

Tempo and mode of performance evolution across multiple independent origins of adhesive toe pads in lizards

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Understanding macroevolutionary dynamics of trait evolution is an important endeavor in evolutionary biology. Ecological opportunity can liberate a trait as it diversifies through trait space, while genetic and selective constraints can limit diversification. While many studies have examined the dynamics of morphological traits, diverse morphological traits may yield the same or similar performance and as performance is often more proximately the target of selection, examining only morphology may give an incomplete understanding of evolutionary dynamics. Here, we ask whether convergent evolution of pad-bearing lizards has followed similar evolutionary dynamics, or whether independent origins are accompanied by unique constraints and selective pressures over macroevolutionary time. We hypothesized that geckos and anoles each have unique evolutionary tempos and modes. Using performance data from 59 species, we modified Brownian motion (BM) and Ornstein–Uhlenbeck (OU) models to account for repeated origins estimated using Bayesian ancestral state reconstructions. We discovered that adhesive performance in geckos evolved in a fashion consistent with Brownian motion with a trend, whereas anoles evolved in bounded performance space consistent with more constrained evolution (an Ornstein–Uhlenbeck model). Our results suggest that convergent phenotypes can have quite distinctive evolutionary patterns, likely as a result of idiosyncratic constraints or ecological opportunities.

KEY WORDS: Anole, Brownian motion, gecko, Ornstein–Uhlenbeck, toe detachment angle.

When investigating how the diversity (or lack thereof) of a trait arose, one of the first steps is to observe the variation present in the trait and investigate how the trait evolved through time, asking whether the trait has thoroughly explored a small part of trait space, or if the trait appears to have freely explored trait space. Thorough coverage of a limited region of trait space can suggest constrained evolution, possibly due to limited developmental or genetic variation, biomechanical constraints, or limited ecolog-

ical opportunity to adapt and change. Alternatively, a trait may appear to have explored trait space in a less constrained fashion. This may be due to fewer developmental, genetic, or biomechanical constraints, the trait accessing more open niches, or the trait being under weak selection, drifting through trait space with little consequence.

Knowledge of how a clade has evolved through trait space can be integrated into a fuller understanding of that clade's evolutionary history. If a clade has exhibited constrained evolutionary patterns, future studies can investigate how the focal trait may be limited by developmental, genetic, or mechanical constraints, or how biotic interactions have influenced the diversification of the

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trait. For example, habitat use/morphology correlations have been reported to differ between Caribbean and South American anoles (Irschick et al. 1997; Macrini et al. 2003). These differences may suggest Caribbean and mainland anoles have filled trait space differently, possibly due to differences in development, genetics, biomechanical considerations, or differences in abiotic or biotic conditions in the Caribbean and mainland South America.

In addition, morphological traits can be constructed in alternative ways to accomplish the same adaptive function, and these alternative constructions may or may not require similar amounts of morphological change to enable the organism to adapt to changing adaptive requirements. For these reasons, studying performance directly as a trait, as is the case in our study, rather than morphology may give a clearer picture of ecological function and evolutionary dynamics (Arnold 1983; Wainwright and Reilly 1994). Evidence of a clade having evolved constrained in performance space could be explained by a variety of situations. Focal clades may not have had the genetic, developmental, or mechanical capabilities to diversify and explore performance space, or there may have been limited niche space available to diversify into, similar to as if a focal trait was a morphological trait. In addition, when considering performance niche space, limited successful performance options do not impose limited underlying morphological diversity. Few adaptive options can lead to convergent or parallel morphological evolution, including many-to-one mapping, when different morphologies perform similarly. Alternately, evidence of unconstrained-performance evolution could be explained by behavioral plasticity, phenotypic plasticity, adaptive change tracking adaptive peaks, as well as weak selection allowing performance to drift through performance space.

Modeling the evolutionary history of a trait also requires some knowledge or assumptions about the origin or origins of the trait in question. While many studies have focused on the relationship between convergent morphology and performance, few studies have compared the tempo and mode of performance evolution in a comparative framework (but see Harmon et al. 2003). By focusing on convergent traits, we can better understand how limiting factors such as constraints or limited ecological opportunities have shaped the evolution of our focal clades.

Evaluating the fit of Ornstein–Uhlenbeck (OU) and Brownian motion (BM) models of trait evolution to a focal clade can identify how constrained (OU) or unconstrained (BM) the evolution of the trait has been (Lande 1976; Hansen 1997). Brownian motion models the diffusion of a trait through trait space with two parameters, the root value and a stochastic rate parameter (σ^2). Alternatively, OU models extend BM models to represent constrained evolution toward a target value (θ). OU has the additional parameter α , which describes the rate of pull toward the target trait value θ . As α gets smaller and approaches zero, an OU model converges toward a BM model. BM models can also be extended

to model a directional trend when a third parameter, μ , is nonzero, modeling the tendency of the trait value to consistently drift in a particular direction (positively or negatively) away from the root value.

In this study, we examine the evolutionary dynamics of performance in two groups of squamates: geckos and anoles. Adhesive toe-pads have evolved at least three times in Squamata: most famously in geckos, but also twice outside of Gekkota, in anoles and skinks. We define adhesive toe pads as having morphological traits such as setae or modified scales that generate both friction and adhesion (frictional adhesion; Autumn et al. 2006a). The results from previous studies have suggested one (Harrington and Reeder 2017) or multiple origins of toe pads within the 1700 described species of geckos (Underwood 1954; Haacke 1976; Russell 1976; Russell 1979; Irschick et al. 1996; Russell 2002; Gamble et al. 2012; Russell et al. 2015; Higham et al. 2016; Gamble et al. 2017). The adhesive system of lizards is an excellent system for investigating patterns of adaptation, constraint, and convergence. Gecko and anole toe pads are morphologically complex, being comprised of modified ventral scales with a free edge (lamellae) covered in small hair-like structures called setae. There is considerable morphological diversity among species at the macroscale, that is toe pad shape, skeletal features, and digital musculature (Russell 1979; Gamble et al. 2012) and at the microscale, that is setal morphology (Ruibal and Ernst 1965; Williams and Peterson 1982; Peattie 2007; Johnson and Russell 2009; Hagey et al. 2014). These structures are responsible for generating adhesion and friction on a variety of surface textures, self-cleaning, and not self-adhering (Hansen and Autumn 2005; Vanhooydonck et al. 2005; Autumn et al. 2006a; Huber et al. 2007; Persson 2007; Russell and Johnson 2007; Pugno and Lepore 2008b; Hu et al. 2012; Autumn et al. 2014; Russell and Johnson 2014) suggesting that while toe pads appear very diverse, there likely exists extensive constraints and limitations on their morphology and performance. It is likely that the evolution and adaptation of adhesive performance in padded lizards has balanced selective pressures and opportunities with mechanical and developmental constraints, likely limiting the options open to evolution and adaptation.

We considered how gecko and anole toe pad adhesive performance evolved by fitting a variety of stochastic models of trait evolution. We fit models with shared or independent parameter values and/or models across geckos and anoles, incorporating ancestral state reconstruction results into our models, to test the hypothesis that independent origins differ in rate (tempo) or pattern (mode). If a single-rate model is a good fit to our entire adhesive performance dataset, this would suggest that the performance of padded lizards and their convergent morphologies evolved under similar processes, shared mechanical, developmental constraints, and/or similar selection dynamics. In contrast, if

clade-specific models or parameters fit our data well, this would reveal a pattern of clade-specific evolutionary dynamics, likely associated with clade-specific constraints or ecological opportunities (Hansen 1997; Butler and King 2004; Yoder et al. 2010; Eastman et al. 2013). Considering patterns of performance evolution in conjunction with ancestral information improves our understanding of how historical processes of adaptation have shaped extant diversity, morphology, and performance.

Methods

ESTIMATION OF THE NUMBER OF ORIGINS OF TOE PADS ACROSS SQUAMATA

To identify independent origins of adhesive toe pads in lizards, we used a large, species-level phylogeny of Squamata (Pyron and Burbrink 2013). While this phylogeny has topological differences as compared to other smaller, group-specific phylogenies (Sadleir et al. 2005; Brown et al. 2012; Gamble et al. 2012; Oliver et al. 2012), we do not feel these differences impacted our results. Also see Title and Rabosky (2016) for comments on the use of large macrophylogenies in diversification studies. We chose a time-scaled, ultrametric phylogeny because our models of trait evolution model trait change in relation to time rather than sequence divergence. We assigned presence or absence of toe pads to each species in the phylogeny (4162 species). Four species of skinks are known to have adhesive pads, *Prasinohaema virens*, *P. flavipes*, *P. prehensicauda*, *Lipinia leptosoma* (Williams and Peterson 1982; Irschick et al. 1996; Pianka and Sweet 2005). Of the three pad-bearing *Prasinohaema* species, only *P. virens* is in the Pyron and Burbrink (2013) phylogeny. In addition, only one species of *Lipinia* is in the phylogeny (*L. pulchella*). We substituted *L. leptosoma* for *L. pulchella* without a loss of phylogenetic information (Austin 1998) for a total of two pad-bearing skink species in our toe pad presence/absence dataset. We assigned the presence of toe pads to all *Anolis* species in the phylogeny (207 species) except *A. onca* (Peterson and Williams 1981; Nicholson et al. 2006). To assign presence/absence to geckos, we modified generic-level assignments from Gamble et al. (2012) adding information from Wilson and Swan (2010) and personal observations (T.H.), to assign toe pad presence (472 species) or absence (188 species) to all 660 species of geckos in the phylogeny (see Fig. 3 and Supplemental Material). The remaining lizard and snake species in the tree were considered padless.

