



Phenotypic integration between claw and toepad traits promotes microhabitat specialization in the *Anolis* adaptive radiation

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The performance of an organism in its environment frequently depends more on its composite phenotype than on individual phenotypic traits. Thus, understanding environmental adaptation requires investigating patterns of covariation across functionally related traits. The replicated adaptive radiations of Greater Antillean *Anolis* lizards are characterized by ecological and morphological convergence, thus, providing an opportunity to examine the role of multiple phenotypes in microhabitat adaptation. Here, we examine integrated claw and toepad morphological evolution in relation to habitat partitioning across the adaptive radiations of Greater Antillean anoles. Based on analysis of 428 specimens from 57 species, we found that different aspects of claw morphology were associated with different perch dimensions, with claw height positively associated with perch diameter and claw curvature positively associated with perch height. Patterns of integration also varied across claw and toepad traits, likely driven by correlative selection for performance on smoother and rougher substrates. Finally, rates of evolution differed between claw and toepad traits, with claw length evolving faster than all other traits despite having no predicted functional importance. Our results highlight the multivariate nature of phenotypic adaptation and suggest that phenotypic integration across Greater Antillean anoles is driven by fine-scale correlative selection based on structural habitat specialization.

KEY WORDS: Convergence, ecomorphology, geometric morphometrics, Greater Antilles, phenotypic evolution, rate of evolution.

Natural selection acts upon whole-organism performance, as determined through multivariate trait complexes (Arnold 1983; Schluter and Nychka 1994; Wainwright and Reilly 1994) and often involves performance trade-offs between multiple aspects of an organism's phenotypes (Reznick and Ghalambor 2001; Ghalambor et al. 2003). Therefore, correlative selection often leads to phenotypic integration or patterns of covariation in functionally related traits that maximize performance across otherwise independent traits (Zelditch 1988; Schluter and Nychka 1994;

Endler 1995; Pigliucci 2003). Such selection-mediated phenotypic integration may be particularly prevalent during adaptive radiations, allowing clades to use previously inaccessible ecological space (Miller 1949; Monteiro and Nogueira 2010; Stroud and Losos 2016).

The replicated adaptive radiations of *Anolis* lizards on the Greater Antilles display remarkable convergent evolution in both habitat use and morphology (Williams 1972; Losos et al. 1998; Losos 2009). The primary axis of divergence in structural habitat

use is facilitated by convergent evolution of overall body plans, including body size, limb dimensions, head dimensions, and adhesive toepad morphology, producing highly integrated whole-organism phenotypes across the radiations (Losos 1990b; Losos et al. 1998; Beuttell and Losos 1999; Kolbe et al. 2011). Based on these ecological, behavioral, and morphological convergences, the Greater Antilles anoles are classified into six ecomorphs, named for their utilization of vertical microhabitat space: grass-bush, trunk-ground, trunk, trunk-crown, crown-giant, and twig anoles (Williams 1972; Losos et al. 1998; Losos 2009). These patterns of convergences indicate strong multitrait selection associated with structural habitat use among Greater Antillean anoles. Functional studies have demonstrated the utility of several convergent phenotypes in exploitation of the structural habitat (Losos and Sinervo 1989; Losos 1990b; Beuttell and Losos 1999; Elstrott and Irschick 2004; Crandell et al. 2014). Yet, the functional linkage between traits that might facilitate phenotypic integration in a correlative selection framework is not always straightforward, and finer-scale phenotypic covariation may be masked by gross morphology.

Here, we examine the integrated evolution of two traits whose functions should be highly linked in anoles: the adhesive toepad and the claw. Both toepads and claws are found in all species of anoles except *Anolis onca*, which lacks toepads. In the adaptive radiation of *Anolis* lizards, adhesive toepads are thought to be a key innovation which allowed them to occupy broader arboreal niche space relative to species without toepads (Irschick et al. 2006; Losos 2009). Certainly, Greater Antillean anoles display remarkable convergent evolution in toepad traits related to habitat use (Macrini et al. 2003; Schaad and Poe 2010), and toepads are functionally important for clinging ability (Elstrott and Irschick 2004; Irschick et al. 2006; Crandell et al. 2014). However, our understanding of the role of claws in habitat partitioning by anoles is limited (Losos 2009; Crandell et al. 2014). Yet, in other arboreal species, claws are often ecologically important for arboreality even when adhesive toepads are present (Zani 2000; Vanhooydonck et al. 2005; Song et al. 2016). Toepads are adapted to provide adhesion to smoother surfaces due to increased surface area contact between setae and underlying substrates (Vanhooydonck et al. 2005; Gillies et al. 2014), whereas the interlocking performance of claws is thought to be facilitated by surface texture (Cartmill 1974). Therefore, previous authors have suggested, although never empirically tested, that integration in claws and toepad traits may have contributed to habitat partitioning by allowing anoles to efficiently navigate a variety of surface textures more effectively than toepads alone would have (Elstrott and Irschick 2004; Crandell et al. 2014).

Across vertebrates, the morphology of claws often reflects their specialized functionality (Tulli et al. 2009; Birn-Jeffery et al.

2012; Tulli et al. 2016). For instance, tree-climbing and branch-perching birds have greater claw curvature than terrestrial species (Feduccia 1993), a pattern that is broadly shared across squamate reptiles as well (Zani 2000; Tulli et al. 2009; D'Amore et al. 2018). Greater claw curvature facilitates climbing by improving penetrative and interlocking performance with climbing surfaces (Cartmill 1974). Arboreal lizards also have taller claws compared to terrestrial lizards, yet claw height and curvature are associated with different aspects of clinging performance (Zani 2000). Claw height improves clinging performance on rougher surfaces but not on smoother surfaces across squamate reptiles, whereas claw curvature improves clinging performance on smoother surfaces (Zani 2000; Tulli et al. 2011). Therefore, correlative selection should lead to phenotypic integration between toepad traits, which provide adhesion to smoother surfaces, and claw curvature but not claw height.

Although another aspect of claw morphology, length, may act as an extension of the toe in more terrestrial species, allowing for greater surface area contact and propulsive force when running on flat surfaces (Glen and Bennett 2007; Tulli et al. 2009; Birn-Jeffery et al. 2012), claw length does not affect clinging performance on any surface (Zani 2000; Tulli et al. 2011) and, thus, should not be under correlative selection with toepad traits related to adhesive performance or other functionally relevant claw traits. Phenotypic integration is also expected to be linked to the rate of evolution across integrated traits (Frédérich et al. 2014; Sherratt et al. 2017). Because traits that are under strong selection and biomechanical constraint often have lower rates of evolution (Adams et al. 2017a), sets of integrated biomechanical traits should evolve more slowly than those not under correlative selection. Therefore, we expect that rates of evolution should be similar across integrated claw and toepad traits but not across traits lacking ecological relevance in our study system.

