ORIGINAL ARTICLE

doi:10.1111/evo.13

The convergent evolution of snake-like forms by divergent evolutionary pathways in squamate reptiles*

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Received June 14, 2018

Accepted November 8, 2018

Convergent evolution of phenotypes is considered evidence that evolution is deterministic. Establishing if such convergent phenotypes arose through convergent evolutionary pathways is a stronger test of determinism. We studied the evolution of snake-like body shapes in six clades of lizards, each containing species ranging from short-bodied and pentadactyl to long-bodied and limbless. We tested whether body shapes that evolved in each clade were convergent, and whether clades evolved snake-like body shapes following convergent evolutionary pathways. Our analyses showed that indeed species with the same numbers of digits in each clade evolved convergent body shapes. We then compared evolutionary pathways among clades by considering patterns of evolutionary integration and shape of relationship among body parts, patterns of vertebral evolution, and models of digit evolution. We found that all clades elongated their bodies through the addition, not elongation, of vertebrae, and had similar patterns of integration. However, patterns of integration, the body parts that were related by a linear or a threshold model, and patterns of digit evolution differed among clades. These results showed that clades followed different evolutionary pathways. This suggests an important role of historical contingency as opposed to determinism in the convergent evolution of snake-like body shapes.

KEY WORDS: Body shape, convergence, determinism, historical contingency, parallelism.

Convergent evolution is the recurrent evolution of similar phenotypes in unrelated taxa (Wake 1991; Hall 2007; Stayton 2015). Convergence is ubiquitous in nature, happening at all levels of biological organization, from molecular to ecological, and it has been documented in all taxa from prokaryotes to plants, fungi, and animals (Brooks 1996; Donoghue 2005; Castoe et al. 2009; McGhee 2011; Azua-Bustos et al. 2012; Parfrey and Lahr 2013). The occurrence of convergent evolution is viewed as evidence of determinism and predictability of evolution, whether it is driven by similar selective pressures or similar constraints on how evolution can happen (Losos 2011; Wake et al. 2011).

A fundamental question in studying convergence is whether convergent phenotypes arise via the same or different evolutionary pathways (Arendt and Reznick 2008; Elmer and Meyer 2011; Conte et al. 2012). These pathways inform us of mechanism because they are the sequence of modifications of genes, developmental programs, or constituent phenotypic traits underlying the evolution of the phenotype (Leinonen et al. 2012; Collar et al. 2014). The concept of evolutionary pathways is more inclusive than parallelism, which is restricted to the same genetic or developmental mechanism (Powell 2007; Scotland 2011). Convergent changes in constituent phenotypic traits may also be suggestive of convergent underlying genetic or developmental mechanism. Hence, convergent phenotypes can evolve through the same or different pathways. Concordant evolutionary pathways provide a much stronger test of determinism than simply observing convergent phenotypes.

Evolutionary pathways address how evolution has proceeded at different levels of biological organization. The same evolutionary pathways in convergent clades means that evolution was concordant at different biological levels, while different pathways means that convergent phenotypes were arrived at through non-convergent mechanisms (Wray 2002; Arendt and

*This article corresponds to Dolezal, M., and J. Lipps. 2019. Digest: How many ways to make a snake? Evidence for historical contingency of the convergence of squamate reptiles. Evolution. https://doi.org/10.1111/evo.13688.

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Reznick 2008; Meyer et al. 2013). A well-studied example is the evolution of light coloration in diverse vertebrates, including lizards, birds, mice, and mammoths (Theron et al. 2001; Hoekstra et al. 2006; Rompler et al. 2006; Rosenblum et al. 2010). Generally, a mutation in the melanocortin-1 receptor (*Mc1r*) gene is associated with this phenotype. Multiple populations of the beach mouse (*Peromyscus polionotus*) have evolved light coloration, and some of these populations have done so through a single nucleotide substitution in *Mc1r*, indicating the same pathway. Other populations have evolved the phenotype through a mutation to a different gene, indicating non-convergent evolutionary pathways (Hoekstra et al. 2006). At higher levels of organization, convergent functional capacities for suction feeding and durophagy in fishes have evolved by changes in different underlying functional phenotypic traits (McGee and Wainwright 2013; Collar et al. 2014). This imperfect convergence again illustrates how convergence at a particular level of organization can arise through different evolutionary pathways (Collar et al. 2014). Nevertheless, we still lack a general understanding of when we might expect convergence across multiple levels of biological organization, and how deterministic convergent evolution is.

An excellent case study of the role of determinism in evolution, and whether convergent phenotypes arise through the same pathways, is the evolution of snake-like body shapes. This is one of the most striking and widespread examples of convergence in vertebrates, and involves the elongation of the body and the reduction and loss of the limbs (Lande 1978; Bejder and Hall 2002; Caldwell 2003). It has evolved at least 14 times in fishes (Ward and Brainerd 2007; Mehta et al. 2010), multiple times in amphibians (Jockusch 1997; Para-Olea and Wake 2001), at least 25 times in squamate reptiles (i.e., lizards and snakes) (Greer 1991; Wiens and Singluff 2001; Wiens et al. 2006; Brandley et al. 2008), and in mammals, with the evolution of mustelids and whales (Gliwicz 1988; Bejder and Hall 2002).

Both body elongation and limb reduction can happen along multiple phenotypic evolutionary pathways. Body elongation can result from the relative lengthening of the trunk, the tail, or both (Ward and Brainerd 2007; Brandley et al. 2008). Because the body axis is composed of vertebrae, elongation of either the body or tail can also evolve through the addition of vertebrae, the lengthening of vertebrae, or both (Ward and Brainerd 2007; Wake et al. 2011; Arnold et al. 2017). Likewise, limb reduction can result from the miniaturization or the loss of limb elements (Shapiro 2002; Caldwell 2003; Shapiro et al. 2007). Limb reduction and loss may or may not be evolutionarily reversible (Dollo 1893; Kohlsdorf and Wagner 2006; Galis et al. 2010). The patterns of integration among body parts, or how closely they co-evolve during the evolution of a snake-like body shape may differ between clades (Klingenberg 2008; Caetano and Harmon 2017). Furthermore, the shape of the relationship between body parts may differ. For example, some body parts may co-evolve in a linear fashion, while others follow a sigmoidal pattern, termed a threshold relationship in studies of snake-like body shape evolution (Lande 1978; Brandley et al. 2008; Siler and Brown 2011). If these patterns differ among convergent clades, then this is evidence that they have evolved a convergent phenotype through different pathways. Importantly, these different phenotypic evolutionary pathways have different underlying developmental mechanisms (see Discussion; Pilbeam 2004; Ward and Mehta 2010).

Evidence from fishes, salamanders, and squamate reptiles suggests that different clades do indeed evolve body elongation and limb reduction in different ways (Brandley et al. 2008; Ward and Mehta 2010; Wake et al. 2011). Different clades of fishes that have evolved elongate phenotypes have done so through the elongation of the head, trunk, and/or tail (Ward and Mehta 2010). In plethodontid salamanders, some taxa have elongated their bodies only by adding vertebrae, while others have done so only by lengthening their vertebrae (Para-Olea and Wake 2001; Wake et al. 2011). In squamate reptiles, surface-dwelling species have tended to elongate their trunks and tails, while fossorial species have only elongated their trunks (Brandley et al. 2008). However, this dichotomy is more of a continuum, with considerable variation in relative tail length (Wiens et al. 2006). Many elongate squamates have increased numbers of vertebrae, but whether they have elongated by the addition and/or lengthening of vertebrae remains untested (Greer 1987; Bergmann and Irschick 2012).

