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DIRECTIONAL EVOLUTION OF STOCKINESS COEVOLVES WITH ECOLOGY AND LOCOMOTION IN LIZARDS

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Although studied in many taxa, directional macroevolution remains difficult to detect and quantify. We present an approach for detecting directional evolution in subclades of species when relatively few species are sampled, and apply it to studying the evolution of stockiness in Phrynosomatine lizards. Our approach is more sensitive to detecting the tempo of directional evolution than other available approaches. We use ancestral reconstruction and phylogenetic mapping of morphology to characterize the direction and magnitude of trait evolution. We demonstrate a directional trend toward stockiness in horned lizards, but not their sister groups, finding that stockier species tend to have relatively short and wide bodies, and relatively short heads, tails, and limbs. Ornstein–Uhlenbeck models show that the directional trend in horned lizards is due to a shift in selective regime and stabilizing selection as opposed to directional selection. Bayesian evolutionary correlation analyses indicate that stockier species run more slowly and eat a larger proportion of ants. Furthermore, species with larger horns tend to be slower and more ant-specialized. Directional evolution toward a stocky body shape has evolved in conjunction with changes in a suite of traits, representing a complex example of directional macroevolution.

KEY WORDS: **Bayesian methods, defensive structures, diet, macroevolution, Phrynosomatinae, sprint speed.**

Recent reviews have demonstrated that directional selection is a prevalent evolutionary force in nature (Kingsolver et al. 2001). From a microevolutionary perspective, directional selection shifts population means and decreases variance (Kingsolver and Pfennig 2007), but from a macroevolutionary perspective its effects are less obvious, particularly when the direction of selection is not static. For example, oscillating directional selection resulting from a changing environment (Losos et al. 2006) can lead to a pattern mimicking weak stabilizing selection and stasis (Gibbs and Grant 1987; Felsenstein 1988; Blomberg et al. 2003; Roopnarine 2003; Kingsolver and Pfennig 2007). Directional evolution, or evolutionary trends are often considered the result of concerted directional selection (Schluter 1996; Kingsolver and Pfennig 2004). However, a pattern of directional macroevolution can arise not only by directional selection, but also by stabilizing selection on an optimum phenotype that is different from the ancestral phenotype (Hansen 1997; Butler and King 2004). Directional macroevolution has been studied in a variety of both extinct and extant organisms (Bell et al. 1985; Wagner

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1996; Poulin 2005; Verdu 2006; Whittal and Hodges 2007), and classic examples include evolutionary increases in body size in horses (MacFadden 1986), the Foraminifera (Arnold et al. 1995), and mollusks (Jablonski 1997).

However, the ability to detect such directional trends is fraught with difficulties when using either extinct or extant taxa. The most direct evidence of directional macroevolution comes from paleontological studies, which often focus on putative ancestor-descendent pairs (Cheetham 1986; MacFadden 1986; Wagner 1996). Studies of the fossil record have also been fruitful in differentiating subtly different patterns, such as true directional trends, where the entire sample distribution shifts through time, from diffusion processes that shift the distribution mean by increased trait variance through time (Jablonski 1997). However, time-averaging, biases in preservation and sedimentation, and small sample sizes all act to obscure directional patterns, making interpretation of fossil data difficult (Cheetham 1986; Bush et al. 2002; Hunt 2004; Hannisdal 2006). Hence, detecting directional evolution even using well-preserved paleontological series can be contentious (Bell et al. 1985; Cheetham 1986; MacFadden 1986). Likewise, in extant taxa the detection of directional evolution can be hampered when taxon sample sizes are modest, particularly when model-based techniques are used (Poulin 2005; Adamowicz and Purvis 2006; e.g., these studies included 11–75 taxa per analysis). When directional evolution occurs only in a subclade of the considered phylogeny, then it is increasingly unlikely to adopt a directional model over one that assumes nondirectional neutral drift. Some authors have argued for directional evolution in their taxon of study, despite failing to reject a neutral drift model (e.g., Poulin 2005).

While many studies of directional evolution have focused on the evolution of body size, fewer have considered the evolution of body shape. Variation in body shape, or the relative sizes of various body parts, is perhaps the most defining characteristic of organismal diversity (Carroll 1997). Body shape presents significant challenges for detecting directional trends in its evolution because of its multivariate nature. Because different body shapes are adaptive in different habitats, one might expect directional evolution in body shape if some lineages tend to occupy successively more specialized habitats. Vertebrates have evolved a diversity of body shapes, leading to the expectation of directional trends in this trait. For example, an elongate body form has repeatedly evolved in fish (Ward and Brainerd 2007), amphibians (Duellman and Trueb 1994), squamate reptiles (Greer 1991; Carroll 1997), and mammals (Bejder and Hall 2002). The evolution of elongation is characterized by lengthening and narrowing of the body and tail and a reduction of limbs, and, at least in squamates, is thought to occur in response to a fossorial or structurally complex habitat (Presch 1975; Greer 1987; Caputo et al. 1995; Melville and Swain 2000; Wiens and Singluff 2001). Stockiness, the converse of elongation, has also evolved multiple times and is observed in frogs (Pugener 2002), tetraodontiform fish (Brainerd and Patek 1998), and horned lizards (Sherbrooke 2003).

We address these issues by building on existing approaches to produce a method for detecting directional evolution. Specifically, we present a quantitative approach using ancestral reconstruction to identify and compare directional macroevolutionary patterns when the number of taxa available for sampling is relatively small or when directional evolution occurs only in a subset of the studied taxa. We demonstrate this method by examining the evolution of body shape in phrynostomatine lizards, compare it to other approaches, evaluate it using simulation, and use a series of evolutionary models to differentiate between directional and stabilizing selection as the mechanism behind any directional trend. Using a multilocus phylogeny constructed for this study from published sequence data, we are able to detect directional evolution in a clade with relatively few extant taxa using our approach, but not other published approaches. The novel aspect of our dataset is that while many studies that examine directional evolution focus on a single trait, we examine a suite of traits (including external morphology, locomotor performance, diet, and investment in defense) and argue that this integrative approach is crucial for interpreting such trends.