Using the complete phylogeny of Pyron and Burbrink (2013), we estimated the number of origins of adhesive toe pads across squamates by combining Bayesian estimates of transition rate matrices with stochastic character mapping. We estimated transition matrices for a binary-state, Mk model with asymmetric transition rates allowing the rates of pad gain and loss to vary (i.e., q_{10} and q_{01} were not constrained to be equal) using the R package

Diversitree (FitzJohn 2012). We then ran a Bayesian MCMC for 10,000 generations sampling every 100 generations, with an initial burn-in of 3000 generations, resulting in a posterior sample of 701 Q matrices. To visualize our reconstructions, monomorphic clades were collapsed, resulting in a phylogeny with 118 tips. Using the posterior sample of Q-matrices, we generated 701 simmap phylogenies using the R function *make.simmap* in the phytools package (Revell 2012). Of particular interest was the number of independent origins of toe pads within geckos (Gamble et al. 2012). We therefore counted the number of estimated origins in Gekkota across the simmap-generated reconstructions to obtain a posterior sample of origins.

COLLECTION OF PERFORMANCE DATA

Previous studies of pad-bearing lizards have quantified adhesive performance in multiple ways (Irschick et al. 1996; Autumn et al. 2006a,b; Pugno and Lepore 2008a; Autumn et al. 2014; Hagey et al. 2014; Hagey et al. 2016). We chose to use the angle of toe detachment, which was first used to quantify adhesive performance in frogs (Emerson 1991; Moen et al. 2013) and subsequently in geckos (Autumn et al. 2006a; Hagey et al. 2014, 2016). The angle of toe detachment is directly related to the adhesive mechanics of setae (Autumn et al. 2006a; Tian et al. 2006) and can be measured easily in the laboratory or field with relatively simple equipment (see Supplemental Material). This approach quantifies the maximum proportion of adhesion (negative normal force), relative to friction, generated by a species' toe pad (see Fig. 1 and Methods). We quantified adhesive performance across three families of geckos (Gekkonidae, Phyllodactylidae, and Diplodactylidae) and the genus *Anolis* (see Supplemental Material). Our toe detachment observations were collected following previous studies, using captive and wild caught specimens from the field (Costa Rica, Panama, Thailand, and Australia) and the lab (Autumn et al. 2006a; Hagey et al. 2014, 2016). We used a variety of equipment setups that included powered rotational stages, stepper motors (including Lego Mindstorm motors), and manual rotational stages. To measure angle of toe detachment, live nonsedated lizards were suspended via the toe pad of a single rear toe from a vertical glass microscope slide (Video links in Supplemental Material; Autumn et al. 2006a; Hagey et al. 2014, 2016). Variation in performance across toes has not been previously investigated and so we strived to always test similar toes. Our trials alternated between the longest left and right rear toes, or the center rear toes if all rear toes were similar in length. Using a single toe eliminated confounding forces that would be generated by multiple toes acting in opposing directions. During each toe detachment trial, the glass substrate was initially vertical with the animal's toe pad generating friction relative to the substrate (and likely little adhesion, that is force perpendicular and toward the glass). The glass substrate was then slowly inverted. When this

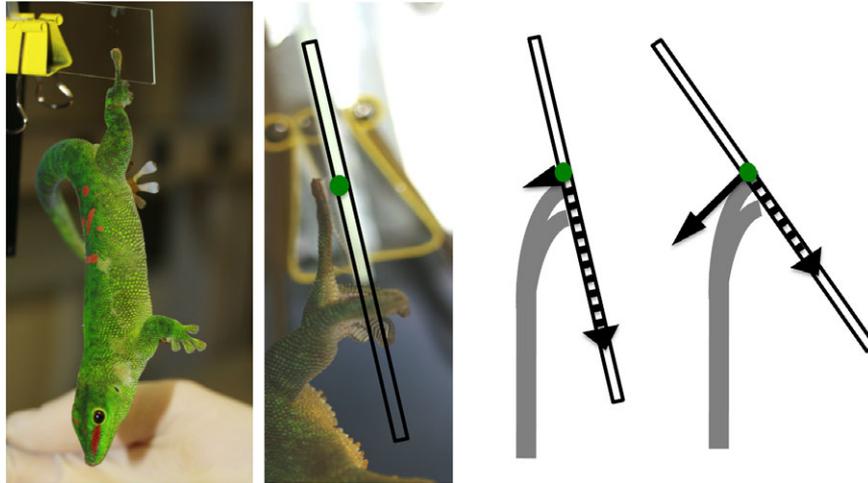


Figure 1. Angle of toe detachment assay. To quantify toe detachment angle, a pad-bearing lizard is suspended from a glass microscope slide by a single rear toe (left images). When the glass substrate is near vertical, the lizard's toe pad, and hence setae, are predominantly generating friction relative to the substrate (see right images, seta illustrated in gray, friction illustrated as dotted arrows). As the substrate is slowly inverted, the setae generate relatively less friction and more adhesion (see far right image, adhesion illustrated as solid arrow). At the angle of toe detachment, the setae can no longer maintain the proper orientation with the substrate to remain attached and the animal falls onto a cushioned base (see video links in Supplemental Material). As a result, the angle of toe detachment quantifies the maximum amount of adhesion, relative to friction, generated. Image modified from Hagey et al. (2014).

occurred, the setal shaft angle increased, generating adhesion, and friction relative to the glass. At the angle of toe detachment, the maximum ratio of adhesion to friction that the toe pad was capable of generating was exceeded, and the animal fell onto a cushioned pad (see Fig. 1 and video links in Supplemental Material). Toe-pad area has previously been shown to correlate with the amount of friction generated by anole toe pads (Irschick et al. 1996), presumably due to the fact that larger pads have more setae interacting with the substrate. This relationship has not been investigated regarding toe detachment angle. While we would not predict toe-pad area to correlate with toe detachment angle, due to the fact that detachment angle is weight independent and likely related to setal morphology (Autumn et al. 2006a) and not the absolute number of setae contacting the surface, this relationship still requires evaluation.

Our performance observations included measurements of over 250 individual lizards from 59 species (13 species of anoles and 46 species of geckos; Fig. 3; see Supplemental Material). Our dataset had a minimum of two observations per individual and maximum of 49, with a mean of 9.1 observations per individual. We collected five or more observations from 91% of the individuals sampled. Observations from each individual lizard were fit to a Weibull distribution, which is often used in “time-to-failure” analyses (McCool 2012). The Weibull scale parameter, with standard error, was then estimated, representing each individual's detachment angle (Hagey et al. 2016). To produce a mean value for each species, we calculated a weighted average using each individual's estimated Weibull scale value, weighting by the inverse of its es-

timated standard error. In six of our 59 focal species, we did not record individual identity for each performance trial; therefore we estimated performance of these species as if all observations were from a single individual (see Table S1).

MODELING TRAIT EVOLUTION

We performed all trait evolution analyses using untransformed performance data. Natural-log transforming our data would artificially emphasize differences between small detachment angles and reduce differences between large detachment angles. Our initial analyses fit single and multiregime BM and OU models of trait evolution via a maximum likelihood approach with the use of a priori assigned clades using the R package OUwie (Beaulieu et al. 2012). We also conducted analyses not requiring a priori clade assignments using the R packages AUTEUR (Eastman et al. 2011), fitting multiregime BM models, and SURFACE (Ingram and Mahler 2013), fitting multi- θ OU models (See Supplemental Material). In our OUwie analyses we considered seven models in total, including species mean errors. Our two simplest models were a Brownian motion model (BM1) and an Ornstein–Uhlenbeck model (OU1) that each fit a single set of parameters. Our other five models fit unique parameter values in various combinations to the gecko and anole clades. The decision to assign unique parameter values to anoles and geckos followed the results obtained from our ancestral state reconstruction, with anoles and geckos representing independent origins of toe pads, although we note that other studies have suggested multiple independent origins within geckos (see Introduction and Discussion). We fit the

following models: a BM model with variable evolutionary rates (σ^2) and single root value ($\text{BM}\sigma^2$), an OU model with single α and σ^2 parameter value and different optima (θ) values ($\text{OU}\theta$), an OU model with a single α but multiple rate (σ^2) and optima (θ) parameter values ($\text{OU}\sigma^2\theta$), an OU model with a single σ^2 but variable α and θ values ($\text{OU}\alpha\theta$), and a OU model ($\text{OU}\sigma^2\alpha\theta$) in which all three parameters, σ^2 , α , and θ , varied (Table 1; Beaulieu et al. 2012). We then compared the fit of our seven models using AICc weights based on relative model likelihoods (Table 1; Burnham and Anderson 2002).

The models we have described so far can sometimes rely on unrealistic assumptions. These models estimate a trait value at the root, which is the phylogenetic weighted mean of tip states for our BM1 and OU1 models. In our case, toe pads have had multiple origins, with the backbone of the squamate phylogeny likely lacking toe pads. Our model assumptions regarding performance at the root of the tree, the most recent shared common ancestor of geckos and anoles, is inferred to have a performance that is near the average of geckos and anoles. This is almost surely in error. Incorrect root-node trait values can affect parameter estimate values and fit comparisons; for example, by allowing less change and/or a weaker α parameter value, mimicking Brownian Motion. To incorporate ancestral state information, we fit a set of BM and OU models that assumed independent origins for geckos and anoles using modified likelihood functions from the R packages *bayou* and *geiger* (Harmon et al. 2008; Pennell et al. 2014; Uyeda and Harmon 2014). We considered the lack of toe pads to have a performance value of 0° . Both the gecko and anole clades were assigned a root state of 0° and shifted to an OU or BM process model along their respective stem branch, with the timing of the initiation of the OU or BM model being allowed to vary along the branch, before diversification. When considering the likely evolution of setae from spinules, simple early structures likely initially generated friction but little adhesion, which would present itself as a low detachment angle. Higher detachment angles were likely achieved after the evolution of more complex setae (see Discussion). As a result, our assignment of detachment angles of 0° to padless species and the assumption that recently evolved toe pads have performance near zero is supported from a biomechanical and evolutionary point of view.

