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Citation	Sanger, Thomas J., Emma Sherratt, Joel W. McGlothlin, Edmund D. Brodie, Jonathan B. Losos, and Arhat Abzhanov. 2013. Convergent Evolution of Sexual Dimorphism in Skull Shape Using Distinct Developmental Strategies. <i>Evolution</i> 67, no. 8: 2180–2193.
Published Version	doi:10.1111/evo.12100
Accessed	February 19, 2015 5:12:02 PM EST
Citable Link	http://nrs.harvard.edu/urn-3:HUL.InstRepos:12559595
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CONVERGENT EVOLUTION OF SEXUAL DIMORPHISM IN SKULL SHAPE USING DISTINCT DEVELOPMENTAL STRATEGIES

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Received December 3, 2012

Accepted February 23, 2013

Data Archived: Dryad doi: 10.5061/dryad.hk2v3.

Studies integrating evolutionary and developmental analyses of morphological variation are of growing interest to biologists as they promise to shed fresh light on the mechanisms of morphological diversification. Sexually dimorphic traits tend to be incredibly divergent across taxa. Such diversification must arise through evolutionary modifications to sex differences during development. Nevertheless, few studies of dimorphism have attempted to synthesize evolutionary and developmental perspectives. Using geometric morphometric analysis of head shape for 50 *Anolis* species, we show that two clades have converged on extreme levels of sexual dimorphism through similar, male-specific changes in facial morphology. In both clades, males have evolved highly elongate faces whereas females retain faces of more moderate proportion. This convergence is accomplished using distinct developmental mechanisms; one clade evolved extreme dimorphism through the exaggeration of a widely shared, potentially ancestral, developmental strategy whereas the other clade evolved a novel developmental strategy not observed elsewhere in the genus. Together, our analyses indicate that both shared and derived features of development contribute to macroevolutionary patterns of morphological diversity among *Anolis* lizards.

KEY WORDS: Allometry, *Anolis*, craniofacial, evo-devo, face length.

The last 20–30 years have witnessed a renewed interest in integrating evolutionary and developmental analyses in search of a deeper mechanistic understanding of morphological evolution. Evolutionary biologists have recognized the need for such synthesis for more than a century (e.g., Darwin 1859; Huxley 1942; Schmalhausen 1949; Gould 1977). Despite the rapid success of this integrated research program (Muller 2007; Carroll 2008), studies among closely related species and descriptions of intraspecific variation remain rare, leaving fundamental questions about developmental evolution unanswered. At the population and species levels, what are the developmental origins of ecologically relevant variation? How do the key components of develop-

mental process—not only genes, but pathways, networks, cells, and tissues—that facilitate morphological evolution themselves evolve? Does development evolve dramatically within species, or are developmental processes relatively stable over “mesoevolutionary” timescales (Abouheif 2008)?

Sexually dimorphic traits represent some of the most striking examples of biological diversity, yet few studies have explored the developmental bases of this variation, particularly among vertebrates (Wilkins 2004; Williams and Carroll 2009). Males and females of many species differ in morphology, physiology, and behavior. Patterns of sexual dimorphism vary widely among species, differing in both pattern and magnitude. Phenotypic differences

between males and females must arise from sex-specific differences in developmental programs, and interspecific differences in sexual dimorphism must be the result of developmental alterations arising during evolution. Despite the extensive literature on the ecology and evolution of sexual dimorphism, studies of its developmental basis, particularly in a comparative context, are rare. Overlaying macroevolutionary patterns of sexual dimorphism with developmental analysis at multiple biological levels promises to shed light on the mechanisms of morphological evolution.

When considering morphometric traits, sexual dimorphism may occur in both size and shape. Sexual size and shape dimorphism often have unique developmental origins and ecological significance and are thus best considered separately in comparative analyses. Although size dimorphism has been studied in a variety of biological contexts (reviewed in Fairbairn et al. 2007), shape dimorphism is far less studied and, at times, conflated with size dimorphism (reviewed by Gidaszewski et al. 2009). Shape dimorphism refers to differences in body proportions between males and females of a species, including exaggerated ornaments (e.g., beetle horns, deer antlers, or the peacock's tail) and more subtle differences in the relative sizes of traits such as limb length (Butler and Losos 2002; Butler et al. 2007), wing shape (Gidaszewski et al. 2009), or facial shape (Leigh and Cheverud 1991). Size and shape dimorphism need not evolve in concert or under the same selective regimes (Butler and Losos 2002; Gidaszewski et al. 2009). Size and shape dimorphisms also likely possess different developmental origins. Size dimorphism is often regulated by the differential production of systemic hormones between the sexes controlling the rate or duration of growth (e.g., Badyaev 2002; Cox et al. 2009; Adkins-Regan 2012). Although the molecular bases of shape dimorphisms have been difficult to discover (Williams and Carroll 2009), they are likely the result of the sex-specific regulation of gene expression in distinct tissues, such as sex hormone receptors expressed in skeletal and muscle tissues (e.g., Ranz et al. 2003; Emlen et al. 2006; McGlothlin and Ketterson 2008; Williams and Carroll 2009).

Proportional shape dimorphisms must arise through sex differences in the growth of particular structures, that is, through sex differences in allometry. Allometric growth trajectories represent the sum of all underlying molecular and cellular processes contributing to organismal growth. For example, the comparison of trait size to body size illustrates the underlying developmental processes regulating local versus global rates of growth (Fig. 1; Cheverud 1982; Klingenberg 1998; Sanger et al. 2012b). Allometry, therefore, serves as a useful framework to compare the developmental bases of proportional differences between males and females.

Allometry may be studied at a number of different biological levels (Cheverud 1982; Klingenberg 1998). Ontogenetic allometry represents the scaling relationships among traits through-

out development, from early development (e.g., morphogenesis, hatching, or birth) through adulthood. Static allometry represents growth during a distinct developmental stage, in most cases a snapshot of adult size and shape. There are often strong correlations between static and ontogenetic allometry (e.g., Cheverud 1982; Klingenberg and Zimmermann 1992; Klingenberg 1996, 1998), particularly among invertebrates where patterns of static allometry are established during larval development (e.g., Emlen et al. 2006; Shingleton et al. 2007; Shingleton et al. 2008). Among vertebrates, however, early and late growth phases are often dissociated; the same developmental processes do not necessarily regulate early and late growth periods and the resultant growth trajectories are, therefore, not necessarily correlated. In both mice and humans, for example, there are weak correlations between growth rates at birth and later stages (Sovio et al. 2009; Sanger et al. 2011). Therefore, when investigating the developmental timing of sexual differentiation, it is critical to appreciate that these differences can arise through temporally restricted processes.

Differences in growth can occur early in ontogeny or late in life, during distinct periods, or through continual divergence (Fig. 1). Parallel allometric trajectories suggest that similar developmental processes regulate growth in males and females whereas divergent growth trajectories represent the sex-specific regulation of growth. Variation in shape dimorphism among species can arise through changes in the slope of the growth trajectory, either of one sex or both, or by changing the duration of differential growth. In species exhibiting continual divergence, shape dimorphism is the greatest at the maximum size of the species. Because sexual dimorphisms can arise through distinct developmental strategies, comparing the timing of sexual divergence among species is a critical step toward understanding the developmental origins of morphological variation and the evolution of the developmental processes themselves.

Lizards are commonly used as models for studies of sexual dimorphism (e.g., Schoener 1967; Shine 1989; Zamudio 1998; Cox and John-Alder 2005, 2007; Cox and Calsbeek 2010). Caribbean *Anolis* lizards, in particular, have been the subjects of both comparative and mechanistic studies of sexual dimorphism (e.g., Butler et al. 2000; Butler and Losos 2002; Butler et al. 2007; Cox et al. 2009) and are an emerging model for comparative developmental analyses (Sanger 2012). Anoles are renowned for their rapid diversification and repeated convergence across the islands of the Greater Antilles: Cuba, Hispaniola, Jamaica, and Puerto Rico (reviewed in Losos 2009). On each of these islands species have converged on similar morphologies, behaviors, and patterns of sexual size and shape dimorphism. Both intra- and interspecific patterns of divergence are thought to reflect adaptations to different portions of arboreal habitat.

