ORIGINAL ARTICLE

ROLES FOR MODULARITY AND CONSTRAINT IN THE EVOLUTION OF CRANIAL DIVERSITY AMONG *ANOLIS* LIZARDS

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Complex organismal structures are organized into modules, suites of traits that develop, function, and vary in a coordinated fashion. By limiting or directing covariation among component traits, modules are expected to represent evolutionary building blocks and to play an important role in morphological diversification. But how stable are patterns of modularity over macroevolutionary timescales? Comparative analyses are needed to address the macroevolutionary effect of modularity, but to date few have been conducted. We describe patterns of skull diversity and modularity in Caribbean *Anolis* lizards. We first diagnose the primary axes of variation in skull shape and then examine whether diversification of skull shape is concentrated to changes within modules or whether changes arose across the structure as a whole. We find no support for the hypothesis that cranial modules are conserved as species diversify in overall skull shape. Instead we find that anole skull shape and modularity patterns independently converge. In anoles, skull modularity is evolutionarily labile and may reflect the functional demands of unique skull shapes. Our results suggest that constraints have played little role in limiting or directing the diversification of head shape in *Anolis* lizards.

KEY WORDS: Craniofacial, evo-devo, macroevolution, morphology, skull.

Modularity, or the partitioning of biological variation into semiindependent blocks of traits, is expected to play a significant role in the evolution of complex morphologies (Olson and Miller 1958; Riedl 1978; Cheverud 1996; Klingenberg 2008, 2010). Modules are present at all biological levels, from molecules to complete organisms, and influence processes across different temporal scales, from individual development to macroevolution (reviewed in Klingenberg 2008). Whether molecular or morphological, modules create evolutionary building blocks by limiting relatively strong correlations to within functionally or developmentally related sets of traits. Although general patterns of modularity and the origin of modular organization have been the subject of extensive research (e.g., Wagner et al. 2007; Klingenberg 2008;

Wagner and Zhang 2011), the macroevolutionary impact of modular organization on morphological diversification has not been as widely studied (Eble 2004, 2005; Klingenberg 2008 and references therein).

The concepts of modularity and integration are closely related. At a given level of organization, a module is defined as a suite of correlated traits that are relatively autonomous with respect to other traits (Wagner et al. 2007). Integration refers to the strength and pattern of covariance within a module (or, alternatively, of an entire morphological structure). For morphological structures, modularity arises due to shared developmental origins or through the actions of pleiotropic loci (reviewed in Klingenberg 2008). Development builds morphological traits

through the proliferation, subdivision, specification, and growth of cellular populations. Sets of traits that arise from the same cellular origins or that are influenced by the same growth factors will covary due to their shared developmental origins. Quantitative genetic theory predicts that patterns of integration and modularity will also be shaped by external selective pressures (Cheverud 1996; Wagner 1996; Wagner and Altenberg 1996). In other words, selection may mold the pleiotropic networks underlying groups of functionally related traits to yield genetically integrated modules that respond to selection as a single unit. Evolutionary modularity is expected to emerge when genetic or developmental integration patterns are maintained over geologic time scales and conserved among descendant species.

If patterns of integration are stable over long time scales and across speciation events, they are expected to bias large-scale patterns of trait evolution. Specifically, traits within the same module will be constrained to evolve in a correlated manner, whereas traits among modules will evolve independently (Olson and Miller 1958; Lande 1980; Wagner 1996). In this case, we expect that much of the observed diversity among related species is due to correlated evolution of traits within modules and there will be little correlated evolution among traits belonging to different modules.

Caribbean *Anolis* lizards, or anoles, are an extensively studied model system of evolutionary diversification (Losos 2009) and are useful to study the evolution of phenotypic modularity during adaptive diversification. Anoles are endemic to the Caribbean, Central America, and South America and have radiated into a variety of arboreal niches during the Cenozoic. Anole species have adapted to specialize on different microhabitats via the evolution of a suite of morphological traits, which confer habitat-specific performance advantages. Anole microhabitats may be distinguished in broad terms by differences in perch height and perch width, and anoles have adapted to these microhabitats primarily through evolutionary changes in limb length, body size, and features of the adhesive toe pads that they use to cling to arboreal substrates. Sets of between four and six habitat specialists, termed ecomorphs, have repeatedly arisen on the islands of the Greater Antilles: Cuba, Hispaniola, Jamaica, and Puerto Rico. The ecomorphs are named for the microhabitat most often inhabited by those species: trunk, trunk-crown, twig, trunk-ground, crown-giant, and grass-bush. These ecomorphs exhibit striking convergence in morphology, behavior, and ecology, and have been the subject of much study (e.g., Losos et al. 1998; Beuttell and Losos 1999; Johnson et al. 2008, 2010; Pinto et al. 2008; reviewed in Losos 2009). In addition to their well-known diversity in body shape, *Anolis* lizards also exhibit impressive head shape diversity, which has been the subject of relatively little study in comparison to postcranial characters (Fig. 1A; Beuttel and Losos 1999; Harmon et al. 2005).

The amniote skull is a well-studied model of evolutionary-developmental biology, in general, and studies of modularity and morphological integration in particular (e.g., Cheverud 1982; Marroig and Cheverud 2001; Marugan-Lobon and Buscalioni 2006; Wroe and Milne 2007; Jamniczky and Hallgrímsson 2009; Kulemeyer et al. 2009; Drake and Klingenberg 2010). In fact, studies of mammalian skull modularity have played a major role in the development of evolutionary theories of modularity. Skull modularity exhibits striking stability among therian mammals (marsupials and placentals), leading some authors to hypothesize that this pattern is the result of conserved developmental processes governing skull morphogenesis (Goswami 2006a,b; Marroig et al. 2009; Porto et al. 2009; Shirai and Marroig 2010).

Our research examines the evolution of skull morphology and modularity in Caribbean *Anolis* lizards. We first quantified anole cranial diversity using geometric morphometric methods. We used phylogenetic methods to uncover general patterns of Caribbean anole skull evolution, and to test whether ecologically similar lineages have converged in overall skull shape. We then tested the hypothesis that patterns of modularity are evolutionarily conservative—that is, that integration patterns correlate more with evolutionary history than with the functional or selective demands associated with particular skull shapes.

Methods
ANOLIS CRANIOFACIAL DIVERSITY

We used geometric morphometrics to identify the primary axes of craniofacial shape variation among *Anolis* lizards. We examined 106 *Anolis* species, primarily representing Caribbean taxa, although also including four representatives of the predominantly South American Dactyloa clade. These species represent all of the major deep lineages of anoles, and include both a wide representation of microhabitat ecomorphs from each of the four islands in the Greater Antilles, as well as many additional species that do not fit into any ecomorph class (referred to as "non-ecomorph" anoles from here on). A total of 638 skulls were examined ranging from one individual for relatively rare species to 44 specimens for one common species. Skulls were obtained from the Museum of Comparative Zoology (MCZ) at Harvard University and the National Museum of Natural History, Smithsonian Institution. New skeletal material was also prepared by TJS from MCZ museum specimens and recently collected material.

We took a scaled digital photograph of each skull in dorsal aspect on a standardized background using an Axiocam camera mounted on a Zeiss Discovery V8 stereomicroscope. Twenty-four landmarks (four median and 20 lateral paired landmarks) were placed on each skull using tpsDIG2 (Fig. 1B, Rohlf 2005). We selected landmarks that covered most of the cranial skeletal elements and could be applied to all species. Several additional steps
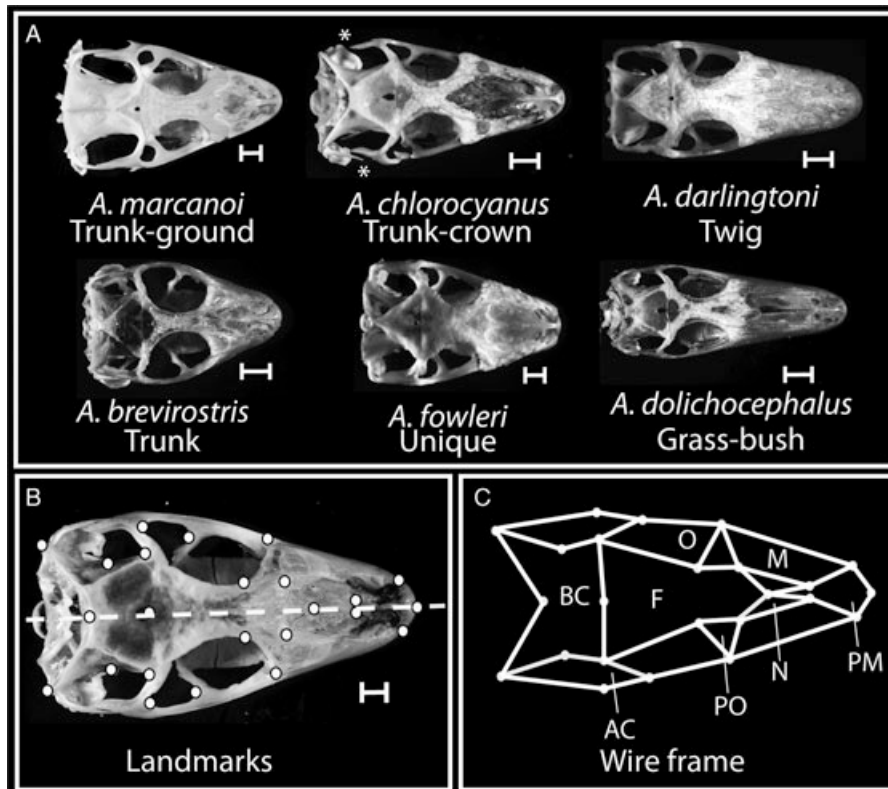
Figure 1. Craniofacial diversity and geometric morphometric landmarks. (A) A sample of *Anolis* skulls representing a wide range of ecomorphological diversity from the island of Hispaniola. Note that in many skulls, the postorbital and squamosal have become disarticulated during preparation leading to an underrepresentation of adductor chamber size (*). (B) Twenty-four evenly spaced landmarks were used in the analysis of skull diversity and object symmetry was taken into account for all analyses (dotted line). (C) We use deviations of wireframes to illustrate variation in skull shape because these allow us to highlight how the shapes of specific skeletal elements or functional units have evolved. BC = parietal (braincase); AC = adductor chamber; O = orbit; F = frontal; PO = preorbital; N = nasal; M = maxilla; PM = premaxilla. Scale bars equal 1 mm.

were taken to accurately place landmarks of crown-giant anoles, which undergo extreme osseous elaboration (Appendix 1, [SA1]). Measurement error associated with photographing the skulls and digitizing landmarks was negligible (repeatability: 0.85–0.99).