Stem branch dates were taken from the Pyron and Burbrink (2013) phylogeny. For geckos, the timing of the shift to an OU or BM process was constrained to occur between 168.8 mya (the timing of the divergence of geckos from other lizards) and 82.3 mya (the ancestral node of Gekkota). For anoles, the timing of the shift was constrained between 76.3 mya (the divergence of anoles from Corytophanidae) and 44.1 mya (the ancestral node of *Anolis*). We again considered single and multiregime models of BM and OU, constraining our OU models to a maximum θ value of 90° (no species has been observed sticking to a surface

with one toe beyond an angle of 45°). A total of nine models incorporating ancestral information were considered (models denoted by an asterisk, Table 1). We did not exhaustively fit all possible combinations of models, but instead let the results of earlier analyses guide our choices: BM with a shared σ^2 for both geckos and anoles (*BM1), Single-optimum OU with shared α and σ^2 parameters (*OU1), Brownian motion with a trend and shared mean, σ^2 , and μ parameter, where μ describes the rate of the trend (*BMT), Brownian motion with a trend and shared σ^2 , but different trend (μ) parameters for each clade (*BMT μ), an OU model with separate θ for each clade (*OU θ), OU with separate α and θ for each clade (*OU $\alpha\theta$), OU with separate σ^2 and θ for each clade (*OU $\sigma^2\theta$), OU with separate α , σ^2 , and θ for each clade (*OU $\sigma^2\alpha\theta$), and lastly a BM model with a trend fit to geckos and an OU model fit to anoles (*BMT $_G$ -OU $_A$). We computed AIC scores and AIC weights for each model using maximum likelihood optimization to evaluate which model was best supported by our data (Table 1). To supplement these analyses assuming one origin of toe pads within geckos, we also conducted a set of limited analyses assuming two origins of toe pads within Gekkota (see Supplemental Material).

In addition to this likelihood analysis, we fit the full *OU $\sigma^2\alpha\theta$ model using a Bayesian implementation in *bayou* (denoted *OU $\sigma^2\alpha\theta_{\text{Bayesian}}$ in Table 1). By considering our most complex model, we can compare posterior probabilities for inferring differences in parameters between clades. We set the following priors on the parameters: $\alpha \sim$ half-Cauchy (scale = 0.1), $\sigma^2 \sim$ half-Cauchy (scale = 0.1), $\theta \sim$ Uniform (min = 0, max = 90). Shift locations were given uniform priors over the length of the stem branches for geckos and anoles. We ran four chains for 1,000,000 generations and discarded the first 30% of the samples as burn-in. We then combined all the chains and estimated the median and 95% highest posterior density (HPD) interval for each parameter value.

For use in our comparative modeling, we modified the Pyron and Burbrink (2013) phylogeny by removing unsampled taxa. In a few cases we replaced closely related unsampled taxa with taxa for which we had performance measurements. We replaced *Afroedura karroica* and one of the closely related *Geckolepis* species with *A. havequensis* and *A. loveridgei*, possibly overestimating the divergence between our two sampled *Afroedura* species. We also had performance observations from the recently described *Oedura bella*, substituting it for the closely related *O. gemmata* (Oliver et al. 2012; Oliver and Doughty 2016).

Results

Regarding our reconstruction of the number of independent origins of toe pads, our posterior sample of transition matrices had negligible autocorrelation for all parameters and high effective

Table 1. Model of trait evolution fits and estimated parameters.

OUwie Models	AICc Weights	Parameter Values				
BM1	0.35	$\sqrt{\sigma^2}$	σ^2			
OU $\sigma^2\alpha\theta$	0.19	θ	σ^2	$\ln(2)/\alpha$		Anoles
		>90.0	3.56	>1000		Geckos
OU θ	0.13	θ	σ^2	$\ln(2)/\alpha$		Anoles
		19.0	0.33	161.7		Geckos
BM σ^2	0.12	$\sqrt{\sigma^2}$	σ^2			Anoles
		25.5	0.28			Geckos
OU1	0.12	θ	σ^2	$\ln(2)/\alpha$		Anoles
		25.5	0.28	>1000		Geckos
OU $\alpha\theta$	0.05	θ	σ^2	$\ln(2)/\alpha$		Anoles
		18.9	0.42	100.8		Geckos
OU $\sigma^2\theta$	0.05	θ	σ^2	$\ln(2)/\alpha$		Anoles
		18.9	0.29	114.0		Geckos
		32.4	0.39			Geckos
BAYOU Models	AICc Weights	Parameter Values				
*BMT _G -OU _A	0.37	$\sqrt{\sigma^2}$	σ^2	$\ln(2)/\alpha$	μ	Shift Time
		19.4	29.7	0.3	–	32.2
		0.0	0.27	–	0.35	82.3
*BMT	0.34	$\sqrt{\sigma^2}$	σ^2	$\ln(2)/\alpha$	μ	Shift Time
		0.0	0.27	–	0.34	23.4
						82.3
*BMT μ	0.18	$\sqrt{\sigma^2}$	σ^2	$\ln(2)/\alpha$	μ	Shift Time
		0.0	0.27	–	0.43	32.2
					0.35	82.3
*OU $\sigma^2\alpha\theta$	0.04	θ	σ^2	$\ln(2)/\alpha$	μ	Shift Time
		19.4	52.1	0.2	–	22.9
		90.0	0.33	208.2		23.6
*OU1	0.04	θ	σ^2	$\ln(2)/\alpha$	μ	Shift Time
		90.0	0.36	117.6	–	18.3
						49.9
*OU θ	0.02	θ	σ^2	$\ln(2)/\alpha$	μ	Shift Time
		90.0	0.36	117.6	–	18.3
		90.0				49.9
*OU $\alpha\theta$	0.01	θ	σ^2	$\ln(2)/\alpha$	μ	Shift Time
		90.0	0.41	2.3	–	21.4
		90.0		98.9		55.3
*OU $\sigma^2\theta$	0.01	θ	σ^2	$\ln(2)/\alpha$	μ	Shift Time
		90.0	0.30	98.9	–	21.4
		90.0	0.41			55.3
*BM1	0.00	$\sqrt{\sigma^2}$	σ^2	$\ln(2)/\alpha$	μ	Shift Time
		0.0	0.59	–	0.00	0.0
						0.0
*OU $\sigma^2\alpha\theta$ _{Bayesian}	–	θ	σ^2	$\ln(2)/\alpha$	μ	Shift Time
		22.6	0.68	12.9	–	18.1
		(17.2, 61.1)	(0.00, 3.08)	(0.1, 65.4)		(1.9, 32.2)
		66.7	0.39	121.5		24.5
		(39.2, 90.0)	(0.22, 0.66)	(37.1, 247.7)		(0.0, 57.2)

We evaluated multiple models of trait evolution using the OUwie, and bayou packages. We ascribed model names based on their use of a BM or OU procedure followed by parameters that were allowed to vary across clades. We display AICc weights and parameter estimates for each model we considered, sorted by their AICc weights. The models considered in our bayou analyses all incorporated constraints (denoted by asterisks) limiting the trait value to 0° prior to the stem branches leading to geckos and anoles. We report the predicted timing of the origins of toe pads in geckos and anoles (Shift Time) in millions of years since the split of the stem segregating the clade from the rest of the phylogeny. OU α values are displayed as phylogenetic half-life values ($\ln(2)/\alpha$) in millions of years. Our bayou Brownian motion models also include root parameter values illustrating the trait value at the root of the phylogeny. In BM models lacking a trend, in which the μ parameter is zero, the root parameter value is also the clade mean. The μ parameter represents the expected change in trait over time. Lastly, results from our *OU $\sigma^2\alpha\theta$ _{Bayesian} model included estimated medians and 95% highest posterior density (HPD) intervals for each parameter, indicated in parentheses under each value, displayed in the last row of the table.

