape, size, and age happens, can induce profound changes in morphological diversity (Gould 1977; Gerber et al. 2008; Bhullar et al. 2012; Foth et al. 2016). Size is the independent variable used in studies of ontogenetic allometry, whereas time is the independent variable in studies of heterochrony. Without data on the age of the individuals, it is not possible to infer information on rates of change, or establishing heterochronic processes driving evolution (Godfrey and Sutherland 1995). Nevertheless, using size as a proxy of age, it is possible to detect the observable outcomes of heterochrony, such as paedomorphosis and peramorphosis, where species become more juvenile or adult looking in respect to the other, respectively (Piras et al. 2011; Gerber and Hopkins 2011). In ontogenetic scaling, a special form of heterochrony, species overlap in size–shape space, where the relationship between size and shape is maintained (i.e., same allometric regression equation), and changes in growth rate will change the juvenile and adult morphologies along the same trajectory (Mitteroecker et al. 2005; Zelditch et al. 2012). Heterochronic changes therefore produce forms that are more peramorphic or paedomorphic in respect to the ancestor (Gould 1977; Alberch et al. 1979; Piras et al. 2011; Gerber and Hopkins 2011). Figure 1 illustrates examples of these patterns, and the workflow used to detect them.

Pythons are a family of Old World constrictor snakes that include 44 species distributed in Africa, Asia, Melanesia, and Australia. They exhibit their maximum taxonomic, phenotypic, and ecological diversity in the Australasian region (Barker et al. 2015). Several aspects of this family make them an excellent model for the study of morphological diversity, adaptation, and allometry. They display one of the most extreme size ranges among any animal family, ranging from the pygmy python (*A. perthensis*) that reach only 65 cm (Esquerré, unpublished data) to the reticulated python (*Malayopython reticulatus*), the longest snake on Earth that can reach almost 9 m in length (Murphy and Henderson 1997)—with difference in mass that spans three orders of magnitude. Their remarkable ecological diversity, particularly in microhabitat choice, has provided diverging selective pressures driving an equally remarkable head shape diversity (Esquerré and Keogh 2016). This combination of features provides an ideal scenario to test hypotheses on the contributions of allometry to clade diversity and how allometry interacts with ecological factors.

Our study focuses on ontogenetic allometry and observable products of heterochrony in python head and body shape. Here, we examine diversity in postnatal ontogenetic allometric trajectories in regard to direction, length (magnitude), and intercept at the family level, and at a genus or clade level, to estimate if closely related species would be more constrained to change shape in the same direction and have similar ontogenetic trajectories. Additionally, we test if among-species morphological dis-

parity increases (indicating ontogenetic divergence) or decreases (indicating ontogenetic convergence) over ontogeny at the whole family level and for each clade separately. In clades where trajectories are overlapping, we explore how the array of phenotypic mega-diversity in pythons is affected by changes along a common ontogenetic trajectory, with the ambition of finding patterns that can be explained by processes of heterochrony. Then, by incorporating ontogenetic allometry into a phylogenetic framework, we evaluate how direction and magnitude of shape change when size evolves. This is achieved by mapping those traits on a phylogeny and treating allometry itself as an evolvable trait (Gerber et al. 2008), which we interpret as a trait that can change during evolutionary timescales. Finally, we determine if selection on microhabitat use, which is known to strongly affect adult head morphology (Esquerré and Keogh 2016), impacts the diversity of ontogenetic trajectories directing phenotypic change through growth in pythons.

Material and Methods

SAMPLING

We visited nine major natural history collections (see Table S1) and sampled 1191 specimens spanning 40 of the 44 recognized species of Pythonidae (Barker et al. 2015), plus *Boa constrictor* from Boidae to have a comparison with this snake family that shows strong convergent evolution with pythons (Esquerré and Keogh 2016). We measured an average of 32.2 specimens per species (range 3–153; see Table S1 for details on sampling). To increase statistical power, we considered some species that are phenotypically and ecologically extremely similar, and have shallow or unassessed genetic divergence, to be a single unit for analyses. This gave us more complete size ranges and did not change our interpretation of the results. Specifically, we grouped *Python breitensteini* with *P. curtus*, *P. natalensis* with *P. sebae*, *Morelia imbricata* with *M. spilota*, *Morelia azurea* with *M. viridis*, all the *Leiopython* species with *L. albertisii*, and *Boa imperator* with *B. constrictor*.

MORPHOMETRICS

The head of each specimen was photographed in the dorsal view with a Canon 7D camera with a Canon 100 mm f/2.8 macro lens and a Canon Twin Lite macro flash (Canon Inc.), mounted on a tripod. We placed a scale bar next to each specimen to quantify size. On each photograph, we digitized a configuration of nine landmarks and 26 semilandmarks to characterize head shape (Fig. S1), as described in Esquerré and Keogh (2016). We digitized the landmark and semilandmark coordinates using tpsDig version 2.17 (Rohlf 2015). Semilandmarks were permitted to slide to minimize bending energy (Gunz and Mitteroecker 2013) on tpsRelw

version 1.54 (Rohlf 2015). To retain only shape variation between the landmark coordinates, the effects of location, scale, and orientation were removed with a generalized Procrustes analysis (Rohlf and Slice 1990), taking into account object symmetry (Klingenberg et al. 2002), performed with the function *bilat.symmetry* in the R package *geomorph* version 3.0.2 (Adams et al. 2016). Head size was calculated as centroid size, the square root of the sum of the squared distance of every landmark to the centroid or “center” of the landmark configuration.

For body shape analyses, we measured head length (from the posterior edge of the jaw, marked with a pin on the specimen, to the tip of the snout) and head width (between the posterior edges of the jaw) from the photographs using tpsDig version 2.17 (Rohlf 2015). For each specimen where the body was preserved and in good condition, we measured the snout–vent length (SVL), tail length, mid-body girth (measured at half of the SVL), and neck girth (measured just posterior to the skull), using a thread that was then measured against a ruler. To avoid overlapping body measurements, we subtracted head length from SVL to get a body length measure. To remove the effect of size while maintaining allometric effects, we calculated the log-shape ratios of the body measurements (Mosimann and James 1979; Claude 2013) where, for each individual, we first computed size as the geometric mean of all measurements. Then, each measurement for each specimen was divided by this size estimation to obtain the shape ratios and then log-transformed. These were used as the data for the subsequent analyses. The log-transformed geometric mean was used as size for the analyses of body shape allometry because it uses all of the body measurements as an estimation of size (Mosimann 1970; Claude, J. 2008) rather than just SVL. Before performing the analyses stated below, we tested for the presence of sexual dimorphism with a distance-based analyses of variance (ANOVA) (Goodall 1991; Anderson 2001) on both head and body shape, and found none. This was performed with the function *procD.lm* in *geomorph*.