We performed geometric morphometric analyses of shape variation in MorphoJ (Klingenberg 2008, 2011). First, we calculated average values of landmark coordinates for each species using species-specific Procrustes superimposition, which removes the effects of position, orientation, and scale from the data (Dryden and Mardia 1998; see also Zelditch et al. 2004). Centroid size, the preferred measure of size in geometric morphometrics, is computed during Procrustes superimposition as the sum of squared distances from the centroid to the series of landmarks. 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). The average configurations for each species were then combined into a single dataset, aligned using Procrustes superimposition, and used for comparative analyses.

Anoles vary substantially in body size, ranging from 33 to 191 mm in snout-to-vent length (Losos 2009). This variation in body size may generate strong allometric correlations that are not fully accounted for during Procrustes superimposition (Zelditch et al. 2004). We tested for allometry in our geometric shape data using a permutation test while accounting for phylogenetic relationships among species (10,000 iterations) and after finding a significant effect (see Results) all subsequent analyses were performed on size-corrected shape variables (Klingenberg 2011). Specifically, we performed a multivariate regression of the independent contrasts of Procrustes coordinates on the independent contrasts of centroid size (Monteiro 1999; Klingenberg et al. 2003; Zelditch et al. 2004; Elmer et al. 2010). Using this regression equation, we then computed shape residuals in the original species space (a similar procedure is described in Revell 2009). We then conducted a principal component analysis (PCA) on the resulting shape residuals. We use deformed wireframes to highlight shape changes described by each principal component (PC hereafter, Fig. 1C). The magnitude of deformation in wire

diagrams (i.e., scale factor) is depicted as a Procrustes distance, corresponding to the degree of shape change along a PC axis for a given distance.

We investigated patterns of skull evolution among *Anolis* ecomorphs in a phylogenetic context using a recently published ultrametric phylogeny of *Anolis* relationships, based on an analysis of mtDNA (the maximum clade credibility phylogeny of Mahler et al. 2010). To visualize phylogenetic patterns of head shape evolution in our species sample, we used maximum likelihood to estimate ancestral trait values for the first four shape PCs 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]). We also tested whether anole skull shape exhibited significantly more phylogenetic signal than expected under a null hypothesis of "no phylogenetic structure" using Klingenberg and Gidaszewski's (2010) phylogenetic permutation test for multivariate shape data (10,000 permutations).

To test whether distantly related species from the same ecomorph class have converged in skull shape, we conducted a discriminant function analysis (DFA) on ecomorph class using phylogenetically subsampled datasets. Many members of the Greater Antillean ecomorph classes have morphologically similar sister species that are assigned to the same ecomorph class (Losos et al. 2006; Losos 2009), and a DFA that includes such species will discriminate ecomorphs on the basis of similarities due to common ancestry as well as those due to convergence. We therefore conducted DFAs on datasets reduced to independently evolved members of the ecomorph classes. To do this, we first conducted a maximum likelihood equal-rates discrete character ancestral reconstruction of ecomorph class on the phylogeny of all 106 species. This reconstruction included the six traditional anole ecomorph categories plus a single non-ecomorph category (data not shown). We examined this reconstruction to identify lineages that had evolved to an ecomorph "state" and then continued to diversify into a monophyletic or in one case paraphyletic lineage of anoles that are all assignable to a single ecomorph state. Across the phylogeny, for each such lineage identified, we randomly retained a single species to represent the independent evolution of that ecomorph state in that lineage, and we conducted our DFAs using this set of species ($n = 58$). We repeated our subsampling procedure 100 times, each time randomly choosing which species to retain from the within-ecomorph radiations. In each iteration, we size-corrected and reduced the dimensionality of our data as described above (retaining four PC axes), and we tested for equal group means between each anole ecomorph class ($n = 6$) and the remaining species using a permutation test (10,000 iterations, Klingenberg 2011). We generated summary statistics for each ecomorph comparison by comparing the average Hotelling T-squared value to the F-distribution (a multivariate generalization of the t-test).

MORPHOLOGICAL INTEGRATION AND MODULARITY

Studies of morphological integration require relatively large sample sizes within each species to adequately detect the covariation among traits. Therefore, to test the impact of modularity on morphological evolution, we examined patterns of modularity in eight species for which we were able to sample large numbers of individuals. Our sample included six species that either independently evolved extremely short (*A. cybotes, A. sagrei, A. barbouri, and A. distichus*) or extremely elongate (*A. bahorucoensis* and *A. carolinensis*) skull morphologies, and two species with intermediate morphologies (*A. marmoratus* and *A. bonairensis*).

We examined four alternative hypotheses of cranial modularity (Fig. 2, Table 1). The "*Anolis* Skull Shape Hypothesis" (ASH) predicts two modules—the rostrum and cranium—and was inspired by the general patterns of variation observed in our analysis of skull diversity (see below). Two hypotheses, "Mammalian Morphometric Hypotheses 1 and 2" (MMH) are derived from previous studies of cranial modularity in a broad diversity of mammal species (e.g., Goswami 2006a,b; Porto et al. 2009), and each also predicts two cranial modules. These divisions may reflect the cellular origins of the skull (with neural crest composing the face and mesoderm composing the neurocranium) or the differential timing of growth of these two regions (e.g., Cheverud 1996; Marroig and Cheverud 2001; Hallgrímsson and Lieberman 2008; also see Noden and Trainor 2005; Chai and Maxson 2006 for reviews of early cranial development). Because we do not yet know the precise landmark homologies between mammalian and squamate skulls, we have tested two alternative sets of landmark configurations (MMH1 and MMH2) emphasizing the partitioning of the skull into facial and neurocranial modules, the predominant pattern observed in mammals. Lastly, we tested a fourth hypothesis, the "tripartite hypothesis" (TH), which divides the skull into its three primary functional units, the rostrum (or snout), orbital region, and the braincase and jaw adductor muscle chambers.

Only fully intact skulls were used for this analysis. To fully represent the shape of the skull, 30 landmarks (four median and 26 paired) were placed on the dorsal image of the skull using tps-DIG2 (Rohlf 2005, Fig. 2B). Differences in size that arise from the somatic growth of the whole organism can mask underlying modules by generating the appearance of global integration (Olson and Miller 1958; Ackermann 2005; Klingenberg 2009; Jamniczky and Hallgrímsson 2009). Therefore, we conducted modularity analyses on covariance matrices generated from residuals of a multivariate regression of Procrustes landmarks on centroid size. Modularity analyses were conducted in MorphoJ, which automatically corrects for object symmetry (Klingenberg et al. 2002; Klingenberg 2009).

As mentioned above, accurate estimates of morphological integration require that sufficient numbers of individuals be sampled to ensure accurate estimation of trait correlation or covariance
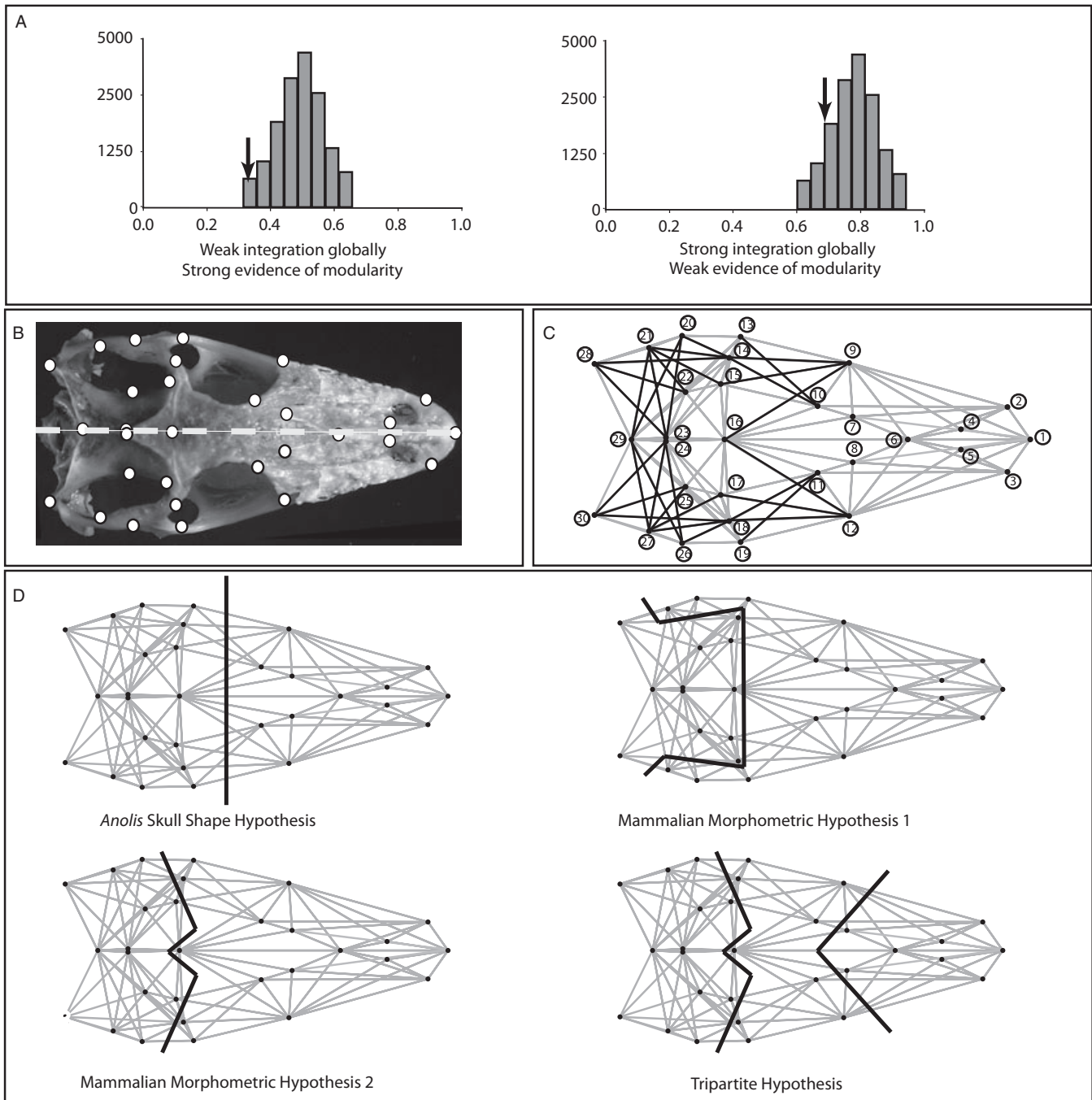
Figure 2. Morphological integration and modularity methods. **(A)** Klingenberg's method contrasts the RV-coefficient of a hypothesized partition scheme (arrow) against a distribution of RV-coefficients from 20,000 random partitions of the skull (histogram). There is strong support for modularity when the RV-coefficient of the hypothesized modularity scheme is significantly lower than random (i.e., within the 5th percentile of random RV-coefficient values; *P* < 0.05). The position of the null histogram along the x-axis represents the degree of integration throughout the structure. To study patterns of craniofacial modularity in anoles, we placed 30 landmarks on the dorsal view of the skull **(B)**. We connected landmarks into adjacency graphs that were used to generate a null distribution of covariation intensity **(C)**. We calculated RV-coefficients among partitions of contiguous landmarks using two partitioning schemes that differed in how landmark contiguity was defined. First, we included all spatially contiguous partitions that connect adjacent landmarks (light and bold lines). We then tested a second set of partitions with noncontiguous partitions removed, those not directly connected by skeletal tissues (bold lines removed). We tested four modularity hypotheses to see which would best explain integration patterns in the anole skull **(D)**. See text for further discussion on Klingenberg's application of the RV-coefficient and descriptions of our modularity hypotheses.