We study the Phrynosomatinae, which includes three clades of lizards (the horned, sand, and fence lizards). This group shows marked variation in stockiness (Sherbrooke 2003), with horned lizards being generally stockier than the other clades. We demonstrate that horned lizards, but not their sister taxa (the sand and fence lizards), have followed a directional trend toward a stockier phenotype. The evolution of stockiness in phrynosomatines has coevolved with the adoption of a relatively sedentary lifestyle of consuming ants, an increased investment in defense (horn size), and reduced body size and locomotor performance. Our findings represent a compelling example of how directional evolution occurs across integrated aspects of the phenotype, behavior, and function, as a result of the invasion of a novel habitat.

Materials and Methods

MORPHOMETRIC DATA

Our morphometric dataset included 835 specimens from 38 species of the Phrynosomatinae (see Fig. 1 for species names and Appendix Table A1 for species sample sizes). We measured snout-vent length (SVL), tail, head, front and hind limb lengths, as well as body width and height, and head width and height to 0.01 mm using a Mitutoyo digital caliper from museum as well as field-collected and released specimens (following Herrel et al. 2002). We log-transformed the dataset and used principal component analysis with a covariance matrix to reduce dimensionality and quantify stockiness using SYSTAT

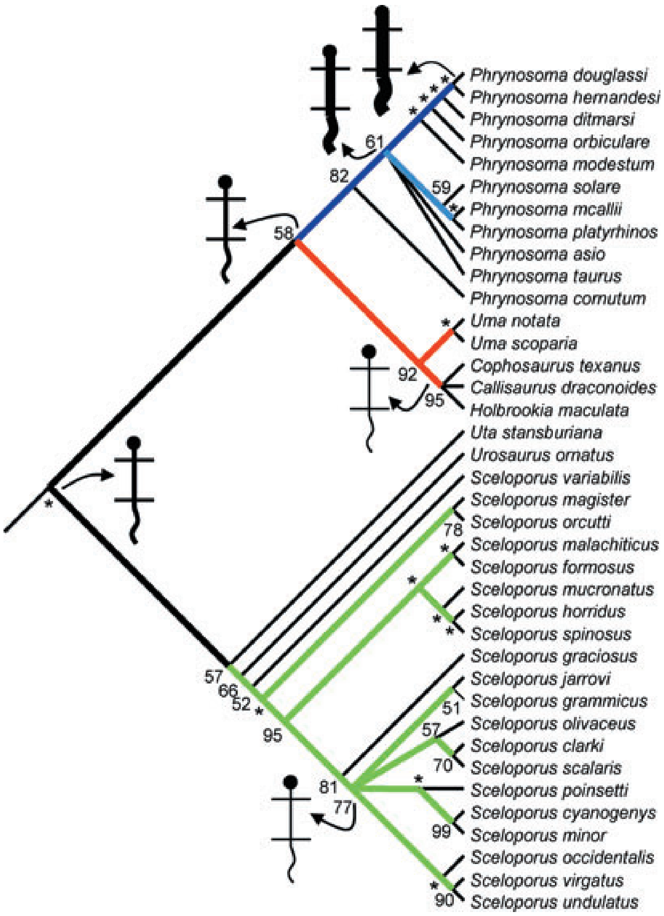

Figure 1. Bayesian mtDNA phylogeny of focal species of the Phrynosomatinae from RJ-MCMC analyses. Numbers at nodes indicate posterior probabilities expressed out of 100, with * indicating 100. Internal branches of the phylogeny are color-coded to match Figure 2, with blue indicating horned lizards, red indicating sand lizards, and green indicating fence lizards. Stick lizard diagrams symbolize relative degree of stockiness at nodes along the phylogeny.

v.10.2 (Wilkinson 2001). We calculated species means for the first two principal components for subsequent comparative analyses (representing measures of body size and stockiness, respectively – see below). We considered this approach to be appropriate because body shape is a complex trait, and all of the included measurements could contribute to characterizing an axis of stockiness.

PHYLOGENETIC HYPOTHESIS

As available, we compiled sequence data for four mitochondrial genes (ND2, ND4, 12s rRNA, and 16s rRNA) consisting of 3043 bp from Genbank for the 38 species included above, plus three outgroup taxa (see Table A1 for accession numbers and outgroup names) and aligned them using Clustal X (Thompson et al. 1997). We checked and edited the alignment manually, removing ambiguous regions.

We analyzed aligned sequences using a Bayesian Markov Chain Monte Carlo (MCMC) approach (Huelsenbeck et al. 2001; Pagel and Meade 2004), applying a mixture model (Pagel and Meade 2004; Brandley et al. 2005). We used a reversible-jump (RJ) algorithm to determine the optimal number of patterns in the data, applying a general time reversible model to each pattern with a discretized gamma distribution used to model variation in rates of evolution across patterns (Pagel and Meade 2004). This approach leads to an increased number of parameters, which is justified because underparameterizing models can bias results (Huelsenbeck and Rannala 2004). We ran the RJ-MCMC analysis, of length 8×10^6 generations and sampled every 1000 generations, in triplicate to increase the chances that the global maximum was found (Lewis 2001) using BayesPhylogenies v1.0 with default priors (Pagel and Meade 2004). All three results concurred, so either a sample of 6000 sample trees from one of these analyses, or the 50% majority-rule consensus tree of that sample was used in subsequent analyses. Trees used in subsequent analyses were additive (not ultrametricized) unless otherwise stated. We also ran one Metropolis-Coupled MCMC analysis because it searches tree space more effectively using BayesPhylogenies (Pagel and Meade 2004), decreasing the chance of getting trapped in a local optimum (Huelsenbeck et al. 2001; Altekar et al. 2004). Although likelihoods of sampled trees appeared to converge after ~50K generations, we used a burn-in of 1.9×10^6 generations because such heuristics may underestimate actual time to convergence (Mossel and Vigoda 2005).