ANALYSES OF ALLOMETRIC ONTOGENETIC TRAJECTORIES

To study allometry at a shallower evolutionary scale and to compare between lineages, we grouped the species in clades which correspond to their genera, except for *Bothrochilus* and *Leiopython* that are sister taxa grouped together here. The following analyses follow the schematic Figure 1 in a step-by-step procedure, where changes in allometry and heterochrony are identified. All the analyses were performed on head shape and body shape variables separately. First, we determined if any species displayed isometric growth (no change in shape with size) by fitting individual regressions of size on shape for each species. This was done with the *procD.lm* function of the R package *geomorph* and we assessed the significance with 10,000 iterations. A significant

association rejects the null hypothesis of isometry and reveals that there is allometry. A nonsignificant relationship indicates isometry or lack of sufficient sampling to provide statistical power to detect allometry. We then assessed the relationship of shape and log-transformed size (i.e., allometry) for all species of pythons using two complementary approaches: (1) a homogeneity of slopes (HOSs) test, and (2) a phenotypic trajectory analyses that are described in detail next. They are both used to establish the patterns of direction and magnitude of shape change with size by testing for differences in the slope angle and length. The tests differ in that the first considers size as a continuous variable and the second considers size as a proxy for estimating ontogenetic stage as a categorical variable. It is useful to perform both because together they provide a robust statistical framework and visualize different aspects of the results, the first being a size–shape space and the second a morphospace visualization.

We performed an HOSs test, using a distance-based analysis of covariance (ANCOVA) on both head and body shape including size, species, and the interaction of the two terms (Collyer and Adams 2013). The test performs statistical assessment of the terms in the model using distances among specimens, rather than explained covariance matrices among variables (Anderson 2001). It quantifies the amount of shape variation explained by size, computes the allometric slopes for each species, and performs pairwise comparisons for the slope angles (direction of shape change with size) and slope lengths (amount of shape change with size). Statistical significance was evaluated with a residual randomization permutation procedure with 10,000 iterations. This was performed using the *advanced.procD.lm* function in *geomorph*. For a simple visualization of the diverse ontogenetic allometric trajectories among the species, we plotted the first principal component (PC1) of the matrix of predicted shapes from the multivariate regression (Adams and Nistri 2010).

We performed a phenotypic trajectory analysis (PTA) (Adams and Collyer 2009; Collyer and Adams 2013), a procedure that quantifies the variation of different attributes of a shape change trajectory between two or more points. We used species as the groups and juveniles and adults as the trajectory points. First, for each species we chose the specimens that clearly represented juveniles or adults (sexually mature), leaving out specimens where sexual maturity was uncertain. We compared the direction and size of the trajectories between juveniles and adults between all taxa and assessed the significance of these comparisons with 10,000 permutations. This analysis was performed with the function *trajectory.analysis* in *geomorph*. To visualize the ontogenetic phenotypic trajectories, we plotted the first two PCs of shape variation. To enable biological interpretation of the PCs from the PTA analysis and the above multivariate regression of head shape data, we used thin plate spline deformation grids (Bookstein 1991). For body shape analyses, we examined the PC loadings.

When pairwise species comparisons of slope angle did not reject the null hypothesis of parallel allometric slopes, indicating similar slopes, we performed an additional test to evaluate whether the slopes are overlapping (H_0) or parallel (H_1). This was done by assessing whether there is a shift on the intercept along the Y (shape) axis using pairwise comparisons of the intercepts of the allometric regression of each species. Significance was assessed by comparing the difference to a set of 10,000 permutations, with the *int.test* R function developed by Piras et al. (Piras et al. 2011). For species where the null hypothesis of overlapping trajectories was accepted, we then investigated the possibility of heterochrony.

It is important to note that due to the limitations of a study using wild-caught rare species, the ontogenetic sampling for all of the species is not complete. This mainly influences the results on magnitude of shape change with size. The broad patterns across the family is clear, but specific comparisons, particularly with *Simalia nauta*, *S. oenpelliensis*, and *Liasis fuscus*, where small juveniles could not be measured, should be taken with caution.

HETEROCHRONY

We performed a test to identify patterns of peramorphosis/paedomorphosis with the null hypothesis that two species do not differ in predicted shape at the maximum size of the species being compared using the R function *peram.test* developed by Piras et al. (2011). A rejection of the null is interpreted as one of them being peramorphic (more “adult-like”) or paedomorphic (more “juvenile-like”) with respect to the other. Statistical significance was assessed by comparing to a randomly generated distribution of the data with 10,000 permutations.

Finally, we performed two tests developed by Gerber and Hopkins (2011), and based on Mitteroecker et al. (2005), to further examine heterochrony by assessing the species trajectories overlap in shape and size–shape space. These tests rely on the fact that heterochrony requires the species shape change trajectories to overlap. They are both based on computing multivariate regressions of shape on size for the species being compared. The first one (Tfh1) is used to identify heterochrony by ontogenetic scaling (maintaining an overlap in size–shape space), which is translated into an extension or truncation of the ontogenetic trajectory. It uses the sum of the squared residuals from the regression as a tests statistic. The second one (Tfh2) is used to identify heterochrony with a size–shape dissociation, and uses the sum of squared distances from each specimen to its nearest point on the regression curve as a test statistic. Statistical significance was assessed with 10,000 permutations for Tfh1 and 500 permutations for Tfh2.

The number of pairwise comparisons from the intercept and peramorphosis tests requires that the P values are corrected with a Benjamini–Hochberg or “false discovery rate” correction (Benjamini and Hochberg 1995). The P values from the Tfh1 and Tfh2 tests from heterochrony were not corrected because we are

interested in accepting the null hypothesis and therefore type II error is a bigger concern than type I error. The P values from the slope angle and length tests were not corrected because the tests are not independent and do not require correction. For all the tests, we considered a P -value below 0.05 (5%) as the threshold for statistical significance.

ONTOGENETIC CONVERGENCE AND DIVERGENCE

Slopes that differ in their angular direction, as defined by the HOS test, may be the result of ontogenetic convergence (e.g., Adams and Nistri 2010) or divergence (i.e., allometric repatterning; Webster and Zelditch 2005). For pythons as a whole, and for each clade where not all species had the same slope, we assessed whether their ontogenetic allometric trajectories display a convergent or divergent pattern, against a null hypothesis of ontogenetic consistency, by comparing the variation among juveniles versus the variation among adults. We calculated and summed the pairwise Euclidean distances between all specimens, using the first PC of the predicted morphologies in both ontogenetic stages separately, to calculate the $D = D_{\text{juv}} - D_{\text{adult}}$ statistic. A positive result implies adults are more similar to each other than juveniles are (convergence) and a negative result implies juveniles more similar to each other than adults are (divergence). The significance of the statistic was assessed with 10,000 permutations of the data where depending on the hypothesis of convergence or divergence, a P -value was obtained based on the proportion of iterated D statistics that were below or above the observed D, respectively (Adams and Nistri 2010).

PHYLOGENETIC HYPOTHESIS

To examine variation in ontogenetic allometry in an evolutionary context, we constructed a time-calibrated ultrametric phylogenetic tree of the pythons. We used the alignment provided for the most recent and complete phylogenetic analysis that included this group (Reynolds et al. 2014). This dataset corresponds to a supermatrix of three mitochondrial and eight nuclear loci of most pythons and boas. Details on how we reconstructed the phylogeny can be found in the Supporting Information.