Table 1. Models of morphological integration and modularity.

Name	Description
Modularity hypotheses	
Anolis Skull Shape Hypothesis (ASH)	Divides skull into anterior and posterior regions based on analysis of morphological diversity.
Mammalian Morphometric Hypotheses 1 and 2 (MM1 and MM2)	Based on mammalian integration patterns, divides the skull into neurocranium and face representing division of cell progenitor sources or temporal differences in growth rate.
Tripartite Hypothesis (TH)	Divides the skull into its major functional components: rostrum, orbits, and braincase/adductor chambers.
Null partition models	
Complete	All partitions connecting landmarks on the same bone, adjacent bones, and connected with soft tissues used.
Contiguous	Only spatially contiguous partitions used, thought to reflect developmental tissue–tissue interactions.

matrices. At low sample sizes, such matrices may become unstable, and stochastic differences among individuals can strongly affect matrix structure. To assess the relationship between sample size and matrix stability in the anole skull, we performed a rarefaction analysis of within-species trait matrix correlations for two species, *A. cybotes* and *A. carolinensis*, using the Mathematica "Modularity" software package (Goswami 2006a; Goswami and Polly 2010; see SA4 for a detailed description of this analysis). Following Goswami (2006a), for each of these species, we used the rarefaction curve to determine the number of individuals for which the mean within-species trait matrix correlation comfortably exceeded the range of pairwise among-species trait matrix correlations, calculated for species with more than 20 individuals. Based on these analyses (Fig. SA4.1), we conducted intraspecific analyses of modularity and morphological integration only for species with 20 or more individuals ($20 < n < 40$; the eight species that met these criteria were listed above). However, comparisons among species with divergent head shapes were performed on species with as few as 12 individuals sampled (15 species met this criterion), as interspecific comparisons among species with different morphologies are not as sensitive to fluctuations in matrix structure. Although results from rarefaction analyses should be interpreted cautiously they do serve as a use-ful approximation of the minimum sample size appropriate for modularity analyses.

We use Klingenberg's application of the RV-coefficient to test among hypothesized modules in the anole skull (Escoufier 1973; Klingenberg 2009). The RV-coefficient is a measure between 0 and 1 that represents the strength of association between two sets of variables (Escoufier 1973). The RV-coefficient can be interpreted as the multivariate extension of the bivariate R^2 value and may be used to test a priori hypotheses of modularity and morphological integration by comparing correlations among sets of landmarks (Fig. 2A–D, Klingenberg 2009). The RV-coefficient is 0 when there is no covariation among sets of landmarks and 1 when these sets exhibit perfect covariance. To determine whether there is support for modularity in anole skulls, we compared the RV-coefficient for our four hypothesized landmark partitioning schemes to RV-coefficients estimated from 20,000 random partitions (Fig. 2A–C). Strong evidence of modularity exists when there is moderate integration of landmarks across the entire structure and the RV-coefficient for a given modularity hypothesis is less than the RV-coefficients of 95% of the random contiguous partitions of the structure (similar to a *P*-value with a 0.05 significance threshold).

Including partitions that are not biologically realistic in the null distribution could bias against finding signatures of modularity by over-representing landmark partition schemes with low correlations (since noncontiguous and distant landmarks are less likely to be strongly correlated). Therefore, we used adjacency graphs based initially on Delaunay triangles to outline the potential partitions of the skull used during randomization (Klingenberg 2009). We tested two different sets of null partitions (Fig. 2C, Table 1). Based on the idea that integration patterns are the direct result of developmental interactions among tissues, we first tested only contiguous partitions, those directly connected by skeletal tissue (Klingenberg 2009; Drake and Klingenberg 2010). But integration patterns continue to develop into late ontogenetic stages and adulthood through interactions among muscles, bones, and other tissues, as described in the Palimpsest Model of modularity (Hallgrímsson et al. 2009). Therefore, we also tested a second set of partitions that includes those that crosssoft tissue compartments, specifically the adductor chambers and orbits (Fig. 2C, bold lines).

To test the hypothesis that closely related species share integration patterns, we examined the relationship between phylogenetic distance and correlation among integration patterns. We calculated pairwise matrix correlations among landmark covariance matrices for all species with greater than 12 individuals (Ackermann and Cheverud 2000; Marroig and Cheverud 2001; Jamniczky and Hallgrímsson 2009). This includes 15 species and 105 pairwise comparisons. To distinguish the effects of phylogenetic similarity from functional similarity, closely related
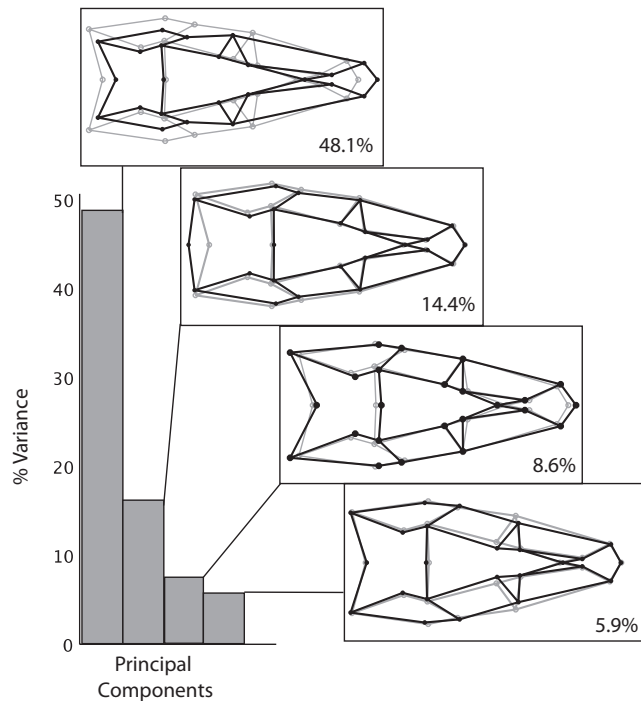
Figure 3. Summary of craniofacial variation among *Anolis* lizards after size correction. Gray wireframe represents the centroid of the shape data and the black wireframe represents the positive deviation in shape along that axis (scale factor = 0.1). PC1 correlates with variation in rostrum length and skull width. PC2 summarizes the size and shape of the adductor chambers, as well as the adductor muscle scar along the posterior ridge of the parietal. PC3 summarizes the displacement of the most anterior and posterior landmarks. PC4 primarily summarizes variation in relative rostrum width. See text for further details.

ecomorphologically similar species were not compared in this analysis. Phylogenetic distances among species were calculated as patristic distances from the ultrametric maximum clade credibility tree of Mahler et al. (2010). Correlations between species-specific covariance matrices were calculated in MorphoJ including values along the diagonal (Klingenberg and McIntyre 1998). Finally, we used a Mantel test to look for correlations between the matrices of pairwise interspecific landmark correlations and pairwise phylogenetic distances. We used permutation tests (10,000 iterations) to assess the significcance of the pair wise comparisons and the Mantel test.

Results
ANOLIS CRANIOFACIAL DIVERSITY
Head size varies greatly among anoles and allometry accounts for 13.3% of total craniofacial shape variation (permutation test: $P < 0.001$; Fig. S1). In the PCA on size-corrected shape data, the first four PC axes account for 77.0% of the variation (Figs.

3 and 4; SA2). Subsequent PCs each explain less than 5% of the variation, are difficult to interpret biologically, and are not considered further. PC1 explains 48.1% of the variation and correlates with variation in head length and head width. Changes in skull length are concentrated in the face and are slightly more exaggerated than changes in skull width. PC2 (14.4% of craniofacial variation) summarizes variation in the size, shape, and position of the adductor chambers, and variation in the posterior margin of the dorsal surface of the parietal along the adductor muscle scars. PC3 and PC4 account for a small fraction of the total variation (<10% each) and the variation along these axes may generally be attributed to relatively few species with unique head morphologies. PC3 explains the displacement of the most anterior and posterior landmarks, particularly in the anoles of the *carolinensis* and *roquet* series. PC4 represents variation in relative snout width, and much of the variation along this axis is explained by several crown-giant anoles and the twig anole *A. insolitus*.

Our maximum likelihood ancestral trait reconstruction of individual shape variables suggests that relatively short-faced anoles (those more than one standard deviation [SD] less than the centroid value on PC1) independently evolved from more intermediate ancestors numerous times: four times in ecomorph lineages and at least three more times in non-ecomorph lineages (Fig. 5). Remarkably elongate skulls (those more than two SDs greater than the centroid value on PC1) evolved at least two times. In all lineages, variation in skull length appears to be primarily due to changes in the rostrum, mostly caused by changes in the nasal bones, maxillae, and premaxilla.

The cranial shape data exhibit significant phylogenetic signal (permutation test: $P < 0.001$; Fig. S3). Nonetheless, the phylogenetically subsampled DFA reveals that anoles belonging to the trunk-ground and twig ecomorph classes are each convergent (Table 2, Fig. 4). Briefly, trunk-ground species tend to have short, robust skulls with large adductor chambers, whereas twig anoles have relatively elongate heads with large adductor chambers. Trunk-crown and grass-bush anoles have highly variable skull morphologies, ranging from relatively elongate to relatively short. Trunk anoles also do not exhibit significant convergence (possibly due to the fact that only two independent lineages are classified as trunk anoles, limiting the power to classify members of this ecomorph), but nonetheless tend to have short faces and relatively small adductor chambers compared to most other species. Similarly, crown-giant anoles are found near the shape centroid but are not statistically convergent.