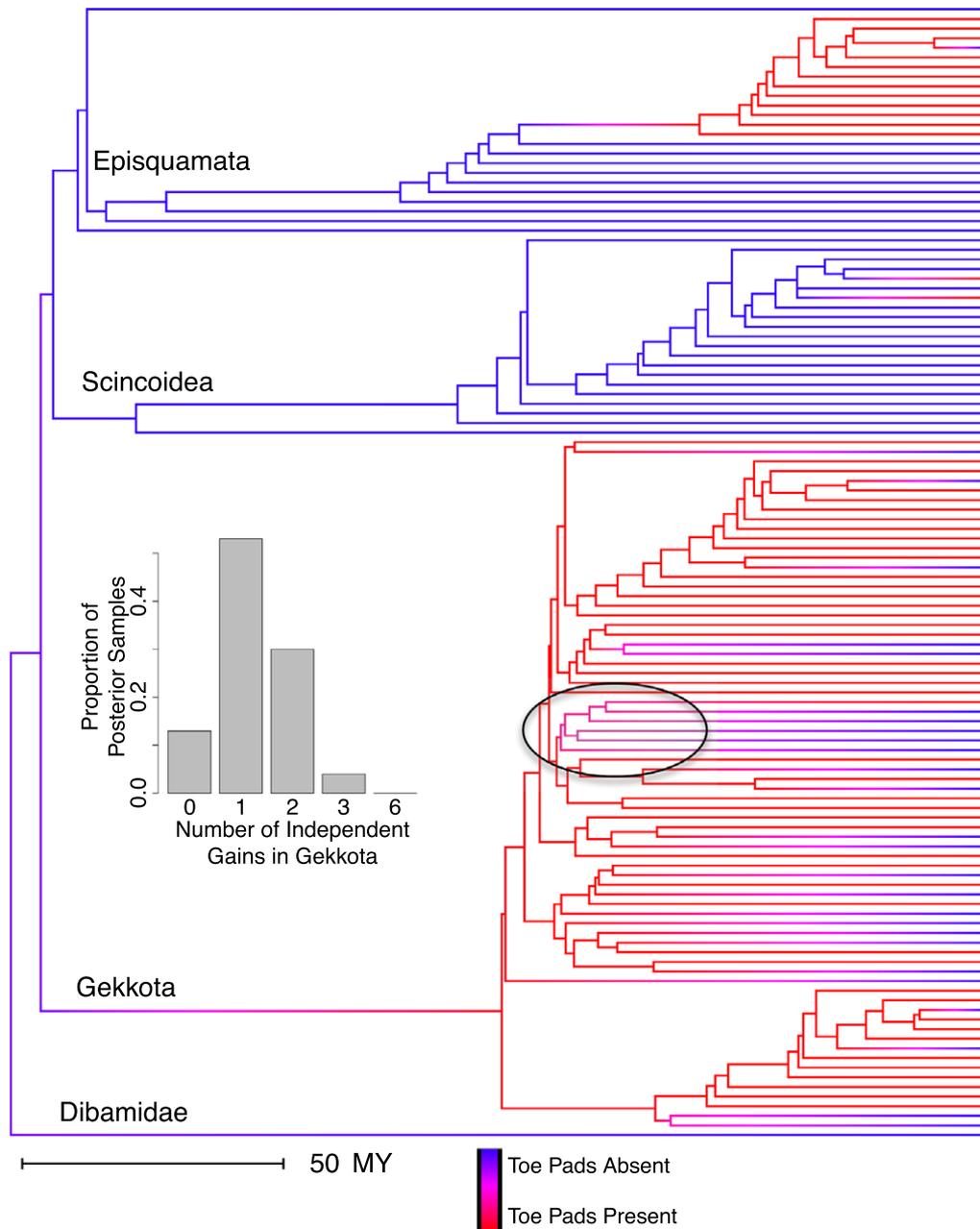


Figure 2. Toe pad ancestral state reconstruction. We reconstructed the presence (red) and absence (blue) of adhesive toe pads across Squamata. We predicted toe pads likely evolved once within geckos, with many losses. The embedded histogram highlights the number of independent origins within Gekkota across our posterior sample of reconstructions (see Methods). Some of the reconstructions in our posterior sample yielded independent origins of toe pads in the stem leading to *Hemidactylus* (see Results). The root of the clade containing *Hemidactylus* is circled. For tip names see Supplemental Material.

sample sizes, indicating convergence and adequate mixing. Transition rates were estimated to be highly asymmetric, with losses of toe pads occurring at rates an average of 16.8 times faster than gains (95% HPD 3.2–41.1). Our reconstruction favored three origins in squamates (geckos, anoles, and skinks, Fig. 2) but we were unable to rule out multiple origins within geckos. Within geckos, our reconstruction favored a single origin (53% of posterior reconstructions), followed by two origins (30%), with only 4% of

reconstructions having three or more origins within geckos. Thirteen percent of our reconstructions contained no origins within geckos, modeling the root of squamates as having pads. It is worth noting that we observed some reconstructions in our posterior sample with transient assignments, in which toe pads transitioned from absent to present, back to absent along a single branch, generating no overall change but possibly inflating the number of origins we observed. In addition, we observed an origin of toe

pads in the branch leading to *Hemidactylus* in 33% of our posterior reconstructions, complementing previous studies of toe pad origins in geckos (Fig. 2; Gamble et al. 2012).

We conducted a Shapiro–Wilk test of normality and found our performance data to not be significantly different from than expected for a normal distribution ($W = 0.98$, $P = 0.32$). We found toe detachment angle to vary widely across padded lizards (Fig. 3, Table S1), ranging from 15° to over 40° . When we consider detachment angle among clades, we note detachment angle in anoles ranged from 15.7° to 23.3° ; lower than in most gecko species. Gekkonid and phyllodactylid geckos showed the greatest variation, with detachment angles ranging from 23.4° to 40.5° (Fig. 3, Table S1). Diplodactylid geckos exhibited intermediate performance between anoles and the gekkonids and phyllodactyls, exhibiting detachment angles between 15.0° and 30.1° (Fig. 3, Table S1).

Considering our trait evolution analyses, our OUwie results did not find clear support for one particular model of trait evolution (Table 1). We found support for a single-rate BM model (BM1, AICc weight of 0.35) with weaker support for an OU model with clade specific σ^2 , α , and θ values, (OU $\sigma^2\alpha\theta$ model, AICc weight of 0.19). When we examine our OU $\sigma^2\alpha\theta$ model parameter estimates, geckos were modeled under an OU model with a very small α value (2.1×10^{-9}), large σ^2 (3.6), and distant θ (>1000), which converges toward BM with a trend (Table 1). It is worth noting again that these models assume unrealistic ancestral states, with a phylogenetic mean performance value for the ancestor of geckos and anoles, which almost certainly did not have toe pads.

For our custom models of trait evolution, which improved upon our OUwie analyses by incorporating constrained root state and timing of parameter shifts, our best-fitting model was one in which geckos evolved under a BM model with a trend, and anoles evolved under an OU model (*BMT_G-OU_A, AIC weight = 0.37; Fig. 4), followed closely by a global Brownian Motion with a trend model (*BMT, AIC weight = 0.35; Table 1). The third best-fitting model assigned unique μ values to geckos and anoles (*BMT μ , AIC weight = 0.18). When independent OU models are fit to geckos and anoles, the estimated gecko phylogenetic half-life was 208.2 million years with an estimated θ of 90° (the maximum allowable performance value), compared to the short half-life estimated for anoles of 0.33 million years and a θ of 19.4° . Support for a BM model with a trend in geckos is indicative of very little statistical signal for bounded evolution, a surprising result given the bounded nature of performance space (detachment angle being constrained between 0° and 90°). This result is supported when assuming one or two origins in Gekkota (see Supplemental Material). By contrast, there is support for an OU model in anoles, in which anoles are very near their estimated θ value and have a very rapid phylogenetic half-life. However, possibly due to the limited

sampling of *Anolis* species in our dataset (14 species), the *BMT and *BM_G-OU_A models are roughly equivalent when accounting for the fact that the *BMT model has only four parameters, while the *BM_G-OU_A model has seven.

Considering our *OU $\sigma^2\alpha\theta$ _{Bayesian} model, although we observed overlap among parameters estimated for geckos and anoles, the results again suggest that the phylogenetic half-life for anoles is shorter than that of the geckos, with anoles much closer to their θ value, whereas gecko evolution is relatively unconstrained (Fig. 5; Table 1). All parameter estimates reached stationarity and had effective sizes of over 200 and were similar to maximum likelihood estimates (Table 1).

Discussion

In this study, we modeled the evolution of adhesive performance considering gecko and anole lizards. In order to incorporate historical information such as the repeated evolution of adhesive toe pads in lizards, we conducted an ancestral state reconstruction. Our reconstruction favored a single origin of toe pads within geckos, which is significantly fewer than previous work (Gamble et al. 2012), although we cannot rule out multiple origins (see Gamble et al. 2017). Our performance observations suggested toe detachment angle to be highly variable across species of padded lizards (14° to 40° , see Supplemental Material). Lastly our modeling results supported our hypothesis that independent toe pad origins would exhibit different tempos and modes of performance evolution. There was no evidence of substantial constraints on the evolution of gecko adhesive performance. In fact, we found consistent support for an unconstrained model of trait evolution in geckos, which indicates adhesive performance in geckos has evolved with ample evolutionary opportunity and few constraints. Conversely, anole performance appears to be limited to relatively low angles of toe detachment, suggesting strong constraints, consistent selection, or limited ecological opportunity.

INDEPENDENT ORIGINS OF TOE PADS

Many previous studies have contributed to our understanding of independent toe pad origins within geckos (Underwood 1954; Haacke 1976; Russell 1976; Russell 1979; Irschick et al. 1996; Russell 2002; Higham et al. 2015; Russell et al. 2015; Higham et al. 2016), with recent studies suggesting between one (Harrington and Reeder 2017) and eleven origins (Gamble et al. 2012), including origins in the Phyllodactylidae family and on the stem of *Hemidactylus*. This is still a very active area of research (Gamble et al. 2017). Our reconstruction suggested a single origin at the base of geckos, although we did find some evidence suggesting *Hemidactylus* may represent an independent origin of toe pads within Gekkota (see Results, Fig. 2, and Supplemental Material), complementing results from Gamble et al. (2012),