ANCESTRAL RECONSTRUCTIONS AND TESTING FOR DIRECTIONALITY

We calculated marginal ancestral reconstructions of PC-1 and PC-2 and their standard errors with ANCML (Schluter et al. 1997) using the multigene estimated phylogeny and branch lengths, a maximum likelihood (ML) approach, and a neutral drift model. This approach gives the same values and standard errors as the root reconstruction from phylogenetically independent contrasts and squared-change parsimony (Garland et al. 1999; Martins 1999; Pagel 1999). The standard errors of these marginal reconstructions are not directly comparable to one another, but nevertheless give a measure of the uncertainty associated with each one (Garland et al. 1999). We also calculated reconstructions using a Bayesian approach with BayesTraits (Pagel et al. 2004), which allowed the incorporation of phylogenetic uncertainty into the estimates. To accomplish this, we used a sample of 6000 trees with branch lengths resulting from the RJ-MCMC analysis (Pagel et al. 2004). We plotted ancestral reconstructions against the distance from the root of the phylogeny, as calculated in the PDAP module (Garland et al. 1992; Midford et al. 2003) of Mesquite (Maddison and Maddison 2006), and redrew the phylogeny onto the plot to trace the rate and direction of evolution. Using this plot, we calculated the slopes for branches of the tree using the distance from the root and PC-2 ancestral reconstructions as coordinates. We then assigned these branch slopes to their respective subclades and used an analysis of variance (ANOVA) with permutation to determine whether rates of evolution in stockiness differed among these clades. For this ANOVA (Hollander and Wolfe 1999), the test statistic distribution was estimated using a Monte Carlo approach with 1 million permutations of the data using the Coin package (Hothorn et al. 2008) in R (R Core Development Team 2008). A permutation ANOVA makes no distributional assumptions about the test statistic and was used to ameliorate, but not fully solve, the effects of nonindependence of the slopes analyzed. Similar approaches to ours have been used for series of fossils, often using ancestor-descendent species pairs (Cheetham 1986; MacFadden 1986; Wagner 1996), a strategy that is unavailable for extant taxa without ancestral reconstruction and requires good fossil records, preferably with large sample sizes (Hannisdal 2006). The same graphical technique as ours has also been used for extant taxa but without quantification of evolutionary patterns (Bonine et al. 2005).

Using an undirected neutral drift model for ancestral reconstruction and then using these reconstructions to detect directional trends, as we have done, might lead to the detection of directional trends in some subclades simply by chance as different clades diverge. To test for this possibility, we simulated 100 replicate datasets on our phylogeny using an undirected neutral drift model in Mesquite (Maddison and Maddison 2006) and the rate of evolution for PC-2 obtained from ANCML (Schluter et al. 1997; A. Ø. Mooers, pers. comm.). We then reconstructed ancestral states for each dataset using squared-change parsimony in Mesquite, and calculated internode slopes as described above, ascribing each to one of the three phrynosomatine subclades. Each dataset was then analyzed with a permutation ANOVA, as described above. We produced a distribution of the resultant test statistics and compared them to the test statistic calculated using our PC-2 data. If directional trends as pronounced as those presented for PC-2 are expected simply by chance, then one would expect a test statistic equal to or greater than that for our data to arise in $> 5\%$ of the simulated datasets (i.e., $\alpha = 0.05$). Similar to our comparison of ancestor-descendent slopes, absolute values of standardized independent contrasts can be compared among clades using standard statistical tests (Clobert et al. 1998) to compare rates of evolution. We did this for the three phrynosomatine clades using ANOVA and Kruskal–Wallis tests to contrast with our approach.

To further explore the mechanism of directional evolution of stockiness, we used the Ornstein–Uhlenbeck (OU) family of models which explicitly account for stabilizing selection in a comparative framework and can differentiate stabilizing from directional selection (Hansen 1997; Butler and King 2004; A. A. King, pers. comm.). We fit a series of OU models to our data and an ultrametricized version of the consensus tree in a likelihood framework using the Ouch package (Butler and King 2004) in R (Core Development Team 2008) and compared them by calculating AICc values (corrected for small sample size) and resultant model weights for the set of models (Burnham and Anderson 1998). These models are characterized by the parameters α (the intensity of selection), σ (the intensity of random processes), θ_o (the root phenotype), and θ_x (the phenotypic optima, x, for different selective regimes) (Hansen 1997; Butler and King 2004). The number of selective optima and the taxa to which each is applied are defined by the investigator and represent alternate selective hypotheses (Butler and King 2004). We considered four models: a neutral Brownian motion model (BM), a single optimum OU model (OU.1), an OU model with one optimum for horned lizards and one for sand and fence lizards (OU.2), and an OU model with different optima for each of the three phrynosomatine clades (OU.3). If $\alpha = 0$, the OU model reduces to a neutral Brownian motion model; if $\alpha \to 0$ and $\theta_x \to \infty$, then a hypothesis of directional selection is supported (Hansen 1997; M. A. Butler, A. A. King, pers. comm.); if $\alpha > 0$ and there are multiple selective optima (θ_x), then a hypothesis of a shift in selective regime and stabilizing selection on each optimum is supported.