A highly debated question within evolutionary biology is whether convergent phenotypes arise through the same, similar,

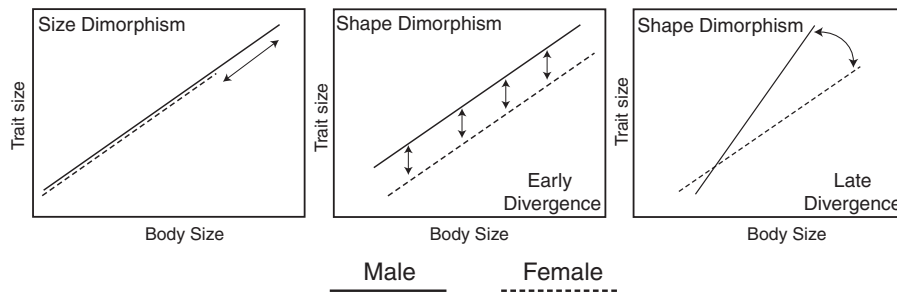


Figure 1. Alternative developmental hypotheses of size and shape variation. Allometric hypotheses contrast rates of local growth compared to growth in body size. Size dimorphism can arise through modification in the rate or duration of systemic growth, but these alternatives have a similar appearance on an allometric plot. Similar patterns of adult shape dimorphism can arise through two alternative developmental strategies: through the early differentiation of the sexes followed by parallel growth trajectories or through the gradual differentiation of males and females through sex-specific growth trajectories.

or different developmental modifications (reviewed in Wake 1991; Gould 2002; Losos 2011; Wake et al. 2011). Despite the great interest in both sexual dimorphism and convergence, the repeated evolution of sex-specific variation has not been analyzed in this light. Furthermore, rarely have alternative developmental strategies underlying sexual characters been analyzed in a rigorous comparative context (Baker and Wilkinson 2001; Voje and Hansen 2012). Here we explore evolutionary patterns of sexual dimorphism in head shape of *Anolis* lizards. We then address whether convergent patterns of extreme sexual dimorphism arise through convergent developmental strategies by tracing the evolutionary history of several distinct developmental strategies. We use two distinct allometric approaches to better understand the developmental bases of sexual dimorphism in skull shape. To determine the timing of divergence, we first examine static allometry for a broad sample of *Anolis* species nearing adult proportions. Then, to better understand the dynamics of craniofacial outgrowth, we compare ontogenetic growth trajectories using longitudinal data collected for males and females of six species exhibiting varying levels of sexual shape dimorphism in adult cranial morphology. Our analyses reveal that two lineages have converged on extreme levels of sexual dimorphism through nearly identical changes in male facial morphology. We also find that both shared and derived developmental strategies underlie the evolution of facial dimorphism among *Anolis*.

Materials and Methods

MACROEVOLUTIONARY PATTERNS OF SEXUAL DIMORPHISM

Our first objective was to trace the evolution of skull shape dimorphism in *Anolis* lizards and to determine where in anole history shifts in dimorphism occurred. We sampled males and females of 50 *Anolis* species (Table S1), including most of the major Caribbean anole lineages and representing the full range of anole

skull diversity (Sanger et al. 2012a). A total of 475 skulls were examined for this analysis (236 females, 239 males). Dried skulls were obtained from the Museum of Comparative Zoology (MCZ) at Harvard University (Cambridge, MA). To increase the breadth of our sample, skulls of several poorly represented species were reconstructed in 3D using microcomputed tomography (μ CT), and digitally aligned to a similar orientation as the dried material. The details of μ CT scanning are described in the online Supplementary Methods. For more common species, new skeletal material was also prepared from MCZ alcoholic specimens.

To extract the primary axes of skull shape dimorphism and develop a metric of sexual shape dimorphism, we used geometric morphometrics similar to Sanger et al. (2012a). Briefly, we digitized 24 landmarks on the dorsal aspect of the skull from scaled digital photographs using TPSdig II (Fig. 2; Rohlf 2006). We then performed geometric morphometric analyses in MorphoJ (Klingenberg 2011) by calculating the average values of landmark coordinates for each species removing the effects of position, orientation, and scale from the data (Dryden and Mardia 1998; Zelditch et al. 2004; Klingenberg 2010). Procrustes superimposition accounted for “object symmetry” of the skull by reflecting lateral landmarks across the midline to find an average landmark position (Klingenberg et al. 2002). Size correction was performed using a multivariate regression of shape data on centroid size, the preferred measure of size in geometric morphometrics (see online Supplementary Appendix for further details). Principal component analysis was then conducted on these shape residuals to extract the primary axes of skull shape variation. Following morphometric analysis, the magnitude of shape dimorphism was calculated as the Euclidean distance between males and females in morphological space taking into account all principal component axes (PCs hereafter) explaining greater than 5% of the variation.

We investigated evolutionary patterns of sexual dimorphism by mapping the magnitude of sexual dimorphism onto the

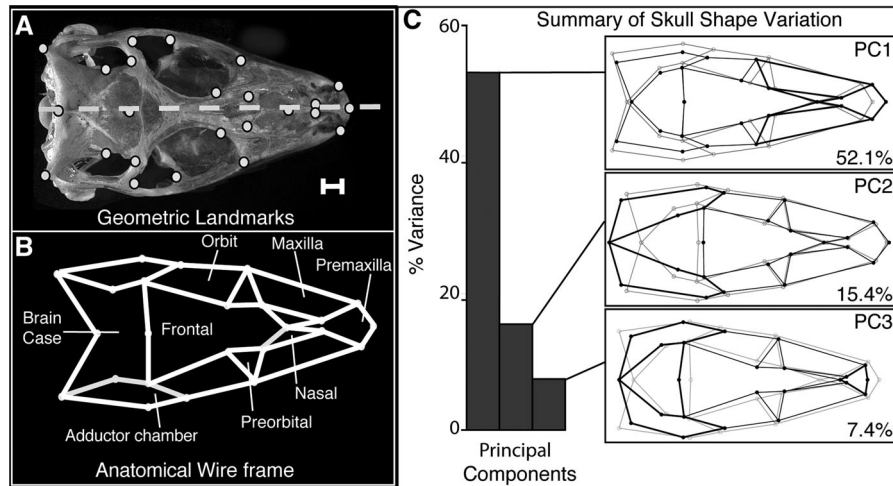


Figure 2. Morphometric landmarks, wire diagram, and results. (A) Skull of *A. aeneus* depicting the geometric landmarks used to analyze skull shape variation among *Anolis* lizards. Skull symmetry was taken into account by reflecting paired landmarks across the midline (dashed line). Scale bar equals 1 mm. (B) To illustrate shape variation, landmarks are converted into wire diagrams that highlight the major functional and skeletal regions of the skull. (C) The first three principal components cumulatively explain 74.9% of variation in anole head shape. Grey wireframes represent the average head shape of anoles. Black wire frames represent a positive deviation of shape change along that axis with the darkened lines highlighting the most variable skeletal elements. See Table S1 for sample sizes and shape scores.

maximum clade credibility tree of Mahler et al. (2010), using maximum likelihood ancestral character state reconstruction. Character change was estimated under a Brownian motion model of continuous trait evolution (Schluter et al. 1997; implemented in the ace function of the R package APE [Paradis et al. 2004]). For descriptive purposes, clades were considered to possess “extreme dimorphism” when they reached levels greater than 1.5 standard deviations beyond the reconstructed root value within their 95% confidence intervals. We also explored the relationship between size and shape traits, both among species and between sexes, using phylogenetic regression (implemented in the R package Phytools using the gls command [Revell 2011]).

STATIC ALLOMETRY

At least two distinct developmental strategies can underlie similar patterns of sexual shape dimorphism (Fig. 1). Our next objective was to explore the evolutionary history of alternative developmental strategies among *Anolis* and to determine whether species that converge on extreme levels of sexual dimorphism also converge on similar developmental strategies. Therefore, to test between alternative hypotheses, we compared growth trajectories for males and females of 27 *Anolis* species, focusing on sexually mature lizards. We measured facial length (from the parietal foramen to the tip of the snout) and regressed this against body size using the standard allometric equation (Huxley 1932; Klingenberg 1998). We typically measured more than 20 individuals per sex per species, but for one species, *A. equestris*, only 17 males and

14 females were available. Snout-to-vent length (SVL) is the typical measure of body size used for herpetological studies (e.g., Beuttell and Losos 1999; Calsbeek and Smith 2007), but this measure confounds our dependent and independent variables. Therefore, as a measure of body size we used body length, defined as total head length subtracted from SVL.

The most important variables distinguishing the developmental hypotheses are differences in slope and intercept (Huxley 1932; also see discussion in Voje and Hansen 2012). Differences in slope and intercept between males and females were evaluated for each species using an analysis of covariance (Sokal and Rohlf 1995). All allometric analyses were performed in PASW Statistics (SPSS v.17: IBM). Measurements were taken from MCZ alcoholic museum specimens using digital calipers. Data were log-transformed before analysis.

To determine the evolutionary history of alternative developmental strategies (Fig. 1), whether one strategy represents a widespread, potentially ancestral, condition or whether the alternatives are used intermittently throughout the anole radiation, we mapped the three models of shape dimorphism onto the phylogeny from Mahler et al. (2010). We categorized each species into one of three alternative developmental strategies—early divergence, late divergence, and no sexual shape dimorphism. Alternative character states were mapped to the phylogeny using maximum likelihood (Schluter et al. 1997) implemented in Mesquite (Maddison and Maddison 2011). Proportional likelihoods obtained for each model are reported for all nodes.