Although squamates have evolved a snake-like body at least 25 times, the majority of these instances lack extant transitional forms, hindering reconstruction of their evolutionary pathways (Lande 1978; Greer 1991; Brandley et al. 2008). Furthermore, the selective pressures that resulted in elongate phenotypes have likely changed after their evolution (Gans 1975, 1986; Shine 1986). Six clades have extant transitional forms ranging from relatively short-bodied and pentadactyl to elongate and limbless, allowing us to study their evolutionary pathways (Fig. 1). These are the gymnophthalmid *Bachia* from South America (Presch 1975; Kohlsdorf and Wagner 2006), and the scincid clades including *Hemiergis* (Shapiro 2002) and *Lerista* from Australia (Greer 1987, 1990; Skinner et al. 2008; Skinner and Lee 2009), *Scelotes* from southern Africa (Whiting et al. 2003; Heideman et al. 2011), *Chalcides* from northern Africa and the Middle East (Caputo et al. 1995; Greer et al. 1998), and *Brachymeles* from the Philippines (Siler and Brown 2011; Siler et al. 2011). All of these cases belong to the short-tailed fossorial forms defined by Brandley et al. (2008), although *Chalcides* contains surface-dwelling and fossorial taxa (Carranza et al. 2008).

Here, we test whether the same phenotypes have evolved in each of these six clades, and whether each of these phenotypes evolved through the same evolutionary pathway. We might expect that each clade has somewhat different starting points, but

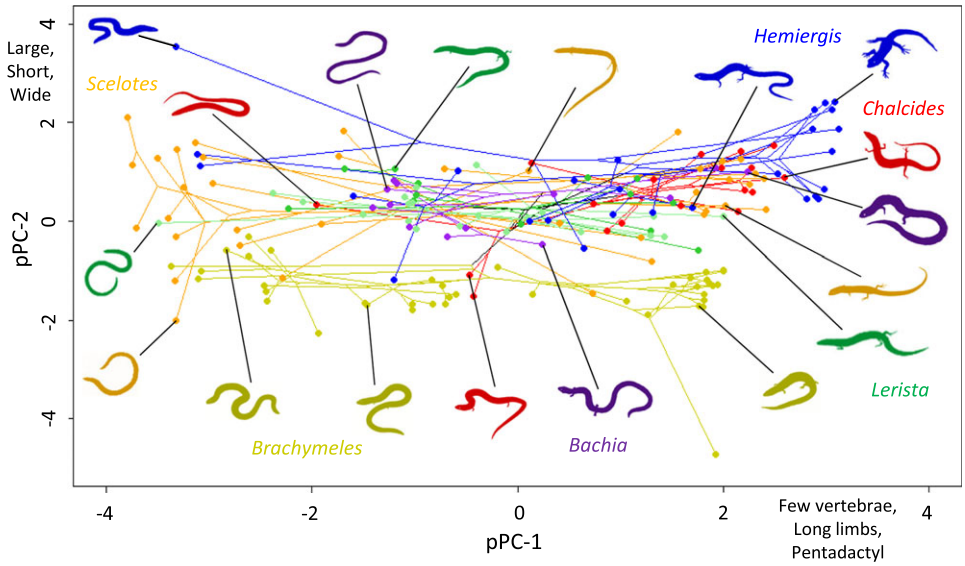

Figure 1. A phylomorphospace based on the first two phylogenetic principal components that include the six focal clades. Phylogenetic branches and dots (signifying species) are color-coded by clade. Silhouettes are also color-coded and show representative body shapes.

has evolved similar snake-like body shapes via similar changes in phenotype (Wiens et al. 2006; Brandley et al. 2008). If the snake-like species in each clade have indeed evolved similar body shapes, then this is evidence for convergence. If they have followed similar evolutionary pathways to get there, then this is evidence of determinism in convergent evolution (Losos et al. 1998). However, if we find considerable variation in the evolutionary pathways to achieve these snake-like phenotypes then this would suggest that the unique evolutionary history of each clade, termed historical contingency, plays an important role in their evolutionary outcomes (Vermeij 2006; Powell 2007). This would call into question whether snake-like body shapes arose convergently, akin to imperfect convergence, in which the same outcome at one biological level coincides with non-convergence, or even divergence, at a lower level (Stayton 2006; Collar et al. 2014). To address these issues, we first test for convergence of body shapes among the six clades. We then compare the evolutionary pathways of each of the six focal clades by (1) measuring the degree of integration among their body parts, (2) quantifying the shape (linear or threshold) of the relationship between those body parts, (3) determining how vertebral number versus length have contributed to the elongation of the body, and (4) modeling their patterns of digit evolution.

Methods

DATA SOURCES AND COLLECTION

We collected data for a total of 231 species represented by 655 museum specimens (range: 1–10, mean: 2.8, median: 1; Table S1) belonging to the six focal clades: *Bachia* and allies ($n = 16$, Gymnophthalmidae), *Hemiergis* and allies ($n = 36$, Scincidae), *Lerista* ($n = 62$, Scincidae), *Brachymeles* ($n = 40$, Scincidae), *Scelotes* and allies ($n = 47$, Scincidae), and *Chalcides* and allies ($n = 30$, Scincidae). When possible, we selected adult specimens with original tails. We measured head length, snout-vent length, original tail length (exclusively), front and hind limb length using Mitutoyo (Kanagawa, Japan) digital calipers to the nearest 0.01 mm three times, and took the average (see Morinaga and Bergmann 2017) for details). We also counted the number of fingers and toes. We used digital X-rays to count the number of presacral and, when possible, caudal vertebrae. We used ImageJ (Rasband 2016) to measure the length and width of the fourth and fifth vertebrae anterior to the sacrum, and the fifth and sixth caudal vertebrae that lacked pronounced transverse processes to the nearest 0.01 mm with the help of a scale object of known length on each X-ray. These vertebrae were representative in dimensions of many presacral and caudal vertebrae, without being near vertebral regional transitions. For species with $n > 1$, we used average measurements to generate species data. We then calculated relative snout-vent length, tail length, and limb lengths by dividing each variable by head length, which is the standard proxy for size in elongate lizards (Stokely 1947; Wiens and Singluff 2001; Siler and Brown 2011; Morinaga and Bergmann 2017). We also calculated the average presacral and caudal vertebral aspect ratios by dividing vertebral length by width. Data were then ln-transformed prior to analysis, except for analysis of thresholds, so as not to linearize the data.

We did all tree manipulations and data analysis using R 3.4.0 (R Core Team 2017). We obtained a phylogeny of all of the species represented in the dataset using the tree of Pyron et al.

(2013) and pruned it using the 'geiger' package in R (Pennell et al. 2014). We thus obtained one tree for each clade plus an overall tree that included all of the species from all six clades. Due to low species representation for *Brachymeles*, we used a revised phylogeny from Siler and Brown (2011) for that clade. We used penalized likelihood with a smoothing parameter of one to ultrametricize each phylogeny (Sanderson 2002) and scale it to a root-to-tip length of one using the 'ape' package (Paradis et al. 2004). The data and phylogeny is available on DataDryad (www.datadryad.org).

TESTING FOR CONVERGENT PHENOTYPES

We quantified overall patterns of relationships among morphometric variables by running a phylogenetic principle component analysis (pPCA) using the 'phytools' package (Revell 2009, 2012). The pPCA included head length as a proxy for size, presacral vertebral number, numbers of fingers and toes, and relative body width, snout-vent, tail, front limb, and hind limb lengths. This analysis included relative tail length, so used 170 species for which we had tail length data (Table S1). We ran the pPCA using a correlation matrix, estimating the phylogenetic signal of the variables as λ (Pagel 1999; Revell 2009). We visualized the phylogenetic pathways taken by each clade by plotting a phylomorphospace (Sidlauskas 2008) of pPC-1 and pPC-2 scores for all species with 'phytools' (Revell 2012). The phylomorphospace uses maximum likelihood ancestral state reconstructions for node values to plot the phylogeny (Revell 2012).

