

Enemy at the gates: Rapid defensive trait diversification in an adaptive radiation of lizards

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Adaptive radiation (AR), the product of rapid diversification of an ancestral species into novel adaptive zones, has become pivotal in our understanding of biodiversity. Although it has widely been accepted that predators may drive the process of AR by creating ecological opportunity (e.g., enemy-free space), the role of predators as selective agents in defensive trait diversification remains controversial. Using phylogenetic comparative methods, we provide evidence for an “early burst” in the diversification of antipredator phenotypes in Cordylinae, a relatively small AR of morphologically diverse southern African lizards. The evolution of body armor appears to have been initially rapid, but slowed down over time, consistent with the ecological niche-filling model. We suggest that the observed “early burst” pattern could be attributed to shifts in vulnerability to different types of predators (i.e., aerial versus terrestrial) associated with thermal habitat partitioning. These results provide empirical evidence supporting the hypothesis that predators or the interaction therewith might be key components of ecological opportunity, although the way in which predators influence morphological diversification requires further study.

KEY WORDS: Body armor, comparative methods, diversification rate, early burst, ecological opportunity, model fitting, predation.

Ever since Darwin’s encounter with finches on the Galápagos Islands, naturalists and evolutionary biologists have been intrigued by the process of *adaptive radiation* (AR)—the rapid diversification of an ancestral species into an array of descendants that occupy a variety of ecological resources and differ in the phenotypic traits used to exploit these resources (Schluter 2000; Gavrillets and Losos 2009; Glor 2010; Losos 2010). Ecological opportunity, in which the ancestral species enters a new environment with abundant, underexploited resources (Muschick et al. 2014), habitat heterogeneity (Mahler et al. 2010), and/or novel

microclimates (Algar and Mahler 2016) is widely thought to regulate the tempo of AR (Simpson 1953; Rainey and Travisano 1998; Losos 2010; Losos and Mahler 2010; Yoder et al. 2010; Wellborn and Langerhans 2015). The entry into newly accessed ecological zones is assumed to shift natural selection acting on ecologically important traits, resulting in an “early burst” (EB) of trait diversification followed by a slow down in diversification rates as interspecific competition increases (Losos and Schluter 2000; Yoder et al. 2010; Ingram et al. 2012). Consequently, recent research has focused on characterizing ecologically relevant



phenotypic traits and determining whether these adaptive traits diversify along similar patterns and at similar rates to disentangle the processes underlying ARs (e.g., Ackerly et al. 2006; Sallan and Friedman 2012; Lopéz-Fernández et al. 2013; Muschick et al. 2014; Colombo et al. 2015; Pincheira-Donoso et al. 2015).

Predation has been suggested to play an important role in macroevolution (Nosil and Crespi 2006; Vamosi 2005; Meyer and Kassen 2007). First, the *enemy release* hypothesis posits that the extinction of predators or absence thereof in a novel environment may create ecological opportunity and initiate ARs, for example, on newly emerged islands (Joyce et al. 2005; Meyer and Kassen 2007; Givnish et al. 2009; Riesch et al. 2013). Second, the evolution of a novel defensive trait could release prey from predators, thereby facilitating radiation into a predator-free space (Schluter 2000). Similarly, the evolution of a defensive trait that allows prey to tolerate predators might lead to a burst of diversification. Under this *escape-and-radiate* scenario, repeated bursts of predator and prey diversification are often predicted as a consequence of their interactions (Ehrlich and Raven 1964; Thompson 1994; Janz 2011), resulting in a constant net diversification rate over time, as predator and prey lineages iteratively coevolve in an arms race. The *escape-and-radiate* scenario frequently involves *escalation*, wherein defensive trait efficacy increases from the ancestral to the derived state (Vermeij 1987).

Predation could also play a secondary role during diversification once ecological opportunity is created, yet, its significance has largely been overlooked in previous research. For instance, resource partitioning (e.g., diet and habitat) might have correlated effects on the vulnerability to predation (Lima and Dill 1990; Lima 1998) and consequently, the niche filling expected of defensive traits should be directly related to niche filling of traits associated with resource exploitation. Under this scenario, rapid diversification of both sets of traits is to be predicted, followed by diversity-dependent slowdowns resulting from interspecific competition for limited resources or niches (Moen and Morlon 2014). The hypothesized defensive trait diversification scenario is most likely an outcome of resource-specific predation risk, associated with the presence of multiple distinct types of predators with a trade-off in the ability of prey to avoid these different predators (Abrams 2000).