ONTOGENETIC ALLOMETRY

To more thoroughly assess the timing of sexual differentiation, particularly among species exhibiting early sexual differentiation, we performed a longitudinal study of male and female facial morphology for six anole species representing varying levels of sexual dimorphism and facial morphology. Parents were collected from free-living populations in Puerto Rico (*Anolis cristatellus*, *Anolis evermanni*, and *Anolis pulchellus*) and South Bimini, Bahamas (*Anolis angusticeps*, *Anolis sagrei*, and *Anolis smaragdinus*), between 2006 and 2009. Adults were paired in the laboratory by placing one male and one female together in a 28 × 36 × 20 cm cage. Eggs, which females placed in a potted plant in the cage, were removed once a week and placed in an incubator at 28°C and 75% relative humidity (RH) until hatching. Hatchlings were transferred to individual 17 × 26 × 15 cm cages, where they were kept in a common laboratory environment (28°C during 12 h of light, 25°C during 12 h of darkness; 65% RH) and fed a diet of pinhead crickets for 6 months. Juvenile lizards were X-rayed four times during growth to assess skeletal development: near hatching (1–12 days), 1, 3, and 6 months of age. Lizards were briefly chilled in small plastic bags (10 min at 4°C) before imaging in a Faxitron 43805N X-ray machine. These bags were secured to X-ray film packets using masking tape to prevent movement.

Face length, head length, and SVL were measured for 12–20 lizards of each sex at hatching (1–12 days), 1, 3, and 6 months of age. SVL was measured using a segmented line as the distance from the tip of the snout to the caudo–sacral junction. Body length was then calculated by subtracting total head length from SVL. Measurements were taken directly from digitized X-ray films using ImageJ (Schneider et al. 2012). The timing of sexual differentiation in head shape was assessed using a mixed model design with “individual” included as a random variable. Differences in the relative rate of facial outgrowth were analyzed by testing for differences in the slope of male and female growth trajectories of each species. Because growth differences can potentially be isolated to distinct periods of ontogeny, data were separately analyzed from hatchling to 1, 1–3, and 3–6 months. Analyses of ontogenetic allometry were performed in JMP v.9 (SAS Institute, Cary, NC, 1989–2011). Data for both static and ontogenetic allometry are available in Dryad (doi:10.5061/dryad.hk2v3).

Results

MACROEVOLUTIONARY PATTERNS OF SEXUAL DIMORPHISM

In the PCA on size-corrected shape data, three PCs were recovered explaining 74.9% of variation in head shape (Fig. 2). Within species, males and females tend to have similar head shapes com-

pared to broader patterns of skull diversity (correlations from phylogenetic regression: 0.82 PC1, 0.85 PC2, and 0.87 PC3). The primary axis of craniofacial variation (PC1, 52.1% of total variation) described facial length, particularly snout length, the region of the face anterior to the orbits. The second axis of variation (PC2, 15.4%) described variation in the size and shape of the adductor chambers, the regions of the skull that surround the jaw musculature including the parietal muscle scar and postorbital bar. The third axis of variation explained only a small portion of variation (PC3, 7.4% of variation), summarizing either extension or compression of the most anterior and posterior landmarks.

Anolis species vary extensively in degree of skull shape dimorphism (Fig. 3). The magnitude of shape dimorphism is not correlated with head size (centroid size) or size dimorphism (Fig. S1). Maximum likelihood ancestral character state reconstruction suggests that anoles possessed low to moderate levels of sexual dimorphism ancestrally (Table S2). This analysis also suggests that two clades, the *carolinensis* and *hendersoni* clades, have independently converged on extreme levels of sexual dimorphism. In addition, extreme dimorphism has been reached through very similar morphological transitions. In both of these lineages, males have evolved highly elongate faces whereas females retain skulls of more moderate proportions. This pattern is unique to the *carolinensis* and *hendersoni* clades; the degree of sexual dimorphism and facial length (PC1) are not correlated among anoles more broadly (Fig. S1).

COMPARATIVE ANALYSIS OF DEVELOPMENTAL STRATEGIES

Comparison among species of the *carolinensis* and *hendersoni* clades reveals that they have converged phenotypically using distinct developmental strategies (Figs. 4 and S2). In all three species of the *hendersoni* clade, males and females exhibit parallel allometric growth trajectories, statistically differing in intercept but not slope. In contrast, all five of the *carolinensis* species examined exhibit statistically significant differences in intercept and diverge in facial morphology relatively late in life.

In the 19 species we examined outside of the *carolinensis* and *hendersoni* clades, 13 exhibited significant facial length dimorphism. All 13 of these species achieve dimorphism via the same developmental strategy, early divergence; that is, they exhibit significant differences in intercept, but not slope (Figs. 4 and S2). Ancestral character state reconstruction of developmental strategies suggests that late divergence of male and female facial morphology is unique to the *carolinensis* clade of anoles (Fig. 5, Table S3). This analysis also suggests that early sexual differentiation is a common strategy of sexual differentiation shared among species, most likely shared because of common ancestry (proportional likelihood of root node: 0.946). Therefore, although

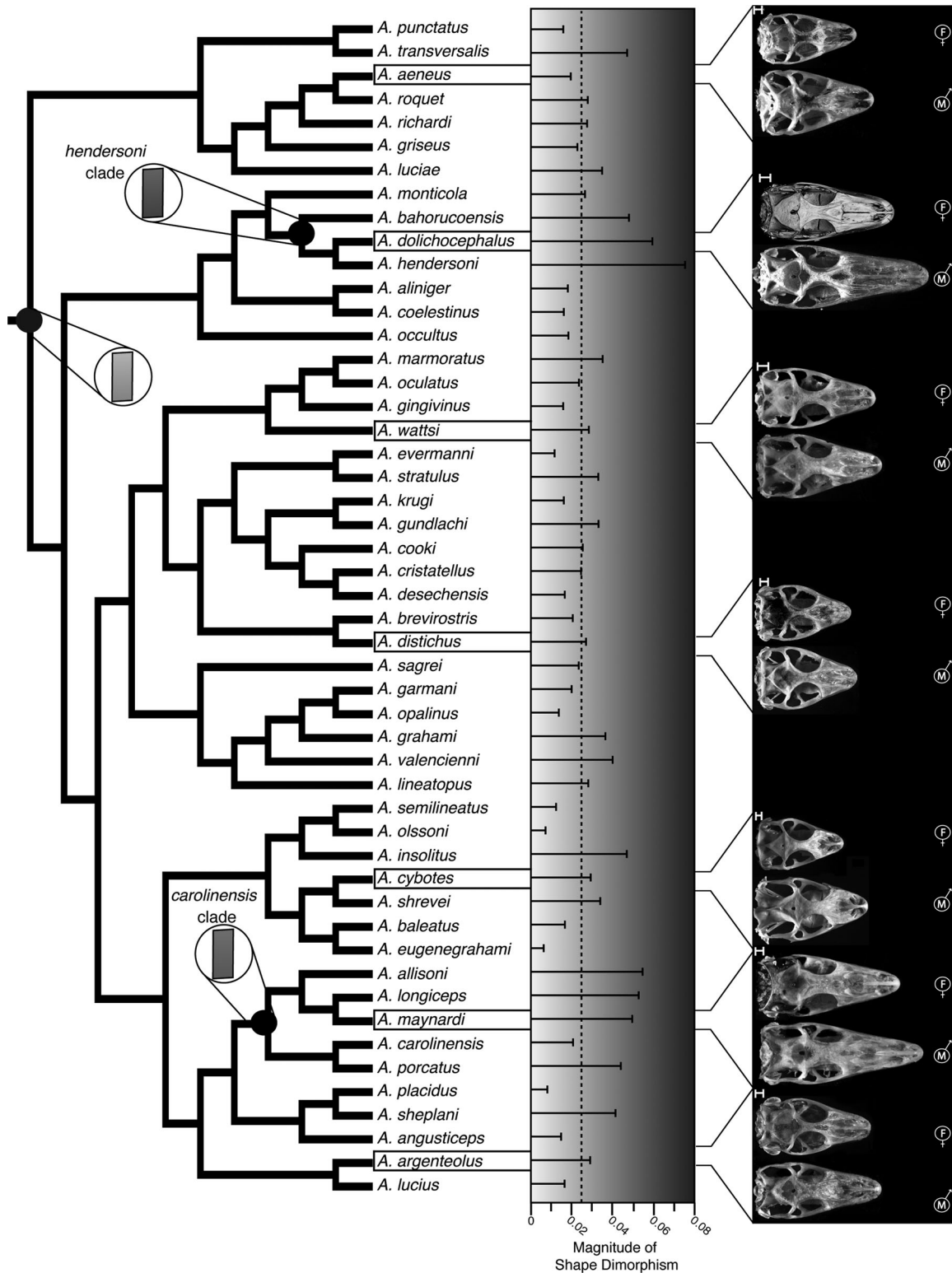


Figure 3. Evolutionary history of sexual skull shape dimorphism. Anoles exhibit substantial variation in sexual dimorphism of skull shape (SShD). Ancestral character state reconstruction suggests that anoles had low to moderate levels of sexual dimorphism ancestrally. Extremely dimorphic skulls—greater than 1.5 standard deviations away from the reconstructed root value (dashed line)—evolved twice in the history of anoles, in the *carolinensis* and *hendersoni* clades. Rectangular gradient insets represent maximum likelihood 95% confidence intervals for the highlighted nodes (black dot) based on the shape dimorphism gradient; light colors represent low levels of dimorphism and dark colors high levels of dimorphism. Skulls for males (m) and females (f) of species with varying levels of shape dimorphism are illustrated in the right margin. See Table S2 for additional details of ancestral character state reconstruction.