To test for convergence of body shapes among clades, we compared the fit of a series Brownian motion (BM) and Ornstein–Uhlenbeck (OU) models (Hansen 1997; Butler and King 2004) that we defined a priori to represent convergent and non-convergent hypotheses. We fitted multivariate models to the first two pPCs because these models take covariation among variables into account (Adams and Collyer 2018), using the 'mvMORPH' package (Clavel et al. 2015). Hypotheses representing convergence were OU models with different optima assigned to species with different numbers of digits. Hence, they tested whether species with the same digit numbers were convergent in body shape. The convergent OU models we considered defined optima by (1) the number of fingers, (2) the number of toes, (3) the number of total digits (fingers + toes), and (4) each unique combination of fingers and toes (digit morphs). We also fitted a model (5) that categorized total digits into three optima: low (0–2), medium (3–6), and high (8–10), to ensure that results were not biased by high numbers of parameters in some of the other models. No species in the dataset had seven total digits. We also fitted a series of models representing non-convergent alternatives to how body shape may have evolved. These included single regime BM and OU models, BM and OU models that assigned each of the focal clades a different regime, and BM models that assigned the number of fingers or toes to different regimes. These last two models allow digit number to affect rate of body shape evolution, but do not force them to converge on similar body shapes. We compared models using AICc (Burnham and Anderson 2002).

INTEGRATION AMONG BODY PARTS

We tested for integration among body parts by calculating evolutionary correlations among the number of presacral vertebrae, number of fingers and toes, relative snout-vent length, relative body width, relative tail length, and relative front and hind limb lengths. Correlations involving tail length were estimated using the 170 species for which we had those data; all other correlations used the complete dataset of 231 species. We calculated pairwise evolutionary correlations that took phylogeny into account for each focal clade from an evolutionary variance–covariance matrix using the 'geiger' package (Pennell et al. 2014). To test whether each correlation was significant, we simulated 10,000 null datasets that followed a Brownian motion model of evolution with the rate parameter, σ, set to that estimated for each original variable in our empirical dataset (following Morinaga and Bergmann 2017). We calculated the *P*-value as the number of null datasets that had an evolutionary correlation equal to or greater than that from the empirical data divided by the number of null datasets. We also tested whether patterns of integration were similar among clades. To do this we ran pairwise Mantel tests on clade evolutionary correlation matrices, each with 10,000 simulations, expecting significant positive correlations if body parts were similarly integrated among clades.

SHAPE OF RELATIONSHIP AMONG BODY PARTS

We also compared evolutionary pathways among clades by testing whether the relationship between untransformed number of presacral vertebrae, number of fingers and toes, and relative snout-vent and limb lengths was linear or followed a threshold (sigmoidal) pattern. We accomplished this by fitting three models to pairs of these variables and comparing them using AICc (following Morinaga and Bergmann 2017). The null model, $y = a$, represents no relationship between variables; a is simply a constant. The linear model, $y = a + bx$, is a regression, representing a gradual relationship. The threshold model, $y = A - (\frac{A}{1+(x/K)^S})$, is a form of logistic equation, where A is the asymptote, K is the location of the inflection along the x-axis, and S is the slope at the inflection (following Morinaga and Bergmann 2017). When we used the number of fingers or toes as y, we assumed the asymptote to be five, reducing the number of estimated parameters by one. This approach takes phylogeny into account while estimating the phylogenetic signal in the residuals as λ, which it estimates simultaneously to the other model parameters (Pagel 1999; Revell 2010; Orme et al. 2013). To choose a more complex model in our hypothesis testing, we required that the

Table 1. Eigenvectors from a phylogenetic PCA on all traits and all species from the six focal clades. The eigenvalue and percentage of variance explained by each component are also presented. Loadings > 0.4 are in bold. "Rel" are relative measures, standardized by head length.

	PC1	PC2	PC3
Head Length	**0.510**	**0.558**	−0.116
Vertebral #	**−0.894**	−0.028	0.063
# Fingers	**0.895**	−0.120	0.149
# Toes	**0.923**	−0.014	0.086
Rel Snout-Vent	**−0.811**	**−0.424**	−0.114
Rel Body Width	0.215	**−0.598**	**−0.727**
Rel Tail	−0.037	**−0.695**	**0.566**
Rel Hind Limb	**0.851**	−0.188	−0.071
Rel Front Limb	**0.856**	−0.316	0.056
Eigenvalue	4.874	1.482	0.916
% Variance	54.16	16.47	10.18

more complex model have an AICc at least two units lower than a simpler model. Otherwise, evidence is equivocal for the more complex model, and parsimony dictates choosing the simpler one.

CONTRIBUTION OF NUMBER AND LENGTH OF VERTEBRAE TO BODY ELONGATION

We tested whether elongation of the body was due to the addition of vertebrae, relative lengthening of vertebrae, or both using PGLS multiple regression, as implemented in the package 'caper' (Orme et al. 2013). To do this, we used relative snout-vent length as the response variable and number of presacral vertebrae and average presacral aspect ratio of the vertebrae as explanatory variables, while taking phylogeny and phylogenetic signal (λ) of the residuals into account. We did this analysis for each clade separately and for the entire dataset. We repeated the analysis on relative tail length as response, and number of caudal vertebrae and their aspect ratio as explanatory. However, sample sizes were very low due to missing tail data ($n = 4$–11 per clade), with no significant patterns detected. Results are presented only for the tail analysis that includes all clades ($n = 39$ species, Table S1).

PATTERNS OF DIGIT EVOLUTION

We modeled evolution of digit number on the front and hind limbs separately using continuous-time Markov models that treated digit number as a discrete variable and estimated a matrix **Q**, containing transitions between numbers of digits, using maximum likelihood (Schluter et al. 1997; Pagel and Meade 2006), as implemented in the package 'geiger' (Pennell et al. 2014). We coded digit number using six states, 0–5, with zero representing an absence of limbs and one representing either one digit or a partial limb without digits. This was done due to difficulty differentiating a single digit from a partial limb that lacked digits in some species, and because we assumed that these two phenotypes would be functionally similar. All models that we considered treated digit number as an ordered character.

We fitted a series of five models to the front and hind digit data, representing different evolutionary hypotheses and chose the most parsimonious using AICc (Burnham and Anderson 2002). The Equal rates model estimated a single rate of evolution for the gain and loss of digits. The Gain/Loss model assigned one rate for the gain of digits and one for their loss. We also fitted two models representing a relaxed Dollo's law, which states that once lost, complex traits cannot re-evolve (Dollo 1893; Galis et al. 2010). These two models were identical to the first two models described, except that if the limbs were lost (character state zero), they could not re-evolve. Finally, we fitted a strict Dollo's model that estimated a rate of evolution for the loss of digits, but disallowed any gain of digits. As described above, we conservatively chose a more complex model over a simpler one only when its AICc was at least two units better than the simpler one. A simpler model was one with fewer parameters, or one that made fewer evolutionary assumptions. For example, Dollo's models restricted how a trait could evolve, so were viewed as more complex than models with the same number of parameters that did not assume Dollo's law. Using the best model for each clade, we calculated the ratio of front to hind limb evolutionary rates. We also calculated the ratios of digit gain to loss for the front and hind limbs using the best Gain/Loss model, facilitating comparison of how digits evolved in each clade.