EVOLUTION OF ONTOGENETIC ALLOMETRIC TRAJECTORIES

There are currently no methods to perform the preceding statistical analyses for allometric ontogenetic trajectories in a phylogenetic comparative context, taking into account the statistical nonindependence of species data when within-species variation is the feature of interest (rather than species averages). However, we performed a similar approach to what Tavares et al. (2016) performed to visualize the evolution of the parameters of the allometric trajectories in a phylogenetic context. Similarly to what is done in a traditional morphospace visualization, this method is

based on plotting the first two PCs of the allometric vector describing the multivariate slopes of the trajectories, similar to what has been called an allometric space (Klingenberg and Froese 1991; Wilson and Sanchez-Villagra 2010). However, in addition to the terminal taxa, the reconstructed ancestral state of each node using a maximum-likelihood approach are added, and the tree topology connecting these, making it analogous to a phylomorphospace (sensu Sidlauskas 2008). We propose this visualization be termed phyloallomospace. This analysis was implemented with the function *plotGMPhyloMorphoSpace* of *geomorph*. To reconstruct the evolution of the magnitude of allometric shape change, we used the slope vector length, or magnitude of shape change per unit of size, to perform an ancestral state reconstruction using maximum likelihood. This was performed with the function *contMap* in the R package *phytools* version 0.5 (Revell 2012). We also removed the three species with missing early ontogenetic stages from the evolutionary analysis to avoid creating any bias in the results.

ECOLOGICAL INFLUENCE ON ONTOGENETIC ALLOMETRY

Finally, to test the effect of ecology on ontogenetic allometry, and accounting for phylogenetic relationships, we performed a phylogenetic ANOVA that uses a generalization of phylogenetic generalized least squares (PGLSs) for high-dimensional and multivariate data (Adams 2014). This was first performed on the allometric slopes using microhabitat use as the predictor variable, first as coded in Esquerré & Keogh (2016) where *Aspidites melanocephalus* and *A. ramsayi* are considered semifossorial and *Liasis mackloti* as semiaquatic, and second where we lumped the semifossorial and semiaquatic taxa as terrestrial, because these shifts only occur a single time in both cases. This was performed with the *procD.pgls* function in *geomorph*. To have a visualization of the coupling between the evolution of ecology and allometric coefficients, we performed an ancestral state reconstruction of microhabitat use by using stochastic character mapping (Huelsenbeck et al. 2003). We ran 10,000 independent stochastic character maps to have an estimate of uncertainty (Revell 2014), using the R package *phytools*.

Results

ONTOGENETIC ALLOMETRY

Most of the species displayed allometric growth (i.e., lack of isometry). The species that did display nonsignificant relationships between size and shape had low samples numbers, so we call caution interpreting results regarding them, namely *Morelia carinata*, *S. oenpelliensis*, *S. nauta*, *Python anchietae*, *P. brongersmai*, and *L. fuscus* (Table S3). Size, species, and the interaction of the two strongly influence python head shape (ANCOVA, size, $F_{(1, 1127)}$

$= 320.663$, $P < 0.0001$; species, $F_{(31, 1127)} = 47.007$, $P < 0.0001$; size \times species, $F_{(31, 1127)} = 4.108$, $P < 0.0001$) and body shape (size, $F_{(1, 1040)} = 560.549$, $P < 0.0001$; species, $F_{(1, 1040)} = 69.167$, $P < 0.0001$; size \times species, $F_{(31, 1040)} = 4.096$, $P < 0.0001$). These results indicate that there is clear ontogenetic allometry in both head and body shape and that allometry differs between species in both head and body shape. The pattern is clearly observed on the difference in slopes of trajectories in both head and body shape (Figs. 2 and 3). The regression scores, common allometric component (CAC; Mitteroecker et al. 2004), and PCs for all the species and separated by clade can be seen in Figures S2 and S3.

The head shape changes associated with increasing size, as described by a multivariate regression of shape on log-transformed size (Fig. 2) and by PC1 of the PTA (Fig. 3), involve a broadening of the snout and eyes that become smaller and more dorsally situated. PC2 of the PTA represents a massive enlargement and lateralization of the eyes and a transition to a shorter and more pointed snout. For body shape, PC1 of the PTA represents an elongation of the tail (tail length eigenvector = 0.8) and a slimming of the body (mid-body girth eigenvector = -0.35). PC2 of the PTA mainly represents a thickening of the body (mid-body girth eigenvector = 0.63), an elongation of the body (body length eigenvector = 0.47), and a proportional reduction in head size (head length eigenvector = -0.41 ; head width eigenvector = -0.43).

Both the HOS test and PTA analyses show equivalent differences of direction and magnitude of shape change in head and body shape, and therefore we only present the results from the first. Head and body shape ontogenetic allometric trajectory diversity is extremely large in pythons. The slope vector lengths and the trajectory path distances, which both translate into the amount of shape change given size, can be seen in Figure S4, but easily interpreted by looking at the steepness of the slopes of shape on size in Figure 2 and the length of the PTA trajectories on Figure 3. Pairwise comparison of slope angles and slope vector lengths for head and body shape can be seen in Tables S4–S7. Because of the large number of taxa included in these analyses and the great diversity in observed patterns, the results are also summarized on a clade-by-clade basis in Figure 4. The main findings supported by the pairwise species comparisons and the visualization of the results can be broken down as follows.

DIRECTION OF SHAPE CHANGE WITH SIZE

In general, species within the same genus or clade tended to differ very little in angle of shape change with size, implying parallel or overlapping allometric trajectories within clades (Fig. 4; Tables S4 and S5). The broad pattern is that pythons tend to get broader heads and proportionally smaller and more dorsally situated eyes as they grow. There are a few exceptions, but the most contrasting is *M. viridis* that has a significantly different head