Surprisingly, studies investigating the diversification rate of phenotypic traits actively involved in defense against predators are scarce (Vamosi 2006) and often limited to plant defenses (Agrawal 2007). Here, we address this issue by examining the rate and patterns of defensive trait evolution in the girdled lizards (Squamata: Cordylidae). Cordylinae, a rather small subfamily of southern African lizards, comprises approximately 56 morphologically and ecologically distinct and diverse taxa (Stanley et al. 2011). Unique to the Cordylinae is the extensive variation in antipredator phenotypes, ranging from an almost complete lack of

body armor to elaborated body armor, including long spines and thick osteoderms (Losos et al. 2002; Broeckhoven et al. 2015). Various authors have proposed that the degree of body armor is related to the type of predation: the lack of body armor and/or long limbs might facilitate escape and reduces the likelihood of getting captured by aerial predators, such as birds of prey, whereas long spines and/or thick osteoderms are more effective against terrestrial predators, including snakes and mongooses (Mouton and Flemming 2001; Losos et al. 2002; Broeckhoven et al. 2015).

We hypothesize that if predators play a key role during ecological opportunity, the diversification rates and evolution of defensive traits are initially rapid, but slow down as the available resources or niches become filled. More specifically, we predict that (1) the net rate of diversification has slowed down over time, consistent with a niche-filling pattern and (2) the diversification of body armor in cordylina lizards will follow an “EB” process, in which the rate of trait evolution declines over time (Blomberg et al. 2003; Harmon et al. 2010). Following Slater and Pennell (2013), we use three approaches for detecting an “EB” of defensive trait evolution: maximum likelihood (Harmon et al. 2010), disparity through time (DTT) analysis (Harmon et al. 2003) and the node height test (Freckleton and Harvey 2006).

Materials and Methods

DATA COLLECTION

We acquired 3D image datasets from micro-CT scans of 28 species (4.29 ± 1.27 specimens per species) representing all of the major clades within the subfamily Cordylinae (except the serpentiform genus *Chamaesaura*) using a GE Phoenix v|tome|x L240 dual tube CT instrument (Phoenix X-ray; General Electric Sensing & Technologies, Wunstorf, Germany) located at Stellenbosch University (du Plessis et al. 2016). Measurements of 20 morphological traits, characterizing spine, limb, and head dimensions, were collected from each lizard using the VGStudio Max 3.0 software (Volume Graphics GmbH, Heidelberg, Germany). Body length, measured as the distance between the posterior end of the skull and the posterior end of the pelvis, was used as a proxy for size (Revell et al. 2007a). Phylogenetic regression analyses were conducted, following Revell (2009) using the “*phyl.resid*” function in the R package PHYTOOLS (Revell 2012). Each log-transformed species’ average trait measurement was regressed against log-transformed body length and the residuals were retained. Next, size-corrected measurements were subjected to a principal component analysis (PCA). Fitting models of evolution to PC scores might increase support for an “EB” scenario (Uyeda et al. 2015), hence we conducted a phylogenetic PCA (pPCA) using the “*phyl.pca*” function in PHYTOOLS (Revell 2009, 2012). The first three principal components obtained using this analysis accounted for 75% of the total variation and were retained for subsequent phylogenetic

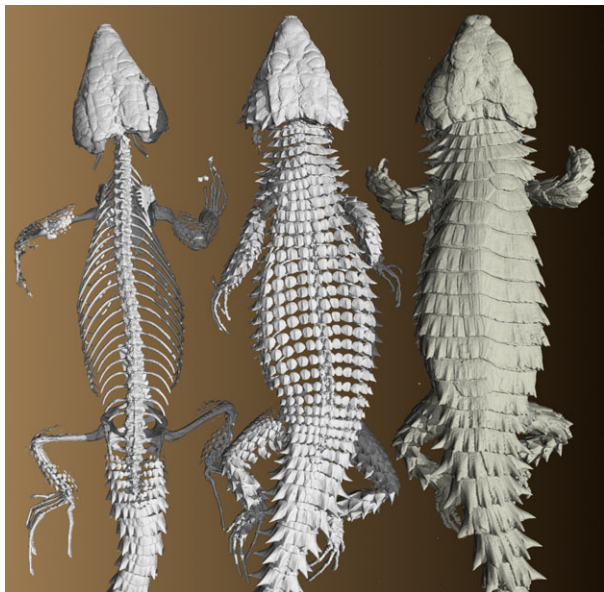


Figure 1. 3D-rendered images of cordylid lizards illustrating the dichotomy in body armor, described by pPC1. Lightly armored fast-moving species have relative long hind limbs and toes, whereas heavily armored slow-moving species possess relatively short hind limbs and toes. From left to right: *Pseudocordylus microlepidotus*, *Smaug breyeri*, and *Ouroborus cataphractus*.