To test these hypotheses about how a multivariate trait complex evolves in response to correlative selection, we examine a suite of claw and toepad characters to study integrated trait evolution in relation to habitat use throughout the adaptive radiations of Greater Antillean anoles. We characterize patterns of claw variation from 57 *Anolis* species to address four major questions that examine the importance of claw and toepad traits at different scales of ecological divergence: (1) Are claw height and curvature correlated with greater arboreality as predicted by functional assays? (2) Is claw morphology associated with microhabitat usage in Greater Antillean anoles? (3) Is there phenotypic integration between toepads and claw curvature but not with height, suggesting correlative selection? (4) Do rates of morphological evolution covary between claw and toepad traits, as predicted for evolution under biomechanical constraint?

Methods

SAMPLING

We collected morphological data from preserved museum specimens maintained by the Museum of Vertebrate Zoology (University of California, Berkeley, CA), the California Academy of Sciences (San Francisco, CA), and the National Museum of Natural History (Smithsonian Institution, Washington, DC) (Table S1). We sampled 428 adult males representing 57 species (Table S2). Our sampling includes at least one species from every ecomorph present on each Greater Antillean island (45 species) plus seven “unique” Greater Antillean species not belonging to an ecomorph class. We also included the mainland species *Anolis carolinensis* as a trunk-crown ecomorph because it represents back colonization from the Greater Antilles to the mainland and retains traits characteristic of Greater Antillean trunk-crown anoles (Losos 2009). Last, because only one truly terrestrial species exists on the Greater Antilles, *Anolis barbouri*, we included following four mainland leaf-litter anoles to examine differences between terrestrial and arboreal species: *Anolis nitens*, *Anolis humilis*, *Anolis uniformis*, and *Anolis quagulus* (Köhler et al. 2006; Losos 2009). Due to ecological and evolutionary differences between the mainland and Greater Antillean anole assemblages (Losos, 2009), these mainland anoles were not included in analyses other than the terrestrial–arboreal comparisons.

MORPHOLOGICAL MEASUREMENTS

After measuring snout-vent length (SVL) for all specimens as a proxy for body size, we imaged the fourth hind toepad for each specimen using a Dino-Lite Digital Microscope (Model AM4115ZT; AnMo Electronics Corporation, Taipei, Taiwan) or an EPSON V19 flatbed scanner (Seiko Epson Corporation, Tokyo, Japan) (Fig. 1). We also sagittally flattened and imaged the fourth hind claw using a Dino-Lite Digital Microscope. The fourth hind digit was chosen because it is the longest digit and has been the predominate focus of morphological and functional studies in anoles (Losos 1990b; Macrini et al. 2003; Kolbe et al. 2011; Mahler et al. 2013; Crandell et al. 2014). Using tpsdig2 (Rohlf 2006), we measured claw height, claw length, and claw curvature from digital images following Zani (2000), so that our data would be comparable to previous studies. Briefly, we measured claw height as the height at the base of the claw, and calculated claw curvature as:

$$\text{curvature} = 57.296 \times \left(\frac{2\sin^{-1}(2A^2B^2 + 2B^2C^2 + 2A^2C^2 - A^4 - B^4 - C^4)^{0.5}}{2AB} \right),$$

where A is the distance from the ventral base of the claw to the vertex of the ventral curve, B is the distance from the ventral

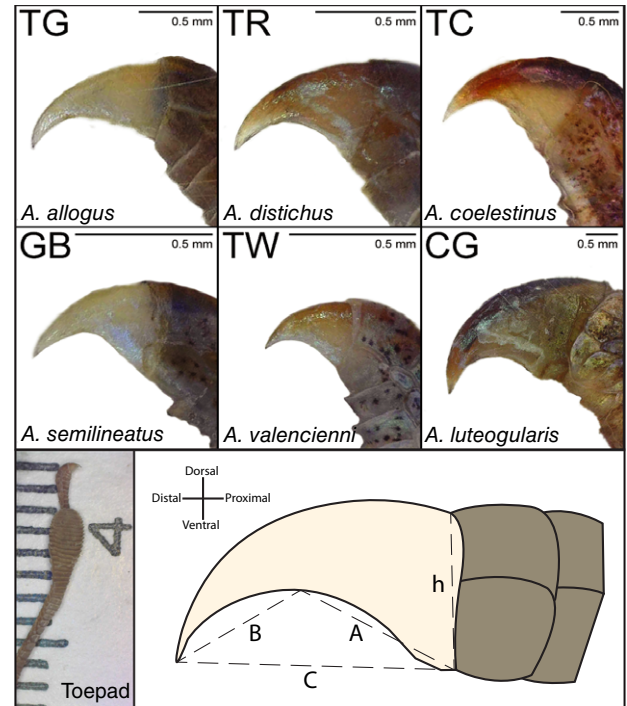


Figure 1. Representative photos of Greater Antillean anole claws imaged using a Dino-Lite Digital Microscope. One species from each ecomorph is depicted with 0.5 mm scale bar (TG = trunk-ground; TR = trunk; TC = trunk-crown; GB = grass-bush; TW = twig; CG = crown-giant). Bottom panels: (left) photo of toepad and claw of an anole and (right) diagram of univariate measurements of claw morphology adapted from Zani (2000). Variables A , B , and C follow equation in text, h = claw height.

vertex to the tip of the claw, and C is the distance from the ventral base of the claw to the tip of the claw (Zani 2000). We calculated claw length as the sum of distances A and B (Fig. 1). For toepad measurements, we defined the toepad as beginning where the next distal lamella is wider than the previous lamella. We counted the number of lamellae, following this definition, and measured toepad width as the widest point of the toepad. We could not measure some toepad traits for three specimens with damaged toepads. We did not measure toepad traits for the mainland leaf-litter species because they were not needed for the relevant analyses.

We conducted all subsequent data analyses in R version 3.3.3 (R Core Team 2017). For all analyses requiring phylogenetic information, we used the most recent comprehensive *Anolis* phylogeny (Poe et al. 2017) pruned to include only our focal taxa. Specifically, the maximum clade credibility tree generated in MrBayes. We natural log transformed all univariate measurements, except claw curvature, to achieve normality and homoscedasticity of our data. To quantify correlations with body size, we performed phylogenetic independent contrasts (PICs; Felsenstein 1985) between log-transformed SVL and all other morphological and ecological traits. PICs use the underlying phylogeny to

transform trait values to achieve statistical independence and identical distributions assuming Brownian motion (Felsenstein 1985). We identified allometric scaling between claw traits and body size by comparing 95% confidence intervals (CIs) of slopes against 1, to test for deviations from isometry, and against 0, to test for deviations from independence. For traits significantly correlated with body size, we regressed log-transformed traits against log-transformed SVL accounting for phylogeny (Revell 2009) using the “*phyl.resid*” function in the *phytools* package (Revell 2017). We used residuals for all downstream analyses involving body size correlated traits.