MORPHOLOGICAL INTEGRATION AND MODULARITY
Species-specific integration histograms tend to be centered between 0.3 and 0.5 (Figs. 6 and 7). No single hypothesis of modularity was supported among all eight species examined but species convergently similar in general head shape also tended to share
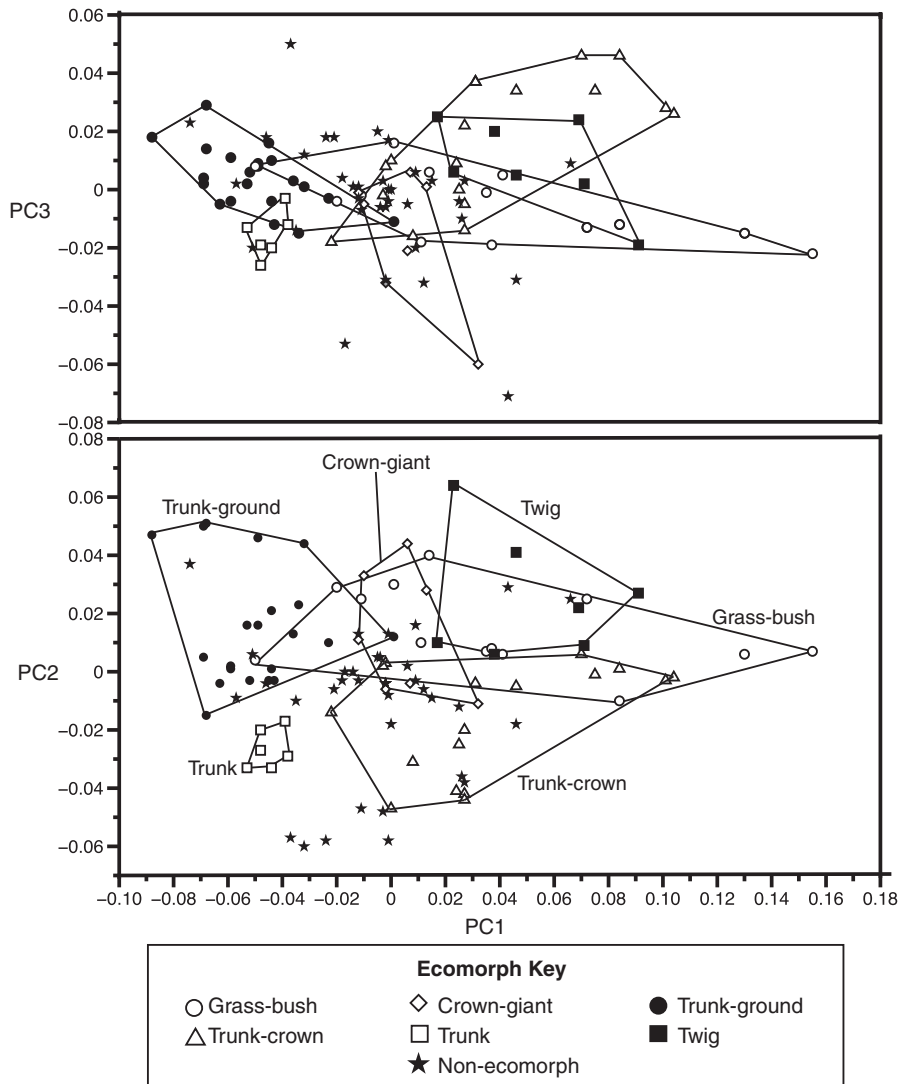
Figure 4. Craniofacial morphospace. Plot of craniofacial morphospace for 106 species of *Anolis* lizard summarized by ecomorph. Note, most of the variation in skull shape is explained by PC1. Trunk-ground anoles tend to have short rostra with large adductor chambers, trunk anoles possess short faces with small adductor muscle chambers, twig anoles exhibit moderately elongate skulls with large adductor chambers, and crown-giant anoles are generally near the centroid of the data. Trunk-crown and grass-bush anoles are highly variable.

similar patterns of modularity (Figs. 6 and 7). Of the models partitioning the head into two modules, ASH and MM1 had the greatest support whereas MM2 was rarely found to be significant (Figs. 6, 7, and SA4.2). In general, modularity hypotheses were poorly supported in anole species with short skulls and large adductor chambers (*A. cybotes*, *A. sagrei*). For the species with elongate skulls, the three-partition modularity hypothesis (TH), which partitions the skull into the snout, orbital region, and braincase/adductor chamber, was the best-supported hypothesis. For the remaining four species, a simple two-partition modularity hypothesis (ASH or MMH1) was most strongly supported. Differences between the two types of partition models tested, complete, and contiguous, do not affect the interpretation of our results (Figs. SA4.3,

SA4.4).

Seven species exhibit unimodal integration histograms, as would be expected, but the histogram of *A. cybotes* is bimodal in the dataset using 30 landmarks. This is the result of extremely strong correlations among landmarks on the neurocranium and adductor chambers, specifically strong correlations between landmarks 22, 23, and 29 (Fig. SA4.5). The bimodality arises because partitions that contain combinations of these landmarks tend to show greater integration than those in which the landmarks are in different modules. Histograms are unimodal when analyses are rerun without landmark 29, and exclusion of this landmark did not qualitatively change our results.

Pairwise interspecific correlations among cranial landmark covariance matrices range from 0.27 to 0.81 (Fig. 8). However,
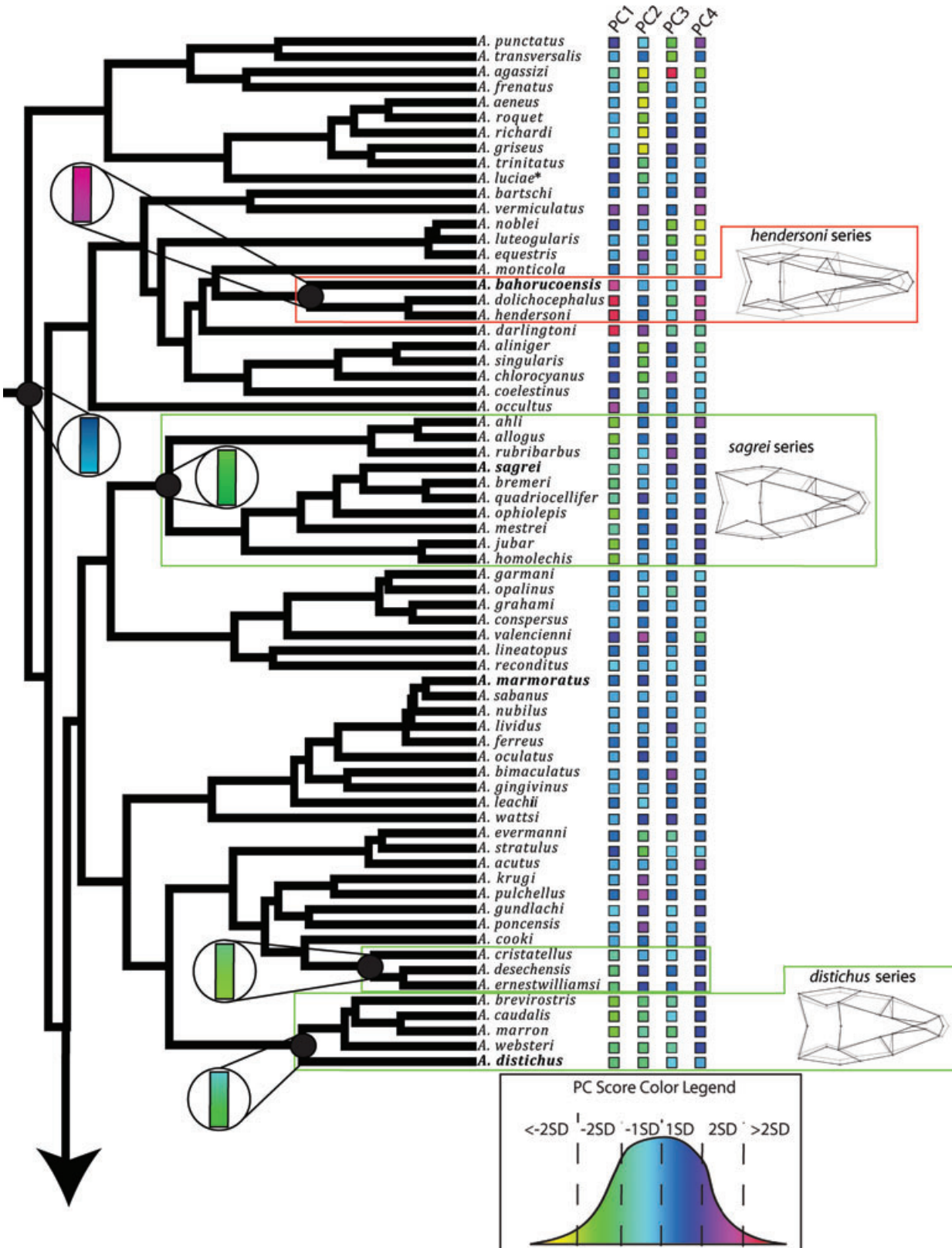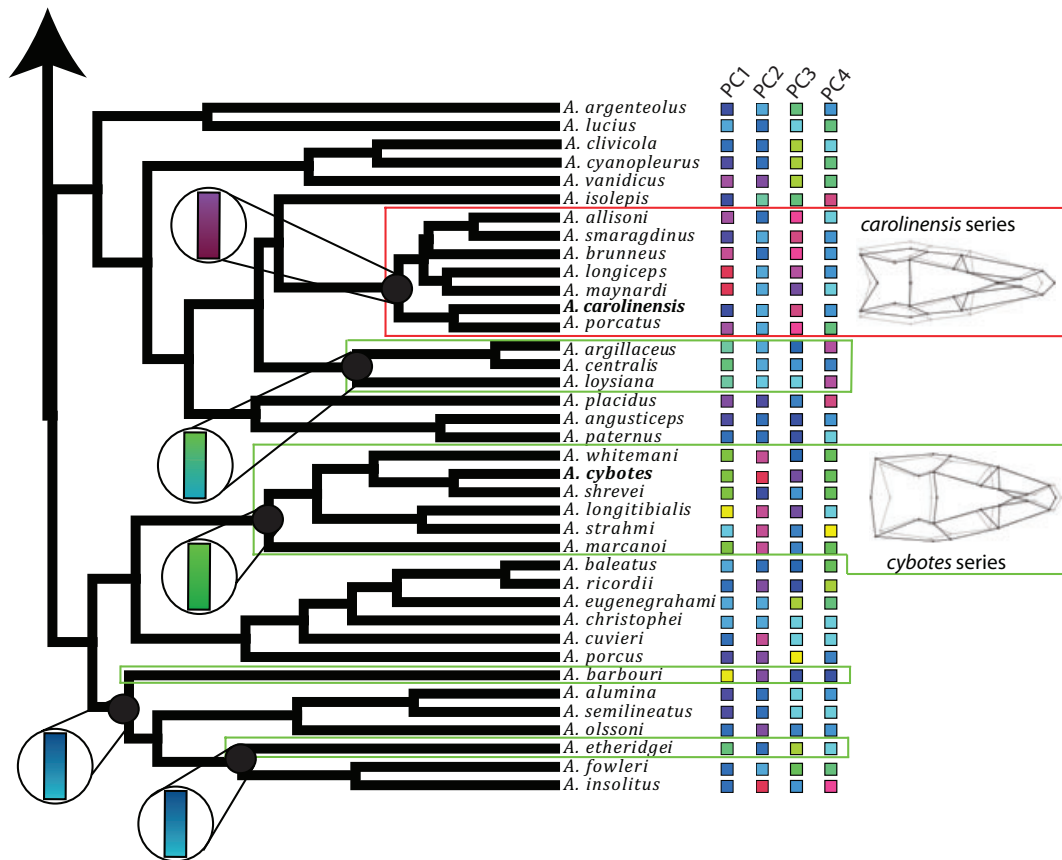
Figure 5. Continued on next page.