comparative analyses (Supporting Information Table S1). pPC1 correlated positively with all spine measurements and negatively with toe and hind limb length, hence it represented a gradient from heavily armored species with relatively long spines to lightly armored species with relatively long hind limbs and toes (Fig. 1). Relative limb size, pelvic, and pectoral width were strongly correlated with pPC2 and are directly associated with structural microhabitat specialization in lizards (Revell et al. 2007b). The third phylogenetic principal component (pPC3) was correlated with head morphology, especially head width and height, and could serve as proxy for dietary specialization (Broeckhoven and Mouton 2014).

PHYLOGENETIC TREE

Partial gene fragments generated by Stanley et al. (2011) were obtained from three mitochondrial (12S, 16S, and ND2) and three nuclear (PRLR, MYH2, and KIF24) DNA gene regions, representing the same 28 species as for the morphological dataset and accounting for approximately half of all known species in Cordylinae. Five outgroup taxa were selected, namely two platysaurids (*Platysaurus capensis* and *Platysaurus intermedius*) and three gerrhosaurids (*Cordylosaurus subtesselatus*, *Gerrhosaurus nigrolineatus*, and *Matobosaurus validus*). Because of the monophyletic nature of the selected taxa (Stanley et al. 2011), a single specimen was chosen to represent each species. All sequences were downloaded from Genbank, aligned, and edited using MEGA version

6 (Tamura et al. 2013). For each of the protein-coding gene fragments (ND2, PRLR, MYH2, and KIF24), a substitution model was calculated for each codon position, whereas a single substitution model was calculated for the nonprotein coding 12S and 16S, implementing the Akaike information criterion with correction (AICc) for sample size using JModeltest (Posada 2008). To obtain an ultrametric tree with relative divergence times between the in-group taxa, the BEAST package version 2.1.3 (Bouckaert et al. 2014) was employed to analyze the concatenated dataset. The models obtained from JModeltest for all six loci, along with their parameters, were used to specify the site models in BEAUti. As we were interested in relative, rather than absolute node ages, a relaxed lognormal clock model was selected, estimating around the clock rate of 1.0. The birth–death (BD) model was selected as tree prior. The Markov Chain Monte Carlo was run for 20 million generations, sampling every 2000 generations. Tracer version 1.6 (Rambaut et al. 2013) was employed to assess the chain convergence before discarding the first 10% as burn-in using TreeAnnotator version 2.1.2 (available within the BEAST software package), while the remaining 9001 trees were summarized as a maximum clade credibility tree. Finally, FigTree version 1.4 (Rambaut 2012) was used to visualize the resulting tree.

PHYLOGENETIC COMPARATIVE ANALYSES

Tempo of lineage diversification

Declining net diversification may reflect diversity-dependent processes, consistent with an EB niche-filling pattern (Burbrink and Pyron 2010; Derryberry et al. 2011; Tran 2014). The summary statistic γ was assessed for the cordylid phylogeny to determine the tempo of lineage diversification. A Monte Carlo constant rates (MCCR) test, taking the number of missing taxa into account, was conducted using the “mccrTest” function (10,000 repetitions) in the LASER package version 2.4-1 (Rabosky 2006a). Furthermore, the fit of a constant rate pure birth and BD was compared with two diversity-dependent diversification models (i.e., exponential or diversity-dependent exponential and linear or diversity-dependent linear) using AIC to test whether the diversification rate has slowed down over time (Rabosky 2006b; Rabosky and Lovette 2008).