ANALYSIS OF ECOLOGICAL CORRELATES

We compiled species mean data on horn length, sprint speed, and degree of ant diet specialization for species from all three phrynosomatine clades (Table 1). One of us (JJM) measured the length of the longest horn and we expressed it relative to head length (following (Leaché and McGuire 2006). We obtained diet data, expressed as percent total stomach volume consisting of ants, from the literature (Kennedy 1956; Roth 1971; Pianka and Parker 1972; Parker and Pianka 1973; Pianka and Parker 1975; Brooks and Mitchell 1989; Goldberg and Bursey 1990; Turner 1998).

We obtained maximal sprint speeds from Bonine and Garland (1999) for most species, and supplemented them using data collected by PJB (Table 1). Sprint speeds for *Phrynosoma platyrhinos* ($n = 7$) and *P. solare* ($n = 3$) were calculated from videos of these lizards running on a racetrack (1.5 m × 15 cm) in the field. All lizards were run within 24 h of capture and were subsequently released at site of capture. Lizards were videotaped using a Fastec (San Diego, CA) Troubleshooter 250 high-speed video camera at 250 frames per second, 2× shutter speed, and at a resolution of 640 × 480 pixels. Videos were imported into Peak Motus 32 software (Englewood, CO) and a point painted mid-dorsally on the occiput of each lizard in nontoxic white paint prior to videotaping was digitized in each frame. Frame numbers were recorded for two frames, representing how long each lizard took to run its quickest 0.25 m, as measured by the coordinates of the digitized point. This was done such that calculated sprint speeds were

Table 1. **Ecological data used in evolutionary correlational analysis.**

Species	Body size	Stockiness	Horn size	A-speed	R-speed	% ants	Reference (speed/diet)
Phrynosoma asio	1.74257	−0.14377	0.21690			31.1	- / (Pianka and Parker 1975)
Phrynosoma cornutum	1.37039	0.84529	0.39909	2.18	23.19	61.2	(Bonine and Garland 1999) / (Pianka and Parker 1975)
Phrynosoma ditmarsi	0.86771	1.35065	0.12781			11.3	- / (Roth 1971)
Phrynosoma douglassi	−0.62441	2.12656	0.10679			51.8	- / (Pianka and Parker 1975)
Phrynosoma mcalli	0.31011	0.90759	0.57180	1.60	22.87	78	(Bonine and Garland 1999) / (Pianka and Parker 1975)
Phrynosoma modestum	−0.20004	1.97549	0.25796	1.45	25.02	66.1	(Bonine and Garland 1999) / (Pianka and Parker 1975)
Phrynosoma orbiculare	0.95144	1.02626	0.23759			25.9	- / (Pianka and Parker 1975)
Phrynosoma platyrhinos	0.55109	0.99740	0.42767	3.01	40.34	56.6	This Study / (Pianka and Parker 1975)
Phrynosoma solare	1.23869	1.11329	0.40021	1.60	17.56	88.8	This Study / (Pianka and Parker 1975)
Phrynosoma taurus	0.71250	1.07717	0.22719			56.5	- / (Pianka and Parker 1975)
Callisaurus draconoides	−0.15520	−1.64828	0.00001	5.72	78.40	3.0	(Bonine and Garland 1999) / (Pianka and Parker 1975)
Cophosaurus texanus	−0.46615	−1.11787	0.00001	4.57	72.14		(Bonine and Garland 1999) / -
Holbrookia maculata	−1.01503	−0.43264	0.00001	3.12	57.65		(Bonine and Garland 1999) / -
Uma notata	−0.19883	−0.78021	0.00001	3.49	47.65	20	(Bonine and Garland 1999) / (Turner 1998)
Uma scoparia	0.23529	−1.07639	0.00001	3.20	37.77		(Bonine and Garland 1999) / -
Sceloporus clarkii	0.96137	−0.67531	0.00001	3.00	32.92	6.2	(Bonine and Garland 1999) / (Brooks and Mitchell 1989)
Sceloporus jarrovi	0.22052	−0.58090	0.00001	1.62	21.01	26.3	(Bonine and Garland 1999) / (Goldberg and Bursey 1990)
Sceloporus magister	1.38770	−0.88926	0.00001	3.02	29.00	23.2	(Bonine and Garland 1999) / (Parker and Pianka 1973)
Sceloporus occidentalis	0.02280	−0.89043	0.00001	2.88	38.57		(Bonine and Garland 1999) / -
Sceloporus olivaceus	0.26092	−1.14874	0.00001			10.4	- / (Kennedy 1956)
Sceloporus undulatus	−0.46748	−0.23954	0.00001	2.51	40.72		(Bonine and Garland 1999) / -
Sceloporus virgatus	−0.90091	0.21650	0.00001	1.64	31.35		(Bonine and Garland 1999) / -
Urosaurus ornatus	−1.35636	−0.07973	0.00001	2.42	46.73		(Bonine and Garland 1999) / -
Uta stansburiana	−1.36519	−0.00112	0.00001	2.55	51.81		(Bonine and Garland 1999) / -

Only species for which sprint speeds or diet data were available are included. All values are species means. Body size and stockiness are PC-1 and PC-2 from this study, respectively. Horn size is the quotient of maximum horn length and head length (J. J. Meyers, unpubl. data), A-speed is absolute sprint speed in ms^{-1}, R-speed is the quotient of A-speed and SVL in m, % ants is the proportion of ants by volume in the diet. Literature citations are given for sprint speed and diet data. The speed reference is separated from the diet reference by a slash. A hyphen is used to indicate instances where there is no reference because there is no data provided.

comparable to other published studies using race-tracks with light sensors placed at intervals of 0.25 m. The number of frames it took the lizard to run 0.25 m divided by 250 (frames per second) allowed us to calculate absolute sprint speed in ms^{-1}. We calculated relative sprint speed in all cases by dividing it by mean SVL for each species.