Because nongeometric data do not fully capture complex shape information, we also collected geometric morphometric data by characterizing claw shape using 30 semilandmarks placed along both the ventral and dorsal curves of the claw (Tinius and Russell 2017) and toepad shape using 50 semilandmarks following the outline of each toepad starting and ending on opposing proximate corners of the first toepad lamella. We placed semi-landmark curves using *tpsDig2*. To remove nonshape features, we performed generalized Procrustes superimposition allowing semi-landmarks to slide to minimize bending energy (Bookstein 1997) using the “*gpagen*” function in *geomorph* package (Adams et al. 2017b). Bending energy does not deform landmarks beyond the endpoints of the curve (Gunz and Mitteroecker 2013). For downstream analyses, we calculated mean shape values as the average coordinate position of each semilandmark for each species using the “*mshape*” function in *geomorph*.

ECOMORPH COMPARISONS

To examine the association between structural habitat usage and claw morphology, we tested for the effects of ecomorph classification on univariate claw traits in Greater Antillean anoles using phylogenetically corrected ANOVAs (Garland et al. 1993) implemented by the “*phylANOVA*” function in *phytools*. If ANOVAs were significant, we then conducted post hoc pairwise comparisons between ecomorph categories. For multivariate claw shape, we analyzed the effects of ecomorph classification using Procrustes ANOVAs in a phylogenetic framework (Adams 2014a) implemented by the “*procD.lm*” function in *geomorph*. We then calculated geometric morphometric shape disparity as the Procrustes variance for each ecomorph using the “*morphol.disparity*” function in *geomorph*. To test whether levels of disparity differed between ecomorphs, we performed pairwise comparisons using absolute differences in Procrustes variances as test statistics compared against null distributions generated by permuting residuals across groups (Zelditch et al. 2012).

ARBOREALITY AND PERCH DIMENSIONS

Although ecomorph classification denotes differences in structural habitat use, no truly terrestrial ecomorph is present in the

Greater Antilles and only a single “unique” species, *A. barbouri*, can be classified as terrestrial. Thus, to evaluate the effects of arboreal and terrestrial ecologies, we classified all Greater Antillean ecomorphs as arboreal and included mainland terrestrial species in comparisons between arboreal and terrestrial claw morphology. We compared terrestrial and arboreal species for each univariate claw trait using phylogenetically corrected ANOVAs. For geometric morphometric claw shape, we tested for differences between terrestrial and arboreal species using Procrustes ANOVAs in a phylogenetic framework.

To examine the association between claw morphology and different characteristics of perch substrate, we compiled mean perch height and perch diameter estimates for 37 species (Losos and Sinervo 1989; Losos 1990a; Losos and Irschick 1996; Losos and de Queiroz 1997; Schettino et al. 2010; J. B. Losos, unpubl. data). Perch data for the remainder of our study species were not readily available. As with morphological traits, if perch traits were significantly correlated with body size, we analyzed residuals after regressing the trait against log-transformed SVL. We then tested if log perch height and diameter were correlated with univariate claw traits using phylogenetic generalized least squares (PGLS). Next, we performed phylogenetic Procrustes regressions (Adams 2014a) to test for relationships between perch height and diameter with multivariate claw shape. We excluded twig, grass-bush, and crown-giant anoles from analyses of perch height because their highly specialized habitat utilizations are not principally differentiated by vertical perch height (Losos 2009). The remaining ecomorphs (trunk-ground, trunk, and trunk-crown anoles) represent different degrees of arboreality through their vertically structured use of tree trunk microhabitat.

PHENOTYPIC INTEGRATION

To test for patterns of phenotypic integration across univariate traits, we performed PGLS between all combinations of univariate claw and toepad traits. We then estimated the degree of phenotypic integration between geometric morphometric claw and toepad shape, accounting for phylogeny, under Brownian motion by partial least squares (Adams and Felice 2014) as implemented by the “*phylo.integration*” function in *geomorph*. To assess statistical significance of integration between geometric morphometric claw and toepad shapes, we compared our observed r_{PLS} against a null distribution of values generated by permuting claw data relative to toepad data.

RATES OF MORPHOLOGICAL EVOLUTION

We examined the rate of morphological evolution for both univariate claw and toepad characteristics and multivariate shape traits. To test whether the rates of evolution are decoupled between toepads and claws, we fit both a single-rate model and an independent-rate model of continuous trait evolution under

Brownian motion for all pairs of univariate traits and compared these fitted models using likelihood ratio tests (Adams 2013) implemented through the “ratebytree” function in *phytools*. Because we used natural log-transformed values to standardize measurements, the variation in rate is the relative change, in proportion to the mean, for each trait.

To examine differences in rates of evolution across ecomorphs, we calculated net rates of morphological evolution for multivariate shape data for each ecomorph using the “compare.evol.rates” function in *geomorph*. Due to the high dimensionality of geometric morphometric shape data, we followed the approach of Adams (2014b), which uses the sum of squared distances between phylogenetically transformed data and the origin in multidimensional morphospace to estimate rates of evolution. We estimated the rates of evolution for toepad and claw shapes separately. The ratio between the rates of evolution was used as a test statistic, with the ratio between the minimum and maximum rate across ecomorphs used to determine overall ecomorph differences (Denton and Adams 2015). We estimated statistical significance using phylogenetic simulation under Brownian motion, assuming a common evolutionary rate across all species, to generate a null distribution of rate ratios.

Results

BODY SIZE CORRELATIONS

We found that both claw length ($F_{1,50} = 160.0$, $R^2 = 0.757$, $P < 0.001$; slope = 1.20, 95% CI [1.01, 1.39]) and claw height ($F_{1,50} = 435.7$, $R^2 = 0.895$, $P < 0.001$; slope = 1.24, 95% CI [1.12, 1.35]) scaled allometrically with body size, but claw curvature scaled independently of body size ($F_{1,52} = 3.667$, $R^2 = 0.050$, $P = 0.061$; slope = 0.106, 95% CI [-0.01, 0.22]). Therefore, we corrected claw length and height, but not curvature, for body size in subsequent analyses.

ECOMORPH COMPARISONS

Consistent with the anole ecomorph hypothesis, we found that both multivariate ($F_{5,40} = 3.24$, $P = 0.001$; Fig. 2A, B) and univariate claw shape were significantly predicted by ecomorph classification. For univariate traits, ecomorph classification significantly predicted relative height ($F_{5,40} = 10.35$, $P = 0.002$), relative length ($F_{5,40} = 8.33$, $P = 0.004$), and curvature of the claw ($F_{5,40} = 4.79$, $P = 0.046$; Fig. 3A–C). Consistent with previous work (Irschick and Losos 1998; Losos et al. 1998; Macrini et al. 2003; Schaad and Poe 2010), we found that toepad shape was predicted by ecomorph classification (all $P < 0.05$).