Rates and patterns of trait evolution

To investigate whether the evolution of body armor in cordylid lizards follows an “EB” model (Blomberg et al. 2003; Harmon et al. 2010), in which the rate of trait evolution decelerates through time, we fitted several evolutionary models to the pPCA scores. The White Noise (WN) model, whereby a trait evolves independently of the phylogeny, served as our reference model. By doing so, we first tested the assumption that EB, which assumes phylogenetic signal, will have a better fit than the WN model, which eliminates phylogenetic signal. Next, we compared the fit of the

EB model to that of two alternative models using the R packages GEIGER version 2.0.6 (Pennell et al. 2014) and PMC version 1.0.2 (Boettiger et al. 2012): (1) the Brownian motion (BM) model in which the trait change follows a constant rate (Felsenstein 1985) and (2) a single-optimum Ornstein–Uhlenbeck (OU) model (Butler and King 2004), in which a trait evolves toward an optimal value α . It must be noted that the OU model is known to suffer from elevated Type I errors, especially for small phylogenetic trees (Cooper et al. 2016) and the result for this model fitting was interpreted cautiously.

To determine the preferred model of evolution, we deployed two procedures. First, the mean AICc, difference in mean AICc among models (Δ), and AICc weights (ω) were calculated using the “fitContinuous” function in the R package GEIGER version 2.0.6 (Pennell et al. 2014) to simultaneously compare the likelihoods of several models of evolution. Second, considering that the low number of taxa ($n = 28$) may reduce the power to differentiate the models using the AICc, we used phylogenetic Monte Carlo power analyses, which allow for a direct estimate of model fit and power to choose between the models (Boettiger et al. 2012). Pairwise comparisons of evolutionary models were performed using the likelihood ratio test approach implemented in PMC version 1.0.2 (2000 bootstrap replicates). In each comparison, the more complex of the two models was considered the “test” model, whereas the other was considered the “null” model. Next, the probability of rejecting the “null” model was determined by the proportion of the simulated likelihood ratios of the “null” model (δ_{null}) greater than the observed likelihood ratio (δ). The power of the test is determined by the proportion of simulated likelihood ratios of the “test” model (δ_{test}) greater than the 95th percentile of the δ null distribution (see Boettiger et al. 2012 for further details).

To assess the adequacy of the best-fit model, we used posterior predictive simulation, implemented in ARBUTUS version 1.1 (Pennell et al. 2015). The maximum likelihood parameter estimates from the preferred model for each PC score were used to construct a unit tree and 10,000 datasets were simulated based on this unit tree. Next, six test statistics were calculated for both the original and simulated datasets, and we tested whether the observed test statistic fell within the distribution of the respective simulated test statistic. For a detailed explanation of the test statistics, see Pennell et al. (2015). Briefly, M_{SIG} is a measure of overall evolutionary rate and provides an indication, if violated, of over- or underestimation of the rate of trait evolution. C_{VAR} detects deviations from rate heterogeneity; S_{VAR} tests whether contrasts are smaller or larger than expected based on their branch lengths; S_{ASR} evaluates whether variation in rates is relative to the trait value; S_{HGT} is used to capture variation with respect to time; D_{CDF} is used to capture deviations from normality.

The time dependence of trait evolution was assessed using the rate change parameter a of the EB model, which measures

acceleration (negative a) or deceleration (positive a) of trait evolution through time. In addition, we estimated the δ parameter of Pagel’s (1999) δ model. If $\delta < 1$, trait disparity is more concentrated toward the roots, whereas if $\delta > 1$, trait disparity is concentrated toward the tips of the tree. Although the Pagel’s δ parameter scales branch lengths, rather than models an evolutionary process, it can imitate the behavior of the EB model (i.e., $\delta < 1$).

Trait disparity

To test the hypothesis that trait values in cordyline lizards slowed down through time, first, a node-height test was performed using the “nh.test” function in GEIGER version 2.0.6 (Pennell et al. 2014). The node-height test calculates the absolute value of standardized independent contrasts and compares those to the contrasts expected under a BM model of evolution (i.e., no covariation between the contrast and age of the respective node). The presence of a statistically significant positive relationship between the absolute contrast value and node age suggest a deceleration of body armor through time, consistent with the ecological niche-filling model (Freckleton and Harvey 2006). Second, we calculated how trait disparity changed through time by using the “dttFullICIs” function implemented in GEIGER version 2.0.6 (Pennell et al. 2014). By simulating body armor evolution 10,000 times across the Cordylinae tree, we calculated the morphological disparity index (MDI) to quantify the difference in observed disparity in relation to the disparity expected under BM (Harmon et al. 2003). High disparity followed by a decline toward the tips, as indicated by a negative MDI value, suggests an EB pattern of diversification (Harmon et al. 2003).