We tested for pair-wise correlations among stockiness (PC-2), body size (PC-1), relative horn length, absolute and relative sprint speed, and percentage of ants in diet using phylogenetically independent contrasts (PIC) implemented in Mesquite (Felsenstein 1985; Garland et al. 1992; Maddison and Maddison 2006), as well as ML and a Bayesian approach implemented in BayesTraits (Pagel et al. 2004). The latter two analyses involved comparing the fit of a generalized least squares neutral drift model that forces traits to evolve in a correlated manner with a neutral drift model where traits evolved independently (Pagel 1999). If these models fit equally well, then the null hypothesis of uncorrelated evolution (the simpler model) is not rejected (Pagel 1999). To account for phylogenetic uncertainty with the Bayesian approach, we again used our sample of 6000 trees. We evaluated PIC results using hypothesis testing and *P*-values, compared ML models using AICc values (Akaike 1973; Burnham and Anderson 1998), and

Table 2. Morphometric variables included in principal component analysis and associated standardized loadings for PC-1 and PC-2.

Variable	PC1	PC2
Snout-vent length	0.290	−0.326
Head length	0.254	−0.326
Head height	0.373	0.275
Head width	0.385	0.116
Hind limb length	0.242	−0.574
Front limb length	0.325	−0.321
Body width	0.496	0.514
Body height	0.392	0.047
% variance	86.15	8.55

compared Bayesian models using Bayes Factors (BF – Raftery 1996). Like AICs used in a likelihood framework (Akaike 1973; Burnham and Anderson 1998), BFs avoid applying absolute cut-offs to significance and penalize models with greater numbers of parameters (Pagel and Meade 2004; Brandley et al. 2005). For interpretation, BF > 2 suggests limited support for a model, BF > 5 indicates strong support, and a BF > 10 indicates very strong support (Raftery 1996). A BF < 2 suggests negligible support for a given model. We ran each Bayesian analysis for 1.01×10^9 generations with 1×10^7 generations of burn-in, sampling every 20,000 generations.

Results

CHARACTERIZATION OF STOCKINESS

Principal component analysis, an approach successful at defining morphospace in similar systems (Wiens and Singluff 2001; Stayton 2006; Wiens et al. 2006), reduced the morphometric dataset to two principal components (PCs) that explained 94.8% of total variation (Table 2). PC-1 explained the majority of variation, represented a size axis, and was used as a proxy for body size. Body width loaded highly positively on PC-2, while snout-vent, head, and front and hind limb lengths loaded highly negatively on PC-2, thus defining an index of stockiness (Table 1). Species with relatively wide bodies also had short bodies, short limbs, and a short head, and can be described as stocky. Stocky animals also had relatively short original tails [correlation between PC-2 and Log(tail length): $R = 0.609$, $P < 0.0001$, $n = 745$], but variation in tail length was large enough to obscure patterns in the other variables, so was not included in the PCA.

PHYLOGENY OF PHRYNOSOMATINAE AND THE DIRECTIONAL EVOLUTION OF STOCKINESS

As expected from published phylogenies (Reeder and Wiens 1996; Harmon et al. 2003), our Bayesian tree divided the phrynosomatines into three clades: the horned lizards, including all *Phrynosoma*; the sand lizards, including *Callisaurus, Cophosaurus, Holbrookia*, and *Uma*; and the fence lizards, including *Uta, Urosaurus*, and all *Sceloporus* (Fig. 1). The horned and sand lizards were sister clades, and together were sister taxa to the fence lizards. The phylogeny had three polytomies, but with most other nodes well supported.

By calculating marginal ancestral reconstructions of stockiness (PC-2) using a neutral drift model of evolution and the phylogeny presented above, and plotting the ancestral values against the distance from the root of the phylogeny for their corresponding nodes, we mapped the phylogeny onto the graph (Fig. 2A). This technique resulted in a graphical representation of evolution of stockiness in the phrynosomatines, and allowed us to quantitatively evaluate directional evolution. From this plot, changes along the ordinate axis represented anagenesis, and the slopes of line segments connecting adjacent ancestors represented the direction and rate of evolution. The evolution of stockiness followed a striking pattern: there was strong directional evolution in the horned lizards toward a stocky body form (Fig. 2A). In

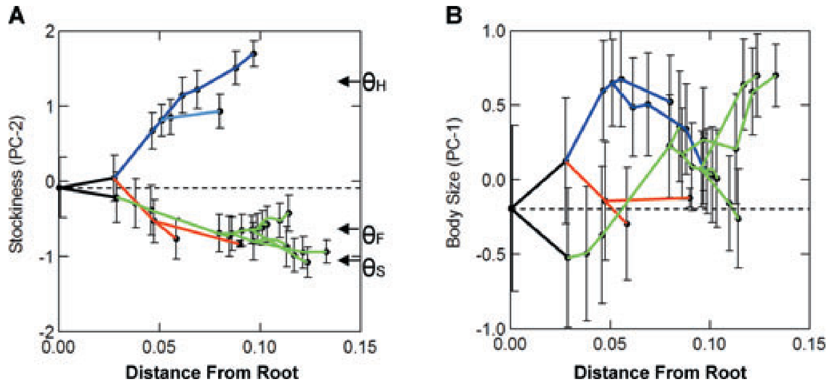

Figure 2. Plots of ancestral reconstructions of (A) stockiness (PC-2) and (B) body size (PC-1). Reconstructions are plotted against the distance from the root of the phylogeny. Internal branches are mapped from the phylogeny onto these plots and color-coded, with blue indicating horned lizards, red indicating sand lizards, and green indicating fence lizards. Terminal taxa are not included. Dashed horizontal lines indicate root ancestral morphology, and arrows labeled θ_X indicate optima for the OU.3 model (see text; θ_H is a similar value for both OU.2 and OU.3 models). Vertical bars indicate standard errors of marginal ancestral reconstructions.