Subsequent post hoc pairwise tests found twig anoles significantly differed in multivariate claw shape from crown-giant ($P = 0.003$), grass-bush ($P < 0.001$), trunk-crown ($P = 0.004$),

and trunk-ground anoles ($P = 0.035$). Trunk-crown anoles also significantly differed in multivariate claw shape from grass-bush ($P = 0.035$) and trunk-ground anoles ($P = 0.033$). For univariate measurements, claw curvatures in twig anoles were significantly different from trunk-crown ($P = 0.002$) and trunk anoles ($P = 0.023$) and claw curvature in trunk-ground anoles was significantly different from trunk-crown anoles ($P = 0.016$). However, only twig anoles were significantly differentiated for both relative claw height and length (all $P < 0.005$) in post hoc pairwise comparisons (Fig. 3).

We also found that levels of morphological disparity or shape variance differed across ecomorphs. Morphological shape disparity for claws was significantly greater in twig anoles than all other ecomorphs (all $P < 0.05$). Twig anoles had 2.8 to 4.7 times greater shape disparity for claws than other ecomorphs, whereas no other ecomorphs differed significantly from each other (all $P > 0.05$; Fig. 2C).

ARBOREALITY AND PERCH DIMENSIONS

We found significant differences in both multivariate claw shape ($F_{2,54} = 3.36$, $P = 0.016$; Fig. 4) and univariate claw curvature ($F_{1,55} = 16.22$, $P = 0.001$) between arboreal and terrestrial anoles. Consistent with predictions from previous functional studies (Zani 2000), claw curvature was greater in arboreal species compared to terrestrial species. However, we found no significant differences between arboreal and terrestrial species for either relative claw height ($F_{1,55} = 0.17$, $P = 0.865$) or length ($F_{1,55} = 1.86$, $P = 0.205$; Fig. 4).

Perch diameter was significantly correlated with body size ($F_{1,35} = 6.95$, $P = 0.012$), whereas perch height was not correlated with body size ($F_{1,20} = 0.79$, $P = 0.384$). Thus, we corrected perch diameter, but not perch height, for body size in tests for correlations between claw morphology and perch dimensions. We did not find a significant positive correlation between log perch height and relative claw height ($F_{1,20} = 1.33$, $P = 0.262$), claw length ($F_{1,20} = 1.07$, $P = 0.313$), or claw curvature ($F_{1,20} = 3.06$, $P = 0.080$), although the latter did approach significance. We did find a significant positive correlation between relative perch diameter and relative claw height ($F_{1,35} = 3.99$, $P = 0.046$) but not with relative claw length ($F_{1,35} = 1.94$, $P = 0.316$) or claw curvature ($F_{1,35} = 0.02$, $P = 0.877$) (Fig. 3D–I). For multivariate claw shape, we found that log perch height was significantly associated with claw morphology ($F_{1,20} = 5.90$, $R^2 = 0.286$, $P = 0.006$), but relative perch diameter was not ($F_{1,35} = 1.51$, $R^2 = 0.041$, $P = 0.213$). Finally, we found that perch height predicted toepad morphology (all $P < 0.05$).

PHENOTYPIC INTEGRATION

We found significant signals of phenotypic integration between some univariate toepad and claw traits. As predicted, claw

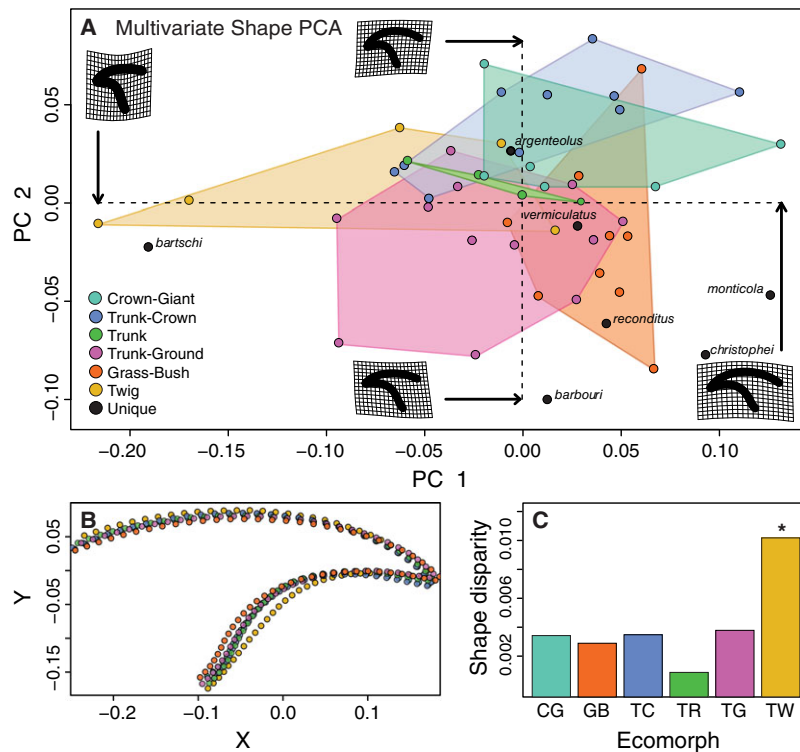


Figure 2. (A) Plot of the first and second Procrustes principal components for geometric morphometric claw shape across Greater Antillean anoles. Each data point represents a single species. Minimum convex hulls for each ecomorph and warp grids for extreme values of PC1 are displayed. (B) Mean claw shapes for each ecomorph. Points represent mean positions for individual semilandmarks. (C) Morphological shape disparity represented as Procrustes variances for each ecomorph (* $P < 0.05$). All plots are color coded by ecomorph. Unique anoles were not compared in plots B and C. CG = crown-giant; TC = trunk-crown; TR = trunk; TG = trunk-ground; GB = grass-bush; TW = twig.

curvature was positively correlated with toepad traits (relative lamellae: $F_{1,51} = 4.765$, $P = 0.033$; relative toepad width: $F_{1,51} = 4.283$, $P = 0.044$; Fig. 5), and we found no correlation between univariate toepad traits and claw height or width ($P > 0.05$). For our geometric morphometric data, we recovered a significant signal of phenotypic integration between claw and toepad shape ($r_{PLS} = 0.691$, $P = 0.001$; Fig. 5).

RATES OF MORPHOLOGICAL EVOLUTION

Comparing rates of morphological evolution between toepad and claw traits, we found no significant differences between relative lamellae number, relative toepad width, relative claw height, and claw curvature (Table 1). However, relative claw length evolved faster than all of these traits. We also found that evolutionary rates for the overall shapes of toepads ($\sigma_1^2/\sigma_2^2 = 6.222$, $\sigma_{all}^2 = 1.30 \times 10^{-3}$, $P = 0.008$) and claws ($\sigma_1^2/\sigma_2^2 = 7.0684$, $\sigma_{all}^2 = 1.70 \times 10^{-3}$, $P = 0.001$) significantly differed among ecomorphs (Fig. S3). Toepad shape in crown-giant anoles evolved faster than in all other ecomorphs except twig anoles (Table 2), and no other ecomorphs differed significantly in the rate of toepad shape evolution. For claw shape, both crown-giant and twig anoles

evolved significantly faster than grass-bush, trunk, and trunk-ground anoles (Table 3), and the rate of claw evolution was significantly slower in trunk anoles compared to all other ecomorphs (Table 3).