Results

CONVERGENT BODY SHAPE PHENOTYPES

The pPCA provided a holistic view of how traits were interrelated and how snake-like body shapes evolved. Phylogeny did affect the pPCA, as $\lambda = 0.804$. pPC-1 explained 54% of variation and represented a continuum from snake-like to lizard-like. Species with high values of pPC-1 were lizard-like, having relatively short bodies with few vertebrae and relatively long limbs with many digits (Table 1). pPC-2 explained 16% of variation, where species with high values were larger and had short bodies and tails (Table 1). pPC-3 explained 10% of variation, but did not have a clear interpretation and is not considered further (Table 1). The phylomorphospace based on pPC-1 and pPC-2 showed that all of the clades covered a similar range of phenotypes from lizard-like to snake-like with numerous intermediate forms, although *Bachia* and *Chalcides* did not have phenotypes as close to the snake-like extreme as the other clades (Fig. 1). However, clades appeared shifted relative to one another on the pPC-2 axis and there was no single snake-like shape that all clades converged

Table 2. Comparison of multivariate models of body shape evolution based on the first two pPCs.

	Model	Optima	*K*	LogL	AICc	Δ_{AICc}
Non-Convergent Hypotheses	BM	1	5	−522	1054	324
	OU	1	8	−518	1053	323
	OU: Clades	7	20	−494	1030	300
	BM: Fingers	6	20	−493	1029	299
	BM: Toes	6	20	−480	1002	272
	BM: Clades	7	23	−474	998	268
Convergent Hypotheses	OU: Fingers	6	18	−391	820	90
	OU: Low, Med, High	3	12	−397	819	89
	OU: Digit Morphs	18	42	−339	774	44
	OU: Toes	6	18	−359	757	27
	OU: Total Digits	**10**	**26**	**−337**	**730**	**0**

The number of optima, parameters (K), the log likelihood, AICc, and Δ_{AICc} are presented for each model. Models are sorted by Δ_{AICc} and the best model is in bold. Models based on the six focal clades also have an ancestral optimum for the phylogeny backbone. Total Digits is the sum of fingers and toes. Digit Morphs represents each combination of fingers and toes. Low, Med, and High models divide total digits into three categories (0-2, 3–6, 8–10). BM is Brownian Motion, OU is Ornstein–Uhlenbeck.

upon, suggesting that the various phenotypes between lizard-like and snake-like differed somewhat among clades (Fig. 1).

Multivariate models of body shape evolution strongly showed that species from different clades with common numbers of digits converged in body shape based on the first two pPCs (Table 2). By far the best supported model assigned a different optimum to species with different numbers of total digits (optima mapped in Fig. 2, parameter estimates in Table S2). Furthermore all of the models representing convergent evolution outperformed all of the non-convergent models by a wide margin (Table 2). The convergent model using total digits for optima showed that the first two pPCs were negatively related and that pPC-1 had a much lower half-life than pPC-2, so evolved toward optima more quickly (Table S2).

INTEGRATION AMONG BODY PARTS

Comparing patterns of integration of body parts among clades was one test of whether clades followed common evolutionary pathways. Most clades had similar patterns of evolutionary correlation, particularly for pairs of traits that showed significant evolutionary correlations (Tables 3 and 4; Table S3). Correlations between pairs of traits were particularly similar for *Lerista* and *Hemiergis*, which are sister clades (Fig. 2; Table 3). The non-scincid *Bachia* deviated the most from the other five clades (Table 3). Mantel tests on pairwise comparisons of clade evolutionary correlation matrices showed that overall patterns of integration were significantly positively correlated, and the magnitudes of Mantel correlations supported the observations from Table 3 (Table 4).

SHAPE OF RELATIONSHIP AMONG BODY PARTS

Evolutionary pathways might also differ among clades in the shape of the relationship between pairs of traits. While traits are often assumed to be linearly related, some traits appear related through threshold relationships in the evolution of snake-like body shapes in squamates. We found that the shape of the relationships between relative SVL, the number of presacral vertebrae, relative limb lengths, and number of digits differed substantially among the six focal clades (Table 5). For example, most traits were related in a linear manner in *Bachia* and *Chalcides*, but all traits had threshold relationships in *Scelotes*. Other clades had more even mixtures of threshold and linear relationships (Table 5). In *Brachymeles*, relative SVL was unrelated to the number of fingers (Table 5). No pair of traits had the same shape of relationship in all six focal clades, although if *Bachia* were excluded, the remaining scincid clades had threshold relationships between number of presacral vertebrae and the number of toes (Table 5). The parameters for the best model for each clade and pair of variables are presented in Table S4.

VERTEBRAL EVOLUTION AND BODY ELONGATION

Whether body elongation in each clade arose through an increase in the number of vertebrae, vertebral length, or both is another specific test of convergent evolutionary pathways. PGLS multiple regressions showed that in each of the six focal clades and when all species were pooled together, presacral vertebral number had a highly significant positive effect on body elongation, but vertebral aspect ratio did not (Table 6). Only in *Brachymeles* was vertebral aspect ratio marginally significantly related to relative body length, but the relationship was negative, so not in the expected direction (Table 6). We carried out similar analyses for relative tail length, but they were inconclusive due to small sample sizes (n = 4–11) resulting from many specimens having autotomized or regenerated tails (Table S1). When all species were pooled, results matched the trunk in that relative

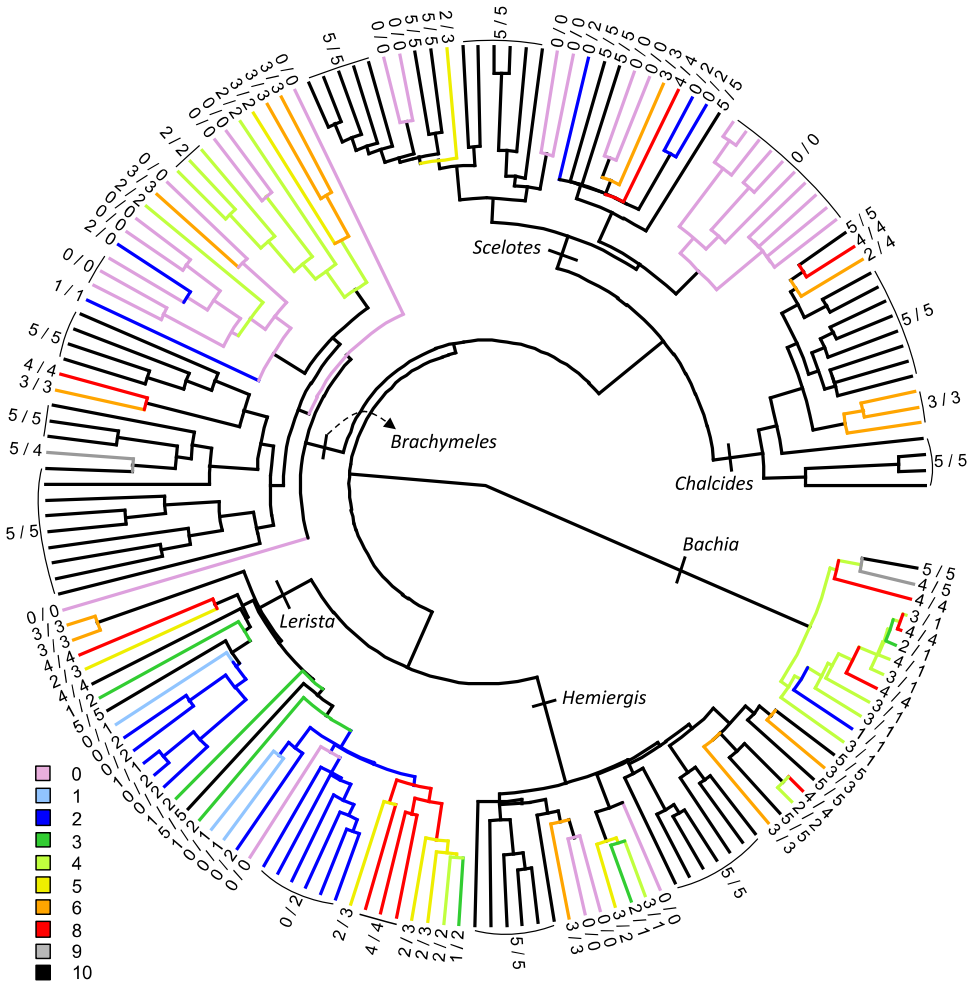

Figure 2. A phylogeny including the six focal clades (labeled). Trait optima based on the total number of fingers and toes are mapped in different colors on the branches. Note that no species in the dataset has seven total digits. Instead of species names, the number of fingers/toes is indicated at the tips. The OU model defining trait optima by total number of digits was the best fitting model. Note that colors do not coincide with those used in Figure 1.