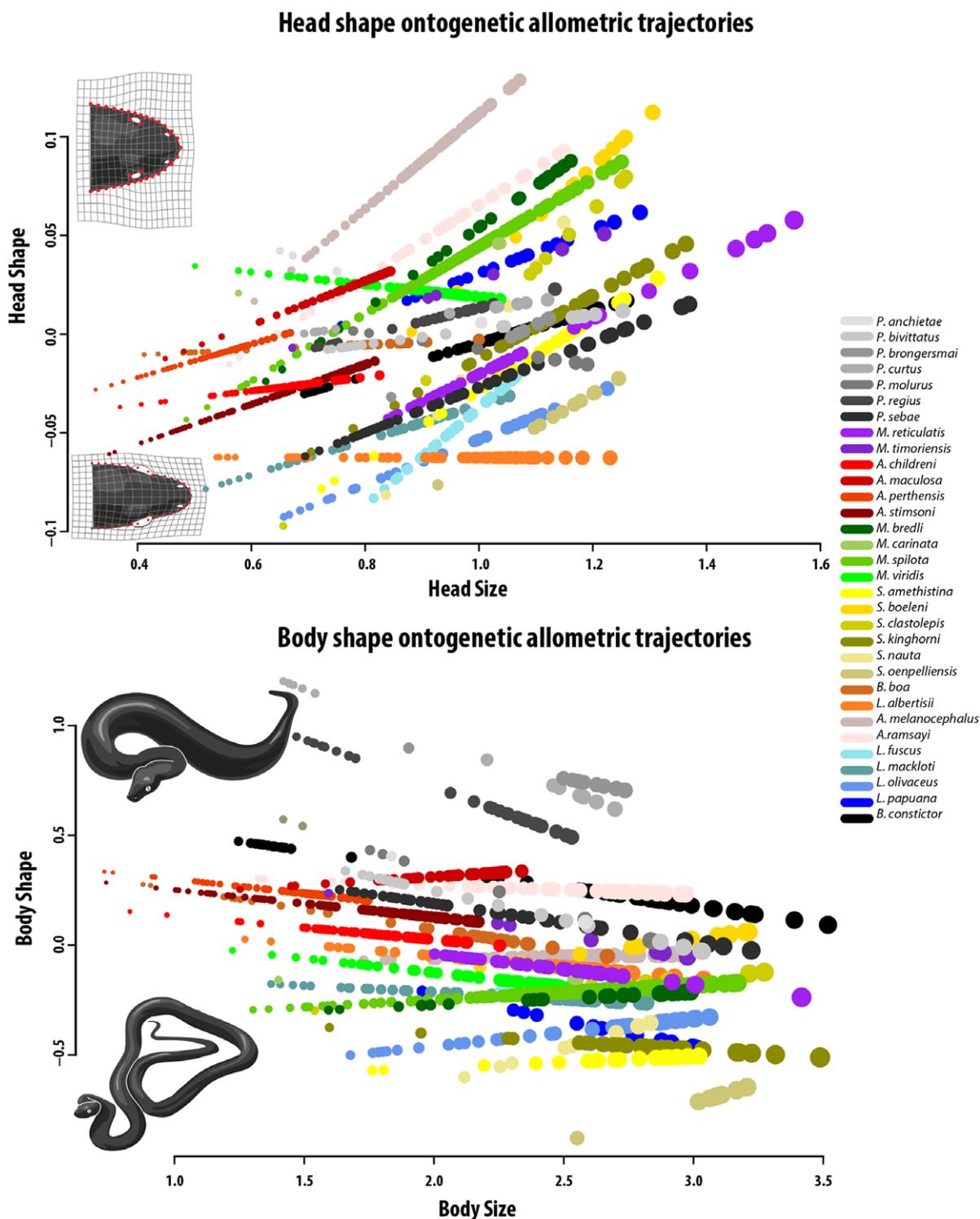


Figure 2. Ontogenetic allometric trajectories derived from the HOS test of head (above) and body (below) shape. The x-axis represents log-transformed centroid size for head and log geometric mean for body. The y-axis on both plots is the first principal component of the predicted values of the multivariate regression of shape on size. Each line of dots represents the predicted allometric trajectory for each species, as per the colored legend on the right. The size of the dots for each specimen is proportional to its size. On the head shape plot, thin plate spline deformation grids show the shape change from the mean shape of the dataset to the highest (above) and the lowest (below) specimen on the y-axis. Similarly, on the body shape plot illustrations based on the specimens that are highest and lowest on the y-axis represent the shape difference along the y-axis.

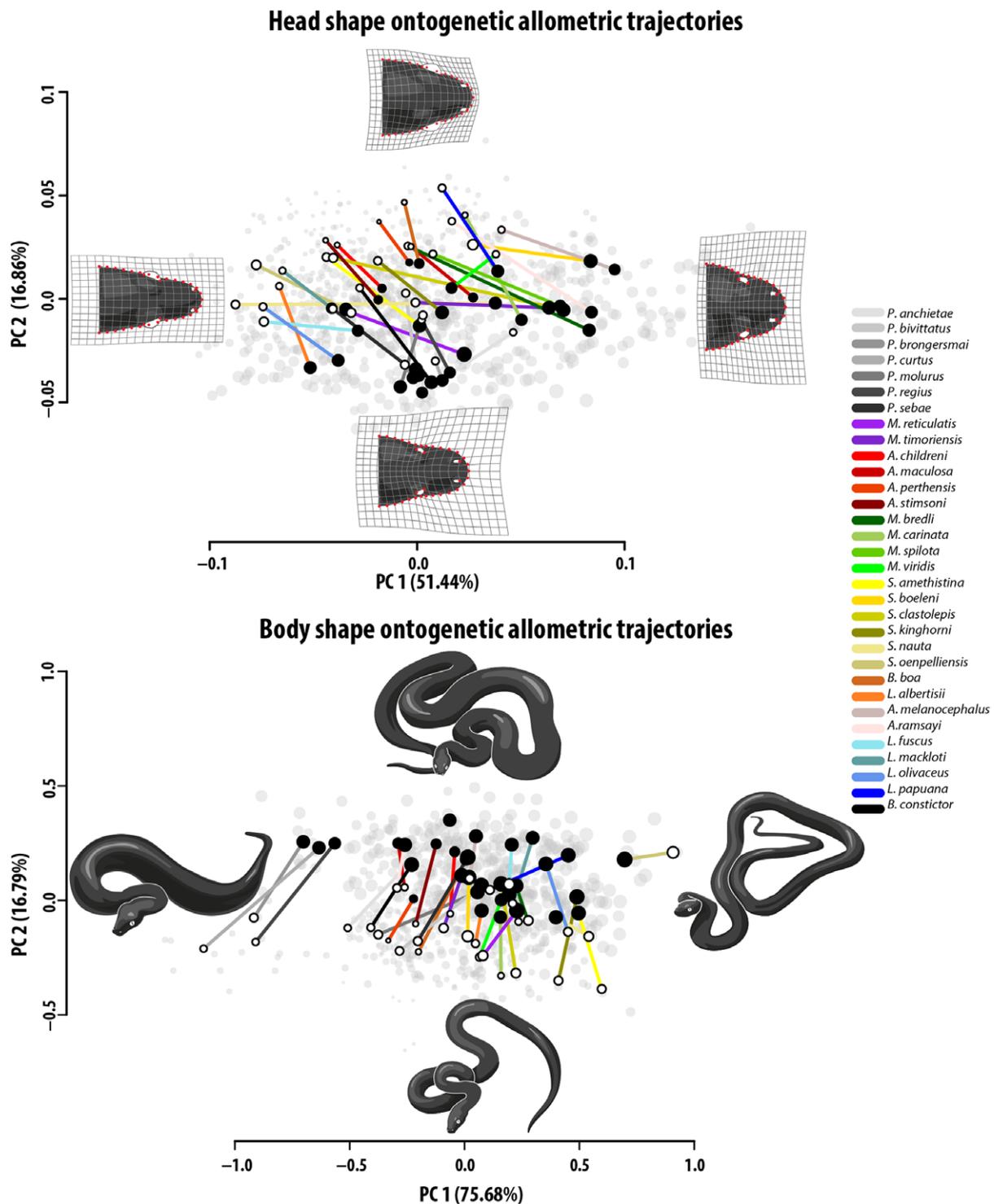


Figure 3. Ontogenetic allometric trajectories derived from the PTA of head (above) and body (below) shape. The specimens are plotted on a morphospace represented by principal components (PC) 1 and 2 on the x- and y-axes, respectively. White dots represent the average shape of juveniles and the black dots the average shape of adults. The lines between them are what we define as trajectories. The color of each line represents the species according to the legend. The gray dots in the background represent the total variation within the sample. The size of the dots for each specimen is proportional to its size. Thin plate spline deformation grids and body shape plot illustrations show the extremes of variation represented by each PC axis.