Discussion

Anoles adaptively radiated on each of the four Greater Antillean islands by vertically partitioning structural habitat (Williams 1972; Losos 2009), and adhesive toepads have been proposed as a key innovation that promoted diversification (Irschick et al. 2006; Losos 2009). Our analyses suggest that not just toepads but a complex evolutionary interplay between toepad and claw traits was involved in adaptation to arboreal environments and allowed for diversification by increasing access to arboreal niche space.

ARE CLAW HEIGHT AND CURVATURE ASSOCIATED WITH GREATER ARBOREALITY?

Arboreal habitats exert different selection on locomotor traits compared to terrestrial habitats because arboreal species must traverse vertical surfaces (Richardson 1942; Cartmill 1974; Glen

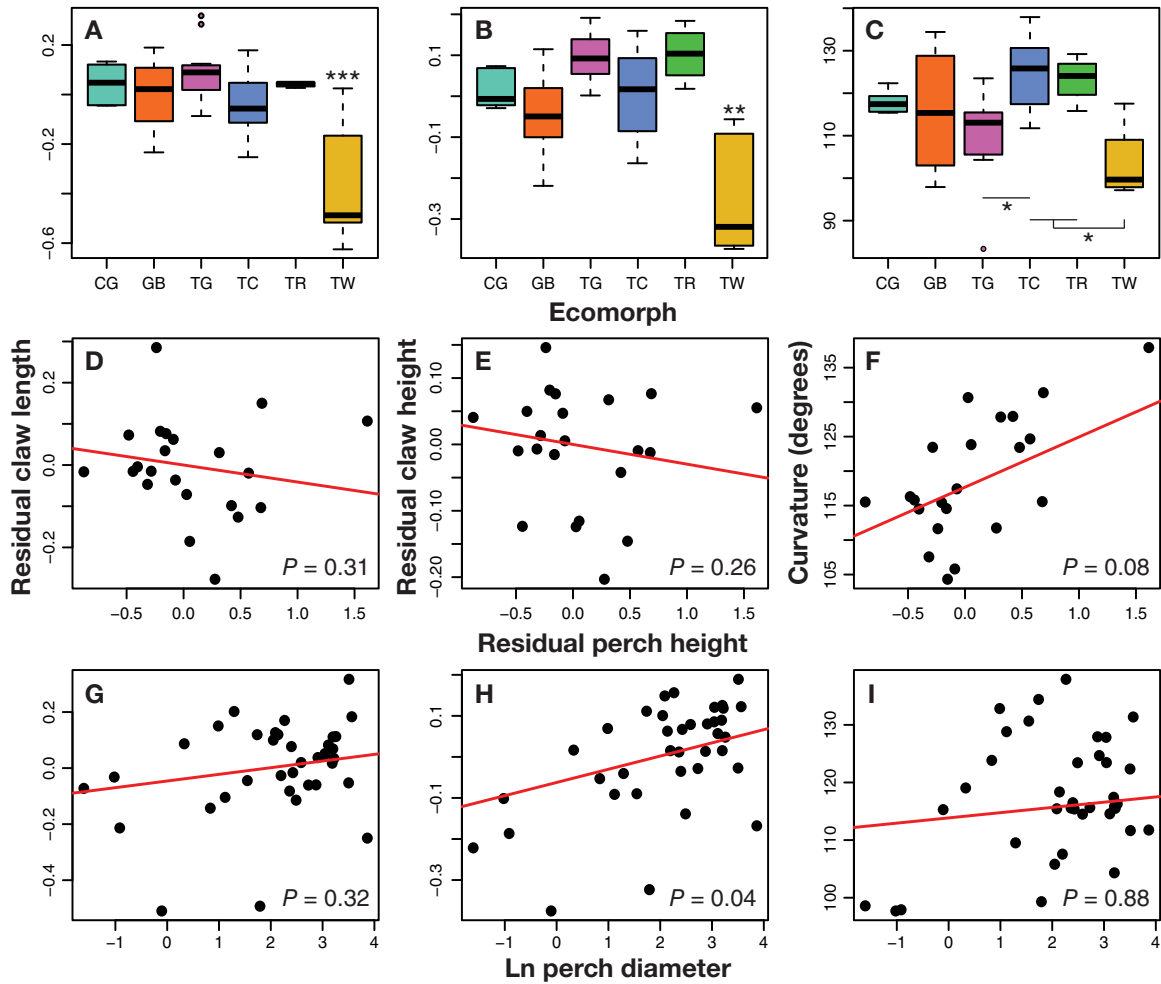


Figure 3. (A–C) Boxplots of univariate measures of claw morphology for each ecomorph (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$). (D–I) Relationship between perch dimensions and claw traits. crown-giant, grass-bush, and twig anoles are not included in models of perch height. For all plots, body size correct claw height and length are represented by residuals of regressions for each trait against SVL. Unique anoles are not depicted. CG = crown-giant; TC = trunk-crown; TR = trunk; TG = trunk-ground; GB = grass-bush; TW = twig.

Table 1. Pairwise comparisons of evolutionary rate between univariate toepad and claw traits.

	Lamellae	TPW	CH	CL	Curvature
Lamellae	4.0×10^{-4}	0.015	0.531	7.783	0.586
TPW	0.903	4.0×10^{-4}	0.368	8.451	0.415
CH	0.466	0.544	3.0×10^{-4}	12.25	0.001
CL	0.005	0.003	<0.001	9.0×10^{-4}	12.40
Curvature	0.444	0.520	0.970	<0.001	3.0×10^{-4}

TPW = toepad width; CH = claw height; CL = claw length.