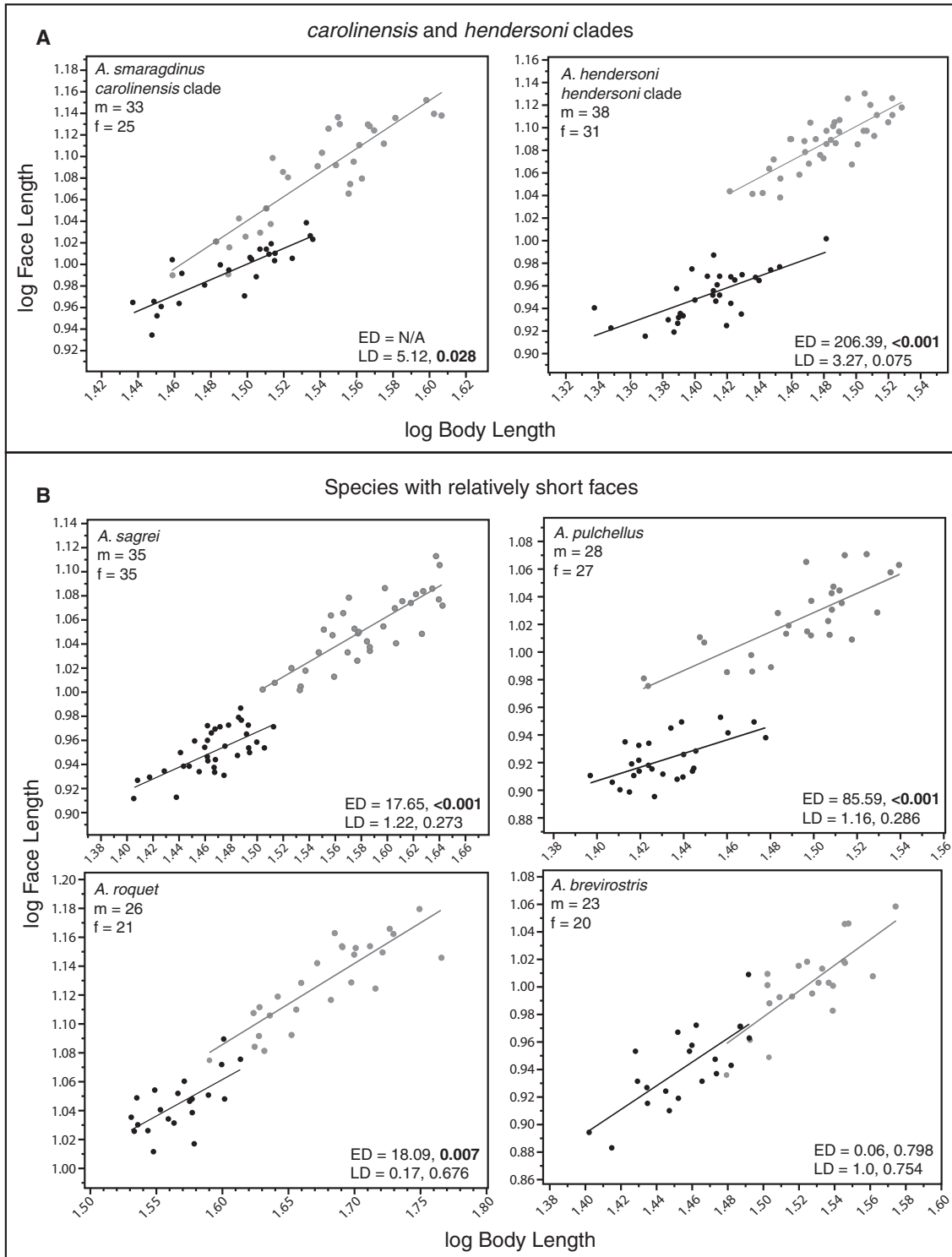


Figure 4. Representative plots of static allometry. (A) Distinct developmental strategies underlie convergence upon extreme craniofacial dimorphism in the *carolinensis* and *hendersoni* clades. Sexual dimorphism in facial length in the *carolinensis* clade—represented here by *A. smaragdinus*—is established late (LD), through sex-specific allometry. Conversely, in the *hendersoni* clade—represented by *A. hendersoni*—sexual dimorphism is established early (ED) and is followed by parallel growth trajectories of the sexes through later life. (B) Species with less sexual shape dimorphism and relatively shorter faces also tend to diverge early in life and follow parallel growth trajectories through sexual maturity. Several species examined exhibit a size dimorphism but not shape dimorphism, such as *A. brevirostris*. See Figure S2 for details on the remaining species. Consistent with previous studies (Butler et al. 2000), all species examined exhibit male-biased body size dimorphism. Male growth data are illustrated in gray and females in black. *F*-statistics and *P*-values for each test are shown.

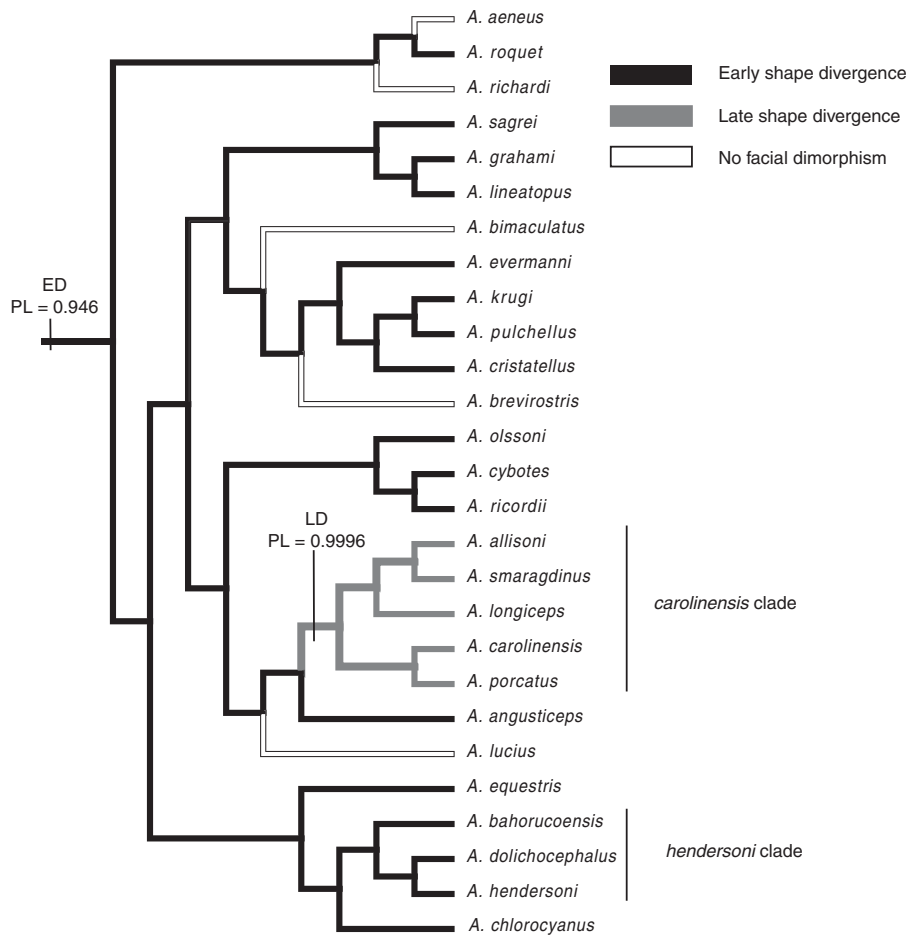


Figure 5. Evolution of developmental strategies underlying facial length dimorphism. Mapping alternative developmental strategies onto the *Anolis* phylogeny suggests that sexual dimorphism was produced ancestrally through the early differentiation of males and females (ED; proportional likelihood [PI] = 0.946). This analysis also reveals that the evolution of sex-specific growth trajectories is unique to the *carolinensis* clade (LD; PI = 0.9996).

a novel developmental strategy underlies extreme dimorphism in the *carolinensis* clade, extreme levels of sexual dimorphism in the *hendersoni* clade represent the exaggeration of an ancestral developmental strategy (Fig. 6).