tail length was significantly explained by number of caudal vertebrae, but not their aspect ratio ($n = 39$, $R^2 = 0.271$; vertebral number: slope $= 0.20 \pm 0.054$, $t = 3.65$, $p = 0.001$; aspect ratio: slope $= -0.04 \pm 0.428$, $t = -0.09$, $p = 0.927$).

PATTERNS OF DIGIT EVOLUTION

A total of 19 digit morphs (out of 36 possible morphs) evolved in the six focal clades, and each clade contained between five and eleven morphs (Fig. 3). Each clade evolved three to six morphs with front-hind limb symmetry, and only *Bachia* had a morph with more than a two digit difference between front and hind limbs (4/1 – *Bachia bicolor*). *Bachia*, *Brachymeles*, and *Hemiergis* evolved morphs with equal or more fingers than toes, while *Chalcides, Lerista*, and *Scelotes* evolved morphs with equal or fewer fingers than toes (Fig. 3). This suggests two different pathways of evolving limb morphology.

Models of evolution also suggested that different clades evolved digit numbers in different ways, but support for many models was equivocal (Table 7). In *Bachia,* both front and hind digits evolved at equal rates, although the more complex gain/loss model fit similarly. In *Hemiergis*, front digit evolution was best modeled by the gain/loss model and hind digit evolution by the Dollo gain/loss model, but these models performed similarly for both limbs. In *Lerista* and *Brachymeles*, front and hind digits were best fit by the equal rates or Dollo equal rates models, although the gain/loss and Dollo gain/loss models also performed similarly. In *Scelotes*, both front and hind digits strongly favored the Dollo gain/loss model. The strict Dollo model performed well only in *Chalcides*, but not much differently from the equal rates and gain/loss models, which made fewer assumptions about digit evolution (Table 7).

Fingers evolved faster than toes in *Lerista, Scelotes*, and *Chalcides*, while toes evolved faster in *Bachia, Hemiergis*, and

Table 3. Evolutionary correlation among body parts for six clades of lizards.

	Presacral Vertebrae								Front Digits								Hind Digits								rSVL								rBW								rTL								rFLL							
	FD		HD		rSVL		rBW		rTL		rFLL		rHLL		HD		rSVL		rBW		rTL		rFLL		rHLL		rSVL		rBW		rTL		rFLL		rHLL		rBW		rTL		rFLL		rHLL		rTL		rFLL		rHLL		rTL		rFLL		rHLL		rFLL	
Bachia		−		−		+		+		−		−		−		+		−		+		+		+		+		−		+		+		+		+		+		−		+		+		+		+		+		+		+		+		+
Hemiergis		−		−		+		−		−		−		−		+		−		+		+		+		+		−		+		+		+		+		+		−		+		+		+		+		+		+		+		+		+
Lerista		−		−		+		−		+		−		−		+		−		+		+		+		+		−		+		+		+		+		+		−		+		+		+		+		+		+		+		+		+
Brachy.		−		−		+		−		−		−		−		+		−		+		+		+		+		−		+		+		+		+		+		−		+		+		+		+		+		+		+		+		+		+
Scelotes		−		−		−		−		−		−		−		+		−		+		+		+		+		−		+		+		+		+		+		−		+		+		+		+		+		+		+		+		+		+
Chalcides		−		−		+		−		+		−		−		+		−		+		+		+		+		−		+		+		+		+		+		−		+		+		+		+		+		+		+		+		+		+

+ and − show direction of correlations, and shaded cells are significant after correction for multiple comparisons. FD = number of front digits; HD = number of hind digits; rSVL = relative snout-vent length; rBW = relative body width; rTL = relative tail length; rFLL= relative front limb length; rHLL = relative hind limb length.

Brachymeles (Table 7), consistent with digit morph observations (Fig. 3)—those with more developed front limbs had higher rates of toe evolution. Rates of digit gain and loss were clade-specific for both limbs. The rate of digit gain was low for *Lerista* and *Chalcides*. Relative rates of digit gain and loss were comparable for front and hind limbs for all clades except *Bachia*, where the rate of digit gain was higher than loss in the front limbs and vice versa for hind limbs (Table 7).

Discussion

DETERMINISM AND HISTORICAL CONTINGENCY IN THE EVOLUTION OF SNAKE-LIKE BODIES

We set out to test whether six clades of squamate reptiles that have evolved snake-like bodies did so following convergent evolutionary pathways, a result that would indicate a strong role of determinism in the evolution of this phenotype. Although we did find that the digit morphs that have evolved in these clades are largely convergent in body form (Table 2), we found variation in body shape among snake-like species (Figure 1) and that each clade evolved following different pathways, pointing to a major role of historical contingency in their evolution. Although patterns of body part integration were positively related among clades (Table 4), there were important differences in specific pairwise comparisons (Table 3). Furthermore, these relationships often differed in shape—some were linear while others were thresholds (Table 5). The relative rates of finger and toe evolution, and rates of digit gain and loss were also clade-specific (Table 7). The main exception to this evidence of historical contingency was that all six clades elongated their bodies by the addition and not the lengthening of their trunk vertebrae (Table 6).

This pattern of convergence on similar phenotypes by different, clade-specific evolutionary pathways may actually be widespread. For example, the convergent evolution of durophagy in Moray eels (Muraenidae) occurred through divergent changes in trophic traits (Collar et al. 2014), something the authors called imperfect convergence, and, similarly to us, interpreted as a hallmark of historical contingency. Likewise, independently derived populations of the benthic ecotype of stickleback (*Gasterosteus aculeatus*) have convergently evolved increased suction performance for feeding while doing so through morphologically divergent pathways (McGee and Wainwright 2013). Different populations of shrew (*Sorex* spp.) have likewise increased bite force capacity through different changes in their musculoskeletal morphology (Young et al. 2010). In these examples, convergent evolution actually resulted from increased disparity of underlying traits, and our findings also suggest this pattern.

It may seem counter-intuitive that diversity of constituent parts results in lack of diversity (convergence) of an emerging

Table 4. Pairwise Mantel test results comparing evolutionary correlation matrices among clades. Lower triangle contains correlation coefficients, upper triangle contains the *P*-values (all are significant after correction for multiple comparisons).

	Bachia	Hemiergis	Lerista	Brachymeles	Scelotes	Chalcides
Bachia		<0.001	<0.001	<0.001	0.015	0.002
Hemiergis	0.763		<0.001	<0.001	<0.001	<0.001
Lerista	0.636	0.926		<0.001	0.002	<0.001
Brachymeles	0.789	0.961	0.906		<0.001	<0.001
Scelotes	0.460	0.667	0.563	0.673		0.003
Chalcides	0.595	0.866	0.847	0.808	0.539	

Table 5. Shapes of relationships between pairs of traits for the six focal clades.