Table 3. Parameter estimates, likelihoods (L), corrected AIC values, and relative model weights (w_i) for Brownian motion (BM) and Ornstein–Uhlenbeck (OU) models fit to stockiness data.

Model	df	α	Σ	Θ_0	θ_{Other}	θ_H	θ_S	θ_F	L	AICc	w_i
BM	2		0.966	−0.221					−35.37	75.08	0.0033
OU.1	4	0.046	0.975	−0.232	−0.011				−35.37	79.95	0.0003
OU.2	5	4.036	1.443	0.011	−0.694	1.333			−26.45	64.77	0.5811
OU.3	6	4.439	1.462	−0.004		1.307	−1.042	−0.607	−25.37	65.44	0.4152

Subscripts on θ parameters indicate different selective optima [0 – root, Other – all tip species (OU.1) or tip species unassigned to other optima (OU.2 and OU.3), H – horned lizards, S – sand lizards, F – fence lizards]. Degrees of freedom (df) for each model are also presented.

contrast, the sand lizards and the fence lizards evolved in parallel, retaining the ancestral level of stockiness for the Phrynosomatinae (Figs. 1, 2A). The rate of evolution within the horned lizards was in the opposite direction and significantly greater than within either of the other two clades (permutation ANOVA: $n = 30$, $F = 3.63$, $P = 0.0001$). This result is more significant than all of 100 datasets simulated under a neutral drift model of evolution (permutation ANOVA simulated data F: mean = 1.35, max = 3.14; 11 of the 100 simulated datasets produced significant differences among clades, with $P < 0.05$, but all $F < 3.63$), indicating that the above result is not an artifact. A similar plot of body size, which was strongly inversely correlated with stockiness (see below), showed no directional patterns of evolution (Fig. 2B). In contrast, when rates of evolution were measured using absolute values of standardized independent contrasts, they did not vary significantly among the three clades (ANOVA: $df_e = 32$, $F = 3.66$, $P = 0.0372$ – becomes not significant after Bonferroni correction; Kruskal–Wallis: df = 2, KW = 5.62, $P = 0.0604$).

The OU.2 model with a selective optimum for horned lizards and another for sand and fence lizards fit considerably better than either the BM model or the single optimum OU model, and marginally better than the OU.3 model with one optimum for each phrynosomatine subclade (Table 3). The parameter estimates for the OU.2 and OU.3 models were consistent and matched PC-2 phenotypes of the tip species (Fig. 2A). For neither of these two models did $\alpha \rightarrow 0$ or $\theta_H \rightarrow \infty$, indicating that directional evolutionary trends documented in our other analyses were consistent with a shift in selective regime for the horned lizards. This suggests that stabilizing selection on the new optimum is a more likely mechanism behind the directional trend, as opposed to persistent directional selection.

CORRELATES OF STOCKINESS

Stockiness (PC-2), body size (PC-1), relative horn length, absolute and relative sprint speed, and proportion of ants in diet were evolutionarily associated in a complex manner. Correlations among these traits when phylogeny was not taken into account were all highly significant, and we do not discuss them further. Results using PIC, ML, and Bayesian approaches were largely in agreement, so are deemed robust and we present only Bayesian evolutionary correlation analyses (Table 4). There was a strong negative correlation between stockiness and body size, with stockier species tending to be smaller. There was also strong evidence for stockier species as well as species with relatively longer horns eating a greater proportion of ants. There was some evidence for stockier animals running more slowly (both in an absolute sense and a relative one). However, there was little evidence that stockier animals also had relatively longer horns.

Measures of sprint speed correlated differently with relative horn length than with degree of ant specialization (Table 4). There was strong support for a negative correlation between relative horn length and relative sprint speed, but no such support for a correlation between relative horn length and absolute sprint speed. Conversely, there was strong evidence that species that eat more ants ran slower on an absolute basis but not a relative basis.

Discussion

HAS DIRECTIONAL EVOLUTION OCCURRED IN HORNED LIZARDS?

Although directional evolution is widely accepted, it remains relatively difficult to document. Furthermore, most classic studies

Table 4. Bayesian analysis of trait correlations showing number of species (n), correlation (R), and Bayes Factor (BF) for each pair of traits (see text for explanation of traits).

Trait 1	Trait 2	N	R	BF
Stockiness	Body size	38	−0.575	14.30
Stockiness	Horn size	24	0.255	2.39
Stockiness	A-speed	18	−0.491	4.72
Stockiness	R-speed	18	−0.148	4.50
Stockiness	% Ants	16	0.630	9.91
Horn size	A-speed	18	−0.359	1.98
Horn size	R-speed	18	−0.422	8.34
Horn size	% Ants	16	0.625	15.26
A-speed	% Ants	10	−0.817	9.32
R-speed	% Ants	10	−0.782	3.44

A-speed and R-speed are absolute and relative sprint speed, respectively.

of directional evolution focus on body size (MacFadden 1986; Arnold et al. 1995), and we lack compelling examples of how use of novel habitats or resources results in directional evolution of a suite of traits. We present such an example, documenting directional evolution toward a stocky body shape in one clade of phrynosomatine lizards (horned lizards, Fig. 2A). We show that this directional evolution has coevolved with other aspects of the phenotype (body size), defense (horn length), and whole-organism performance (sprint speed).