Morphometric analyses suggest that extreme sexual dimorphism is the result of changes in male morphology. To determine whether the developmental changes are also male-specific, we compared growth trajectories among species but within males and females. Comparison of sex-specific developmental trajectories among species reveals that the changes in development are male specific (Fig. S3, Table S4). Of the comparisons among non-*carolinensis* males, few exhibit significant differences in the slope of their facial growth trajectories (10.5%), which indicates that most species have similar growth trajectories at the time of sexual maturity. In contrast, 76.4% of comparisons between *carolinensis* clade males and males of other species exhibit significant differences in slope. Among females only 9.3% of comparisons exhibit significant differences.

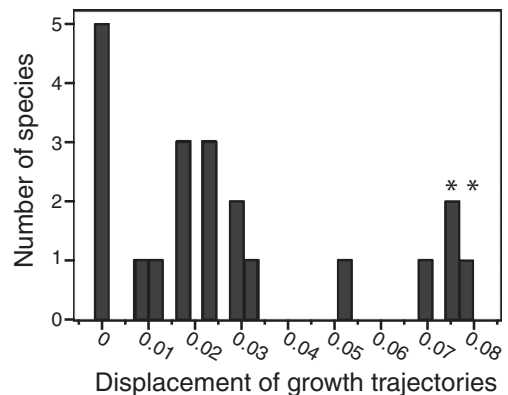


Figure 6. Plot of y-intercept differences of species with early sexual differentiation. Extreme facial length dimorphism in the *hendersoni* clade (*) is achieved by elaboration on the ancestral developmental strategy of early sexual differentiation.

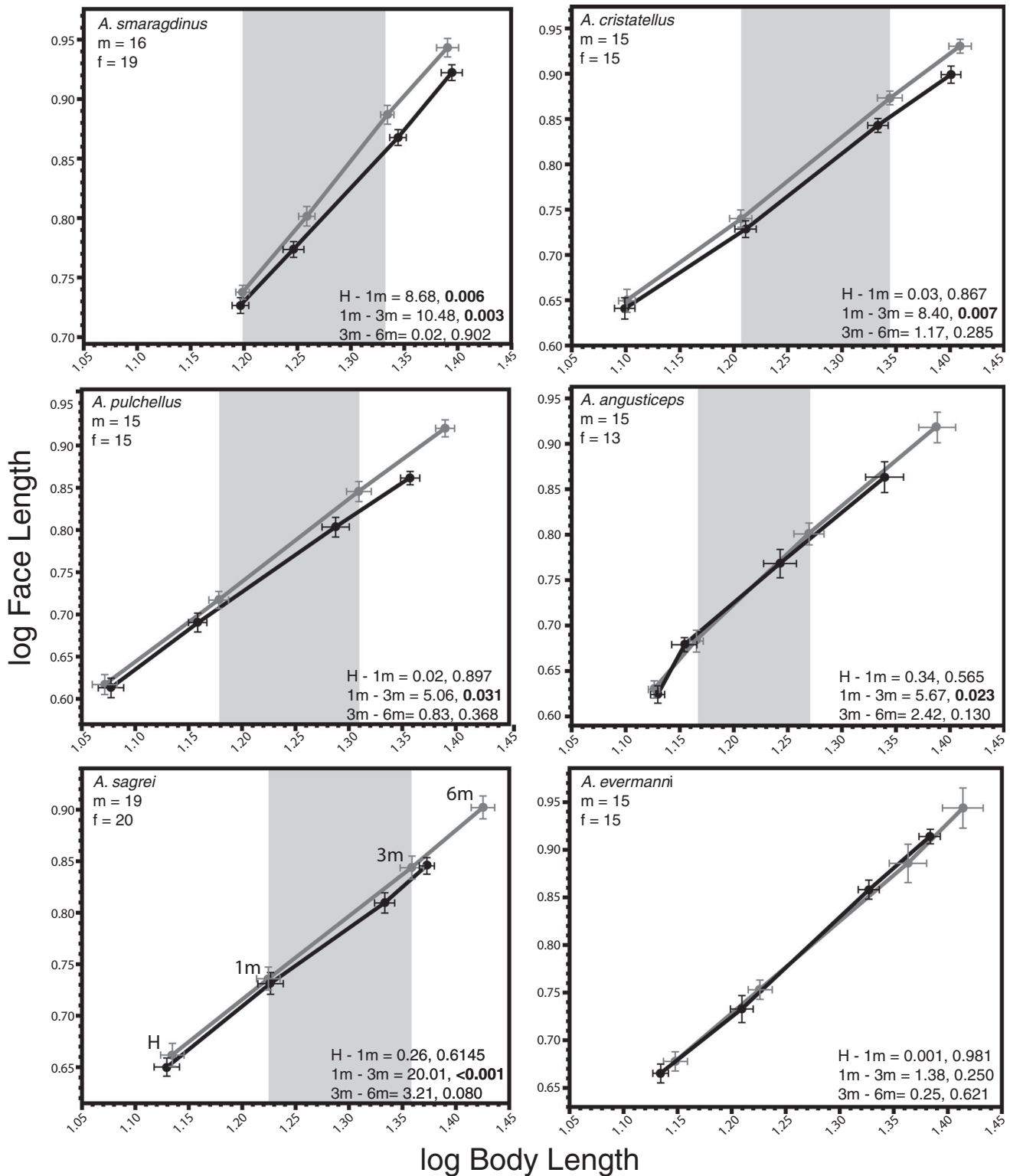


Figure 7. Summary of ontogenetic allometry results. Five of six species examined develop facial length dimorphism at approximately the same size (gray shaded boxes). For four non-carolinensis clade anoles—*A. cristatellus*, *A. pulchellus*, *A. angusticeps*, and *A. sagrei*—sexual divergence occurs between 1 and 3 months of age. The carolinensis clade anole, *A. smaragdinus* hatches larger than the other species and develops dimorphism from hatching to 3 months. The exception is *A. evermanni*, which only exhibits size dimorphism, not shape dimorphism by 6 months of age. *F*-statistics and *P*-values for each test are shown for a test for difference in slope for each time increment.

ONTOGENETIC ANALYSIS OF SEXUAL DIFFERENTIATION

Longitudinal growth data were collected for individuals of six species: five species exhibiting early sexual differentiation (*A. sagrei*, *A. angusticeps*, *A. evermanni*, *A. cristatellus*, and *A. pulchellus*; Fig. S2) and one member of the *carolinensis* clade, *A. smaragdinus*, exhibiting late sexual differentiation (Fig. 4). In all four species exhibiting early divergence, males and females exhibit similar and overlapping growth trajectories from 1 to 3 months of age (Fig. 7). In these four species, facial length dimorphism develops between 1 and 3 months of age through differential elongation of the face. *Anolis smaragdinus* also exhibits a period of subtle, but statistically significant, sexual differentiation early in ontogeny, from 0 to 3 months, despite the emergence of pronounced sexual dimorphism late in ontogeny (Fig. 5). Despite our findings in the comparative analyses, *A. evermanni* did not exhibit a statistical pattern of facial length differentiation at any growth interval. It is unclear whether this is because of technical differences between the static and ontogenetic allometric analyses or population-level differences in facial morphology between the specimens examined.

Discussion

CONVERGENT PATTERNS OF SEXUAL DIMORPHISM

The evolution of sexual dimorphism, particularly the evolution of exaggerated male phenotypes, is of great interest to biologists from many disciplines (Fairbairn et al. 2007; Williams and Carroll 2009). However, most evolutionary studies of sexual dimorphism among vertebrates have focused on differences in body size (e.g., Fairbairn and Roff 2006; Cox et al. 2009), color (Price and Birch 1996; Siefferman and Hill 2005), or weapons and ornaments (e.g., Badyaev 2004; Ord and Stuart-Fox 2006). Pronounced dimorphism in body proportion has rarely been reported among vertebrates (reviewed in Gidaszewski et al. 2009), particularly for facial skeleton morphology. For example, sexual dimorphism in primate facial morphology is the secondary consequence of differences in size (i.e., ontogenetic scaling; Leigh and Cheverud 1991; Schaefer et al. 2004). Dimorphism in bird beak length is widespread (e.g., Burton 1974; Gill and Martinson 1991; Grant and Grant 2003; Freed et al. 2009), but the proportion of this dimorphism that is because of differences in the rhamphotheca, the outer keratinized sheath of the beak, and the underlying bony skeleton has not been quantified. Among lizards, skull dimorphism is mainly restricted to differences in the size and shape of the adductor chambers (Herrel et al. 2007; Kaliontzopoulou et al. 2007; Ljubicavljević et al. 2010).

In contrast to previous studies, we show that two *Anolis* clades, *carolinensis* and *hendersoni*, have converged on similar patterns of sexual dimorphism through nearly identical modi-

fications in male facial morphology (Fig. 3). Although most species have relatively low to moderate levels of shape dimorphism, these clades independently evolved extreme dimorphism through sex-specific changes in morphology. The changes in skull shape evolved independent of changes in head size and size dimorphism (Fig. S1), underscoring the importance of considering size and shape as independent factors in studies of sexual dimorphism.