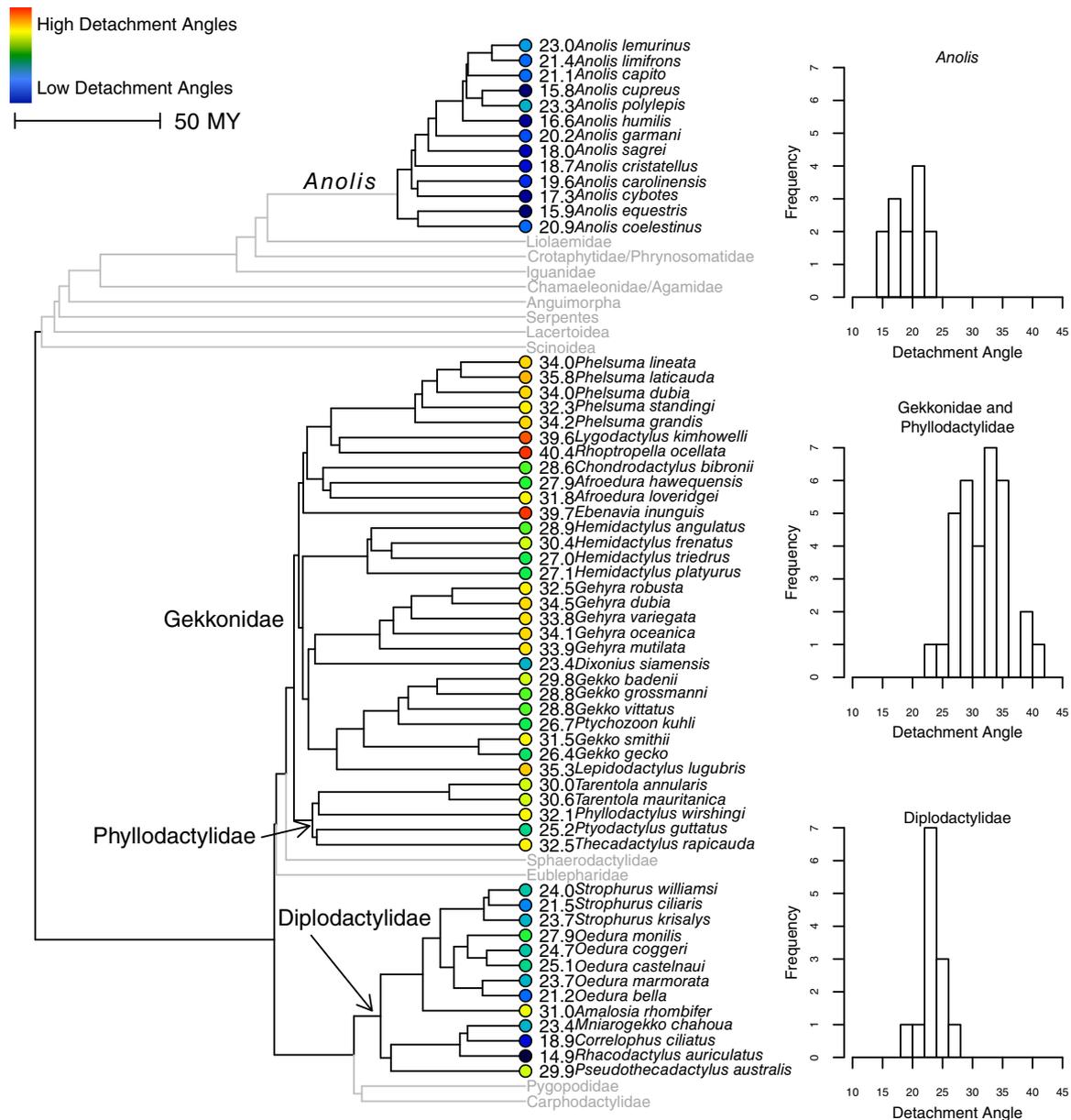


Figure 3. Phylogeny of focal padded species with performance data. We quantified toe-detachment angle across 46 species of geckos and 13 species of anoles. Colored circles and numbers at the tips of the phylogeny represent each species' estimated detachment angle. Warmer colors represent higher detachment angles. We display prominent nonpadded lizard groups to emphasize the evolutionary distance between anoles and geckos and to highlight the fact that not all families of geckos have toe pads (Carphodactylidae and Eublepharidae lack pads, Pygopodidae lacks limbs). Sphaerodactyls do possess adhesive toe pads, but we did not quantify their performance. Histograms to the right of the phylogeny illustrate the observed variation in performance within anoles, diplodactyls, and gekkonids and phyllodactylids. We found *Anolis* lizards to have the lowest detachment angles, followed by diplodactylids. Gekkonids and phyllodactylids had the highest and broadest range of detachment angles.

despite topological differences between the Gamble et al. (2012) and Pyron and Burbrink (2013) phylogenies regarding genera closely related to *Hemidactylus* (see Title and Rabosky 2016 regarding the use of macrophylogenies in comparative analyses). While neither our study nor the Gamble et al. (2012) study allowed the rate of pad gain or loss to vary across clades, some clades may be predisposed to evolving or losing adhesive toe pads, resulting

in clade-specific rates or gain or loss. There are multiple distantly related genera of geckos that exhibit adhesive structures on the tips of their tails strikingly similar to those on their toes such as *Lygodactylus* in the Gekkonidae family and New Caledonia and New Zealand genera in the Diplodactylidae family (Bauer 1998). These independent origins of adhesive tail pads may suggest that geckos are predisposed to evolve adhesive pads, possessing

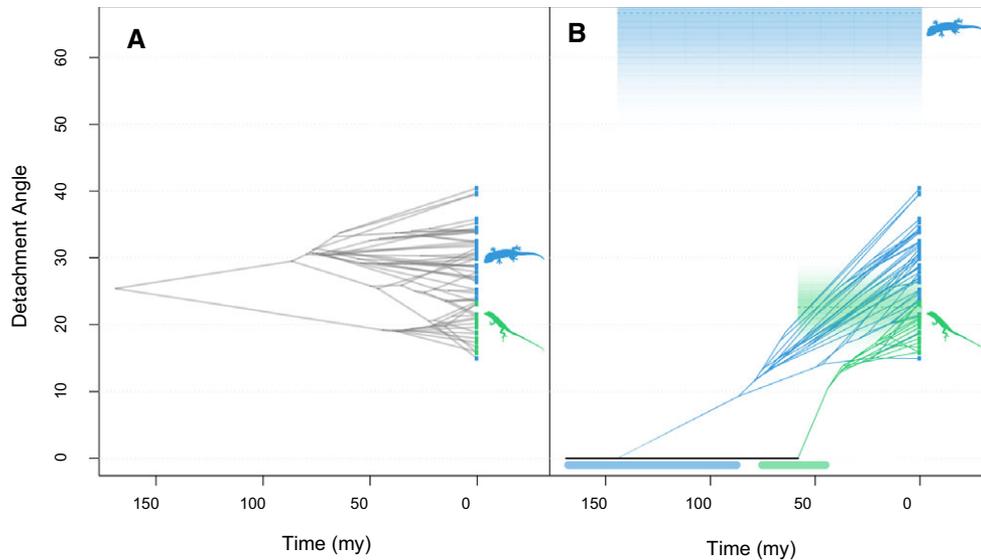


Figure 4. Ancestral state reconstructions using a single-regime BM model (A) and the median posterior parameter estimates for the $*OU\sigma^2\alpha\theta_{\text{Bayesian}}$ model (B) in *bayou*, which assumes independent origins of toe pads geckos and anoles. Anole data are displayed in green and gecko data in blue. (B) Median parameter estimates for the OU target value are indicated by colored dotted lines within the shaded bands indicating the expected densities of the stationary distributions. Horizontal bars below the X-axis indicate the constrained shift regions. Note the median predicted ancestral performance in plot A is estimating a toe detachment angle of approximately 25° for the shared ancestor of geckos and anoles, which likely lacked toe pads. See Supplemental Material for additional analyses assuming two origins of toe pads in *Gekkota*.

easily co-optable developmental pathways as compared to other lizards.

In addition, if toe pad state is correlated with diversification rate, this may impact ancestral reconstruction results (Maddison 2006). Gamble et al. (2012) found toe pads to be associated with slightly higher rate of diversification, although this was not the case for Garcia-Porta and Ord (2013). Considering state-correlated diversification rate alongside an ancestral state reconstruction, Harrington and Reeder (2017) concluded a single origin of toe pads using a “hidden states” binary-state speciation and extinction model (Maddison et al. 2007; Beaulieu et al. 2013; Beaulieu and O’Meara 2016), although Gamble et al. (2017) dispute these results due to potentially high Type 1 error rates (Davis et al. 2013; Maddison and FitzJohn 2015; Rabosky and Goldberg 2015). Future studies may want to consider incorporating character-state correlated diversification information into ancestral state reconstructions using the recently published nonparametric FiSSE (Fast, intuitive, State-dependent, Speciation-Extinction) approach (Rabosky and Goldberg 2017; Zenil-Ferguson and Pennell 2017).

When considering other lines of evidence such as the variation in toe hyperextension anatomy within geckos (Russell 1979), it is likely that the true number of origins within geckos lies somewhere between one and many (Gamble et al. 2017). Future studies investigating the origins of adhesive toe pads in lizards will benefit from considering multiple lines of evidence

(Gamble et al. 2017). The adhesive toe pads of lizards vary in toe pad shape, spinule/seta morphology, skin-to-bone digital tendon system characteristics (Russell 2002), and the presence/absence of internal blood sinuses and paraphalanges (Russell 1976; Russell and Bauer 1988; Gamble et al. 2012). The presence of epidermal spinules may predispose lizards to express adhesive setae, with epidermal spinules having likely evolved into adhesive setae (Maderson 1970; Stewart and Daniel 1972; Russell 1976; Peterson 1983; Peattie 2008). Epidermal spinules appear to be common across geckos and other lizards, including Chamaeleonidae, Iguanidae, Leiocephalidae, and Polychrotidae (Maderson 1964; Ruibal 1968; Maderson 1970; Stewart and Daniel 1975; Peterson 1984; Bauer and Russell 1988; Irish et al. 1988; Peattie 2008; Vucko 2008). Russell et al. (2015) provide a stunning example in *Gonatodes*, highlighting variation in both setal and toe pad morphology suggesting that *Gonatodes* may represent an example of elongated spinules and enlarged ventral scales performing as a friction-generating pad.