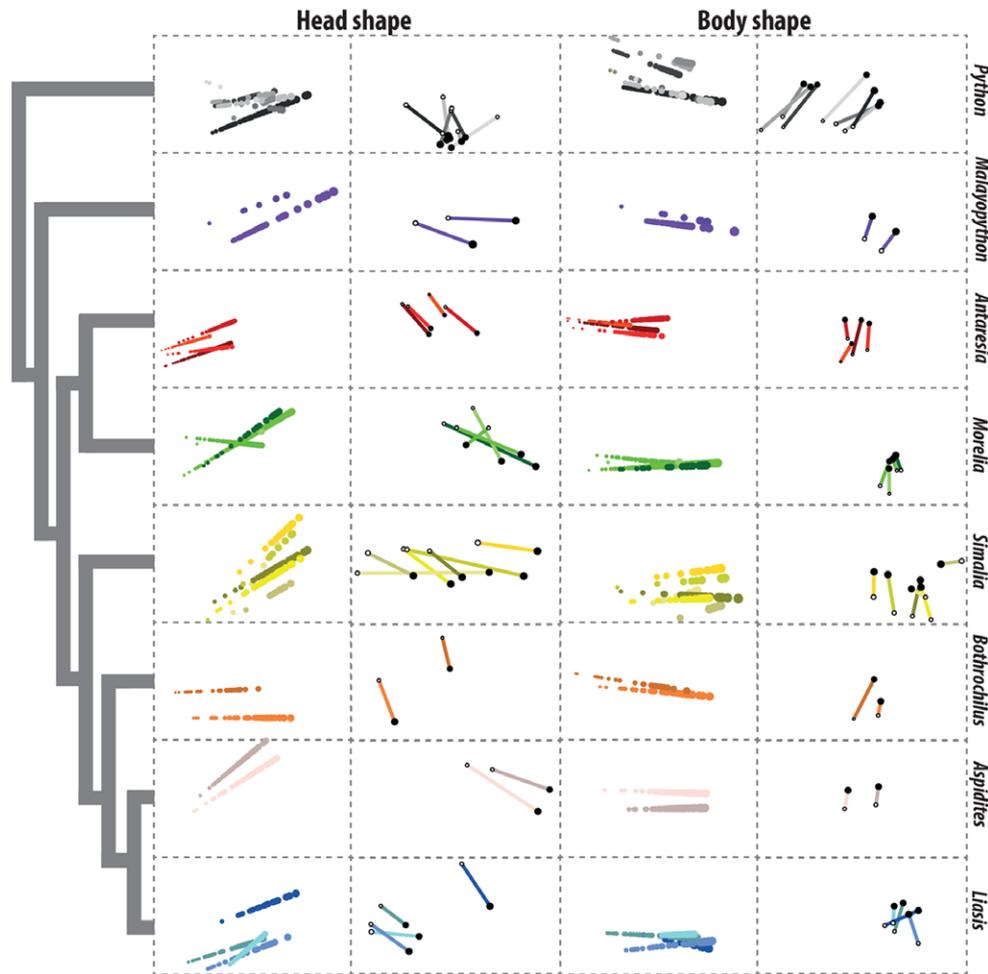


Figure 4. Ontogenetic allometric trajectories derived from the HOS (first and third columns) and PTA (second and fourth columns) tests of head and body shape of the pythons, separated by clade. The rows of figures at each tip of the tree based on our phylogenetic analysis represent the trajectories of the python species within the clade that is labeled on the right edge of the figure. These are the same trajectories illustrated in Figures 2 and 3 therefore for details on the meaning of the axes and how to interpret them see the respective legends.

shape slope orientation from almost all of the species. There are also slope differences in head shape within *Python* and body shape within *Morelia* and *Liasis*.

MAGNITUDE OF SHAPE CHANGE WITH SIZE

Similarly to what is observed with the direction of shape change, species within the same clade tend to differ very little in the amount of shape change they go through with size, implying a strong phylogenetic effect on the magnitude of change (Tables S6 and S7). *Simalia clastolepis* has a significantly larger head shape change than most other pythons (Fig. S4 and Table S6). Species in *Simalia* and *Aspidites* tend to show larger amounts of head shape change, whereas other taxa such as *L. albertisii*, *Bothrochilus boa*, and *P. curtus* display little head shape change with size, getting closer to isometry (Fig. S4 and Table S6), which in multivariate allometry is represented as a flat line parallel to the x-axis (Fig. 4).

Python curtus, *P. regius*, and *P. anchietae* display large amounts of body shape change with size, whereas *L. albertisii*, *M. spilota*, *M. bredli*, and the two species in the genus *Aspidites* display small amounts of change (Fig. S4 and Table S7).

INTERCEPTS OF ONTOGENETIC ALLOMETRY

Among the species with a similar allometric slope, we did not identify parallel allometric trajectories (different allometric intercepts) within clades for head or body shape, but there were a few cases among species of different clades (Tables S8 and S9). This indicates that most species with a shared allometric slope have overlapping trajectories.

HETEROCHRONY

Within clades, the most common pattern explaining morphological diversity in pythons is peramorphosis/paedomorphosis

Table 1. Test for ontogenetic convergence/divergence in head and body shapes in all pythons (Pythonidae) and each clade that displays allometric slope differences, separately.

Clade	Head shape			Body shape			Conclusions
	D	P_{con}	P_{div}	D	P_{con}	P_{div}	
Pythonidae	-1,489	0.0001	1	3,594	0.14	0.86	Overall no convergence or divergence
<i>Python</i>	55.66	0.0005	1	–	–	–	Convergence in head shape
<i>Morelia</i>	15.39	0.99	0.008	184.83	0.024	0.98	Convergence on body shape
<i>Simalia</i>	–	–	–	-321.6	0.86	0.14	No convergence or divergence in body shape
<i>Liasis</i>	–	–	–	36.78	0.014	0.99	Divergence on head shape

A positive D statistic means that juveniles are more variable than adults (convergence) and a negative D statistic means that adults are more variable than juveniles (divergence). P values are drawn from the number of times of 10,000 permutations that the observed D is higher (P_{con} , for convergence) and lower (P_{div} , for divergence) than the randomized D .

(Tables S10 and S11), where slopes and intercepts between species are equivalent, but they differ on the maximum size phenotype, one being more “adult-like” or peramorphic than the other. Between clades, both differences in slopes and shifts along the ontogenetic trajectories are responsible for changes in phenotypes. However, within clades many species pairs have equivalent ontogenetic trajectories. The Thf1 and Tfh2 tests found overlapping trajectories in size-shape and shape space mostly within clades. These tests were more conservative in their results, and most species pairs do not display heterochrony according to the test’s definition of heterochrony. However, in both head and body shape, some species pairs within *Python*, *Antaresia*, *Morelia*, and *Simalia* display ontogenetic scaling and/or heterochrony via size-shape space dissociation. Furthermore, *Antaresia maculosa*/*A. perthensis* (the largest and smallest species within their clade) and *Simalia boeleni*/*S. clastolepis* display overlap in size-shape space and significantly different maximum size phenotypes in head and body shape, respectively, suggesting a strong case of ontogenetic scaling (Tables S12–S15).