Our comparative analyses of development illustrate that the *carolinensis* and *hendersoni* clades converge morphologically using distinct developmental strategies (Figs. 4 and 5). Our analyses also show that although most species have highly conserved craniofacial developmental strategies these processes are not immune to modification; both ancestral and derived developmental strategies underlie sexual dimorphism among *Anolis* lizards. In most species, sexual shape dimorphism is established through differential rates of facial elongation at a distinct interval of ontogeny. In the *carolinensis* clade, however, extreme dimorphism in facial length evolved through the coincident evolution of a novel, male-specific developmental strategy added on to the late stages of development. In summary, by analyzing alternative developmental hypothesis in a broad phylogenetic context our analyses shed novel light on the pattern of developmental evolution that would not be found studying the extreme phenotypes alone.

Phenotypic convergence is often interpreted from two perspectives: shared developmental biases and similarities in selection. Below we briefly discuss our findings in the light of these alternative viewpoints.

SIZE, SHAPE, AND THE DEVELOPMENTAL BIASES UNDERLYING SEXUAL DIMORPHISM

Size and shape dimorphism can arise before or after hatching, through differential maternal investment (e.g., Cordero et al. 2001; Lovorn and Wade 2003), sex-specific differences in morphogenesis (e.g., Drea et al. 1998; Zheng and Cohn 2011), or through differential growth at distinct life stages (e.g., Humphrey 1998; O'Higgins et al. 2001). Although multiple mechanisms operating throughout development can potentially generate sexual dimorphism, *Anolis* species repeatedly develop sexual skull shape dimorphism through differential facial growth at a distinct period of ontogeny (Fig. 5). The broad phylogenetic distribution of early sexual differentiation suggests that it is shared because of common ancestry. Even the *carolinensis* clade species, *A. smaragdinus*, maintains the signature of this more broadly shared strategy despite developing exaggerated sexual dimorphism later in life through a derived developmental strategy (Fig. 7). This indicates that the late stage growth trajectory observed in the *carolinensis* clade is an evolutionary novelty. Late-stage sexual differentiation is definitively not the result of shift in the timing of sexual differentiation from earlier events.

Our observations are consistent with those of Haldane (1932) and Vavilov (1922), who observed that closely related species tend to vary along similar dimensions. In other words, the developmental-genetic architecture of trait variation is conserved among closely related species and is not necessarily remodeled following speciation, adaptation, or changes in morphology. A similar pattern was recently reported for limb length variation among anoles (Sanger et al. 2012b), suggesting that conservation of the developmental processes contributing to macroevolutionary patterns of variation may represent a general pattern in anole evolution. Furthermore, similar observations of conservation in the processes underlying phenotypic variation have also recently been reported in other distantly species groups, suggesting that this pattern may be a common property of morphological evolution more generally. For example, despite the many ways beak shape diversity could evolve, a relatively simple molecular network consistently underlies the diversity of Darwin's finch beaks (Abzhanov et al. 2006; Mallarino et al. 2012).

Overlaying our findings with an understanding of the molecular bases of sexual dimorphism will uncover deeper levels of convergence, parallelism, and developmental conservation underlying the evolution of craniofacial diversity in *Anolis*. It remains unknown whether the similar developmental patterns widely observed in anoles are generated by similar molecular mechanisms. Similarly, it is yet unknown whether morphological convergence in the *carolinensis* and *hendersoni* clades result from temporal shifts of similar molecular pathways, despite their appearance as a distinct developmental strategies at higher levels of organization. However, our results suggest that natural selection can readily “tinker” with broadly conserved regulatory pathways to produce varying levels of sexual dimorphism rather than drastically modifying the mechanisms of development among species (Jacob 1977). The distinct facial growth trajectories (i.e., differences in slope) that underlie facial length dimorphism in anoles does, however, indicate that there must be temporal and sex-specific regulation of developmental process in the nasal cartilage driving facial outgrowth (Wealthall and Herring 2006). We hypothesize, therefore, that a similar molecular “switch” (Williams et al. 2008) controls the broadly shared pattern of early sexual dimorphism, but that a novel form of male-specific regulation evolved at the base of the *carolinensis* clade, which controls the development of late-stage sexual differentiation.

NATURAL OR SEXUAL SELECTION

The traditional approach in studying convergence from an adaptationist perspective is to look for shared selective conditions that might account for the repeated evolution of the same trait. On one hand, convergent patterns of sexual dimorphism can result from similar patterns of natural selection. However, the *carolinensis* and *hendersoni* clades represent two different habitat specialists,

notably different in ecology and behavior: the *carolinensis* anoles are “trunk-crown” anoles, whereas *hendersoni* species are “grass-bush” anoles (Losos 2009). This suggests no common ecological cause associated with microhabitat for the evolution of dimorphism between these clades. Moreover, many other lineages have evolved to become members of both ecomorph classes without evolving extreme dimorphism. On the other hand, convergence in shape dimorphism could result from similar patterns of sexual selection. However, field studies in either *Anolis* or other lizard taxa have not yet elucidated a viable form of sexual selection that can explain male facial elongation. For example, although the colorful dewlap (throat fan) is involved with signaling to potential mates and conspecific males, there is as of yet no evidence that facial morphology is involved in this signal. More detailed research on the social structure and degree of intersexual ecological niche partitioning is needed to investigate whether common selective conditions have driven convergent dimorphism in these clades. Further comparative analyses of different axes of shape dimorphism (PCs2 and 3) may also shed further light on the selective forces that shape craniofacial variation *Anolis* species.

In addition to the broader patterns of convergence, we may also ask whether novel selective conditions are responsible for the evolution of a novel developmental strategy in the *carolinensis* clade. These species differ from all others in that the degree of dimorphism increases throughout the later stages of ontogeny. At this point, we can only speculate why this might be favored by selection. One possibility revolves around the observation that small “sneaker” males occur in *Anolis carolinensis* (Lailvaux et al. 2004). It may be that selection favors smaller males to appear more like females, and thus extreme dimorphism might only be favored in larger adults, potentially leading to the novel developmental trajectory seen in this clade. Whether, in fact, sneaker males are limited to this clade remains to be investigated, as does the pattern of selection on males at different life stages.

Future Directions

The field of evolutionary developmental biology (or evo-devo) has led the recent charge to understand the developmental origins of morphological variation (Carroll et al. 2005). Although originally relying heavily on experimentally tractable model systems (e.g., *Drosophila*, mouse, zebrafish), research into the evolutionary mechanisms of morphological change is now becoming common in diverse clades that have, historically, been more the domain of ecologists and evolutionary biologists (e.g., Chan et al. 2010; Manceau et al. 2011; Emlen et al. 2012; Mallarino et al. 2012; Rajakumar et al. 2012). Analyses of development in an explicit evolutionary (i.e., phylogenetic) context provides an opportunity to determine the polarity of developmental changes and to show whether shifts in development correlate with

changes in morphology or ecology. These analyses will also determine whether changes in the same or similar developmental processes contribute to morphological variation among closely related species or whether the developmental foundations of phenotypic variation are evolutionarily labile. And at a broader scale still, these analyses provide an opportunity to test whether convergent morphologies arise through convergent developmental changes. In total, integrated analyses across biological and evolutionary scales—from molecules to morphology and from micro- to macroevolution—will serve to solidify the synthesis of evolutionary and developmental biology and shed new light on the mechanisms of morphological diversification.

Our macroevolutionary analyses of sexual skull shape dimorphism have illustrated that variable levels of dimorphism among *Anolis* lizards are often the result of tinkering with a similar developmental program whereas one clade develops a similar pattern of dimorphism using a novel strategy. Further examination of the potential mechanisms of ecological and sexual selection will shed light on whether the evolution of this novel growth pattern is also correlated with a novel form of selection that repatterned the developmental architecture of facial growth. Comparing the molecular causes of shape dimorphism in species possessing distinct developmental strategies will also more precisely determine the relationship between these developmental alternatives. Together, these ecological, developmental, and molecular analyses will create a synthetic analysis of morphological variation and the mechanisms that shape its evolution.

ACKNOWLEDGMENTS

This research has benefited greatly from discussions with M. Johnson, C. Extavour, H. Hoekstra, R. Mallarino, M. Manceau, S. Donoughe, B. Ewen-Campen, and M. Brent Hawkins. The authors thank D. Luke Mahler, L. Revell, C. Klingenberg, and L. Monteiro for technical assistance with morphometric techniques and comparative methods. The ultrametric *Anolis* phylogeny was provided by D. Luke Mahler. J. Brocca of the Sociedad Ornitología de la Hispaniola provided valuable field assistance. For access to museum collections and curatorial assistance, the authors thank the Harvard University Museum of Comparative Zoology curatorial staff: J. Rosado, J. Martinez, J. Woodward, T. Takahashi, and M. Woolley. Computed tomography scan data were collected at the Harvard University Center for Nanoscale Systems, a member of the National Nanotechnology Infrastructure Network, which is supported by the National Science Foundation (NSF) under NSF award no. ECS-0335765. Permission to collect specimens included in this project came from the Cayman Islands Department of Environment, the Bahamas Environment, Science & Technology Commission, and the Dominican Republic Secretaría de Estado de Medio Ambiente y Recursos Naturales. Generous funding for this project came from Harvard University Department of Organismic and Evolutionary Biology awarded to AA, and JBL, the Harvard University Museum of Comparative Zoology awarded to TJS, the David and Lucille Packard Foundation awarded to JBL, and NSF DEB-0519777 and DEB-0519658 to EDBIII and JBL. The authors declare no conflicts of interest.