TRAIT EVOLUTION

We used angle of toe detachment as a measure of adhesive performance because it has a well-supported mechanistic basis (Autumn et al. 2006a; Tian et al. 2006), although other metrics exist (Irschick et al. 1996, 2006; Stark et al. 2012; Crandell et al. 2014). Using this measure of performance, we saw striking differences between our focal clades. Species with the lowest

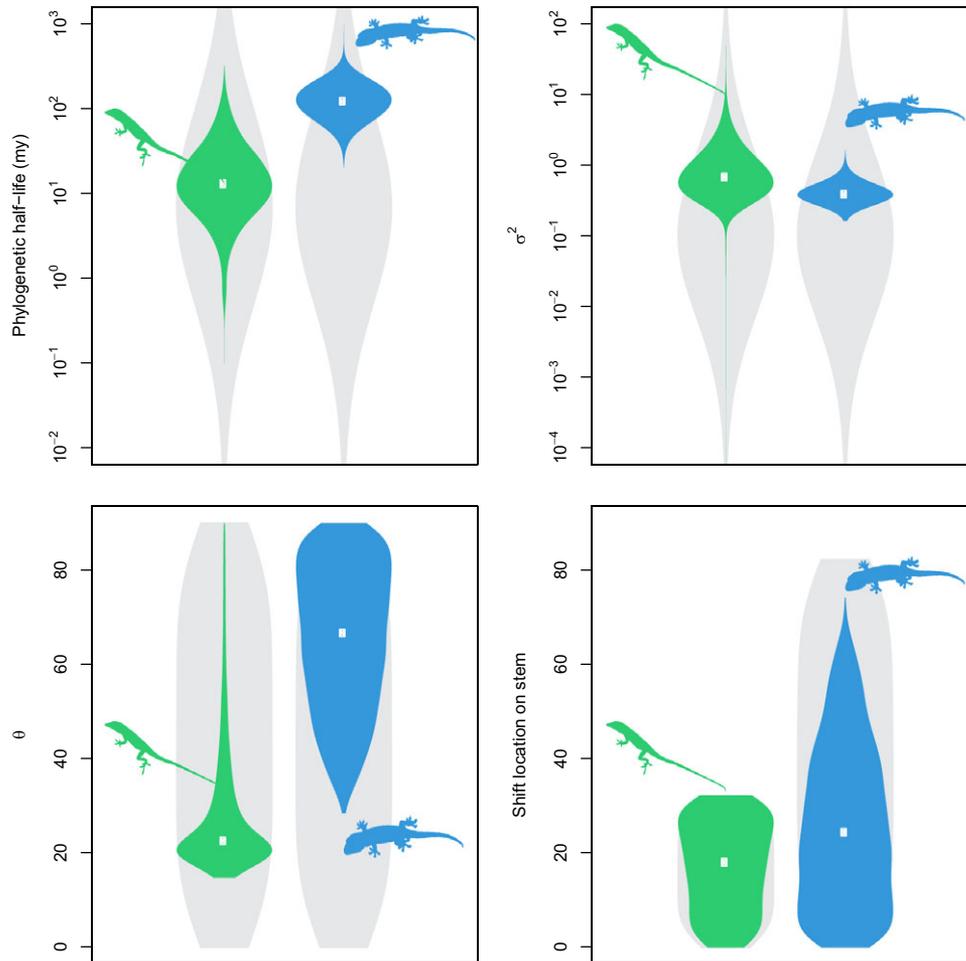


Figure 5. Posterior distributions from the $*OU\sigma^2\alpha\theta$ Bayesian model. Anole data are displayed in green on the left of each plot. Gecko data are in blue on the right of each plot. White dots indicate median estimates for each parameter while black rectangles and whiskers indicate quartiles of the distribution. Gray violin plots indicate the prior distribution. The upper dotted line on the phylogenetic half-life plot indicates the root age of the Squamata phylogeny corresponding roughly to the value at which the OU model approaches a Brownian Motion model. The lower dotted line represents the value of phylogenetic half-life at which no two species in either phylogeny would have more than a 0.05% phylogenetic correlation, that is the values at which our model simplifies into a white-noise model with independent, identically distributed trait values with no effect of phylogeny.

detachment angles (mostly anoles, near 15°) only produce a maximum of 0.27 units of adhesion for one unit of friction, [using $\text{tangent (detachment angle)} = \text{adhesion/friction}$ (Autumn et al. 2006a; Hagey et al. 2014)], whereas particular Gekkonidae geckos have detachment angles over 40° and produce up to 0.84 units of adhesion for every unit of friction, over three times as much as our lowest performing species.

Our trait evolution modeling analyses, which used modified models of trait evolution and our ancestral state reconstruction results, suggested that our observed pattern of gecko performance is well described by a BM with a trend model or a weak OU model with parameters converging toward a BM with a trend (large σ^2 , distant θ , and small α values; Table 1; Figs. 4, 5). Both models suggest adhesive performance in geckos has evolved

directionally, yet relatively unbounded. Conversely, our results suggest anoles, which are much younger than geckos, evolved rapidly in a bounded subsection of performance space, similar to a conventional OU model (short phylogenetic half-life and a θ value near observed values; Table 1; Figs. 4, 5). However, likely due to limited sample size, we have only weak evidence against a Brownian Motion with a trend model.

These observed differences in performance and evolutionary tempo and mode mirror anole and gecko macro- and micro-adhesive morphology, ecology, and the fossil record. For example, geckos were found to be more variable in adhesive performance (Fig. 3) and also have a much wider range of toe pad shapes, setal morphology (Peattie 2007; Gamble et al. 2012), and ecology as compared to anoles. Geckos live in tropical, arid, and

temperate environments on rocks, vegetation, and terrestrial substrates, whereas anoles are generally found in arboreal microhabitats in the Caribbean and South America. Mainland anoles have more detachment angle diversity as compared to Caribbean anoles. These differences may be related to mainland and Caribbean lizard community structure and ecological opportunity (Macrini et al. 2003; Losos 2009). As a result, geckos may be evolving within many different adaptive zones, while the limited variation in the ecology of anoles may be driving them toward one or a few adaptive zones without selecting for novel adhesive morphology. Further work exploring the relationship between adhesive performance and habitat use of padded lizards is also crucial to place performance reported here in an ecological context. Conversely, the evolvability of the gecko and anole adhesive systems may be a driving factor, allowing geckos to diversify extensively, and limiting anole toe pad shape, setal morphology, or performance and hence limiting them to one or few adaptive zones. Our trait modeling results also complement studies of the fossil record. Studies of trait evolution can sometimes underestimate ancestral trait diversity (Mitchell 2015), but recent fossil evidence from anoles preserved in amber suggests a model in which anoles rapidly evolved their current phenotypes, with anole ecomorphs having changed little since the Miocene (Sherratt et al. 2015). The gecko fossil record is unfortunately less informative (Daza et al. 2014, 2016).

Our results provide an example of convergent traits evolving under different evolutionary histories, highlighting the importance of considering macroevolutionary dynamics when inferring historical contingency and ecological opportunity during adaptation. Our study also describes the evolution of a performance trait instead of morphological traits. Despite our results detailing strong evolutionary constraints on anole evolution that we did not find in geckos, there remain many open questions as to how lizard adhesive toe pads have evolved, how they work, and how they are used in the wild. Our results highlight the need to conduct more biomechanical, ecological, and developmental studies of padded lizards with an explicit consideration of their origins. Our results also illustrate the value in incorporating additional information into comparative phylogenetic methods. Without the use of our modified *bayou* model, we would not have identified differences between the evolution of performance in geckos and anoles and we strongly encourage researchers to investigate their model assumptions.

AUTHOR CONTRIBUTIONS

TJH collected performance and toe pad presence data, performed analyses, and write the initial drafts of the manuscript. JCU conducted the modified models of trait evolution analyses. KEC and JAC assisted with performance data collection. KA and LJH assisted with experimental design, statistical analyses, and provided logistical support. All authors contributed to revising the manuscript.

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DATA ARCHIVING

Data are available in the Supporting Information and from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.45623>.

LITERATURE CITED

- Arnold, S. J. 1983. Morphology, performance and fitness. *Am. Zool.* 23:347–361.
- Austin, C. C. 1998. Phylogenetic relationships of *Lipinia* (Scincidae) from New Guinea based on DNA sequence variation from the mitochondrial 12S rRNA and nuclear c-mos genes. *Hamadryad* 23:93–102.
- Autumn, K., A. Dittmore, D. Santos, M. Spenko, and M. Cutkosky. 2006a. Frictional adhesion: a new angle on gecko attachment. *J. Exp. Biol.* 209:3569–3579.
- Autumn, K., S. T. Hsieh, D. M. Dudek, J. Chen, C. Chitaphan, and R. J. Full. 2006b. Dynamics of geckos running vertically. *J. Exp. Biol.* 209:260–272.
- Autumn, K., P. H. Niewiarowski, and J. B. Puthoff. 2014. Gecko adhesion as a model system for integrative biology, interdisciplinary science, and bioinspired engineering. *Annu. Rev. Ecol. Evol. S* 45:445–470.
- Bauer, A. M. 1998. Morphology of the adhesive tail tips of carphodactylid geckos (Reptilia: Diplodactylidae). *J. Morphol.* 235:41–58.
- Bauer, A. M., and A. P. Russell. 1988. Morphology of gekkonid cutaneous sensilla, with comments on function and phylogeny in the Carphodactylini (Reptilia: Gekkonidae). *Can. J. Zool.* 66:1583–1588.
- Beaulieu, J. M., D. C. Jhwueng, C. Boettiger, and B. C. O'Meara. 2012. Modeling stabilizing selection: expanding the ornstein-uhlenbeck model of adaptive evolution. *Evolution* 66:2369–2383.
- Beaulieu, J. M., and B. C. O'Meara. 2016. Detecting hidden diversification shifts in models of trait-dependent speciation and extinction. *Syst. Biol.* 65:583–601.
- Beaulieu, J. M., B. C. O'Meara, and M. J. Donoghue. 2013. Identifying hidden rate changes in the evolution of a binary morphological character: the evolution of plant habit in campanulid angiosperms. *Syst. Biol.* 62:725–737.
- Brown, D., J. W. Wilmer, and S. Macdonald. 2012. A revision of *Strophurus taenicauda* (Squamata; Diplodactylidae) with the description of two new subspecies from central Queensland and a southerly range extension. *Zootaxa* 3243:1–28.
- Burnham, K. P., and D. R. Anderson. 2002. Model selection and multi-model inference: a practical information-theoretic approach. Springer, New York, NY.
- Butler, M. A., and A. A. King. 2004. Phylogenetic comparative analysis: a modeling approach for adaptive evolution. *Am. Nat.* 164:683–695.