ONTOGENETIC CONVERGENCE AND DIVERGENCE

In the test for ontogenetic convergence/divergence, we find convergence in head shape within *Python*, and convergence in body shape within *Morelia* and *Liasis* (Table 1), which is clearly observable from the ontogenetic trajectories (Fig. 4), and from the fact that there is divergence in slopes in those clades (Tables S4 and S5). This means that juveniles of these species are very different to each other, but they become very similar as they approach adulthood. More broadly, species with stocky and short-tailed body shapes as juveniles (e.g., *P. curtus*, *P. brongersmai* and *P. regius*) elongate as they grow, whereas species that are thin and long-tailed as juveniles (e.g., *Simalia*) get stockier and shorter tailed with size, almost converging on body shape with the former.

PHYLOGENETIC HYPOTHESIS

Unsurprisingly, the topology recovered by our analysis is identical to the one recovered by Reynolds et al. (2014). The basal split between the Afro-Asian *Python* genus and the rest of the Pythonidae is dated at the early Oligocene with 33.62 Mya, with a 95% highest posterior density interval between 26.85 and 40.9 Mya. Most nodes are supported with a posterior probability higher than 0.95. *Morelia viridis* is not recovered with *Morelia* but with its sister clade *Antaresia* (with low posterior probability). However, preliminary results from a phylogenomic analysis using hundreds of nuclear loci supports the inclusion of this species in *Morelia* (D. Esquerré et al., unpubl. ms.), therefore it is considered with this clade in the analyses of this article. For details on the tree topology, divergence times, and support see Figure S5.

EVOLUTION OF ONTOGENETIC ALLOMETRY

The biplot of the inferred evolutionary history of direction of allometric shape change (Fig. 5) highlights the phylogenetic structure found on the grouping of the slopes. For both head and body shape, the genus *Python*, which is sister to all other pythons, displays a distinct set of slopes from the rest, despite being itself very diverse. On both head and body shape, other clades like *Simalia*, *Aspidites*, and *Morelia* (except again by *M. viridis*) occupy the opposite side of the biplot, whereas the clades *Antaresia*, *Malayopython*, *Bothrochilus/Leiopython*, and most of *Liasis* occupy a middle area of the space.

The ancestral state reconstruction of the magnitude of shape change with size shows a similar pattern of separation in groups of clades as the evolution of direction of shape change. *Simalia*, *Aspidites*, and *Morelia* (except *M. viridis*) experience a great amount of head shape change but generally small amounts of body shape change with size. The opposite is true for *Python*, and again, the

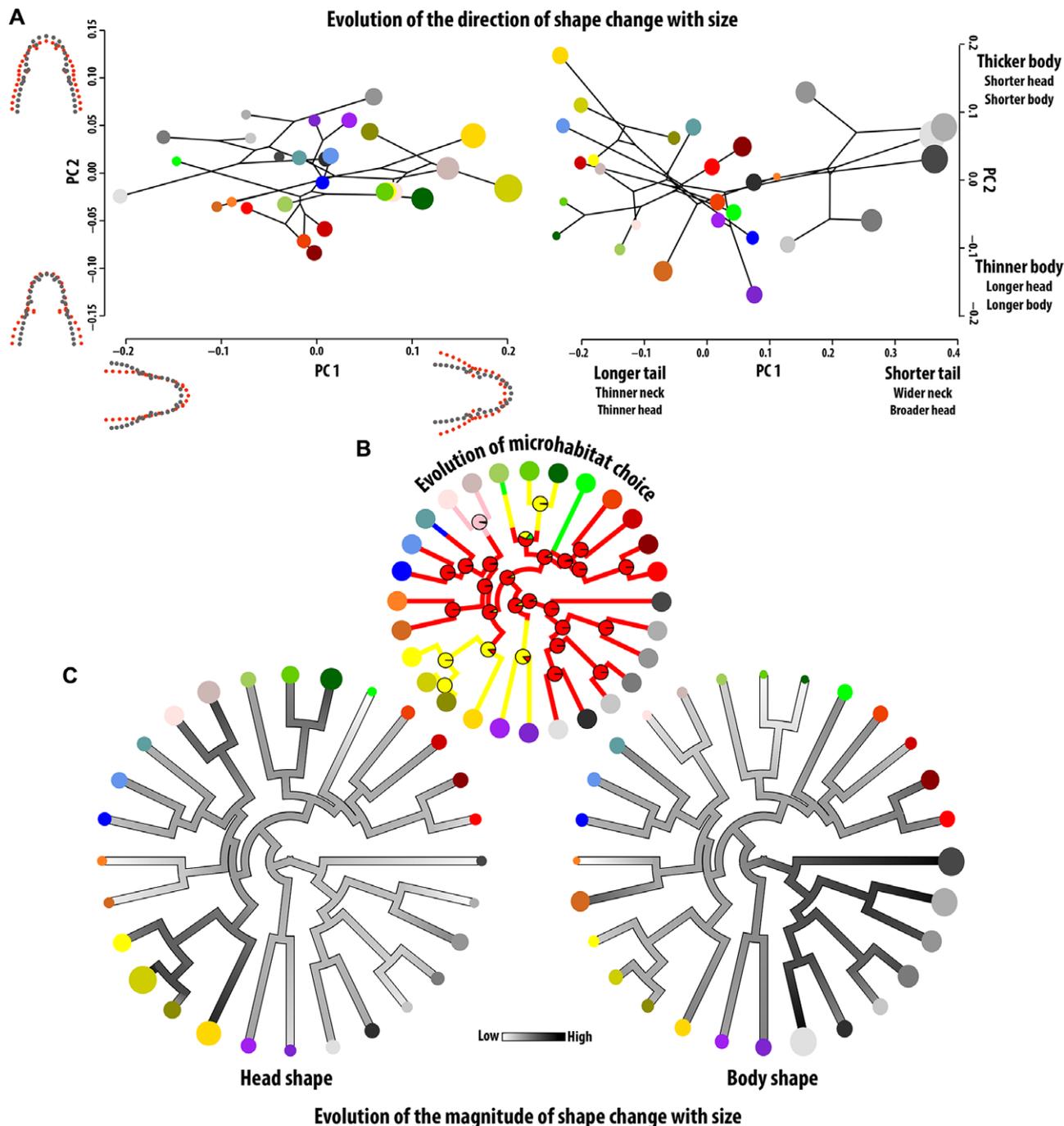


Figure 5. (A) Phylomorphospace and ancestral state reconstruction of the slopes of allometric shape change in each python species, left side shows head shape and right side shows body shape. Besides the PC axes for head shape are illustrations describing the shape changes from the mean configuration (gray) to the extremes (red) of each axis. Besides the PC axes for body shape are summaries of the body shape changes occurring with size from the mean shape to the extremes of each axis, with the amount of change approximately proportional to the size of the text. (B) Stochastic character mapping of the evolution of ecology in pythons. The base tree is a random of 10,000 replicates and the pie charts represent the percentage of time each state is reconstructed at that node. Branch and node colors correspond to green: arboreal, yellow: semiarboreal, red: terrestrial, blue: semiaquatic, and pink: semifossorial. (C) Ancestral state reconstruction of the slope vector length (magnitude of shape change with size), with black representing high phenotypic change and white representing low. Tips of all the figures are colored according to species and clade as in Figures 2 and 3 and on (A) and (C) they are sized proportionally to the magnitude of shape change with size.

other clades show intermediate amount of phenotypic change with growth.