Likelihood ratio tests were conducted to compare a single-rate model with an independent two-rate model of phenotypic evolution. The estimated independent rate of evolution for each trait is denoted on the diagonal. P values from likelihood ratio tests are shown in the lower off-diagonal with statistically significant values bolded. Likelihood ratios are shown in the upper off-diagonal.

and Bennett 2007). We found that arboreal anoles have more curved claws relative to terrestrial species (Fig. 4), consistent with the pattern of claw curvature and arboreality in other squamate reptiles (Zani 2000; Tulli et al. 2009, 2011; D’Amore

et al. 2018) and in birds (Feduccia 1993; Zeffer et al. 2003; Glen and Bennett 2007; Birn-Jeffery et al. 2012). We also observed this pattern, at finer levels of differentiation, when comparing species with different degrees of arboreality, either between

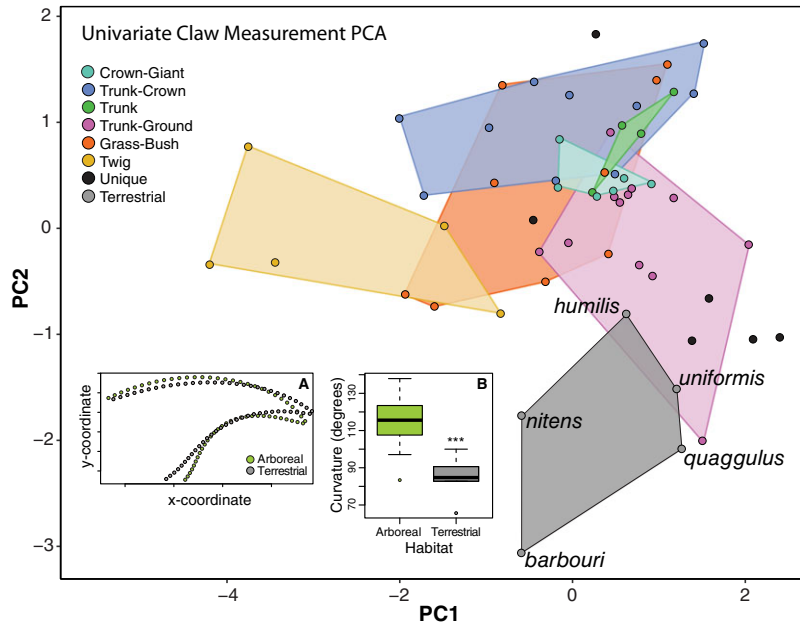


Figure 4. Principal component analysis (PCA) plot of first and second principal components for univariate claw measurements. Convex hulls are depicted for each Greater Antillean ecomorph and terrestrial species (both Greater Antillean and mainland) are labelled. Insets: (A) superimposed means of geometric morphometric shape for terrestrial and arboreal species; (B) boxplots of claw curvature measurements for arboreal and terrestrial species (***P* < 0.001).

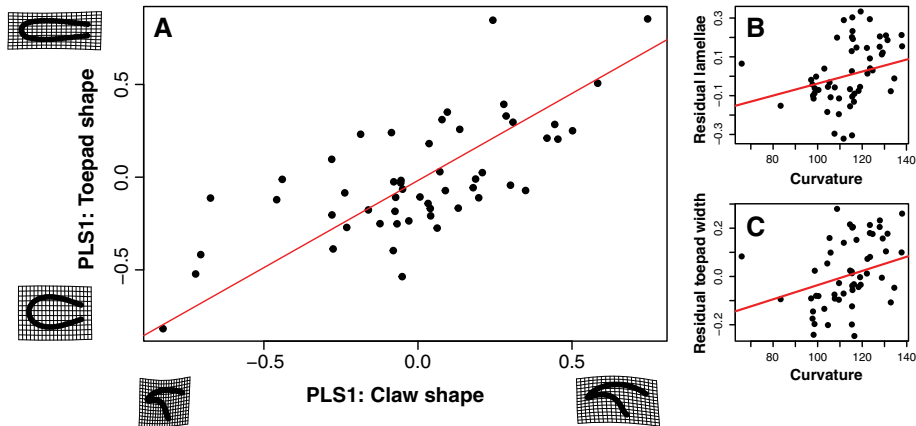


Figure 5. (A) Partial least squares plot of phenotypic integration between overall claw and toepad traits ($r_{PLS} = 0.691$, $P = 0.001$). Warp grids depict extreme shapes for each trait. Positive correlation between claw curvature and both (B) body size-corrected lamellae number and (C) body size-corrected toepad width. Body size-corrected values are residuals from regressing log trait values on log SVL.

Table 2. Pairwise comparisons of evolutionary rate for multivariate toepad shape across ecomorphs.

	CG	GB	TC	TR	TG	TW
CG	2.95×10^{-3}					
GB	0.010	8.53×10^{-4}				
TC	0.023	0.550	1.09×10^{-3}			
TR	0.001	0.246	0.105	4.74×10^{-4}		
TG	0.001	0.644	0.238	0.412	7.17×10^{-4}	
TW	0.121	0.357	0.711	0.067	0.206	1.31×10^{-3}

CG = crown-giant; TC = trunk-crown; TR = trunk; TG = trunk-ground; GB = grass-bush; TW = Twig.

The estimate rate of evolution for each ecomorph is denoted on the diagonal. The overall rate of evolution, $\sigma_{all}^2 = 1.30 \times 10^{-3}$. *P* values for comparisons of rate ratios between ecomorphs are denoted on the off-diagonal. Significant comparisons are bolded.

Table 3. Pairwise comparisons of evolutionary rate for multivariate claw shape between ecomorphs.

	CG	GB	TC	TR	TG	TW
CG	3.13×10^{-3}					
GB	0.003	9.95×10^{-4}				
TC	0.075	0.154	1.60×10^{-3}			
TR	0.001	0.057	0.003	4.43×10^{-4}		
TG	0.005	0.676	0.278	0.018	1.14×10^{-3}	
TW	0.760	0.011	0.172	0.001	0.033	2.74×10^{-3}

CG = crown-giant; TC = trunk-crown; TR = trunk; TG = trunk-ground; GB = grass-bush; TW = twig.

The estimated rate of evolution for each ecomorph is on the diagonal. The overall rate of evolution is $\sigma_{\text{all}}^2 = 1.70 \times 10^{-3}$. *P* values for comparisons of rate ratios between ecomorphs are below the off-diagonal. Significant comparisons are bolded.

ecomorph specialists (i.e., trunk-crown vs. trunk-ground ecomorphs; Fig. 3C) or between species utilizing different perch heights (claw curvature vs. perch height; Fig. 3F). One study of within-species variation in an anole (*Anolis cybotes*) also found reduced claw curvature associated with lower perch height (Wollenberg et al. 2013). However, our results contradict with those previously reported in mainland anoles (Crandell et al. 2014). Yet, we note that curvature values reported in Crandell et al. (2014) are inconsistent with our data, as well as those shown for other taxa (Birn-Jeffery et al. 2012; Wollenberg et al. 2013). For example, we measured a mean curvature of 100.27 ± 4.6 degrees in *A. humilis*, whereas Crandell et al. (2014) reported 34.67 ± 1.0 degrees. Therefore, we believe the results of Crandell et al. (2014) are likely to be inaccurate. Due to the widespread nature of the pattern of greater claw curvature in more arboreal species, some have hypothesized that curved claws provide functional benefits on vertical surfaces, whereas flattened claws provide functional benefits on horizontal surfaces (Glen and Bennett 2007; Tulli et al. 2009; Birn-Jeffery et al. 2012). The dramatically flattened claws of terrestrial anoles relative to arboreal species and performance assays demonstrating that more curved claws facilitate increased clinging force on vertical surfaces (Zani 2000; Dai et al. 2002; Tulli et al. 2011; Song et al. 2016) provide strong evidence that claw curvature plays an adaptive role in habitat utilization.