- Crandell, K. E., A. Herrel, M. Sasa, J. B. Losos, and K. Autumn. 2014. Stick or grip? Co-evolution of adhesive toepads and claws in *Anolis* lizards. *Zoology* 117:363–369.
- Davis, M. P., P. E. Midford, and W. Maddison. 2013. Exploring power and parameter estimation of the BiSSE method for analyzing species diversification. *Bmc Evol. Biol.* 13:38.
- Daza, J. D., A. M. Bauer, and E. D. Snively. 2014. On the fossil record of the Gekkota. *Anat. Rec.* 297:433–462.
- Daza, J. D., E. L. Stanley, P. Wagner, A. M. Bauer, and D. A. Grimaldi. 2016. Mid-cretaceous amber fossils illuminate the past diversity of tropical lizards. *Sci. Adv.* 2:e1501080.
- Eastman, J. M., M. E. Alfaro, P. Joyce, A. L. Hipp, and L. J. Harmon. 2011. A novel comparative method for identifying shifts in the rate of character evolution on trees. *Evolution* 65:3578–3589.
- Eastman, J. M., D. Wegmann, C. Leuenberger, and L. J. Harmon. 2013. Simpsonian ‘evolution by jumps’ in an adaptive radiation of *Anolis* lizards. arXiv preprint arXiv 1305.4216.
- Emerson, S. B. 1991. The ecomorphology of Bornean tree frogs (Family Rhacophoridae). *Zool. J. Linn. Soc-Lond.* 101:337–357.
- FitzJohn, R. G. 2012. Diversitree: comparative phylogenetic analyses of diversification in R. *Methods Ecol. Evol.* 3:1084–1092.
- Gamble, T., E. Greenbaum, T. R. Jackman, A. P. Russell, and A. M. Bauer. 2012. Repeated origin and loss of adhesive toepads in geckos. *PLoS ONE* 7:e39429.
- Gamble, T., E. Greenbaum, T. R. Jackman, A. P. Russell, and A. M. Bauer. 2017. Repeated evolution of digital adhesion in geckos, a reply to Harrington and Reeder. *J. Evol. Biol.* 30:1429–1436.
- Garcia-Porta, J., and T. J. Ord. 2013. Key innovations and island colonization as engines of evolutionary diversification: a comparative test with the Australasian diplodactyloid geckos. *J. Evol. Biol.* 26:2662–2680.
- Haacke, W. 1976. The burrowing geckos of southern Africa, 5 (Reptilia: Gekkonidae). *Annals Trans. Museum* 30:71–89.
- Hagey, T. J., J. B. Puthoff, K. E. Crandell, K. Autumn, and L. J. Harmon. 2016. Modeling observed animal performance using the Weibull distribution. *J. Exp. Biol.* 219:1603–1607.
- Hagey, T. J., J. B. Puthoff, M. Holbrook, L. J. Harmon, and K. Autumn. 2014. Variation in setal micromechanics and performance of two gecko species. *Zoomorphology* 133:111–126.
- Hansen, T. F. 1997. Stabilizing selection and the comparative analysis of adaptation. *Evolution* 51:1341–1351.
- Hansen, W. R., and K. Autumn. 2005. Evidence for self-cleaning in gecko setae. *Proc. Natl. Acad. Sci. USA* 102:385–389.
- Harmon, L. J., J. Baumes, C. Hughes, J. Soberon, C. D. Specht, W. Turner, C. Lisle, and R. W. Thacker. 2013. Arbor: comparative analysis workflows for the tree of life. *PLoS Curr.* 5:ecurrents.tol.099161de099165eabdee099073fd099163d099121a044518dc.
- Harmon, L. J., J. A. Schulte, A. Larson, and J. B. Losos. 2003. Tempo and mode of evolutionary radiation in iguanian lizards. *Science* 301:961–964.
- Harmon, L. J., J. T. Weir, C. D. Brock, R. E. Glor, and W. Challenger. 2008. GEIGER: investigating evolutionary radiations. *Bioinformatics* 24:129–131.
- Harrington, S., and T. W. Reeder. 2017. Rate heterogeneity across Squamata, misleading ancestral state reconstruction and the importance of proper null model specification. *J. Evol. Biol.* 30:313–325.
- Higham, T. E., A. V. Birn-Jeffery, C. E. Collins, C. D. Hulse, and A. P. Russell. 2015. Adaptive simplification and the evolution of gecko locomotion: morphological and biomechanical consequences of losing adhesion. *Proc. Natl. Acad. Sci. USA* 112:809–814.
- Higham, T. E., T. Gamble, and A. P. Russell. 2016. On the origin of frictional adhesion in geckos: small morphological changes lead to a major biomechanical transition in the genus *Gonatodes*. *Biol. J. Linn. Soc.* 120:503–517.
- Hu, S. H., S. Lopez, P. H. Niewiarowski, and Z. H. Xia. 2012. Dynamic self-cleaning in gecko setae via digital hyperextension. *J. R. Soc. Interface* 9:2781–2790.
- Huber, G., S. N. Gorb, N. Hosoda, R. Spolenak, and E. Arzt. 2007. Influence of surface roughness on gecko adhesion. *Acta Biomater.* 3:607–610.
- Ingram, T., and D. L. Mahler. 2013. SURFACE: detecting convergent evolution from comparative data by fitting Ornstein-Uhlenbeck models with stepwise Akaike Information Criterion. *Methods Ecol. Evol.* 4:416–425.
- Irish, F. J., E. E. Williams, and E. Selig. 1988. Scanning electron microscopy of changes in epidermal structure occurring during the shedding cycle in squamate reptiles. *J. Morphol.* 197:105–126.
- Irschick, D. J., C. C. Austin, K. Petren, R. N. Fisher, J. B. Losos, and O. Ellers. 1996. A comparative analysis of clinging ability among pad-bearing lizards. *Biol. J. Linn. Soc.* 59:21–35.
- Irschick, D. J., A. Herrel, and B. Vanhooydonck. 2006. Whole-organism studies of adhesion in pad-bearing lizards: creative evolutionary solutions to functional problems. *J. Comp. Physiol. A* 192:1169–1177.
- Irschick, D. J., L. J. Vitt, P. A. Zani, and J. B. Losos. 1997. A comparison of evolutionary radiations in mainland and Caribbean *Anolis* lizards. *Ecology* 78:2191–2203.
- Johnson, M. K., and A. P. Russell. 2009. Configuration of the setal fields of *Rhoptropus* (Gekkota: Gekkonidae): functional, evolutionary, ecological and phylogenetic implications of observed pattern. *J. Anat.* 214:937–955.
- Lande, R. 1976. Natural-selection and random genetic drift in phenotypic evolution. *Evolution* 30:314–334.
- Losos, J. B. 2009. Lizards in an evolutionary tree: the ecology of adaptive radiation in anoles. California Univ. Press, Berkeley.
- Macrini, T. E., D. J. Irschick, and J. B. Losos. 2003. Ecomorphological differences in toepad characteristics between mainland and island anoles. *J. Herpetol.* 37:52–58.
- Maddison, W. P. 2006. Confounding asymmetries in evolutionary diversification and character change. *Evolution* 60:1743–1746.
- Maddison, W. P., and R. G. FitzJohn. 2015. The unsolved challenge to phylogenetic correlation tests for categorical characters. *Syst. Biol.* 64:127–136.
- Maddison, W. P., P. E. Midford, and S. P. Otto. 2007. Estimating a binary character’s effect on speciation and extinction. *Syst. Biol.* 56:701–710.
- Maderson, P. 1970. Lizard hands and lizard glands: models for evolutionary study. *Forma et Functio* 3:179–204.
- Maderson, P. F. A. 1964. Keratinized epidermal derivatives as an aid to climbing in gekkonid lizards. *Nature* 203:780–781.
- McCool, J. I. 2012. Using the Weibull distribution: Reliability, modeling and inference. Wiley, Hoboken, NJ.
- Mitchell, J. S. 2015. Extant-only comparative methods fail to recover the disparity preserved in the bird fossil record. *Evolution* 69:2414–2424.
- Moen, D. S., D. J. Irschick, and J. J. Wiens. 2013. Evolutionary conservatism and convergence both lead to striking similarity in ecology, morphology and performance across continents in frogs. *P R Soc. B* 280.
- Nicholson, K. E., A. Mijares-Urrutia, and A. Larson. 2006. Molecular phylogenetics of the *Anolis onca* series: a case history in retrograde evolution revisited. *J. Exp. Zool. B Mol. Dev. Evol.* 306:450–459.
- Oliver, P. M., A. M. Bauer, E. Greenbaum, T. Jackman, and T. Hobbie. 2012. Molecular phylogenetics of the arboreal Australian gecko genus *Oedura* Gray 1842 (Gekkota: Diplodactylidae): another plesiomorphic grade? *Mol. Phylogenet. Evol.* 63:255–264.
- Oliver, P. M., and P. Doughty. 2016. Systematic revision of the marbled velvet geckos (*Oedura marmorata* species complex, Diplodactylidae) from the Australian arid and semi-arid zones. *Zootaxa* 4088:151–176.