Figure 5. Phylogenetic patterns of *Anolis* skull shape evolution. A comparison of patterns of variation in PCs 1–4 (right columns) to the phylogenetic relationships of anoles reveals that short skulls (scores on PC1 with values at least one standard deviation lower than the centroid value) have evolved a minimum of seven times (green, species-rich lineages highlighted). Extremely elongate skulls (PC1 scores 2.0 or more standard deviations greater than the centroid value) have evolved at least twice (red). Colored gradients in rectangular insets represent 95% confidence intervals for maximum likelihood ancestral character state reconstruction for PC1 for nodes of particular interest. Taxa shown in bold are those used in the rarefaction analysis. *Anolis bonairensis* is not included in the phylogeny but is the sister species to *A. luciae* (*).

there is no significant relationship between phylogenetic distance and the strength of the correlation (slope not statistically different than 0, Mantel test $P = 0.224$), indicating that closely related species are not more likely to share common patterns of modularity and morphological integration.

Discussion

The study of modularity and morphological integration at both micro- and macroevolutionary scales in the same system has the potential to shed new light on the long-term significance of trait correlations. Correlations among traits may hinder morphological evolution by limiting the ability of traits to evolve independently (Olson and Miller 1958; reviewed in Merilä and Björklund 2004). Conversely, modularity is thought to facilitate evolution by enabling coordinated variation in functionally or developmentally related traits and limiting adverse changes in unrelated traits. Modularity also allows for high degrees of variability in

certain traits, potentially giving those traits greater evolutionary flexibility (Hendrikse et al. 2007). However, these theories presume that patterns of trait correlations are conserved over long evolutionary time scales. In the short term, trait correlations can influence rates of morphological evolution (e.g., Wagner 1988; Arnold 1992; Cheverud 1996; Schluter 1996), but it is less clear whether such correlations persist over geological time scales and affect morphological diversification. One way to assess whether trait correlations constrain morphological diversification is to test the hypothesis that patterns of integration are related to the degree of evolutionary relationship rather than the functional demands of a species.

MODULARITY, MORPHOLOGICAL DIVERSITY, AND CONVERGENCE OF CORRELATION PATTERNS
Modularity and morphological integration are two related concepts that have received much attention in recent years (e.g.,

Table 2. Summary of results from discriminant function analysis on 100 phylogenetically subsampled datasets testing for convergence in skull shape among *Anolis* ecomorphs. Bold values represent significant values indicating strong evidence of convergence.

	Number of individual origins	Mean Hotelling $T^2 \pm 1$ SD	F	P-value	Proportion of significant permutation tests
Trunk-ground	5*	98.18 ± 19.58	2.44	**0.008**	**0.87**
Trunk-crown	4	48.40 ± 11.40	1.20	0.311	0.02
Grass-bush	4	42.89 ± 14.45	1.07	0.427	0.00
Twig	5	108.23 ± 22.12	2.34	**<0.001**	**0.99**
Trunk	2	42.68 ± 9.41	1.06	0.431	0.01
Crown-giant	4	55.71 ± 10.44	1.38	0.191	0.02

*Ancestral character state reconstruction is ambiguous whether trunk-ground anoles evolved four or five times. This uncertainty does not qualitatively change the interpretation of this analysis.

Wagner et al. 2007; Klingenberg 2008, 2010; Hallgrímsson et al. 2009). Integration refers to the tendency for particular traits within a structure to covary (Hallgrímsson et al. 2009). Modularity refers to the partitioning of variation among functionally or developmentally related traits. Modularity and integration are relative terms that can only be interpreted in context with other traits and related species. For example, general patterns of cranial integration are conserved among therian mammals, but trait complexes vary in degree of integration (Goswami 2006a,b; Marroig et al. 2009; Porto et al. 2009 Drake and Klingenberg 2010).

We hypothesized that if integration patterns constrained morphological evolution, patterns of modularity should be evolutionarily conservative and correlate more with evolutionary history than with cranial shape, which exhibits substantial homoplasy in Caribbean anoles, and which has evolved convergently in numerous lineages of *Anolis* microhabitat specialists. In other words, the greatest changes among closely related species should be within cranial modules, not across the skull as a whole. We did not find support for this hypothesis (Figs. 6–8). Instead, we found that lineages that have converged upon similar skull morphologies have also converged on similar integration patterns. The short-faced trunk-ground anoles, *A. sagrei* and *A. cybotes*, have converged on a skull integration pattern that lacks modularity whereas the long-faced species, *A. carolinensis* and *A. bahorucoensis*, have evolved a novel, rostrum-specific module. The correlation between morphology and modularity suggests that adult integration patterns are the result of selection pressures, not historical constraints, and that developmental modularity played little constraining role in the diversification of this genus.

The evolutionary history of anoles appears to be marked by both the breakdown of modules in several relatively short-faced species and the emergence of novel modules in the relatively long-faced lineages. Modularity and integration can arise through direct selection, indirect selection, or neutral mechanisms (for detailed discussions of the origin of modularity, see Wagner et al.

[2007] and Hallgrímsson et al. [2009]). Below, we discuss several possible explanations for the evolution of convergent patterns of modularity in anoles, given our current knowledge of their ecology and natural history.

One possible explanation for the convergent patterns of cranial modularity in anoles is that the convergent features are the result of adaptation in response to similar selective demands. Examining the craniofacial traits that have converged, we hypothesize that shortening the jaws and increasing adductor muscle size will result in increased bite force. If selection favored anoles with greater bite force, it is logical to assume that both an increase in adductor muscle size and a decrease in lever arm length were selected simultaneously, potentially leading to a restructuring of historical patterns of modularity to meet new functional demands. Although it is less clear why anoles might be selected to have long snouts, one possibility is that common selection pressures were strong enough to modify facial integration patterns in both lineages possessing this morphology. This did not necessarily involve the breakdown of ancestral modularity patterns, but rather the addition of another layer of complexity to the variability patterns in these species (Figs. 6 and 7; Young and Hallgrímsson 2005; Kenney-Hunt et al. 2008; Hallgrímsson et al. 2009; Young et al. 2010).