X	Y	*Bachia*	*Hemiergis*	*Lerista*	*Brachy.*	*Scelotes*	*Chalcides*
rSVL	rFLL	**L: 12**	**L: 16**	N	L: 3	**T: 15**	L: 5
rSVL	rHLL	**L: 15**	**L: 12**	**T: 11**	T: 3	**T: 11**	L: 4
rSVL	FD	L: 2	T: 4	T: 4	N	**T: 15**	L: 5
rSVL	HD	**L: 10**	T: 5	**L: 33**	L: 6	**T: 15**	**L: 13**
rSVL	Vert	L: 5	**L: 15**	**L: 43**	**L: 17**	T: 8	**L: 13**
Vert	rFLL	L: 7	**L: 28**	**T: 49**	**T: 10**	**T: 27**	**L: 10**
Vert	rHLL	**L: 15**	T: 3	**T: 23**	**T: 14**	**T: 18**	L: 7
Vert	FD	N	**L: 30**	**T: 44**	**T: 21**	**T: 56**	**L: 11**
Vert	HD	**L: 13**	T: 8	**T: 20**	**T: 17**	**T: 39**	T: 6
rFLL	FD	T: 4	**T: 17**	**L: 87**	**T: 30**	**T: 110**	L: 4
rHLL	HD	**T: 28**	**L: 14**	**L: 23**	**T: 25**	**T: 25**	N

In each case, the preferred model is specified with a letter and shading (T/dark: Threshold, L/light: Linear, N/unshaded: Null). Numbers indicate the smallest Δ_{AICc} value to a simpler model. Entries in Bold indicate strong evidence for a model ($\Delta_{AICc} \geq 10$). rSVL = relative snout-vent length, Vert = number of presacral vertebrae, rFLL = relative front limb length, rHLL = relative hind limb length, FD = number of front digits, HD = number of hind digits.

Table 6. Phylogenetic least squares multiple regression results relating number of presacral vertebrae and thoracic vertebral aspect ratio to relative snout-vent length for each of the six focal clades and for all species from those clades pooled together.

				Presacral Vertebrae			Presacral Aspect Ratio		
Clade	*n*	λ	R^2	Slope	*t*	*P*	Slope	*t*	*P*
Bachia	12	0	0.863	0.21 ± 0.029	7.52	**<0.001**	0.01 ± 0.691	0.01	0.996
Hemiergis	21	0	0.896	0.25 ± 0.021	11.88	**<0.001**	−0.83 ± 0.558	−1.49	0.154
Lerista	15	1	0.664	0.23 ± 0.050	4.67	**0.001**	−0.50 ± 1.270	−0.39	0.701
Brachymeles	31	0.74	0.776	0.24 ± 0.027	8.95	**<0.001**	−1.32 ± 0.587	−2.24	**0.033**
Scelotes	25	0	0.872	0.27 ± 0.027	10.31	**<0.001**	0.45 ± 0.759	0.59	0.561
Chalcides	11	0	0.776	0.18 ± 0.034	5.26	**0.001**	1.07 ± 1.531	0.70	0.504
All species	115	1	0.728	0.25 ± 0.015	17.21	**<0.001**	−0.21 ± 0.284	−0.75	0.456

n is number of species, and λ is phylogenetic signal of the residuals. Slopes are presented with their standard error estimates. Significant *P*-values are in bold.

phenotype. In all of the cases above, the authors invoked redundancy or many-to-one mapping as the mechanism, allowing the convergent phenotype to evolve in many different ways. Indeed, simulated evolution studies have shown that many-to-one mapping can lead to divergent evolutionary pathways to achieve similar function, and that the starting point, reflected by historical contingency, plays an important role in how evolution proceeds (Wainwright et al. 2005). One way in which our work differs is that it relates underlying phenotypic traits to an overall phenotype (snake-like bodies), rather than to a functional trait like bite force or suction. However, redundancy still applies here because it is a general principle that explains how one level of biological

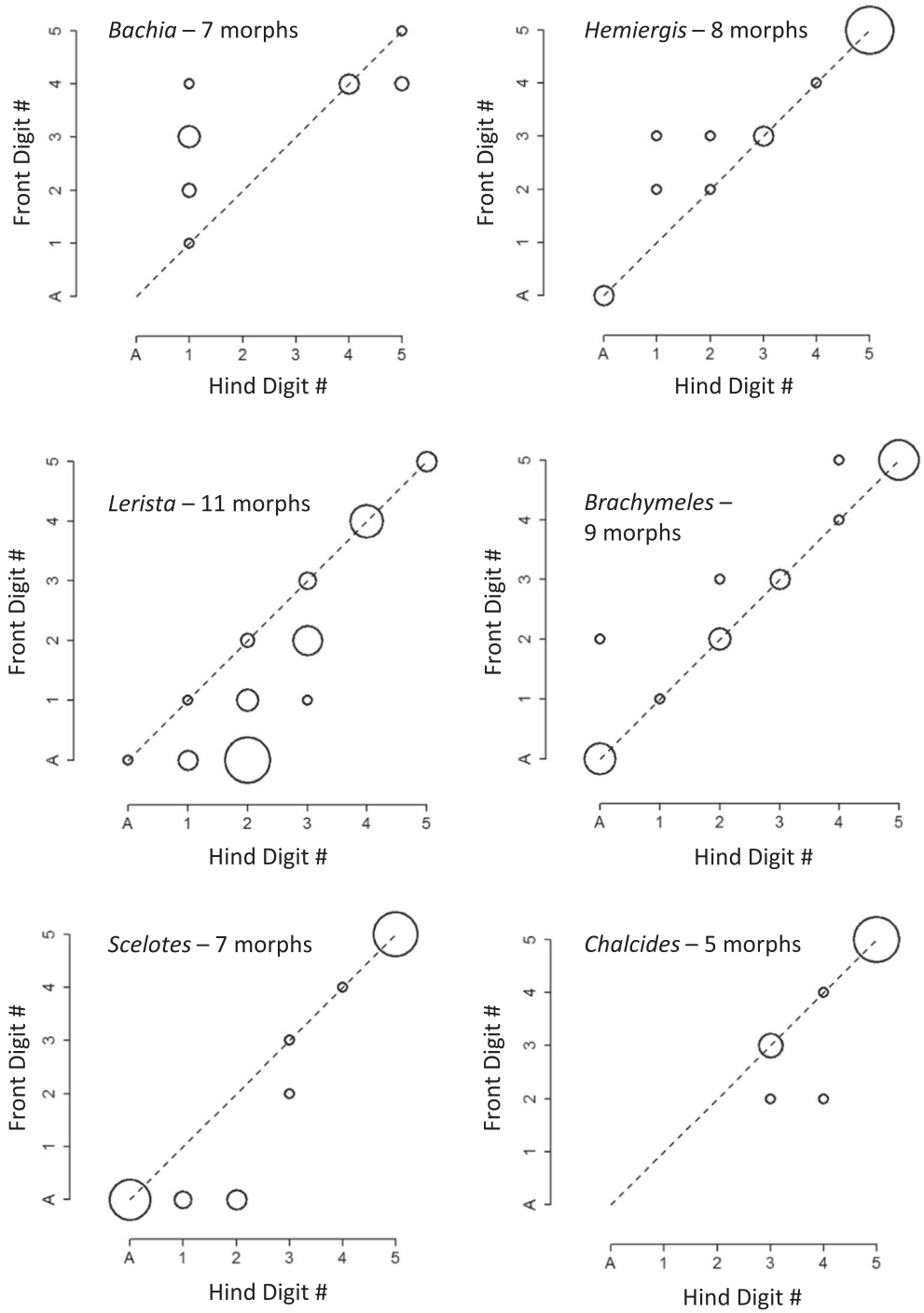

Figure 3. Plots of evolved digit morphs in each of the six focal clades. The size of the circles indicates the number of species in each category. The diagonal line indicates front-hind limb symmetry. (A) indicates that limbs are absent.

organization maps onto a higher level (see below). Hence, a general finding of these studies, including ours, is that evolutionary pathways and initial conditions are all subject to contingency, but that the outcomes, particularly from a functional or ecological standpoint, may be deterministic (Thomas and Reif 1993; Vermeij 2006).