LITERATURE CITED

- Abouheif, E. 2008. Parallelism as the pattern and process of mesoevolution. *Evol. Dev.* 10:3–5.
- Abzhanov, A., W. P. Kuo, C. Hartmann, B. R. Grant, P. R. Grant, and C. J. Tabin. 2006. The Calmodulin pathway and the evolution of elongated beak morphology in Darwin's Finches. *Nature* 442:563–567.
- Adkins-Regan, E. 2012. Hormonal organization and activation: evolutionary implications and questions. *Gen. Comp. Endocrinol.* 176:279–285.
- Badyaev, A. V. 2002. Growing apart: an ontogenetic perspective on the evolution of sexual size dimorphism. *Trends Ecol. Evol.* 17:369–378.
- . 2004. Developmental perspective on the evolution of sexual ornaments. *Evol. Ecol. Res.* 6:975–991.
- Baker, R. H., and G. S. Wilkinson. 2001. Phylogenetic analysis of sexual dimorphism and eye-span allometry in stalk-eyed flies (Diopsidae). *Evolution* 55:1373–1385.
- Beuttell, K., and J. B. Losos. 1999. Ecological morphology of Caribbean anoles. *Herpetol. Monogr.* 13:1–28.
- Burton, P. J. K. 1974. Anatomy of head and neck in the Huia (*Heteralocha acutirostris*) with comparative notes on other Callaeidae. *Bull. Br. Mus. (Nat. Hist.) Zool.* 27:1–48.
- Butler, M. A., and J. B. Losos. 2002. Multivariate sexual dimorphism, sexual selection, and adaptation in Greater Antillean *Anolis* lizards. *Ecol. Monogr.* 72:541–559.
- Butler, M. A., S. A. Sawyer, and J. B. Losos. 2007. Sexual dimorphism and adaptive radiation in *Anolis* lizards. *Nature* 447:202–205.
- Butler, M. A., T. W. Schoene, and J. B. Losos. 2000. The relationship between sexual size dimorphism and habitat use in Greater Antillean *Anolis* lizards. *Evolution* 54:259–272.
- Calsbeek, R., and T. B. Smith. 2007. Probing the adaptive landscape using experimental islands: density-dependent natural selection on lizard body size. *Evolution* 61:1052–1061.
- Carroll, S. B. 2008. Evo-devo and an expanding evolutionary synthesis: a genetic theory of morphological evolution. *Cell* 134:25–36.
- Carroll, S. B., J. K. Grenier, and S. D. Weatherbee. 2005. From DNA to diversity: molecular genetics and the evolution of animal design. Blackwell Scientific, Malden, MA.
- Chan, Y., M. Marks, F. Jones, G. Villarreal, M. Shapiro, S. Brady, A. Southwick, D. Absher, J. Grimwood, J. Schmutz, et al. 2010. Adaptive evolution of pelvic reduction in sticklebacks by recurrent deletion of a Pitx1 enhancer. *Science* 327:302–305.
- Cheverud, J. M. 1982. Relationships among ontogenetic, static, and evolutionary allometry. *Am. J. Phys. Anthropol.* 59:139–149.
- Cordero, P. J., J. Viñuela, J. M. Aparicio, and J. P. Veiga. 2001. Seasonal variation in sex ratio and sexual egg dimorphism favouring daughters in first clutches of the spotless starling. *J. Evol. Biol.* 14:829–834.
- Cox, R. M., and R. Calsbeek. 2010. Cryptic sex-ratio bias provides indirect genetic benefits despite sexual conflict. *Science* 328:92–94.
- Cox, R. M., and H. B. John-Alder. 2005. Testosterone has opposite effects on male growth in lizards (*Sceloporus* spp.) with opposite patterns of sexual size dimorphism. *Science Signal.* 208:4679–4687.
- . 2007. Increased mite parasitism as a cost of testosterone in male striped plateau lizards *Sceloporus virgatus*. *Funct. Ecol.* 21:327–334.
- Cox, R. M., D. Stenquist, and R. Calsbeek. 2009. Testosterone, growth and the evolution of sexual size dimorphism. *J. Evol. Biol.* 22:1586–1598.
- Darwin, C. 1859. On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life. John Murray, London.
- Drea, C. M., M. L. Weldele, N. G. Forger, E. M. Coscia, L. G. Frank, P. Licht, and S. E. Glickman. 1998. Androgens and masculinization of