- Peattie, A. M. 2007. The function and evolution of gekkotan adhesive feet. Ph. D. diss. Pp. 61. University of California, Berkeley.
- . 2008. Subdigital setae of narrow-toed geckos, including a eublepharid (*Aeluroscalabotes felinus*). *Anat. Rec.* 291:869–875.
- Pennell, M. W., J. M. Eastman, G. J. Slater, J. W. Brown, J. C. Uyeda, R. G. FitzJohn, M. E. Alfaro, and L. J. Harmon. 2014. geiger v2.0: an expanded suite of methods for fitting macroevolutionary models to phylogenetic trees. *Bioinformatics* 30:2216–2218.
- Persson, B. N. J. 2007. Biological adhesion for locomotion: basic principles. *J. Adhesion Sci. Technol.* 21:1145–1173.
- Peterson, J. A. 1983. The evolution of the subdigital pad in *Anolis*. I. Comparisons among the anoline genera. *Advances in herpetology and evolutionary biology: essays in honor of Ernest E. Williams*. 1983:245–283.
- . 1984. The microstructure of the scale surface in iguanid lizards. *J. Herpetol.* 1984:437–467.
- Peterson, J. A., and E. E. Williams. 1981. A case history in retrograde evolution: the *Onca* lineage in anoline lizards. II. Subdigital fine structure. *Bull. Mus. Comp. Zool.* 149:215–268.
- Pianka, E. R., and S. L. Sweet. 2005. Integrative biology of sticky feet in geckos. *BioEssays* 27:647–652.
- Pugno, N. M., and E. Lepore. 2008a. Living Tokay geckos display adhesion times following Weibull Statistics. *J. Adhesion* 84:949–962.
- . 2008b. Observation of optimal gecko's adhesion on nanorough surfaces. *BioSystems* 94:218–222.
- Pyron, R. A., and F. T. Burbrink. 2013. Early origin of viviparity and multiple reversions to oviparity in squamate reptiles. *Ecol. Lett.* 17:13–21.
- Rabosky, D. L., and E. E. Goldberg. 2015. Model inadequacy and mistaken inferences of trait-dependent speciation. *Syst. Biol.* 64:340–355.
- . 2017. FiSSE: a simple nonparametric test for the effects of a binary character on lineage diversification rates. *Evolution* 71:1432–42.
- Revell, L. J. 2012. phytools: an R package for phylogenetic comparative biology (and other things). *Methods Ecol. Evol.* 3:217–223.
- Ruibal, R. 1968. The ultrastructure of the surface of lizard scales. *Copeia* 1968:698–703.
- Ruibal, R., and V. Ernst. 1965. The structure of the digital setae of lizards. *J. Morphol.* 117:271–293.
- Russell, A. P. 1976. Some comments concerning interrelationships among gekkonine geckos. Pp. 217–244 in A. d. A. B. a. C. B. Cox, ed. *Morphology and biology of the reptiles*. Linnean Society Symposium Series Number 3. Academic Press, London.
- . 1979. Parallelism and integrated design in the foot structure of gekkonine and diplodactylid geckos. *Copeia* 1979:1–21.
- . 2002. Integrative functional morphology of the gekkotan adhesive system (Reptilia: Gekkota). *Integr. Comp. Biol.* 42:1154–1163.
- Russell, A. P., J. Baskerville, T. Gamble, and T. E. Higham. 2015. The evolution of digit form in *Gonatodes* (Gekkota: Sphaerodactylidae) and its bearing on the transition from frictional to adhesive contact in gekkotans. *J. Morphol.* 276:1311–1332.
- Russell, A. P., and A. M. Bauer. 1988. Paraphalangeal elements of gekkonid lizards: a comparative survey. *J. Morphol.* 197:221–240.
- Russell, A. P., and M. K. Johnson. 2007. Real-world challenges to, and capabilities of, the gekkotan adhesive system: contrasting the rough and the smooth. *Can. J. Zool.* 85:1228–1238.
- . 2014. Between a rock and a soft place: microtopography of the locomotor substrate and the morphology of the setal fields of Namibian day geckos (Gekkota: Gekkonidae: *Rhoptropus*). *Acta Zool.* 95:299–318.
- Sadler, R. A., D. O'Meally, and G. M. Shea. 2005. A new species of spiny-tailed gecko (Squamata: Diplodactylidae: *Strophurus*) from Inland Queensland. *Mem. Queensl. Mus.* 51:573–582.
- Sherratt, E., M. del Rosario Castañeda, R. J. Garwood, D. L. Mahler, T. J. Sanger, A. Herrel, K. de Queiroz, and J. B. Losos. 2015. Amber fossils demonstrate deep-time stability of Caribbean lizard communities. *Proc. Natl. Acad. Sci.* 112:9961–9966.
- Stark, A. Y., T. W. Sullivan, and P. H. Niewiarowski. 2012. The effect of surface water and wetting on gecko adhesion. *J. Exp. Biol.* 215:3080–3086.
- Stewart, G. R., and R. S. Daniel. 1972. Scales of lizard gekko-gecko—surface-structure examined with scanning electron-microscope. *Copeia* 1972:252–257.
- . 1975. Microornamentation of lizard scales: some variations and taxonomic correlations. *Herpetologica* 31:117–130.
- Tian, Y., N. Pesika, H. Zeng, K. Rosenberg, B. Zhao, P. McGuiggan, K. Autmn, and J. Israelachvili. 2006. Adhesion and friction in gecko toe attachment and detachment. *Proc. Natl. Acad. Sci. USA* 103:19320–19325.
- Title, P. O., and D. L. Rabosky. 2016. Do macrophylogenies yield stable macroevolutionary inferences? An example from squamate reptiles. *Syst. Biol.* 2016:syw102.
- Underwood, G. 1954. On the classification and evolution of geckos. *Proc. Zool. Soc. Lond.* 124:469–492.
- Uyeda, J. C., and L. J. Harmon. 2014. A novel Bayesian method for inferring and interpreting the dynamics of adaptive landscapes from phylogenetic comparative data. *Syst. Biol.* 63:902–918.
- Vanhooydonck, B., A. Andronescu, A. Herrel, and D. J. Irschick. 2005. Effects of substrate structure on speed and acceleration capacity in climbing geckos. *Biol. J. Linn. Soc.* 85:385–393.
- Vucko, M. J. 2008. The dynamics of water on the skin of Australian carphodactylid and diplodactylid geckos. Pp. 166. *School of marine and tropical biology*. Masters thesis. James Cook University, Townsville, Queensland, Australia.
- Wainwright, P. C., and S. M. Reilly. 1994. *Ecological morphology*. Chicago Univ. Press, Chicago, IL.
- Williams, E. E., and J. A. Peterson. 1982. Convergent and alternative designs in the digital adhesive pads of scincid lizards. *Science* 215:1509–1511.
- Wilson, S., and G. Swan. 2010. *Complete guide to reptiles of Australia*, 3rd ed. New Holland Publishers, Chatswood, N.S.W.
- Yoder, J. B., E. Clancey, S. Des Roches, J. M. Eastman, L. Gentry, W. Godsoe, T. J. Hagey, D. Jochimsen, B. P. Oswald, J. Robertson et al. 2010. Ecological opportunity and the origin of adaptive radiations. *J. Evol. Biol.* 23:1581–1596.
- Zenil-Ferguson, R., and M. W. Pennell. 2017. Digest: trait-dependent diversification and its alternatives. *Evolution* 71:1732–1734.

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Supporting Information

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

File S1. A .xlsx file listing our toe pad presence/absence assignments for all 4162 tips in the squamate phylogeny from Pyron and Burbrink (2013).

Figure S1. Ancestral state reconstruction with tip names (see Fig. 2, Methods, and Results for additional information).

Table S1. Performance observations. Species mean toe detachment angle and variance (displayed in parentheses). The number of individuals tested was not recorded for some species of anoles (number of individuals = NA) and were treated as observations from a single individual in our analyses.

File S2. Performance observations .xlsx file

Figure S2. Toe detachment field equipment. We build a field-capable TAD device consisting of a force sensor, stepper motor, and multi-axis accelerometer. The upper frame of our apparatus acts as a lever with the fulcrum, allowing the force sensor (left side of image) to detect when a lizard detaches from the glass (right side of image). Our glass slide and accelerometer were attached to a large flat plate. The accelerometer was positioned to measure acceleration in the Y direction (vertical in our image) and Z direction (perpendicular to the mounting surface, out of the plane of the image, toward the reader).

Figure S3. Representative toe detachment performance trial. Representative data output from a single toe detachment trial is displayed. Time is on the X-axis. Raw force data (upper plot) displays our two estimated y-intercepts (red horizontal lines) and time of detachment (red vertical line, approximately 30 seconds in this example) estimated by a broken regression analysis. Raw acceleration data were used to estimate the angle of the glass slide through time (lower plot, gray points). The black line in our lower plot is the estimated substrate angle over the course of the trial. Our estimated angle of toe detachment is the point in which our estimated time of detachment intersects with our estimated angle, slightly under 25° in this example.