Previous studies of directional evolution in extant taxa have often been frustrated because modest numbers of species have been available to fit models of directional evolution (Poulin 2005; Verdu 2006), and some authors have argued that a directional model is necessary to detect such a pattern (Pagel 1999; Oakley and Cunningham 2000; Blomberg and Garland 2002). However, support for a directional model is unlikely when directional evolution occurs in only a subset of studied taxa, and when the root ancestral value is within the range of tip values (Pagel 1999), as is the case with stockiness in the phrynosomatines. Our approach of plotting ancestral values against distance from the root of the phylogeny and calculating internode slopes as relative rates of evolution allows us to detect directional evolution in portions of the studied phylogeny under a neutral drift model. The ability to detect a directional trend in a subclade of the taxa studied is not expected by chance, as demonstrated by simulations. In finding directional evolution in horned lizards but not their sister taxa, we clearly detected directional evolution in a subclade consisting of only 11 species (Fig. 1). In contrast, we were unable to detect differences in rates of evolution among subclades when direction of evolution was not taken into account.

Through basic statistical analysis of our ancestor-descendent slopes, we were able to quantitatively detect and compare the rate and direction of evolution among clades. This is both more informative and more sensitive than methods simply comparing rates (Clobert et al. 1998) because it accounts for direction and rate. Hence, if two clades are undergoing trait evolution in opposite directions, then differences appear larger than when comparing absolute values of rates. Until now, taking direction of evolution into account using ancestor-descendent pairs was the domain of paleontology, using relatively complete fossil series (Cheetham 1986; MacFadden 1986). Like many phylogenetically based approaches, our method assumes that ancestor-descendent slopes are independent of one another, that the phylogeny is correct and that the ancestral reconstructions are accurate. Furthermore, we note that ancestral reconstructions are marginal, so should be adjusted for multiple comparisons (Garland et al. 1999). We used a permutation ANOVA to help ameliorate these problems, although this approach still assumes independence of our slopes, an assumption that remains violated. By focusing on ancestral values as opposed to the model of evolution, our approach can be used under any model of evolution (e.g., it would be equally relevant when using a directional neutral drift model), and is particularly applicable when few species are available for sampling, such as when species are rare or when a clade of interest is not very diverse.

The sensitivity of our approach is further demonstrated by our finding of strong evidence for directionality in stockiness in horned lizards (Fig. 2A) but not body size (Fig. 2B), despite a high correlation between the two traits (Table 4). Our simulations, along with these results, indicate that the methodology will not always give the impression of directional evolution arising from ancestors being mere weighted averages of descendent values. These contrasting findings suggest that stockiness and body size have undergone both qualitatively and quantitatively different patterns of evolution.

WHY HAS DIRECTIONAL EVOLUTION OF STOCKINESS OCCURRED?

A pattern of directional macroevolution can arise as a result of several coexisting factors including the type of selection (e.g., directional vs. stabilizing – Hansen 1997; Butler and King 2004), the strength and direction of selection over evolutionary time (Kingsolver and Pfennig 2007), and the nature of internal constraints on genetic and phenotypic change. Constraints on phenotypic change that lead to directional trends can be described as orthogenesis (Blomberg and Garland 2002; Gould 2002), but documented examples are rare (e.g., coat color in tamarins – Jacobs et al. 1995). Many traits are likely to exhibit phenotypic change along axes with the greatest amount of variation (Schluter 1996). While we cannot comment on the nature of internal constraints channeling phenotypic change in these lizards, we can discuss the potential selective mechanisms that have led to the directional trend in body shape observed in horned lizards.

The exploitation of novel habitats or resources often promotes selection on a suite of morphological, behavioral, and functional traits. For example, in *Anolis* lizards, occupation of habitats with different perch characteristics quickly leads to evolution in limb proportions, locomotor performance and display behavior, resulting in the evolution of ecomorphs, each of which appear best suited for their respective habitats (Losos 1990a,b; Losos et al. 2006). In Darwin's finches, the size of available seeds exerts a strong selective pressure on beak size and shape, which influences both bite force, which is important in seed consumption, and song characteristics, which are important in intraspecific communication (Gibbs and Grant 1987; Podos 2001; Herrel et al. 2005; Huber and Podos 2006).

Similar to the above examples, our analyses indicate extensive trait coevolution, where the evolution of increased stockiness is associated with increased dietary specialization on ants, reduced body size and locomotor performance, and increased investment in defenses in horned lizards (Table 4). A commitment to ant-eating in horned lizards entails significant modifications to feeding morphology and kinematics, including a reduced dentition, more gracile jaws and a faster feeding cycle (Montanucci 1989; Meyers et al. 2006). Because ant-eating lizards spend large amounts of time close to ant mounds, this sedentary lifestyle may expose them to higher levels of predation, necessitating investment in defenses (Sherbrooke 1987; Young et al. 2004). A stocky body shape makes an animal look larger and less ingestible, but tends to limit an animal's locomotor performance (Austin and Arnold 2001; Bergstrom 2002; Losos et al. 2002). Muscle physiology has also evolved in concert with the locomotor needs of these lizards. The m. iliofibularis, a major hindlimb locomotor muscle, primarily consists of fast-twitch oxidative-glycolytic fibers in horned lizards, but of fast-twitch glycolytic fibers in sand lizards (Bonine et al. 2005). The former work aerobically, consistent with slower locomotion, while the latter work anaerobically. Furthermore, more robust lizards tend to run slower than more gracile lizards (Irschick and Jayne 1998) and tend to adopt a sit-and-wait foraging strategy (Huey and Pianka 1981). Our analyses provide support of an adaptive explanation (see Larson and Losos 1996) to the evolution of stockiness because of evolutionary trait associations that link stockiness to locomotor performance and ecological traits. Detailed functional studies illustrating extensive coevolution with other traits (Bonine et al. 2005; Meyers et al. 2006) further support this explanation (Larson and Losos 1996), but studies that examine genetic and phenotypic correlations among these traits (Cheverud 1988) or make direct estimates of selection in the wild (Husak et al. 2006; Calsbeek and Irschick 2007; Irschick et al. 2008) would yield insight into how selection might act. The OU model fitting suggests that the observed pattern of directional evolution toward increasingly stockier body shapes in horned lizards occurred as a result of a shift in selective regime followed by stabilizing selection on a new phenotypic optimum (Hansen 1997; Butler and King 2004). Hence, horned lizards might be expected to continue expressing their current stocky morphology while they maintain their current lifestyle, as opposed to becoming ever stockier, a pattern expected if concerted directional selection was the mechanism behind the observed directional trend.