SNAKE-LIKE CONVERGENCE ACROSS VERTEBRATE CLADES

Given that the evolution of a snake-like body is a major recurring theme in vertebrate evolution, it begs the question of how other instances compare to our findings for squamate reptiles. We found evidence of determinism in that body elongation in all six of

Table 7. Δ_{AICc} values for models of digit evolution for front and hind limbs for each clade.

		Rates	*Bachia*	*Hemiergis*	*Lerista*	*Brachy.*	*Scelotes*	*Chalcides*
Front Limb	Equal	1	0	4.51	7.68	2.35	49.04	1.45
	Dollo Equal	1	N/A	2.54	0	0.42	18.39	N/A
	Gain/Loss	2	1.97	1.76	3.99	1.09	49.74	2.30
	Dollo G/L	2	N/A	0	1.66	0	0	N/A
	Strict Dollo	1	7.54	8.10	2.56	12.16	53.22	0
Hind Limb	Equal	1	0.76	6.69	0.03	5.25	26.16	1.35
	Dollo Equal	1	N/A	5.01	0	0.60	4.71	N/A
	Gain/Loss	2	0	2.68	1.93	6.03	28.02	2.30
	Dollo G/L	2	N/A	0	1.92	0	0	N/A
	Strict Dollo	1	10.19	13.51	6.39	10.98	36.53	0
		F/H	0.32	0.82	2.93	0.96	1.11	1.58
	Front	G/L	1.16	4.57	0.07	2.95	4.52	<<0.01
	Hind	G/L	0.55	3.87	0.83	2.13	2.31	<<0.01

N/A indicates that a model was not fitted because the clade has not evolved a limbless form. Values in grey identify most parsimonious model, used in calculating ratios of rates of front to hind limb evolution (F/H). Ratios of front and hind limb gains to losses (G/L) are calculated based on the Gain/Loss model, except for *Scelotes*, in which the far superior Dollo G/L model is used.

our focal clades was due to the addition of vertebrae, but not their lengthening, particularly in the trunk (Table 6). This has also been observed by others who have studied squamates (Stokely 1947; Greer 1987; Caputo et al. 1995; Schmitz et al. 2005; Bergmann and Irschick 2012), and seems to be the case for various extinct tetrapods (Caldwell 2003; Müller et al. 2010). However, this pattern appears to break down in fishes. Although most elongation is still due to the addition of vertebrae, the relative lengthening of vertebrae also plays a role in some clades (Ward and Brainerd 2007; Ward and Mehta 2010). This pattern breaks down even more in plethodontid salamanders, where some species elongate exclusively by adding vertebrae, while others elongate exclusively by lengthening vertebrae (Para-Olea and Wake 2001; Wake et al. 2011). Elongation through only the lengthening of vertebrae is a glaring exception in the evolution of snake-like body shapes, yet is not completely isolated, as giraffes (*Giraffa camelopardalis*) have also only lengthened the vertebrae in their neck (Solounias 1999).

In considering patterns of integration in snake-like bodies, beyond the expected and documented negative relationship between relative body length and limb lengths, the role of the tail has received some attention. Relative tail length evolves independently in *Brachymeles* (Siler and Brown 2011), *Lerista* (Skinner and Lee 2009), and anguid lizards (Wiens and Singluff 2001), a clade that we did not study due to a lack of clear intermediate forms. Similarly, we found that relative tail length had no significant relationship with other body parts in any of our focal clades (Table 3; Table S3). This may be due to the repeated evolution of short-tailed and long-tailed elongate morphs (Brandley et al. 2008). However, this explanation is only part of the story, as most of our focal clades consist solely of what would be considered short-tailed and fossorial, yet do not have exceedingly short tails. Indeed the short- and long-tailed morphs in squamates are simply extremes in a continuum (Bergmann and Irschick 2012; Bergmann 2012).

Within squamate reptiles, there has long been a fascination with whether traits are related following a linear or a threshold pattern (e.g., Stokely 1947; Lande 1978). We showed clade-specific patterns of which traits were related in each way (Table 5). However, we also showed that all clades except *Bachia,* the only non-skink, had a threshold relationship between the number of trunk vertebrae and the number of toes. This pattern was previously found in *Brachymeles* (Siler and Brown 2011) and *Lerista* (Greer 1987). Brandley et al. (2008) documented a threshold between relative body length and relative limb lengths across elongate squamates. Thus, in the Scincidae there may be a threshold degree of body elongation beyond which taxa quickly reduce and lose their limbs. Threshold covariation between traits is relatively common at the population level, especially in polymorphic insects (Roff 1996; Moczek and Nijhout 2003). The underlying mechanism tends to be that when a gene product is expressed at a level exceeding a critical amount, then this triggers a different pattern of gene expression in the individual (Roff 1994; Quinn et al. 2007). For example, in beetles (*Onthophagus taurus*) males exceeding a certain body size have altered expression of juvenile hormone, leading to horn outgrowth (Moczek and Nijhout 2003). However, in all of these cases, the level of gene expression is affected by environmental factors, such as temperature or diet, and is an example of phenotypic plasticity. Another mechanism that might lead to a threshold relationship between traits is dynamic allometry, where a trait increases relative to another by strong positive allometry for most phenotypes, except extreme ones (e.g., large and small; Tomkins and Moczek 2008).

These principles may apply to macroevolutionary patterns, like the evolution of snake-like shapes, if alternate morphs are subject to different selective pressures, which is often the case (Roff 1996), or if copy number of a critical developmental gene evolves during the evolutionary transition.

DEVELOPMENTAL MECHANISMS UNDERLYING EVOLUTIONARY PATHWAYS TO SNAKE-LIKE FORMS

Selective and developmental explanations to evolutionary trends are not mutually exclusive, and scientists are increasingly interested in integrating the two (Bejder and Hall 2002; Shapiro et al. 2007; Leinonen et al. 2012). Developmental mechanisms of vertebral and digit evolution can be induced from observed patterns of variation in non-model organisms (Pilbeam 2004; Ward and Brainerd 2007; Head and Polly 2015). Changes in vertebral number and relative length represent meristic and homeotic/identity changes, respectively (Pilbeam 2004; Buchholtz and Stepien 2009), and these changes are under control of different developmental genetic regulatory networks. Vertebral number is dictated by developmental rate, length of time during which somites form from paraxial mesoderm, and the rate of elongation of the anterior–posterior (A–P) axis, including tail bud outgrowth (Gomez et al. 2008; Beck 2015; Benazeraf et al. 2017). This is controlled by *Wnt*, Retinoic Acid, and *Notch* signaling, via the segmentation clock (Pourquie 2003; Eckalbar et al. 2012), although some of the genes involved from the *Notch* signaling pathway differ among taxa (Kusumi et al. 2013). In contrast, vertebral identity, including the size shape of each vertebra is controlled by the amount of presomitic mesoderm remaining for somite formation (Ishimatsu et al. 2018) and the *Hox* gene clusters, which are expressed differentially along the A–P axis of the animal (Burke et al. 1995; Burke and Nowicki 2001). Hence, one can infer the developmental genetic mechanism of elongation from vertebral number and measurement data (Pilbeam 2004; Ward and Mehta 2010).