ECOLOGICAL INFLUENCE ON ALLOMETRY

Microhabitat use was found to have a significant effect on the variation of allometric slopes in head shape with the original microhabitat groupings from Esquerré and Keogh (2016) ($F_{(4, 23)} = 1.736$, $P = 0.0062$) and considering the semifossorial and semiaquatic species as terrestrial ($F_{(4, 25)} = 2.376$, $P = \mathbf{0.0033}$). Microhabitat use was not found to have significant effect on body shape allometric slopes ($F_{(4, 23)} = 0.759$, $P = 0.232$) nor when semifossorial and semiaquatic species are coded as terrestrial ($F_{(4, 25)} = 1.087$, $P = 0.076$). The stochastic mapping of microhabitat predicts terrestriality as the ancestral state of pythons, with at least three independent origins of semiarborality, two of arboreality, and one of semiaquatic and semifossorial microhabitat preferences (Fig. 5). Comparing the plots representing the evolution of allometry and ecology in Figure 5 reveals that there is a tendency of species that have the same ecology to share regions of allometric space and patterns of magnitude of shape change with size. Figure S6 displays the ontogenetic allometric trajectories separated by ecology, showing how there are trends of allometry for each microhabitat choice.

Discussion

Pythons are a morphologically and ecologically mega-diverse group of vertebrates that display phenotypes that are highly adapted to their ecological lifestyle (Esquerré and Keogh 2016). We have revealed that their postnatal ontogenetic allometry is evolutionarily labile and they have great diversity in developmental trajectories. Other published studies of ontogenetic allometric trajectories have focused on variation within genera or closely related species; as far as we are aware, this is the first study looking into the evolution ontogenetic allometry at a family level incorporating almost every taxon. Within the Pythonidae, we show that the shifts in ontogenetic allometric trajectories that change a groups' morphological diversity are not consistent across all clades. Some clades show ontogenetic convergence, others equivalent trajectories, but most often the differences observed among phenotypes of a clade are derived from heterochronic processes (i.e., peramorphosis/paedomorphosis). By the sheer diversity and lability of allometry, and its correlation with ecology in pythons, we suggest that allometry is a highly labile, evolvable, and adaptive trait.

Studies within genera normally observe one type of ontogenetic trajectory shift driving the phenotypic diversity: for example, ontogenetic convergence in plethodontid salamander foot morphology (Adams and Nistri 2010) or heterochrony in *Podarcis* lizard head shape (Piras et al. 2011). The species-rich and

phenotypically conserved old world lacertid lizards also display conserved ontogenetic allometries (Hipsley and Müller 2017). Pythons exhibit these and other patterns too, suggesting that different ontogenetic allometric changes can generate morphological diversity in a relatively short time period. Our data demonstrates that patterns in allometric trajectories in pythons differ within and between clades. Within clades there is a strong pattern of overlapping (similar) trajectories between species, where changes are mostly heterochronic and along the same trajectories. Between clades however, there are discernible shifts in both the direction and magnitude of ontogenetic allometry. This is in agreement with the hypothesis that allometries evolve at million-year time scales and closely related species will tend to show less divergence in their trajectories (Voje et al. 2013). For some clades, like lacertid lizards, phenotypic differences between species are developed prenatally (observed as shifts in their allometric intercepts), and in others like pythons, differences mostly develop after birth (observed as conserved intercepts but shifts in the extension or slope of the trajectories). In pythons, there is also substantial variation in the magnitude of shape change, where some *Python* species undergo large body shape transformation as they grow, while some *Morelia* and *Aspidites* species experience milder changes.

Heterochronic changes can rapidly increase disparity and induce dramatic changes in the morphology of lineages (Gould 1977). Birds evolved their unique cranial morphologies by combination of cranial paedomorphosis and beak peramorphosis in relation to theropod dinosaurs (Bhullar et al. 2012; Foth et al. 2016) and heterochrony is a common feature in morphological evolution in squamate reptiles (Piras et al. 2011). Exploring allometric trajectories in size-shape space makes it possible to infer heterochronic products of processes such as paedomorphosis and peramorphosis (Piras et al. 2011) and to distinguish between heterochrony by ontogenetic scaling (with species sharing size-shape space) or by size-shape dissociation (Mitteroecker et al. 2005; Gerber and Hopkins 2011). We identified these two types of heterochrony within four python genera. Most notably, *A. perthensis*, the smallest python in the world, is completely paedomorphic in relation to *A. maculosa*, the largest species within its clade. We identify that the most common form of developmental change fueling initial evolution of pythonid morphologies are heterochronic changes along the trajectories determining the shape at maximum size, where a species changes its phenotype by growing bigger or smaller. Without information on the age of individual specimens though, we can only identify the products and not the processes of heterochronic perturbations (Klingenberg and Spence 1993; Godfrey and Sutherland 1995; Piras et al. 2011), because paedomorphosis or peramorphosis can originate from modifications on age at onset, age at offset, and/or growth rate (Reilly et al. 1997). However, snakes generally display indeterminate growth (Andrews 1982; Shine et al. 1998) and size is strongly correlated

with age (Gignac and Gregory 2005), which make them a much better model for detecting heterochrony than most animal groups. Our data suggests that heterochrony is the process responsible for morphological evolution at the early stages of diversification because it is responsible for divergence within clades. It seems that longer evolutionary times are required for allometric slopes to evolve, allowing the ontogenetic allometric trajectories to explore new areas of morphological space (Weston 2003; Wilson and Sánchez-Villagra 2011). Similarly, some closely related dinosaur species tend to have more similar and conserved ontogenies and that these diverge as phylogenetic relatedness decreases (Bhullar et al. 2012; Mallon et al. 2015). Shifts in ontogenetic allometric slopes increase the disparity in a group and aids in finding new phenotypes that better suit the selective pressures.

The analyses of allometric slope, intercept, and peramorphosis/paedomorphosis reveal a clear pattern of conserved allometric trajectories and hypermorphosis, where differences in the shape attained at maximum size suggest that heterochrony is mostly the cause of morphological evolution at the start of divergence. On the other hand, the Tfh1 and Tfh2 analyses of overlap in size–shape and shape space suggest a much weaker pattern of heterochrony. Nonetheless, given the strong support of heterochronic patterns found by the previous battery of analyses, and the clear morphological differences in phenotypes between most species of pythons of the same clades, we conclude that the Tfh1 and Tfh2 tests are very conservative in detecting patterns of heterochrony.