Convergence of cranial integration patterns could also be the secondary result of convergent developmental changes. In other words, similar developmental mechanisms could have been independently recruited in different lineages leading to convergences in both morphology and modularity. Development structures variation in adult morphology because traits arise from a common developmental origin or share common growth trajectories later in life. During morphogenesis, the skull develops from two distinct cell populations—neural crest cells contribute to the face and mesoderm contributes to the neurocranium (Noden and Trainor 2005). For species with elongate skulls, the evolution of novel snout-specific modules might reflect significant
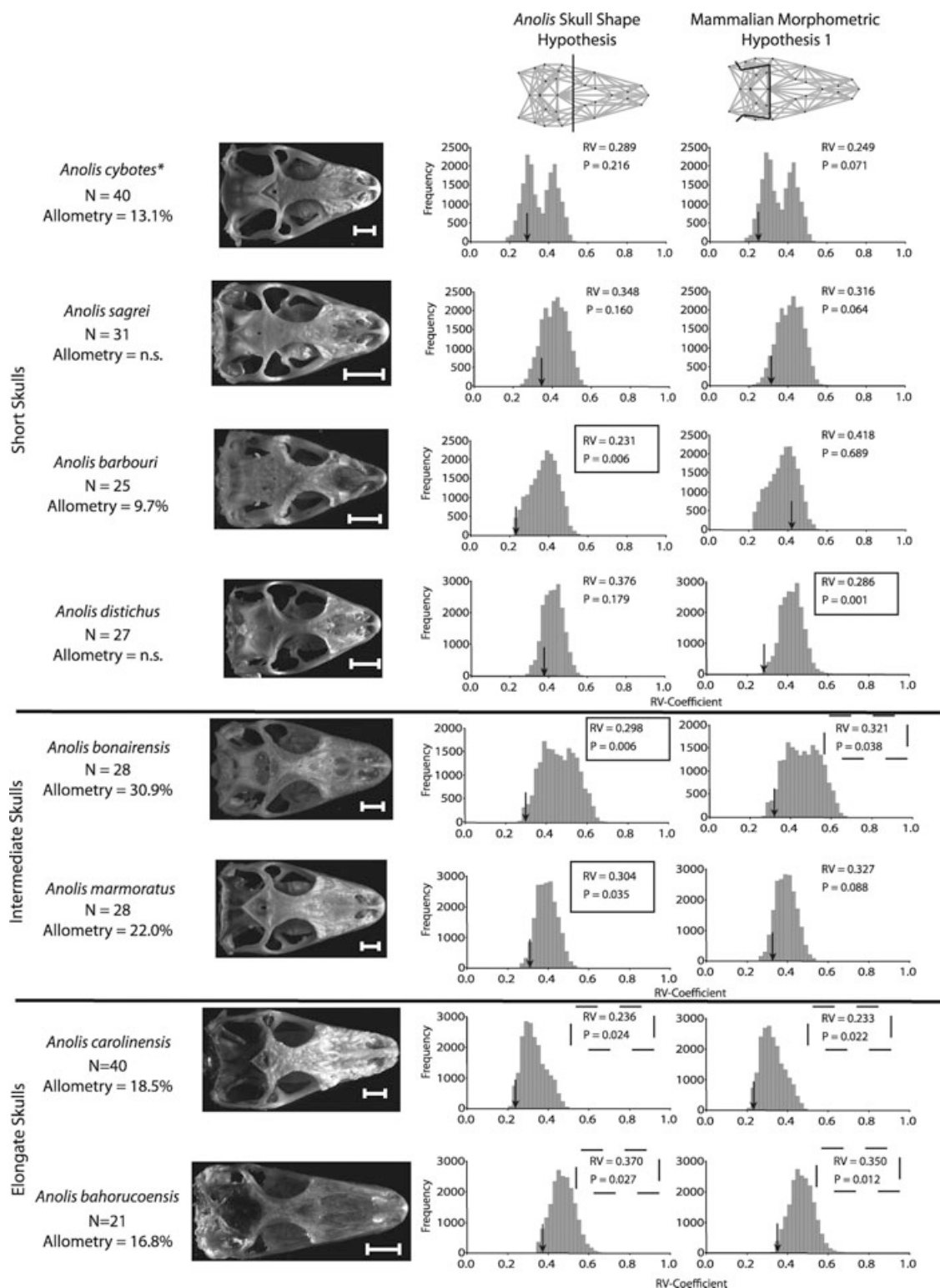
Figure 6. Analyses of craniofacial modularity using complete partitions. Histograms represent null distributions of RV-coefficients from random partitions obtained from adjacency graphs, and arrows represent the RV-coefficient of the modularity hypothesis being tested. Solid boxes highlight the best-supported model of modularity for each species and dashed boxes illustrate other models that also received statistically significant support. MM1 and ASH models are the best supported whereas MM2 (shown in SA4) was never found to be the best model. The histogram of *A. cybotes* appears bimodal due to strong correlations among landmarks surrounding the adductor chamber (SA4, Fig. 5).
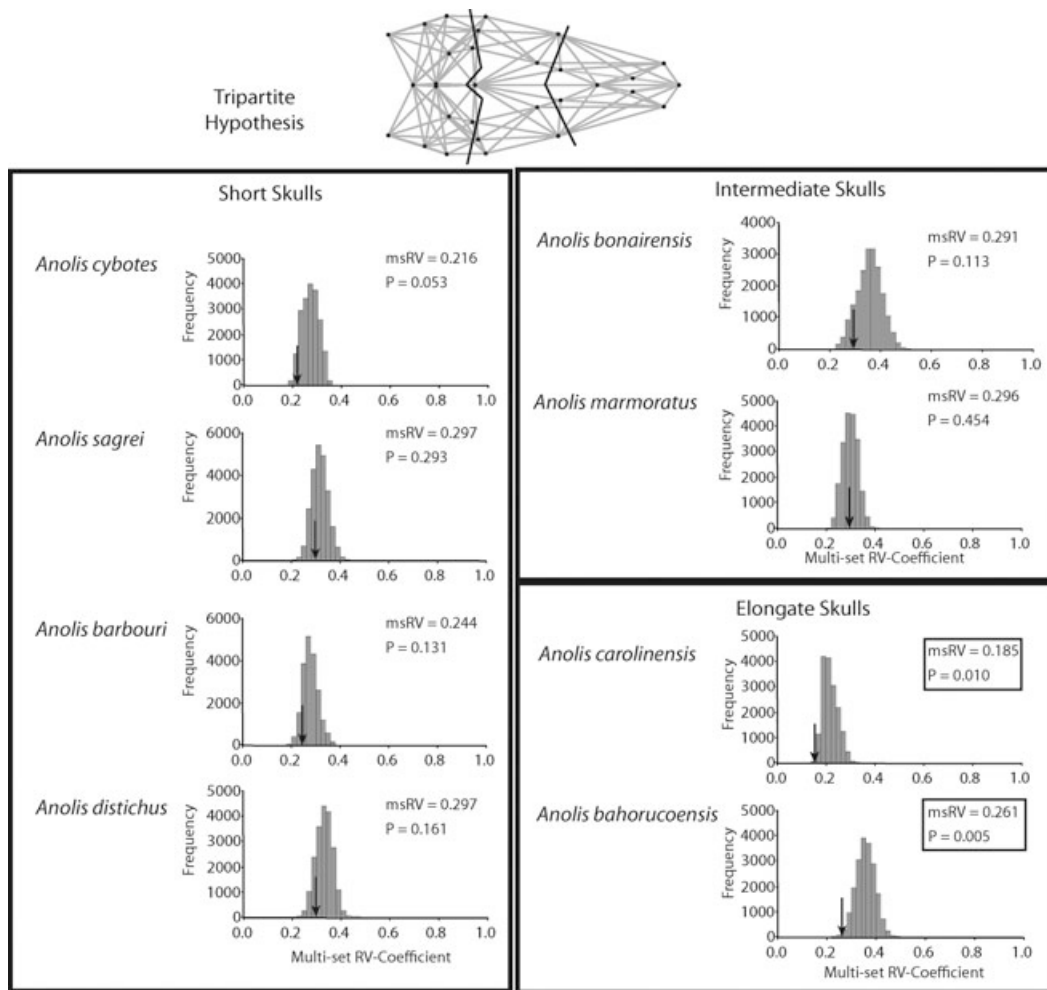
Figure 7. Tripartite modularity hypothesis. There is strong support for the tripartite hypothesis of modularity, which divides the skull into rostrum, orbit, and braincase, in species with elongate skulls, *A. carolinensis* and *A. bahorucoensis*. This hypothesis is not supported in any of the other species examined.

modifications to the mechanisms regulating morphogenesis or later allometric growth of the face. However, based on the cellular fate map of the skull, it is unclear how changes in morphogenesis could generate a more thoroughly integrated structure that crosses these deeply conserved developmental modules, such as that observed in the short-faced lineages.

The mammalian skull has maintained its pattern of integration over 100 million years of diversification that have produced approximately 4500 extant species (Goswami 2006a,b; Porto et al. 2009; values from Pough et al. 2004). Nonetheless, this study on anole cranial integration joins a growing body of evidence from a diversity of organisms illustrating that this finding appears to be the exception; in most cases, patterns of integration appear malleable as species adapt to specific selective demands (e.g., Beldade et al. 2002b; Young et al. 2005, 2010; Jamniczky and Hallgrímsson 2009; Monteiro and Nogueira 2010). For example, *Bicyclus* butterfly eyespots share deeply conserved devel-

opmental programs and are genetically correlated (Beldade et al. 2002a,b). Despite these correlations, artificial selection on single species can rapidly create novel phenotypes that decouple the anterior and posterior eyespots and generate the entire range of variation found within *Bicyclus*. Similarly, artificial selection can also rapidly change the scaling relationships between hindwing, forewing, and body sizes in *Bicyclus* (Frankino et al. 2005, 2007). Patterns of covariation in some vertebrate traits can also evolve relatively rapidly, such as beak dimensions in the genus *Geospiza* (Grant and Grant 1994), even when conserved molecular mechanisms control morphological changes (Abzhanov et al. 2004, 2006; Mallarino et al. 2011). Similar to our findings, there also appears to be no long-term stability to the integration patterns in trilobite heads (Webster and Zelditch 2011) and in bat mandibles (see below; Monteiro and Nogueira 2010). These studies all lend support to the idea that integration patterns do not constrain morphological evolution over long time scales. Rather it seems more
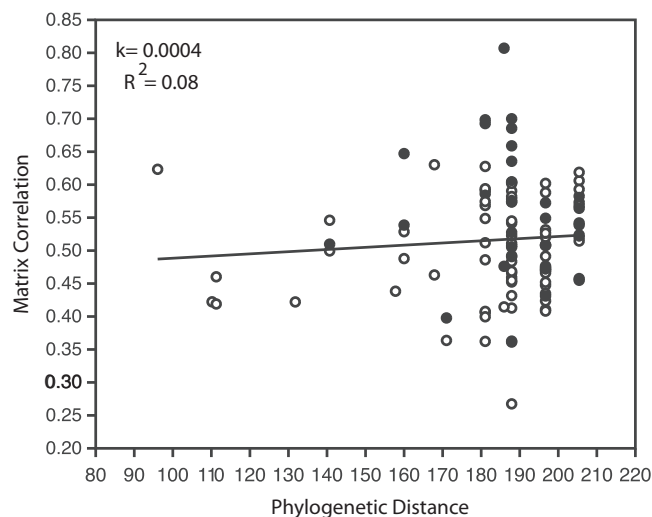
Figure 8. Matrix correlation versus phylogenetic distance. We find no relationship between phylogenetic distance and cranial landmark matrix correlations between species. Filled circles denote comparisons between species represented by greater than 20 individuals, and open circles denote comparisons in which one or both species had 12–19 individuals. There does not appear to be a sample size effect for comparisons between species with different numbers of individuals.

likely that integration patterns influence a species' response to shifting selection pressures over ecological time scales.

It is worth noting, however, that although the most cranially divergent anoles have evolved novel integration patterns, the species we investigated that exhibited more intermediate morphologies appear to possess similar anterior and posterior cranial modules, although the precise boundary appears to fluctuate. Intermediate skull morphologies are common among anoles, and one possibility is that many lineages share the basic modular structure of the intermediate-type species we studied, and that this structure is modified in lineages that evolve more extreme skull morphologies. It will be of interest to examine patterns of integration in additional lineages of anoles, and in squamate lizards more broadly to determine whether a more general and conserved cranial integration pattern exists and whether convergence of morphology and modularity can also be found at larger scales (e.g., Stayton 2006). It may also be informative to examine certain mammalian lineages that show large morphological or ecological transitions at a finer phylogenetic level to see whether their integration ground plan has been broken at some of the extremes of mammalian cranial diversity. For example, bats (order Chiroptera) show extensive craniofacial and ecological diversity, such as insectivorous, nectarivorous, piscivorous, frugivorous, and other ecomorphological forms (e.g., Wetterer et al. 2000; Ruedi and Mayer 2001), and the phyllostomid radiation exhibits most of this variation (Monteiro and Nogueira 2011). Consistent with

our results, Monteiro and Nogueira (2010) found that integration patterns in the bat mandible were remodeled during relatively recent ecomorphological transitions and convergence of morphology and integration pattern was recently found in a suite of cranial and postcranial traits in trunk-ground anoles, suggesting a strong effect of selection history on the patterns of covariation among traits (Kolbe et al. 2011). In future studies, it will be interesting to see whether such patterns are also observed in phyllostomid crania, as well as the skulls of other relatively recent mammalian adaptive radiations. Such comparative studies done at both higher and lower taxonomical levels may show that integration patterns may frequently be remodeled by natural selection.