Our finding that squamates elongate their bodies purely through the addition of vertebrae (Table 6) would at first suggest simply an increase in the rate of the segmentation clock (Pourquie 2003; Gomez et al. 2008). However, the underlying clock and wavefront model predicts that such a change would lead to more but smaller vertebrae. Indeed, snakes (Gomez et al. 2008) and caecilians (Renous and Gasc 1989) show such a tradeoff between vertebral number and size, but we found evidence of this only for *Brachymeles* (Table 6), suggesting that other factors are also involved. One possibility might be an increase in the posterior regression of the determination front at which somite boundaries form, along with prolonged outgrowth of the paraxial mesoderm (Ward and Brainerd 2007; Gomez et al. 2008). Indeed, new evidence and a revised underlying model show that somite size depends on the amount of presomitic mesoderm remaining at the time of somite boundary determination (Ishimatsu et al. 2018). Furthermore, the outgrowth of the tail bud may also contribute to this (Beck 2015). Another possibility might involve subtle changes in vertebral identity via *Hox* gene expression. Recent work shows that snakes and limbless lizards have less variable vertebral shape than their limbed counterparts, but that their vertebral column remains regionalized to a similar degree to limbed species, and that the *Hox* code functions similarly across these species (Head and Polly 2015).

A hallmark of our six focal clades is the existence of transitional forms between pentadactyl and limbless, yet limb reduction and loss are thought to be distinct processes (Caldwell 2003; Shapiro et al. 2007). The 5′ *Hox* genes determine digit identity, but *Sonic Hedgehog* (*Shh*), its upstream regulators, and downstream targets have been implicated in affecting digit reduction and loss, as well as limb loss (Tanaka and Tickle 2007; Zeller et al. 2009). Decreased spatial and temporal *Shh* expression in the posterior margin of the limb bud leads to reduction and loss of digits, generating intermediate forms, as seen in *Hemiergis* skinks (Shapiro et al. 2003; Sagai et al. 2005). Complete limb loss is associated with loss of *Shh* expression in the limb bud, often mediated by upstream regulators like *Tbx* and *Fgf* (Leal and Cohn 2018). In these cases, the limb bud and its apical ectodermal ridge degenerate (Leal and Cohn 2018). Recent work shows that the limb-specific *Shh* enhancer ZRS is critical for proper *Shh* expression and its mutation is implicated with limb loss in snakes (Kvon et al. 2016).

Our work did not consider individual digit identities, something that has undergone complex patterns of evolution (Young et al. 2009). However, the existence of multiple intermediate digit morphs suggests differential spatial and temporal reduction in the expression of *Shh* in the posterior limb bud. We also found that clades differed in whether they had morphs with more fingers than toes or vice versa (Figure 3), and this might be explained by differential regulation of *Shh* by the upstream *Tbx5* in the front limb and *Tbx4* in the hind limb (Leal and Cohn 2018). The study of limb patterning genes in these non-model systems represents both a challenge and a compelling opportunity to understanding limb reduction and loss. Studies such as ours can generate testable hypotheses for this endeavor.

CONVERGENCE ACROSS LEVELS OF BIOLOGICAL ORGANIZATION

Our main finding that each clade has evolved a snake-like body shape following a different morphological pathway joins a growing body of work that shows that convergence at one level of biological organization does not necessitate convergence at other levels (Brooks 1996; Arendt and Reznick 2008; Losos 2011; Conte et al. 2012). The study of convergence inherently requires a hierarchical approach because examples range from

the genetic to the community level (Harmon et al. 2005; Shapiro et al. 2006; Wake et al. 2011; Conte et al. 2012). The unanswered question is whether there are rules governing how convergence maps across levels of organization. The prevalence of redundancy mapping suggests that we should often see different evolutionary pathways leading to convergent outcomes (Wainwright et al. 2005). Such redundancy is present at many levels of biological organization. For example, many genes affect single phenotypic traits (Arendt and Reznick 2008), many body parts contribute to single organismal shapes (this study; Ward and Brainerd 2007; Bergmann et al. 2009), and a single function (Alfaro et al. 2005), and performance at different tasks helps determine niche use (Losos 2009). As such, we do not necessarily expect convergence at lower levels when there is convergence at a higher level.

However, one would expect that convergence at one level means convergence at higher levels (Losos 2011: Fig. 6). For example, herbivorous lizards have convergently evolved skulls with higher mechanical advantage (Stayton 2006), and *Anolis* lizards of specific body shapes inhabit similar microhabitats (Losos 2009). We suggest that the biological details of what we mean by convergent phenotype in each case are important, and that a simplification of this can bias our understanding of this question. Many studies, including this one, begin with a simplified premise of convergence at the focal level of organization. For example, a snake-like body shape is elongate and lacks limbs, and thus, convergence can be identified at a glance. In reality, there is considerable variation in the role of the tail in elongation and this translates into important ecological differences that are poorly understood functionally: long-tailed snake-like species tend to live in dense vegetation while short-tailed ones tend to be fossorial (Wiens and Singluff 2001; Brandley et al. 2008). All six of our focal clades primarily belong to the short-tailed fossorial form, yet there is important ecological variation among these as well. *Hemiergis, Lerista, Scelotes,* and *Chalcides* inhabit dry deserts, burrowing in loose sand (Branch 1998; Wilson and Swan 2005; Carranza et al. 2008). In contrast, *Brachymeles* inhabit wet tropical soils and plant detritus that is often well packed (P.J.B. Pers. Obs.; Siler et al. 2011). Similar reasoning may also be applied to other systems.

In conclusion, we document a case of imperfect convergence, where each of our focal clades evolved slightly different snake-like shapes from slightly different starting points, via largely different evolutionary pathways, indicating an important role of historical contingency in the evolution of snake-like shapes. Our work shows that even for one of the best-documented and widespread patterns of convergent evolution, the details suggest different mechanisms and outcomes. This suggests that convergence does not map simply across levels of biological organization, something that must be examined in further detail across more levels of organization. Although the details of evolution are historically contingent, how predictable the overall outcomes are (Thomas and Reif 1993; Vermeij 2006) begs further consideration.

AUTHOR CONTRIBUTIONS

P.J.B. and G.M. conceived of the study, P.J.B. collected data and conducted analysis, G.M. wrote code for some analyses, P.J.B. wrote the manuscript, and P.J.B. and G.M. edited the manuscript.

ACKNOWLEDGMENTS

The authors would like to thank Cameron Siler for a phylogeny and data for *Brachymeles*. The authors would like to thank the California Academy of Sciences, Kansas University Museum, Museum of Comparative Zoology, Museum of Vertebrate Zoology, Sam Nobel Museum of Natural History, US Museum of Natural History, and the Western Australian Museum for access to specimens. The authors would like to thank José Rosado, Joe Martinez (MCZ), and Luke Welton (KU) for help with X-rays. The authors thank Amy Cheu, Jordan Majka, Graham Slater, and three anonymous reviewers for comments on an earlier version of the manuscript. This work was supported by Clark University and an NSF grant to PJB (IOS-1353703).

DATA ARCHIVING

The doi for our data is https://doi.org/10.5061/dryad.5rh14nc.

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Associate Editor: G. Slater
Handling Editor: P. Tiffin

Supporting Information

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

Table S1. List of species and specimens used.
Table S2. Estimated parameters for best fitting OU model of body shape evolution.
Table S3. Evolutionary correlation coefficients for patterns of integration among body parts for the six focal clades.
Table S4. Model parameters for analysis testing for threshold, linear or null relationships between pairs of traits.