Studies of static allometry, where size–phenotype relationships are studied across different organisms at the same developmental stage, have proposed that size imposes strong limitations and constrains on phenotypes (Huxley 1932; Simpson 1944; Gould and Lewontin 1979). What we observe as developmental constraints in studies of ontogenetic allometry and development can be expressions of different processes, like a common development–genetic architecture or limitations in the number of ways development can be produced due to physiological/mechanical limitations (Sanger et al. 2012). Nevertheless, in recent years, studies of ontogenetic allometry (where size–phenotype relationships are studied across developmental stages within a species) have compared the ontogenetic allometric trends between species. Some authors (Wilson and Sanchez-Villagra 2010; Klingenberg 2010) have concluded that allometry can be an adaptive and evolvable trait that can lead to complex patterns of phenotypic diversity, rather than a constraint on phenotypes imposed by size and ontogeny. Evaluating allometric coefficients in the context of a phylogeny can provide insights into how it evolves and how it is constrained by phylogenetic relatedness (Giannini 2014). Methods do not exist yet that allow for a proper incorporation of phylogenetic information into analyses comparing within-species patterns like ontogenetic allometry, where there are often more individuals than taxa. Nevertheless, examining the

evolution of the patterns of allometric trajectories under a phylogenetic framework, we observe that the developmental patterns of head and body shape change differ greatly and that there are clade-specific trends that seem to be related to their ecology. For example, the species in the lineage that includes the terrestrial Afro-Asian *Python*, which is sister to all other pythons, display small amounts of head shape change, but immense body shape transformation as they grow, including an elongation of the tail and decrease in body girth. The opposite direction and magnitude of allometric shape change is observed in some of the Australo-Papuan clades including the semiarboreal *Simalia*, *Morelia*, and the semifossorial *Aspidites*. These results suggest that allometric coefficients are more labile than previously thought. More studies at a macroevolutionary scale are needed to better understand exactly how evolvable allometry is, and how important it is in shaping the diversity of a group.

The lability of allometry provides evidence that it can be highly evolvable (Pélabon et al. 2014), but a correlation with ecology suggests it also may be adaptive. In pythons, microhabitat use drives phenotypic evolution (Esquerré and Keogh 2016) and our results suggest that it also can have an effect on ontogenetic allometry. In the Australo-Papuan genus *Morelia*, two semiarboreal species, *M. spilota* and *M. bredli*, show the same ontogenetic allometric trajectory. The closely related *M. viridis*, on the other hand, is unique among pythons in most aspects of its ecology and phenotype. It is the only completely arboreal species in the family and it goes through an extreme ontogenetic color change from bright red or yellow juveniles to completely green adults. This coloration change has been found to be strongly associated with changes in head shape allometry and dietary shift from ectothermic to endothermic prey (Natusch and Lyons 2012). This is accompanied by a shift in microhabitat use, from the edge of rainforest closer to the ground to the upper and inner rainforest canopy (Wilson et al. 2007). This ontogenetic shift toward arborality is the opposite to what is commonly observed on other python species that get increasingly terrestrial with size and age (Stafford 1986; Luiselli et al. 2007). Because head shape is predicted by microhabitat, it is expected under the hypothesis that allometry is a trait under natural selection that the head shape ontogenetic trajectories of *M. viridis* and the rest of the pythons also go in opposite directions. As additional evidence, the enigmatic *M. carinata* has been described as ecologically intermediate between *M. viridis* and *M. spilota*/*M. bredli* (Porter et al. 2012) and it also has an intermediate ontogenetic trajectory between the two.

Phenotypic adaptations to terrestrial and semiarboreal ecological niches have evolved independently more than once in pythons, but the remaining ecologies have a single origin. To fully appreciate the role of ecological factors on the evolution of ontogeny, a comparative study with the boas, a group that has convergently evolved the same ecomorphological diversity observed

in pythons (Esquerré and Keogh 2016), would shed further light on this topic.

Data on the ontogeny of ecology in pythons is still anecdotal and incomplete for many species. It is understood that many pythons, and snakes in general, display a dietary shift from ectothermic to endothermic prey as they grow (Slip and Shine 1988; Greer 1997; Shine et al. 1998; Luiselli and Angelici 1998; Natusch and Lyons 2012), but detailed studies on habitat use and other ecological factors are necessary. Several findings of this study need this information to establish the mechanisms behind shifts in ontogenetic trajectories. For example, the strong ontogenetic convergence in *Python* head shape and *Morelia* body shape, where juvenile phenotypes are disparate but adults are extremely similar, needs to be understood in the light of the way their natural history changes with growth, like ontogenetic convergence driven by adaptation in European plethodontid salamanders (Adams and Nistri 2010). Nevertheless, our study adds to a growing body of evidence. Artificial selection on insect allometry has shown that the direction of change with growth can be shifted by evolution (e.g., Weber 1990; Wilkinson 1993; Emlen 1996; Frankino et al. 2005) and adaptive shifts in the allometries of organisms to match their ecomorphological needs (Adams and Nistri 2010) demonstrate the possible adaptive properties of allometry.

Pythons are not only immensely diverse in their shapes and ecologies, but as we have demonstrated here, also in the way they transform their phenotypes through their lives. Different clades of pythons that display different ecological attributes also have different ontogenetic allometric trajectories. Some of them display strong ontogenetic convergence, for example, others have very similar trajectories and many evolve their differences by extending or truncating their trajectories evolving forms that are paedomorphic or peramorphic. As we compare between clades, the slopes of the trajectories change, suggesting it is more challenging for biological forms to shift the direction of their change rather than the starting and finishing points or the rate of change. This enabled pythons to evolve more novel forms as they also shifted in their ecologies, requiring phenotypes better adapted to their needs. Maybe the remarkably diverse array of developmental pathways of pythons is not particularly unique. This calls for studies on broader taxonomic groups on the evolution of ontogenetic allometry and postnatal development. It may be that allometry is generally more evolvable, and that the constraints it imposes are often adaptations. This would underscore the notion that allometry is often only a static constraint that funnels phenotypic variation in a certain direction, and highlight its role in increasing phenotypic diversity in living organisms. This study provides strong evidence of ontogenetic allometry as a highly evolvable trait and calls to evolutionary biologists, to rethink “allometry as a universal constraint on biological traits” and instead as a trait by itself (Klingenberg 2010).

AUTHOR CONTRIBUTIONS

DE and SK conceived the project; DE collected the data and prepared the figures; DE and ES analyzed the data; DE, ES, and SK wrote the manuscript.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Table S1. Summary of the species used in this study, the sample size for each species, their snout–vent length (SVL) range, and head size range.

Table S2. Fossil calibrations used for the time-calibrated phylogeny.

Table S3. Tests statistics from the test for allometry for each species.

Table S4. Head shape slope angle differences.

Table S5. Body shape slope angle differences.

Table S6. Head shape slope length differences.

Table S7. Body shape slope length differences.

Table S8. Head shape intercept differences.

Table S9. Body shape intercept differences.

Table S10. Head shape peramorphosis test.

Table S11. Body shape peramorphosis test.

Table S12. Head size–shape space overlap (ontogenetic scaling) tests for heterochrony (Tfh1).

Table S13. Head shape space overlap tests for heterochrony (Tfh2).

Table S14. Body size–shape space overlap tests for heterochrony (Tfh1).

Table S15. Body shape space overlap tests for heterochrony (Tfh2).

Figure S1. Landmark and semilandmark configuration used to describe dorsal head shape in pythons.

Figure S2. Regression scores (top), common allometric components (CAC; middle), and principal component analysis (PCA; bottom) of python head shape (left) and body shape (right).

Figure S3. Regression scores, CAC, and PCA as described in Fig. S2 for head and body shape, separated by clade.

Figure S4. Slope vector lengths (the magnitude of shape change with unit of size change) for each species of python for head and body shape.

Figure S5. Maximum clade credibility time-calibrated tree of the pythons used in this study.

Figure S6. Ontogenetic allometric trajectories derived from the HOS (first and third columns) and PTA (second and fourth columns) tests of head and body shape of the pythons, separated by microhabitat choice.