In addition, a posterior predictive simulation approach was employed to assess the adequacy of the EB and BM models (Slater and Pennell 2013). For both DTT and node-height test, summary statistics were produced and compared to the predictive distributions. Posterior predictive simulation was performed using the “pp.mcmc” function implemented in GEIGER version 2.0.6 (Pennell et al. 2014). Each chain was ran for 1,000,000 generations, sampling every 1000 generations and the first 200 samples were discarded as burn-in. The fit of the BM or EB model to the simulated datasets was assessed using posterior predictive P -values generated for each statistic. If trait diversification follows an EB-like process, P -values of ≈ 0.5 are expected for simulations under EB, while P -values < 0.05 are expected for simulations under BM.

Results and Discussion

LINEAGE DIVERSIFICATION AND AR

The cordyline tree used for our phylogenetic comparative analyses (Fig. 2) closely resembles a recent study of Cordylidae phylogeny

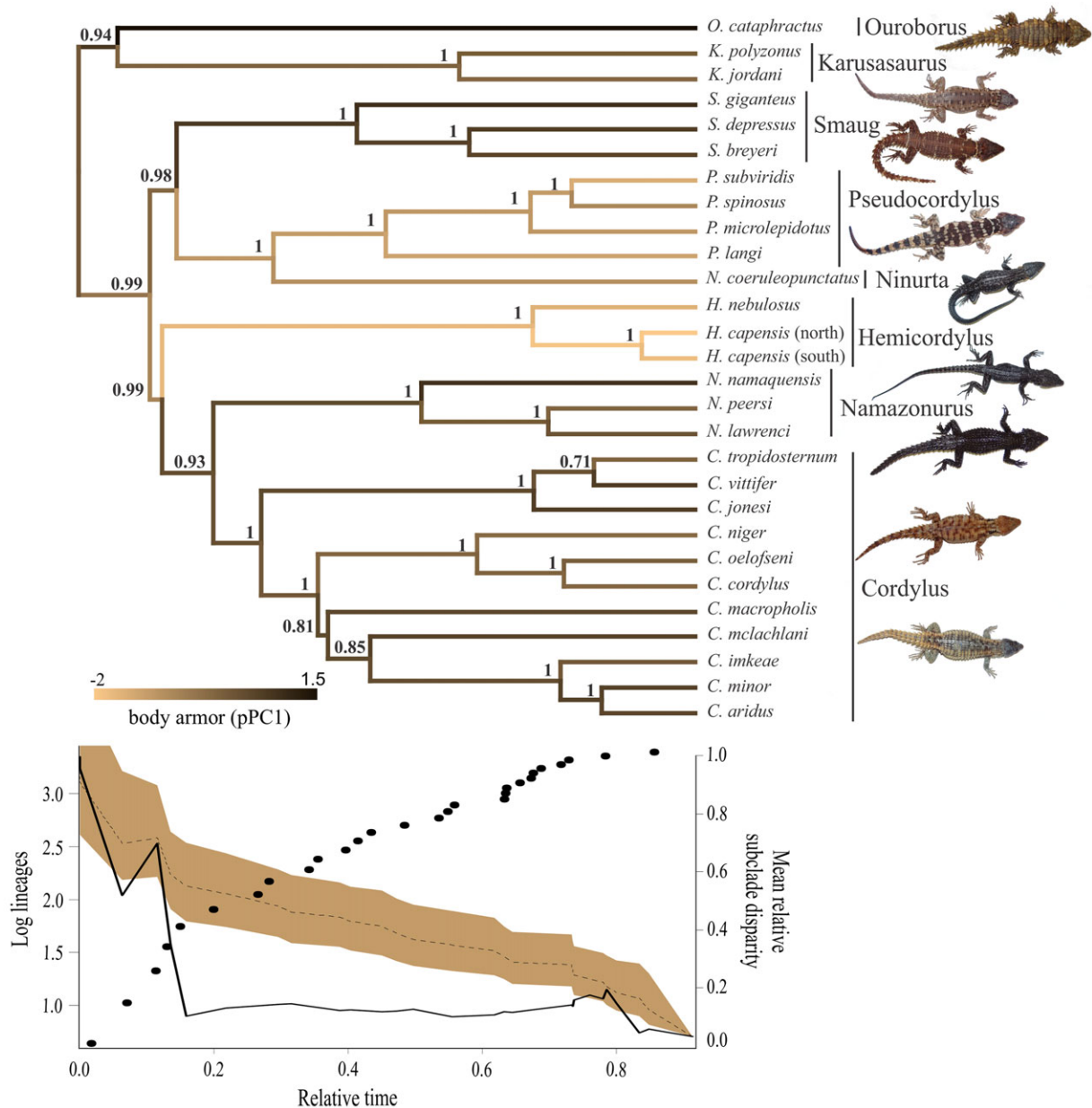


Figure 2. The maximum clade credibility tree attained for the BEAST analysis indicating relative divergence times. Posterior probabilities are indicated at each node. Branches are colored according to the values obtained from the first phylogenetic principal component axis (pPC1). Negative values (light) are representative of lightly armored species, whereas positive values (dark) are representative of heavily armored species. Images on the right illustrate representatives of the major clades of cordylid lizards. From top to bottom: *Ouroborus cataphractus*, *Karusasaurus polyzonus*, *Smaug breyeri*, *Pseudocordylus microlepidotus*, *Ninurta coeruleopunctatus*, *Hemicordylus capensis* (south), *Namazonurus peersi*, *Cordylus tropidosternum*, and *Cordylus cordylus*. The disparity through time (DTT) curve for body armor in cordylid lizards shows a decline in subclade disparity toward the tips, indicative of a slowdown. The lower solid line represents the actual data, while the median DTT based on 10,000 simulations across the Cordylinae tree and following a BM model of evolution is indicated by the upper dashed line. The 95% confidence interval for the simulated data is indicated by the shaded area. In addition, a lineage-through-time plot of the cordylid phylogeny is presented and shows a declining diversification rate following a rapid increase.