The evolution of a stocky body shape occurs not only in horned lizards, but also in tetraodontiform fish, and in frogs. Although untested in the framework used here, the evolution of stockiness in tetraodontiforms may have occurred for similar reasons as in horned lizards. The tetraodontiform fish vary in the number of vertebrae that they posses and have the shortest vertebral columns among fish (Brainerd and Patek 1998). Species with shorter vertebral columns have reduced axial flexibility and locomotor performance (Brainerd and Patek 1998; Walker 2000). In conjunction with this short vertebral column, armature and the ability to inflate have evolved multiple times (Wainwright and Turingan 1997; Brainerd and Patek 1998). However, the evolution of a stocky body shape does not universally result in an increased investment in defense and reduced locomotor performance. For example, the evolution of stockiness and a reduced vertebral column in frogs appears to be an adaptation for making the axis more rigid (O'Reilly et al. 2000; Pugener 2002), which appears to provide better control during jumping (O'Reilly et al. 2000). Even within the Anura, however, toads may represent a stocky, toxin-defended, slow-moving clade.

Sprint speed and endurance can determine the outcome of predator-prey interactions, thereby affecting fitness of the participants (Huey and Stevenson 1979; Hertz et al. 1988; Irschick et al. 2007). However, the relevance of absolute versus relative sprint speed to the outcome of such interactions has been questioned (Van Damme and Van Dooren 1999). Both measures correlating negatively with stockiness is predictable from a biomechanical perspective, as stocky animals are generally less flexible, limiting the body's role in locomotion (Brainerd and Patek 1998; Walker 2000). Our finding that only relative speed correlates with relative horn length (Table 4) adds to evidence that high relative speed is important for evading predators (Van Damme and Van Dooren 1999). However, our finding that absolute sprint speed correlates with the degree of ant specialization suggests that this metric is more relevant when a predator is over-taking prey. Because ants are relatively slow, there is apparently relaxed selection on absolute sprint speed in these lizards, when compared to species that consume a wider variety of insect prey. Although studies testing this idea directly are needed, the observed asymmetry builds on the idea that the behavior of both predators and prey is important in studying their interactions (Lima 2002).

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Appendix Table A1. **A list of species included in the phylogeny, including three outgroup taxa (marked with *), sample sizes for the morphometric dataset, and GenBank sequence accession numbers.**

Species	*N*	ND2	ND4	12s	16s
*Liolaemus pictus**	N/A	AF099226	AY367848		
*Oplurus cuvieri**	N/A	U82685	U66225	U39587	U39587
*Sauromalus obesus**	N/A	U82687	U66232		
Sceloporus clarkii	31	AY297511	AF210344	L40452	L41459
Sceloporus cyanogenys	10	AY297524	AF154193	L40453	L41460
Sceloporus formosus	12	AY297498		L40455	L41462
Sceloporus graciosus	42	AF049860		L40456	L41463
Sceloporus grammicus	11	AY297509	AF210363	L40457	L41464
Sceloporus horridus	12		EF025747	AF000804	AF000844
Sceloporus jarrovi	32	AY297512	AF154209	L40458	L41465
Sceloporus magister	15	AF528741	AF210345	AF440092	L41466
Sceloporus malachiticus	11	AY297518		L41417	L41467
Sceloporus minor	12		AF154232	AF154186	AF000866
Sceloporus mucronatus	11	AY297497		L41419	L41469
Sceloporus occidentalis	9	AY297515		L41420	AF000893
Sceloporus olivaceus	12	AY297521	AF210361	L41421	L41471
Sceloporus orcutti	11	AY297508		L41422	L41472
Sceloporus poinsettii	9	AY297510	DQ358011	L41423	L41473
Sceloporus scalaris	12	AF528742		L41424	L41474
Sceloporus spinosus	12	AY297525	EF025748	L41425	L41475
Sceloporus undulatus	46	AY297514		L41428	L41478
Sceloporus variabilis	11	AY297507	AF210365	L41429	L41479
Sceloporus virgatus	34	AY297516	DQ358009	L41430	L41480
Urosaurus ornatus	33	AY297493	AY141065	L41436	L41487
Uta stansburiana	80	AF049863	AY141066	L41438	L41489
Phrynosoma asio	36	DQ385351	AY141048	L40446	L41452
Phrynosoma cornutum	42	AY297487	AY141049	L40447	L41453
Phrynosoma ditmarsi	33	DQ385353	AY141051	DQ385400	
Phrynosoma douglassii	45	U82686	AY141052	L40448	L41454
Phrynosoma hernandesi	3	DQ385343	AY141053	DQ385389	
Phrynosoma mcalli	21	AY297486	AY141054	AF346840	
Phrynosoma modestum	12	AY297484	AY141055	L40449	L41455
Phrynosoma orbiculare	31	DQ385352	AY141056	DQ385399	
Phrynosoma platyrhinos	28	AY297488	AY141057	AF346842	
Phrynosoma solare	11	AF528739	AY141058	AF346843	
Phrynosoma taurus	6	DQ385356	AY141059	AF346844	
Callisaurus draconoides	35	AY297492		L40437	L41441
Cophosaurus texanum	11	AY297489	AY141062	L40438	L41442
Holbrookia maculata	20	AY297490	AY141063	L40440	L41445
Uma notata	15	DQ385357		L41432	L41483
Uma scoparia	6	AF049861		AF194260	