HEAD SHAPE AND PATTERNS OF CONVERGENCE

Anole diversification is marked by convergence in a wide variety of characters, not only in traits involved in microhabitat use, but also in traits such as sexual dimorphism and behavior (Losos 2009). In regard to head shape, two lineages have independently converged on an extremely elongate morphology, the trunk-crown anoles of the *carolinensis* series and the Hispaniolan grass-bush anoles of the *hendersoni* series (Figs. 5 and 6). The *carolinensis* series anoles also have further elongated their skulls through additional extension of the most anterior and posterior cranial elements (PC3, Fig. 3). Relatively, short skulls have evolved at least eight times, most notably in the trunk-ground and trunk anoles and in the terrestrial *A. barbouri*. Two out of six ecomorph classes exhibit significant convergence, suggesting a relationship between microhabitat and head shape for relatively few anoles (members of the trunk and crown-giant anoles are similar, but not significantly convergent). Two of the ecomorphs that do not converge on a common head shape, the trunk-crown and grass-bush, are those that possess both species with extremely elongate skulls and species with more intermediate phenotypes. This may suggest that certain lineages have complex natural histories with unique, lineage-specific selection pressures that shaped present day craniofacial diversity.

At this time, little is known about the relationship between head shape and ecological factors such as foraging behavior, biomechanics, or prey choice (reviewed in Losos 2009; Johnson et al. 2010), but changes in skull morphology likely have important functional consequences (Dayeh et al. 2009; Moazen et al. 2008). *Anolis* lizards are primarily insectivorous, but also use their jaws for male–male combat (e.g., Lailvaux et al. 2004; Losos 2009), which suggests that the functional basis of skull diversity may be complex. *Anolis* cranial diversity may also have evolved due to additional factors, such as sexual selection or intersexual resource partitioning (e.g., Jenssen et al. 1984; Butler and Losos 2002; Butler 2007; Herrel et al. 2007). Determining the selective basis of snout elongation will require both observational data collected on these species in their native environments

and manipulative experiments that evaluate feeding performance and biomechanics on prey items of varying size and hardness, as well as differential mating success of anoles that differ in snout length.

Conclusions

We tested the hypothesis that cranial integration patterns affected diversification of the anole skull. We found no support for this hypothesis. Instead, our results are consistent with the hypothesis that natural selection has repeatedly restructured integration patterns to generate particular skull shapes. We do not yet know whether convergence of morphology and modularity patterns is primarily the result of functional changes, which may arise relatively late in ontogeny, or is due to similar developmental changes independently modifying the developmental architecture of the cranium in different lineages. Understanding the biological basis of integration patterns observed in adult form will provide deeper insight into their robustness and potential effect on morphological diversification (see Olson and Miller 1958, Chapter 7).

Because of the hierarchical, nested nature of developmental modules—the Palimpsest Model of modularity (Hallgrímsson et al. 2009)—the relative contributions of external selective factors and developmental factors to adult integration patterns are difficult to dissect. Examining temporal changes in integration patterns, from hatching to adulthood, of species with different morphologies is one way to test whether adult integration is primarily the result of morphogenetic changes or interactions among traits relatively late in ontogeny. Investigating the integration patterns of anoles that experienced novel environmental circumstances, perhaps in human-disturbed areas or in experimental studies (e.g., Losos et al. 2001; Losos 2007; Marnocha et al. 2011) is another approach that can determine the flexibility of adult integration patterns (also see Hallgrímsson and Lieberman 2008). Trait correlations that are retained in the face of experimental manipulation are likely the result of genetically determined, developmental interactions whereas labile trait correlations are likely a result of late-acting functional interactions. Based on our results, we hypothesize that the adult integration patterns of short-snouted anoles arise late in ontogeny and, therefore, are primarily based on the functional interactions of different skeletal elements and their connecting muscles. Conversely, we hypothesize that the novel patterns of modularity in species with elongate rostra are the result of developmental changes occurring earlier in ontogeny.

Evolutionary theory predicts that correlations among traits will affect the rate or direction of a species' response to natural selection (e.g., Cheverud 1984, 1996; Wagner 1988, 1996; Arnold 1992; Schluter 1996). But patterns of integration, like other biological properties, have histories; they emerge, evolve, and dis-

appear (Schwenk and Wagner 2004). Although these correlations may affect processes occurring at ecological time scales, many are likely transient on geological time scales. As research on morphological integration progresses, it will be important to not only determine which traits are integrated, but also the robustness and longevity of those correlations. Perhaps more importantly, it will be necessary to determine what factors maintain or degrade integration patterns over evolutionary time. The greatest insight into these difficult questions may come using an integrative approach, one that determines the biological basis of integration patterns, compares them among closely related species, and examines their flexibility using experimental approaches. With a strong history of comparative biology, experimentation, and the recently published genome of *A. carolinensis*, anoles are an ideal model for such research.

LITERATURE CITED
Abzhanov, A., W. P. Kuo, C. Hartmann, B. R. Grant, P. R. Grant, and C. J. Tabin. 2006. The calmodulin pathway and evolution of elongated beak morphology in Darwin's finches. Nature 442:563–567.

Abzhanov, A., M. Protas, B. R. Grant, P. R. Grant, and C. J. Tabin. 2004. *Bmp4* and morphological variation of beaks in Darwin's finches. Science 305:1462–1465.

Ackermann, R. R. 2005. Ontogenetic integration of the hominoid face. J. Hum. Evol. 48:175–197.

Ackermann, R. R., and J. M. Cheverud. 2000. Phenotypic covariance structure in tamarins (genus *Saguinus*): a comparison of variation patterns using matrix correlation and common principal component analysis. Am. J. Phys. Anthropol. 111:489–501.

Arnold, S. J. 1992. Constraints on phenotypic evolution. Am. Nat. 140:S85–S107.

Beldade, P., K. Koops, and P. M. Brakefield. 2002a. Developmental constraints versus flexibility in morphological evolution. Nature 416:844–847.

———. 2002b. Modularity, individuality, and evo-devo in butterfly wings. P. Natl. Acad. Sci. USA 99:14262–14267.

Beuttell, K., and J. B. Losos. 1999. Ecological morphology of Caribbean anoles. Herpetological Monographs 13:1–28.

Butler, M. A. 2007. *Vive le difference*! Sexual dimorphism and adaptive patterns in lizards of the genus *Anolis*. Integr. Comp. Biol. 47:272–284.

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.

Chai, Y., and R. E. Maxson. 2006. Recent advances in craniofacial morphogenesis. Dev. Dyn. 235:2353–2375.

Cheverud, J. M. 1982. Phenotypic, genetic, and environmental morphological integration in the cranium. Evolution 36:499–516.

———. 1984. Quantitative genetics and developmental constraints on evolution by selection. J. Theor. Biol. 110:155–171.

———. 1996. Developmental integration and the evolution of pleiotropy. Am. Zool. 36:44–50.

Dayeh, A. A., K. L. Rafferty, M. Egbert, and S. W. Herring. 2009. Deformation of nasal septal cartilage during mastication. J. Morphol. 270:1209–1218.

Drake, A. G., and C. P. Klingenberg. 2010. Large-scale diversification of skull shape in domestic dogs: disparity and modularity. Am. Nat. 175:289–301.

Dryden, I. L., and K. V. Mardia. 1998. Statistical shape analysis. John Wiley & Sons, New York, NY.

Eble, G. J. 2004. The macroevolution of phenotypic integration. Pp. 253–273 *in* M. Pigliucci and K. Preston, eds. Phenotypic integration: the evolutionary biology of complex phenotypes. Oxford Univ. Press, Oxford, U.K.

———. 2005. Morphological modularity and macroevolution: conceptual and empirical aspects. Pp. 221–238 *in* W. Callebaut and D. Rasskin-Gutman, eds. Modularity. The MIT Press, Cambridge, U.K.

Elmer, K. R., H. Kusche, T. K. Lehtonen, and A. Meyer. 2010. Local variation and parallel evolution: morphological and genetic diversity across a species complex of neotropical crater lake cichlid fishes. Philos. Trans. R. Soc. Lond. B. 365:1763–1782.

Escoufier, Y. 1973. Le traitement des variables vectorielles. Biometrics 29:751–760.

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

———. 2007. Internal and external constraints in the evolution of morphological allometries in a butterfly. Evolution 61:2958–2970.

Goswami, A. 2006a. Cranial modularity shifts during mammalian evolution. Am. Nat. 168:270–280.

———. 2006b. Morphological integration in the carnivoran skull. Evolution 60:169–183.

Goswami, A., and P. D. Polly. 2010. Methods for studying morphological integration and modularity. Pp. 213–243 *in* J. Alroy and G. Hunt, eds. Quantitative paleontology, vol. 16. Paleontological Society Special Publications. Boulder, CO.

Grant, P. R., and B. R. Grant. 1994. Phenotypic and genetic effects of hybridization in Darwin's finches. Evolution 48:297–316.

Hallgrímsson, B., H. Jamniczky, N. M. Young, C. Rolian, T. E. Parsons, J. C. Boughner, and R. S. Marcucio. 2009. Deciphering the palimpsest: studying the relationship between morphological integration and phenotypic covariation. Evol. Biol. 36:355–376.

Hallgrímsson, B., and D. E. Lieberman. 2008. Mouse models and the evolutionary developmental biology of the skull. Integr. Comp. Biol. 48:373–384.

Harmon, L. J., J. J. Kolbe, J. M. Cheverud, and J. B. Losos. 2005. Convergence and the multidimensional niche. Evolution 59:409–421.

Hendrikse, J. L., T. E. Parsons, and B. Hallgrímsson. 2007. Evolvability as the proper focus of evolutionary developmental biology. Evol. Dev. 9:393–401.

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.

Jamniczky, H. A., and B. Hallgrímsson. 2009. A comparison of covariance structure in wild and laboratory muroid crania. Evolution 63:1540–1556.

Jenssen, T. A., D. L. Marcellini, C. A. Pague, and L. A. Jenssen. 1984. Competitive interference between two Puerto Rican lizards, *Anolis cooki* and *Anolis cristatellus*. Copeia 1984:853–861.