(Stanley et al. 2011), with the exception of the basal phylogenetic positioning of the *Karusasaurus*–*Ouroborus* clade. However, the current placement is in accordance with the revised squamate classification by Pyron et al. (2013) and Zheng and Wiens (2016).

Compared to the sister clade Platysaurinae, the diversification of cordylid lizards (Cordylinae) has generally been described as rapid, conforming to the prediction of an AR (Mouton and Van Wyk 1997; Stanley et al. 2011). Indeed, the γ -statistic calculated

Table 1. Results from fitting four models of diversification models to the cordyline tree.

Model	ln <i>L</i>	AIC	Δ	ω
DDL	123.3	-242.6	0	0.66
DDX	122.6	-241.2	1.4	0.33
PB	118.2	-234.4	8.2	0.01
BD	118.2	-232.4	10	0

Models are ranked from best to worst according to the log-likelihood (ln *L*), mean AIC value, difference in mean AIC value among models (Δ) and Akaike weights (ω).

DDL, diversity-dependent linear; DDX, diversity-dependent exponential; PB, pure birth; BD, birth–death model.

by the MCCR test indicates a significant decline in diversification rate ($\gamma = -2.78$, $P = 0.03$), despite our incomplete taxon sampling (critical γ -value = -2.55), as illustrated by a lineage through time plot (Fig. 2). Comparison of diversification rate models revealed more support for diversity-dependent diversification models than for those that assume a constant rate (Table 1). The model fitting results provide evidence of a declining diversification rate following rapid increase (Fig. 2), and corroborate the theory of AR that predicts that declining diversification rates are a consequence of ecological niche filling (Schluter 2000; Rabosky and Lovette 2008).

DEFENSIVE TRAIT DIVERSIFICATION IN CORDYLID LIZARDS

Extensive variation in body armor between species is present in cordyline lizards, ranging from relatively elaborate spines, short hind limbs, and toes in *Ouroborus cataphractus* and the genus *Smaug* to relatively long hind limbs, toes, and an almost complete lack of armor in the genera *Hemicordylus* and *Pseudocordylus* (Figs. 1 and 2). The first axis of the phylogenetically informed PCA (pPC1) represented the dichotomy between armor and speed (Mouton and Flemming 2001; Losos et al. 2002). Hence, degree of body armor was subjected to various phylogenetic comparative methods to test the prediction that the evolution of defense mechanisms in cordyline lizards follows an EB process. Following Slater and Pennell (2013), maximum likelihood analysis (Harmon et al. 2010), DTT analysis (Harmon et al. 2003), and the node-height test (Freckleton and Harvey 2006) were deployed to detect an EB in defensive trait evolution.