Contrary to patterns observed in varanid lizards (D'Amore et al. 2018) and squamates more broadly (Zani 2000; Tulli et al. 2009), we did not find evidence of differences in relative claw height between arboreal and terrestrial anoles. Because the ancestral state of anoles is arboreal rather than terrestrial (Losos 2009), taller claws in terrestrial species may reflect retention of the ancestral condition, particularly, if thinner claws do not offer performance benefits for terrestrial locomotion. If the ancestral claw height of anoles was sufficient to withstand the forces exerted by climbing, selection may not have favored subsequent evolution of claw height.

Beyond differences between arboreal and terrestrial species, our data suggest that different aspects of claw morphology are

associated with different aspects of perch substrates. Greater claw height was correlated with greater average perch diameter, whereas greater claw curvature was correlated with greater average perch height (Fig. 3). Because bark texture is often smoother higher in trees (Ferrenberg et al. 2013), highly curved claws may be favored in species with greater perch heights due to their increased performance on smoother substrates (Zani 2000; Tulli et al. 2011). Similarly, the positive relationship between claw height and perch diameter may be because taller claws improve performance on rougher surfaces (Zani 2000; Tulli et al. 2011) common in trees of larger diameter (Ferrenberg et al. 2013).

IS CLAW MORPHOLOGY ASSOCIATED WITH MICROHABITAT USAGE IN GREATER ANTILLEAN ANOLES?

Anole ecomorphs are characterized by convergent ecologies, behaviors, and morphologies related to microhabitat use (Williams 1972; Losos et al. 1998). Hence, the repeated evolution of anoles into ecomorph classes on each of the Greater Antillean islands provides an opportunity to examine the importance of claw and toepad traits at a finer level of niche divergence, that is, structural habitat partitions. Consistent with the convergence of other morphological traits (Williams, 1972; Losos, 2009; Kolbe et al. 2011), we found that ecomorph classification is associated with claw morphology for both univariate and multivariate shape analyses (Figs. 2–4), suggesting that claws are ecologically important for microhabitat partitioning.

Differences in claw shape were most pronounced in twig anoles, which were characterized by reduced (shorter and thinner, relative to body size) and flattened claws (Fig. 3). These results are consistent with other morphological studies across Greater Antillean anoles, which demonstrate that twig anoles have the most derived body plans of all anole ecomorphs, including elongate bodies and heads, reduced limbs, altered gaits, and in some cases prehensile tails (Hedges and Thomas 1989; Losos 1990b; Diaz et al. 1996; Beuttell and Losos 1999; Huyghe et al.

2007). Because twig anoles make use of a highly derived habitat relative to many other anole species—narrow twigs rather than tree trunks and large branches—these behavioral and morphological phenotypes likely represent a high degree of habitat specialization. The attachment capabilities of claws through frictional interlocking are partly determined by the relative size of surface protrusions and claw height (Song et al. 2016); thus, maintaining taller claws may be unnecessary in twig anoles due to the highly reduced diameter of their preferred perches. Loss of function is common in traits that do not provide fitness benefits in particular populations or species (Fong et al. 1995) and may explain the rapid evolutionary rate of claws, but not of toepads, in twig anoles (Tables 2 and 3; Fig. S3) through release from biomechanical constraint (Adams et al. 2017a; Sherratt et al. 2017). Although claw loss has never been observed in anoles, dramatic claw reduction and even complete loss are observed in toepad-bearing geckos (Russell and Bauer 2008; Khannoon et al. 2015). Still, we cannot rule out the possibility that reduced claw morphology is beneficial to twig anoles either through improved functional interactions with thin perches or because relatively larger claws are deleterious to their mode of locomotion, for instance, by interfering with toepad function.

Although less pronounced than differences between twig anoles and all other ecomorphs, trunk-ground anoles also significantly differ from trunk-crown anoles in claw curvature and overall shape (Figs. 2, 3A–C, and 4). These differences may reflect trade-offs in claw morphology due to relative performance on different substrates. Highly curved claws have been shown to be important for clinging in arboreal species (Zani 2000; Tulli et al. 2011), whereas flattened claws may facilitate propulsive force when running on flat surfaces in terrestrial species (Glen and Bennett 2007; Tulli et al. 2009; Birn-Jeffery et al. 2012). Thus, trunk-ground anoles may have flatter claws because they benefit from sacrificing clinging performance for greater ground sprint speed, although empirical tests confirming the relationship between flattened claws and ground sprint performance are still lacking in squamate reptiles.

IS THERE PHENOTYPIC INTEGRATION BETWEEN CLAW AND TOEPAD TRAITS?

Our results support the hypothesis that covariation between toepad traits and claw curvature results from correlative selection due to microhabitat use. Consistent with predictions based on functional studies of fine-scale claw and toepad morphology (Zani 2000; Tulli et al. 2011; Crandell et al. 2014), we observe strong covariation between traits adapted to similar functions and a lack of covariation between traits adapted to different functions. Specifically, we observe phenotypic integration across fine-scale claw and toepad attributes associated with performance on either rough or smooth surfaces. Claw curvature is positively correlated with

toepad width and lamella number, likely representing adaptation for performance on smoother surfaces. Previous work has shown that greater claw curvature, greater toepad size, and more lamellae are all associated with increased clinging performance particularly on smoother surfaces (Zani 2000; Vanhooydonck et al. 2005; Tulli et al. 2011; Gillies et al. 2014). Conversely, the lack of correlation between toepad traits and claw height or length in Greater Antillean anoles may be a product of differential selection due to their different individual functions. Claw height and toepad characteristics may be evolutionarily decoupled because greater claw height predominantly improves function on rougher surfaces where toepads are less effective (Zani 2000; Song et al. 2016), thus, reducing correlative selection. Similarly, claw length does not affect clinging performance on any surface (Zani 2000; Tulli et al. 2011) and, thus, is likely not under correlative selection with toepad traits related to adhesive performance. Still, overall elongation of the claw appears to be correlated with elongation of toepads or, at least, reduced toepad width in respect to length (Fig. 2A). We lack a functional hypothesis for this observed pattern because no relevant performance consequences have yet been demonstrated for claw or toepad elongation.

Alternatively, patterns of integration may also be driven by genetic or developmental linkage between toepads and claw curvature but not between toepads and other claw dimensions. However, across vertebrates the formation of terminal digit structures is controlled by developmental programs distinct from the remainder of the digit (Casanova and Sanz-Ezquerro 2007), with several genes only expressed in digit tips (Macias et al. 1997; Sanz-Ezquerro and Tickle 2003). Additionally, tip development is often unimpeded by disruptions to the developmental pathways controlling formation of the proximate and medial portions of digits (Baur et al. 2000; Kraus et al. 2001). Consequently, the strong developmental linkage between toepad and claw structures is unlikely to produce our observed relationships. Therefore, we suggest that patterns of phenotypic integration across Greater Antillean anoles are driven by correlative selection based on differential performance on specific attributes of the structural habitat rather than underlying developmental linkage.