Johnson, M. A., M. Leal, L. R. Schettino, A. C. Lara, L. J. Revell, and J. B. Losos. 2008. A phylogenetic perspective on foraging mode evolution and habitat use in West Indian *Anolis* lizards. Anim. Behav. 75:555–563.

Johnson, M. A., L. J. Revell, and J. B. Losos. 2010. Behavioral convergence and adaptive radiation: effects of habitat use on territorial behavior in *Anolis* lizards. Evolution 64:1151–1159.

Kenney-Hunt, J. P., B. Wang, E. A. Norgard, G. Fawcett, D. Falk, L. S. Pletscher, J. P. Jarvis, C. Roseman, J. Wolf, and J. M. Cheverud. 2008. Pleiotropic patterns of quantitative trait loci for 70 murine skeletal traits. Genetics 178:2275–2288.

Klingenberg, C. P. 2008. Morphological integration and developmental modularity. Annu. Rev. Ecol. Evol. Syst. 39:115–132.

———. 2009. Morphometric integration and modularity in configurations of landmarks: tools for evaluating a priori hypotheses. Evol. Dev. 11:405–421.

———. 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. Res. 11:353–357.

Klingenberg, C. P., and G. S. McIntyre. 1998. Geometric morphometrics of developmental instability: analyzing patterns of fluctuating asymmetry with Procrustes methods. Evolution 52:1363–1375.

Klingenberg, C. P., and N. A. Gidaszewski. 2010. Testing and quantifying phylogenetic signals and homoplasy in morphometric data. Syst. Biol. 59:245–261.

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

———. 2003. Body shape variation in cichlid fishes of the *Amphilophus citrinellus* species complex. Biol. J. Linn. Soc. 80:397–408.

Kolbe, J. J., L. J. Revell, B. Szekely, E. D. Brodie III, and J. B. Losos. 2011. Convergent evolution of phenotypic integration and its alignment with morphological diversification of Caribbean ecomorphs. Evolution doi:10.1111/j.1558–5646.2011.01416.x

Kulemeyer, C., K. Asbahr, P. Gunz, S. Frahnert, and F. Bairlein. 2009. Functional morphology and integration of corvid skulls—a 3D geometric morphometric approach. Front. Zool. 7:1–14.

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 morphs in the green anole lizard (*Anolis carolinensis*). Proc. R. Soc. Lond. B. 271:2501–2508.

Lande, R. 1980. Sexual dimorphism, sexual selection, and adaptation in polygenic characters. Evolution 34:292–305.

Losos, J. B. 2007. Detective work in the West Indies: integrating historical and experimental approaches to study island lizard evolution. BioScience 57:585–597.

———. 2009. Lizards in an evolutionary tree: ecology and adaptive radiation of anoles. Univ. of California Press, Berkeley, CA.

Losos, J. B., T. R. Jackman, A. Larson, K. de Queiroz, and L. Rodríguez-Schettino. 1998. Contingency and determinism in replicated adaptive radiations of island lizards. Science 279:2115–2118.

Losos, J. B., T. W. Schoener, and D. A. Spiller. 2001. Experimental studies of adaptive differentiation in Bahamian *Anolis* lizards. Genetica 112–113:399–416.

Losos, J. B., R. E. Glor, J. J. Kolbe, and K. Nicholson. 2006. Adaptation, speciation, and convergence: a hierarchical analysis of adaptive radiation in Caribbean *Anolis* lizards. Ann. MO Bot. Gard. 93:24–33.

Mahler, D. L., L. J. Revell, R. E. Glor, and J. B. Losos. 2010. Ecological opportunity and the rate of morphological evolution in the diversification of Greater Antillean anoles. Evolution 64:2731–2745.

Mallarino, R., P. R. Grant, B. R. Grant, A. Herrel, W. P. Kuo, and A. Abzhanov. 2011. Two developmental modules establish 3D beak-shape variation in Darwin's finches. Proc. Nat. Acad. Sci. USA 108:4057–4062.

Marnocha, E., J. Pollinger, and T. B. Smith. 2011. Human-induced morphological shifts in an island lizard. Evol. Appl. 4:388–396.

Marroig, G., and J. M. Cheverud. 2001. A comparison of phenotypic variation and covariation patterns and the role of phylogeny, ecology, and ontogeny during cranial evolution of New World monkeys. Evolution 55:2576–2600.

Marroig, G., L. T. Shirai, A. Porto, F. B. de Oliveira, and V. De Conto. 2009. The evolution of modularity in the mammalian skull II: evolutionary consequences. Evol. Biol. 36:136–148.

Marugan-Lobon, J., and A. D. Buscalioni. 2006. Avian skull morphological evolution: exploring exo- and endocranial covariation with two-block partial least squares. Zoology 109:217–230.

Merilä, J., and M. Björklund. 2004. Phenotypic integration as a constraint and adaptation. Pp. 107–129 *in* M. Pigliucci and K. Preston, eds. Phenotypic integration: studying the ecology and evolution of complex phenotypes. Oxford Univ. Press, New York, NY.

Moazen, M., N. Curtis, S. E. Evans, P. O'Higgins, and M. J. Fagan. 2008. Combined finite element and multibody dynamics analysis of biting in a *Uromastyx hardwickii* lizard skull. J. Anat. 213:499–508.

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

Monteiro, L. R., and M. R. Nogueira. 2010. Adaptive radiations, ecological specialization, and the evolutionary integration of complex morphological structures. Evolution 64:724–744.

———. 2011. Evolutionary patterns and processes in the radiation of phyllostomid bats. BMC Evol. Biol. 11:137.

Noden, D. M., and P. A. Trainor. 2005. Relations and interactions between cranial mesoderm and neural crest populations. J. Anat. 207:575–601.

Olson, C., and R. L. Miller. 1958. Morphological integration. Univ. of Chicago Press, Chicago, IL.

Paradis, E., Claude, J., and K. Strimmer. 2004. APE: analyses of phylogenetics and evolution in R language. Bioinformatics 20:289–290.

Pinto, G., D. L. Mahler, L. J. Harmon, and J. B. Losos. 2008. Testing the island effect in adaptive radiation: rates and patterns of morphological diversification in Caribbean and mainland *Anolis* lizards. Proc. R. Soc. Lond. B. 275:2749–2757.

Porto, A., F. B. de Oliveira, L. T. Shirai, V. De Conto, and G. Marroig. 2009. The evolution of modularity in the mammalian skull I: morphological integration patterns and magnitudes. Evol. Biol. 36:118–135.

Pough, F. H., C. M. Janis, and J. B. Heiser. 2004. Vertebrate life, 6th ed. Prentice Hall, Upper Saddle River, NJ.

Riedl, R. 1978. Order in living organisms: a systems analysis of evolution. Wiley, New York, NY.

Rohlf, J. 2005. tps DIG version 2.04. Department of Ecology and Evolution, State University of New York, Stony Brook, NY.

Ruedi, M., and F. Mayer. 2001. Molecular systematics of bats of the genus *Myotis* (Vespertilionidae) suggests deterministic ecomorphological convergences. Mol. Phylogenet. Evol. 21:436–448.

Schluter, D. 1996. Adaptive radiation along genetic lines of least resistance. Evolution 50:1766–1796.

Schluter, D., T. Price, A. Ø. Mooers, and D. Ludwig. 1997. Likelihood of ancestral states in adaptive radiation. Evolution 51:1699–1711.

Schwenk, W., and G. P. Wagner 2004. The relativism of constraint. Pp. 390–408 *in* M. Pigliucci and K. Preston, eds. Phenotypic integration:studying the ecology and evolution of complex phenotypes. Oxford Univ. Press, Oxford, U.K.

Shirai, L. T., and G. Marroig. 2010. Skull modularity in neotropical marsupials and monkeys: size variation and evolutionary constraint and flexibility. J. Exp. Zool. 314B:663–683.

Stayton, C. T. 2006. Testing hypotheses of convergence with multivariate data: morphological and functional convergence among herbiverous lizards. Evolution 60:824–841.

Wagner, G. P. 1988. The significance of developmental constraints for phenotypic evolution by natural selection. Pp. 222–229 *in* G. de Jong, ed. Population genetics and evolution. Springer, New York, NY.

———. 1996. Homologues, natural kinds and the evolution of modularity. Am. Zool. 36:36–43.

Wagner, G. P., and L. Altenberg. 1996. Complex adaptations and the evolution of evolvability. Evolution 50:967–976.

Wagner, G. P., and J. Zhang. 2011. The pleiotropic structure of the genotype–phenotype map: the evolvability of complex organisms. Nat. Rev. Genet. 12:204–213.

Wagner, G. P., M. Pavlicev, and J. M. Cheverud. 2007. The road to modularity. Nat. Rev. Genet. 8:921–931.

Webster, M., and M. L. Zelditch. 2011. Evolutionary lability of integration in Cambrian ptychoparioid trilobites. Evol. Biol. 38:144–162.

Wetterer, A. L., M. V. Rockman, and N. B. Simmons. 2000. Phylogeny of phyllostomid bats (Mammalia, Chiroptera): data from diverse morphological systems, sex chromosomes, and restriction sites. Bull. Am. Mus. Nat. Hist. 248:1–200.

Wroe, S., and N. Milne. 2007. Convergence and remarkably consistent constraint in the evolution of carnivore skull shape. Evolution 61:1251–1260.

Young, N. M., and B. Hallgrímsson. 2005. Serial homology and the evolution of mammalian limb covariation structure. Evolution 59:2691–2704.

Young, N. M., G. P. Wagner, and B. Hallgrímsson. 2010. Development and the evolvability of human limbs. Proc. Natl. Acad. Sci. USA 107:3400–3405.

Zelditch, M. L., D. L. Swiderski, H. D. Sheets, and W. L. Fink. 2004. Geometric morphometrics for biologists: a primer. Elsevier Science, Amsterdam, NL.

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Supporting Information

The following supporting information is available for this article:

Figure S1. (A) *Anolis* skull centroid size summarized by ecomorph.

Figure S2. We investigated patterns of skull evolution among *Anolis* ecomorphs in a phylogenetic context using maximum likelihood to estimate ancestral trait values for the first two PCs under a Brownian motion model of continuous trait evolution.

Figure S3. Phylomorphospace of *Anolis* skull shape.

Figure S4. Example of morphometric analysis on a phylogenetically subsampled dataset.

Table S1. Interspecific correlations.

Appendix S1: Additional methods and figures from analyses of skull shape diversity.

Appendix S2: Centroid size and shape scores for 106 Anolis species.

Appendix S3: Maximum likelihood reconstructions of PCs 1-4.

Appendix S4: Additional methods and figures from analyses of morphological integration.

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

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