- genitalia in the spotted hyaena (*Crocuta crocuta*). 2. Effects of prenatal anti-androgens. *J. Reprod. Fertil.* 113:117–127.
- Dryden, I. L., and K. V. Mardia. 1998. *Statistical shape analysis*. John Wiley & Sons, New York.
- Emlen, D. J., Q. Szafran, L. S. Corley, and I. Dworkin. 2006. Insulin signaling and limb-patterning: candidate pathways for the origin and evolutionary diversification of beetle ‘horns’. *Heredity* 97:179–191.
- Emlen, D. J., I. A. Warren, A. Johns, I. Dworkin, and L. Corley-Lavine. 2012. A mechanism of extreme growth and reliable signaling in sexually selected ornaments and weapons. *Science* 337:860–864.
- Fairbairn, D., and D. Roff. 2006. The quantitative genetics of sexual dimorphism: assessing the importance of sex-linkage. *Heredity* 97:319–328.
- Fairbairn, D. J., W. U. Blanckenhorn, and T. Székely. 2007. *Sex, size and gender roles*. Evolutionary studies of sexual dimorphism. Oxford Univ. Press, New York.
- Freed, L. A., R. L. Cann, and K. Diller. 2009. Sexual dimorphism and the evolution of seasonal variation in sex allocation in the Hawaiian akera. *Evol. Ecol. Res.* 11:731–757.
- Gidaszewski, N. A., M. Baylac, and C. P. Klingenberg. 2009. Evolution of sexual dimorphism of wing shape in the *Drosophila melanogaster* subgroup. *BMC Evol. Biol.* 9:110.
- Gill, B., and P. Martinson. 1991. *New Zealand’s extinct birds*. Random Century, Auckland.
- Gould, S. J. 1977. *Ontogeny and phylogeny*. Belknap Press of Harvard Univ. Press, Cambridge, MA.
- . 2002. *The structure of evolutionary theory*. Belknap Press of Harvard Univ. Press, Cambridge, MA.
- Grant, P. R., and B. R. Grant. 2003. Reversed sexual dimorphism in the beak of a finch. *Ibis* 145:341–343.
- Haldane, J. 1932. *The causes of evolution*. Harper’s, New York.
- Herrel, A., L. D. McBrayer, and P. M. Larson. 2007. Functional basis for sexual differences in bite force in the lizard *Anolis carolinensis*. *Biol. J. Linn. Soc.* 91:111–119.
- Humphrey, L. T. 1998. Growth patterns in the modern human skeleton. *Am. J. Phys. Anthropol.* 105:57–72.
- Huxley, J. S. 1932. *Problems of relative growth*. Methuen, London.
- . 1942. *Evolution: the modern synthesis*. Allen & Unwin, London.
- Jacob, F. 1977. Evolution and tinkering. *Science* 196:1161–1166.
- Kalioztopoulou, A., M. A. Carretero, and G. A. Llorente. 2007. Multivariate and geometric morphometrics in the analysis of sexual dimorphism variation in *Podarcis* lizards. *J. Morphol.* 268:152–165.
- Klingenberg, C. P. 1996. Multivariate allometry. Pp. 23–49 in L. F. Marcus, M. Corti, A. Loy, G. J. P. Naylor, and D. E. Slice, eds. *Advances in morphometrics*. Plenum Press, New York.
- Klingenberg, C. P. 1998. Heterochrony and allometry: the analysis of evolutionary change in ontogeny. *Biol. Rev.* 73:79–123.
- . 2010. Evolution and development of shape: integrating quantitative approaches. *Nat. Rev. Genet.* 11:623–635.
- . 2011. MorphoJ: an integrated software package for geometric morphometrics. *Mol. Ecol. Resour.* 11:353–357.
- Klingenberg, C. P., and M. Zimmermann. 1992. Static, ontogenetic, and evolutionary allometry: a multivariate comparison in nine species of water striders. *Am. Nat.* 140:601–620.
- Klingenberg, C. P., M. Barluenga, and A. Meyer. 2002. Shape analysis of symmetric structures: quantifying variation among individuals and asymmetry. *Evolution* 56:1909–1920.
- Lailvaux, S. P., A. Herrel, B. VanHooydonck, J. J. Meyers, and D. J. Irschick. 2004. Performance capacity, fighting tactics and the evolution of life-stage male morphs in the green anole lizard (*Anolis carolinensis*). *Proc. R. Soc. B* 271:2501–2508.
- Leigh, S. R., and J. M. Cheverud. 1991. Sexual dimorphism in the baboon facial skeleton. *Am. J. Phys. Anthropol.* 84:193–208.
- Ljubisavljević, K., A. Urošević, I. Aleksić, and A. Ivanović. 2010. Sexual dimorphism of skull shape in a lacertid lizard species (*Podarcis* spp., *Dalmatolacerta* sp., *Dinarolacerta* sp.) revealed by geometric morphometrics. *Zoology* 113:168–174.
- Losos, J. B. 2009. *Lizards in an evolutionary tree: ecology and adaptive radiation of anoles*. University of California Press, Berkeley.
- . 2011. Convergence, adaptation, and constraint. *Evolution* 65:1827–1840.
- Lovern, M. B., and J. Wade. 2003. Yolk testosterone varies with sex in eggs of the lizard, *Anolis carolinensis*. *J. Exp. Zool.* 295A:206–210.
- Maddison, W. P., and D. R. Maddison. 2011. Mesquite: a modular system for evolutionary analysis.
- Mahler, D. L., L. J. Revell, R. E. Glor, and J. B. Losos. 2010. Ecological opportunity and therate of morphological evolution in the diversification of Greater Antillean anoles. *Evolution* 64:2731–2745.
- Mallarino, R., O. Campàs, J. A. Fritz, K. J. Burns, O. G. Weeks, M. P. Brenner, and A. Abzhanov. 2012. Closely related bird species demonstrate flexibility between beak morphology and underlying developmental programs. *Proc. Natl. Acad. Sci.* 109:743–763.
- Manceau, M., V. S. Domingues, R. Mallarino, and H. E. Hoekstra. 2011. The developmental role of Agouti in color pattern evolution. *Science* 331:1062–1065.
- McGlothlin, J. W., and E. D. Ketterson. 2008. Hormone-mediated suites as adaptations and evolutionary constraints. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 363:1611–1620.
- Muller, G. B. 2007. Evo-devo: extending the evolutionary synthesis. *Nat. Rev. Genet.* 8:943–949.
- O’Higgins, P., P. Chadfield, and N. Jones. 2001. Facial growth and the ontogeny of morphological variation within and between the primates *Cebus apella* and *Cercocebus torquatus*. *J. Zool.* 254:337–357.
- Ord, T. J., and D. Stuart-Fox. 2006. Ornament evolution in dragon lizards: multiple gains and widespread losses reveal a complex history of evolutionary change. *J. Evol. Biol.* 19:797–808.
- Paradis, E., J. Claude, and K. Strimmer. 2004. APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* 20:289–290.
- Price, T., and G. L. Birch. 1996. Repeated evolution of sexual color dimorphism in passerine birds. *The Auk* 113:842–848.
- Rajakumar, R., D. San Mauro, M. B. Dijkstra, M. H. Huang, D. E. Wheeler, F. Hiou-Tim, A. Khila, M. Courmoyea, and E. Abouheif. 2012. Ancestral developmental potential facilitates parallel evolution in ants. *Science* 335:79–82.
- Ranz, J. M., C. I. Castillo-Davis, C. D. Meiklejohn, and D. L. Hartl. 2003. Sex-dependent gene expression and evolution of the *Drosophila* transcriptome. *Science* 300:1742–1745.
- Revell, L. J. 2011. phytools: an R package for phylogenetic comparative biology (and other things). *Methods Ecol. Evol.* 3:217–223.
- Rohlf, F. 2006. tpsDig, version 2.10. Department of Ecology and Evolution, State University of New York, Stony Brook, NY.
- Sanger, T. J. 2012. The emergence of squamates as model systems for integrative biology. *Evol. Dev.* 14:231–233.
- Sanger, T. J., E. A. Norgard, L. S. Pletscher, M. Bevilacqua, V. R. Brooks, L. J. Sandell, and J. M. Cheverud. 2011. Developmental and genetic origins of murine long bone length variation. *J. Exp. Zool. Part B Mol. Dev. Evol.* 316B:146–161.
- Sanger, T. J., D. L. Mahler, A. Abzhanov, and J. B. Losos. 2012a. Roles for modularity and constraint in the evolution of cranial diversity among *Anolis* lizards. *Evolution* 66:1525–1542.

- Sanger, T. J., L. J. Revell, J. J. Gibson-Brown, and J. B. Losos. 2012b. Repeated modification of early limb morphogenesis programmes underlies the convergence of relative limb length in *Anolis* lizards. *Proc. R. Soc. B* 279:739–748.
- Schaefer, K., P. Mitteroecker, P. Gunz, M. Bernhard, and F. Bookstein. 2004. Craniofacial sexual dimorphism patterns and allometry among extant hominids. *Ann. Anat.* 186:471–478.
- Schluter, D., T. D. Price, A. O. Mooers, and D. Ludwig. 1997. Likelihood of ancestor states in adaptive radiation. *Evolution* 51:1699–1711.
- Schmalhausen, I. I. 1949. *Factors of evolution: the theory of stabilizing selection*. Blakiston, Philadelphia, PA.
- Schneider, C. A., W. S. Rasband, and K. W. Eliceiri. 2012. NIH Image to ImageJ: 25 years of image analysis. *Nat. Meth.* 9:671–675.
- Schoener, T. W. 1967. The ecological significance of sexual dimorphism in size in the lizard *Anolis conspersus*. *Science* 155:474–477.
- Shine, R. 1989. Ecological causes for the evolution of sexual dimorphism: a review of the evidence. *Q. Rev. Biol.* 64:419–461.
- Shingleton, A. W., W. A. Frankino, T. Flatt, H. F. Nijhout, and D. J. Emlen. 2007. Size and shape: the developmental regulation of static allometry in insects. *BioEssays* 29:536–548.
- Shingleton, A. W., C. K. Mirth, and P. W. Bates. 2008. Developmental model of static allometry in holometabolous insects. *Proc. R. Soc. B* 275:1875–1885.
- Siefferman, L., and G. E. Hill. 2005. Evidence for sexual selection on structural plumage coloration in female eastern bluebirds (*Sialia sialis*). *Evolution* 59:1819–1828.
- Sokal, R. R., and F. J. Rohlf. 1995. *Biometry*. Freeman and Co., New York, NY.
- Sovio, U., A. J. Bennett, I. Y. Millwood, J. Molitor, P. F. O'Reilly, N. J. Timpson, M. Kaakinen, J. Laitinen, J. Haukka, and D. Pillas. 2009. Genetic determinants of height growth assessed longitudinally from infancy to adulthood in the northern Finland birth cohort 1966. *PLoS Genet.* 5:e1000409.
- Vavilov, N. I. 1922. The law of homologous series in variation. *J. Genet.* 12:47–89.
- Voje, K. L., and T. F. Hansen. 2012. Evolution of static allometries: adaptive change in allometric slopes of eye span in stalk-eyed flies. *Evolution* 67:453–467.
- Wake, D. B. 1991. Homoplasy: the result of natural selection, or evidence of design limitations? *Am. Nat.* 138:543–567.
- Wake, D. B., M. H. Wake, and C. D. Specht. 2011. Homoplasy: from detecting pattern to determining process and mechanism of evolution. *Science* 331:1032–1035.
- Wealthall, R. J., and S. W. Herring. 2006. Endochondral ossification of the mouse nasal septum. *Anat. Rec. A Discov. Mol. Cell. Evol. Biol.* 288:1163–1172.
- Wilkins, A. S. 2004. Deciphering the swordtail's tale: a molecular and evolutionary quest. *BioEssays* 26:116–119.
- Williams, T. M., and S. B. Carroll. 2009. Genetic and molecular insights into the development and evolution of sexual dimorphism. *Nat. Rev. Genet.* 10:883–883.
- Williams, T. M., J. E. Selegue, T. Werner, N. Gompel, A. Kopp, and S. B. Carroll. 2008. The regulation and evolution of a genetic switch controlling sexually dimorphic traits in *Drosophila*. *Cell* 134:610–623.
- Zamudio, K. R. 1998. The evolution of female-biased sexual size dimorphism: a population-level comparative study in horned lizards (*Phrynosoma*). *Evolution* 52:1821–1833.
- Zelditch, M. L., D. L. Swiderski, H. D. Sheets, and W. L. Fink. 2004. *Geometric morphometrics for biologists: a primer*. Elsevier Science, Amsterdam.
- Zheng, Z., and M. J. Cohn. 2011. Developmental basis of sexually dimorphic digit ratios. *PNAS* 108:16289–16294.

Associate Editor: E. Abouheif

Supporting Information

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S1. Additional methods, tables, and figures.