DO RATES OF EVOLUTION COVARY BETWEEN CLAW AND TOEPAD TRAITS?

In addition to our morphological results, our analyses of evolutionary rates also support the hypothesis that claws and toepads are functionally integrated. Across ecomorphs, the rates of evolution for both claw and toepad shape were similar, except for the faster rate of claw shape evolution relative to toepads in twig anoles (Fig. S3). The only trait that had a significantly different rate of evolution was claw length, which evolved faster than all other claw and toepad traits (Table 1). Because biomechanically constrained traits experience strong selection, they often have

slower rates of evolution (Hertz et al. 2013; Adams et al. 2017a). Thus, the faster rate of evolution in claw length is likely due to its lack of biomechanical importance in arboreal anoles (Zani 2000). Similarly, the faster rate of claw evolution in twig anoles relative to toepads may be due to release from biomechanical restraint given their highly specialized form and locomotion.

Although diversifying selection can drive higher rates of morphological evolution in traits, such as those under sexual selection (Arnegard et al. 2010; Klaczko et al. 2015), high rates of morphological evolution are not always necessary to produce corresponding shifts in functional performance, particularly, in ecologically constrained traits (Gunderson et al. 2018). Small changes can produce large functional differences in some traits but not in others. Thus, it may be inappropriate to expect the magnitude of trait divergence to map directly onto changes in performance. For instance, relatively small and evolutionarily slow changes in physiology have been shown to play an important role in thermal habitat partitioning in Greater Antillean anoles (Gunderson et al. 2018). The slower rate of evolution for ecologically important traits (i.e., claw and toepad morphology) observed here suggests that rapid, large changes in morphology are not required to generate corresponding shifts in performance and, subsequently, ecological diversity.

Faster rates of claw evolution in crown-giants and twig anoles are consistent with their more derived overall body plans (Williams 1972; Beuttell and Losos 1999; Losos 2009) and may be linked to their more derived habitat use. However, grass-bush anoles do not show increased rates of evolution as would be expected if differences in habitat use and overall body plan strongly predicted rates of claw and toepad evolution. In agamid lizards, saxicolous and arboreal lineages have slower rates of evolution, suggesting that these ecologies constrain morphological evolution (Collar et al. 2010). Similar constraints may explain the relatively slower rates of morphological evolution observed for claws and toepads in most ecomorphs of anoles. However, semiarboreal agamids evolve faster (Collar et al. 2010), whereas trunk-ground and grass-bush anoles do not, suggesting that the influence of microhabitat use on rates of diversification is not universal.

Conclusions

Vertical habitat partitioning is a hallmark of the adaptive radiations of Greater Antillean anoles and is associated with a suite of convergent morphological adaptations characterized by ecomorph classification (Williams 1972; Losos et al. 1998; Losos 2009). We provide evidence that, at broad levels of ecomorphological divergence, claw morphology is similarly associated with macrohabitat (terrestrial and arboreal) and microhabitat (arboreal habitat partitions) use in Greater Antillean anoles and that patterns of covariation in claw and toepad morphology are likely driven by

correlative selection. In particular, the association of claw morphology with perch dimensions, arboreality, and ecomorph generally align with predictions from previous performance studies in other squamates (Zani 2000; Tulli et al. 2011). Moreover, we also demonstrate that, at a finer scale, specific claw traits are differentially associated with toepad traits and microhabitat features, leading to a pattern of phenotypic integration among traits that is strongly associated with their functional roles. Specifically, correlative selection for performance on smoother surfaces likely drove the integration of claw curvature with toepad traits (and associations between claw curvature and perch height), whereas selection for claw height on rougher surfaces likely led to it being decoupled from toepad traits, which have more limited functional roles on rougher surfaces (Zani 2000; Tulli et al. 2011). Additional functional studies are necessary to confirm the adaptive value of claw shape variation and to tease apart alternative hypotheses about the evolution of claws. Nevertheless, our results suggest that claws, in conjunction with toepads, are likely to have directly facilitated access to greater arboreal niche space and structural habitat partitioning across the replicated adaptive radiations of *Anolis* lizards and, thus, highlight the multivariate nature of adaptive phenotypic evolution.

AUTHOR CONTRIBUTIONS

M. L. Y. and M. H. W. conceived the project. I. J. W. and M. L. Y. provided financial support. M. L. Y. collected and analyzed the data with input from M. H. W. and I. J. W. M. L. Y. wrote the manuscript with important contributions from all coauthors.

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DATA ARCHIVING

All data from this study are archived in Dryad (<https://doi.org/10.5061/dryad.127894k>).

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Poe et al. (2017) phylogeny pruned to include only our focal taxa (left) and UPGMA tree generated from univariate claw measurements (right). Lines connect identical taxa on each tree and are colored by ecomorph classification.

Fig. S2 Plot of the first and second Procrustes principal components for geometric morphometric toepad shape of Greater Antillean anoles. Warp grids for extreme values of each PC are displayed. Inset: mean claw shapes for each ecomorph. Points represent mean positions for individual semilandmarks. Plots are color coded by ecomorph. Mean shapes for unique anoles are not depicted in inset.

Fig. S3 Estimated rates of evolution for claw (left, light) and toepad (right, dark) multivariate shape. Significant differences among rates are denoted by letters: lower case for claws and upper case for toepads. *P* values are indicated in Tables 2 and 3. Solid line represents overall rate of evolution for claw shape and dashed line represents overall rate of evolution for toepad shape.

Table S1 Institutional catalogue numbers for all individual specimens included in this study. Specimens are housed at the California Academy of Sciences (CAS; San Francisco, California), the Museum of Vertebrate Zoology (MVZ; University of California, Berkeley, California), and the National Museum of Natural History (USNM; Smithsonian Institution, Washington, District of Columbia).

Table S2 Measurements for all Greater Antillean species included in this study. Sample size (*N*) and mean values for snout-vent length (SVL), toepad width (TPW), lamella number, claw height (CH), claw length (CL), claw curvature, perch height (PH), and perch diameter (PD) are shown. For all measurements taken for this study, standard error is denoted following the mean value. Ecomorphs are abbreviated as follows: CG = crown-giant; GB = grass-bush; TC = trunk crown; TR = trunk; TG = trunk ground; TW = twig. Islands are abbreviated as follows: CUB = Cuba; HIS = Hispaniola; PUR = Puerto Rico; JAM = Jamaica; BAH = Bahamas; MNA = Mona; MLD = mainland Americas.