The results of the model fitting, based on the AICc weights (ω), show support for the EB model and suggest that rapid diversification in defensive traits occurred early in the evolutionary history of the cordyline lizards, followed by relative stasis (Table 2). The slowdown in defensive trait values over time is supported by a highly negative rate change parameter of the EB model ($a = -25.9$) and the δ parameter of Pagel's δ transformation

($\delta = 0.35$). These findings are corroborated by the parametric bootstrapping method, which indicated that the observed likelihood ratio clearly falls within the distribution of the simulated likelihood ratios of the EB model ($P = 0.02$; Fig. S1). The observed power (i.e., 0.48) of the pairwise comparison of the EB and BM model is, however, relatively low. The adequacy of the EB model was further explored using posterior predictive simulation (Pennell et al. 2014). Although a good absolute fit of the EB model was indicated, a significant departure from the M_{SIG} statistics was detected (Supporting Information Fig. S2): the EB fitted model seems to underestimate the actual rate of evolution (two-tailed $P < 0.0001$), suggesting that the actual process of trait evolution is more complex than one explained by EB.

Early diversification, coupled with rapid defensive trait diversification, is further supported by the DTT analyses. The DTT analysis of defensive trait morphology recovered a MDI value of -0.20. It is very unlikely that this is the result of morphological evolution following a Brownian model because the probability (P) of obtaining a negative MDI when the underlying evolutionary process is BM was <0.0001 (Fig. 2). Posterior predictive distribution for MDI confirms that EB is much better fit than the BM model, where the expected value of 0 falls at the 99th percentile (Supporting Information Fig. S3). In addition, the node-height test showed a positive, but nonsignificant relationship between relative node age and the absolute values of the PC1 score contrasts ($\beta = 0.31$, $r = 0.31$, $P = 0.11$; Supporting Information Fig. S4). However, this trend needs to be interpreted cautiously as the absence of data for several cordyline species at the tips could have biased the results of the node-height test (Freckleton and Harvey 2006). Closer inspection of the scatter plot of the absolute contrast values against time revealed large contrast values for two young splits: *Cordylus vittifer* and *Cordylus tropidosternum*, on the one hand, and *Pseudocordylus spinosus* and *Pseudocordylus melanotus*, on the other hand (Figs. 2 and Supporting Information Fig. S4). By excluding these potential outliers from the node-height test, a statistically significant positive relationship was revealed ($\beta = 0.47$, $r = 0.47$, $P = 0.02$). The posterior predictive P -values for the simulated slopes under an EB model using least squares and robust regression were $P = 0.03$ and $P = 0.16$, respectively, for the exponential model (Supporting Information Fig. S3) and $P = 0.78$ and $P = 0.81$, respectively, for the linear model (Supporting Information Fig. S3). In contrast, P -values for BM simulations were statistically significantly (OLS: $P = 0.001$, RLM: $P = 0.01$; Supporting Information Fig. S3), suggesting that EB provides a better fit than BM (Slater and Pennell 2013).

PREDATORS AS SELECTIVE AGENTS IN TRAIT DIVERSIFICATION

It is well known that the extinction of predators or the absence thereof in new environments might provide opportunities for

Table 2. Summary of the results of the model fitting analysis of body armor (pPC1), microhabitat specialization (pPC2), and dietary specialization (pPC3) in cordyline lizards.

Model	σ^2	θ	a	α	$\ln L$	AICc	Δ	ω
pPC1								
EB	29.9	0.16	-25.9	-	-24.2	55.4	0	0.56
BM	7.46	0.09	-	-	-25.9	56.3	0.98	0.34
OU	7.46	0.09	-	0	-25.9	58.8	3.50	0.10
WN	0.87	-0.08	-	-	-37.8	80.0	24.7	0
pPC2								
BM	6.74	0.05	-	-	-24.50	53.47	0	0.57
OU	10.0	0	-	9.00	-24.11	55.21	1.73	0.24
EB	6.74	0.05	0	-	-24.50	55.99	2.52	0.16
WN	0.22	0.04	-	-	-18.83	42.14	5.94	0.03
pPC3								
OU	10.4	0.02	-	22.8	-17.22	41.44	0	0.40
WN	0.22	0.04	-	-	-18.53	41.54	0.10	0.38
BM	4.66	-0.02	-	-	-19.33	43.15	1.71	0.17
EB	4.66	-0.02	0	-	-19.34	45.67	4.23	0.05

Models are ranked from best to worst according to the mean AICc value, difference in mean AICc value among models (Δ), and Akaike weights (ω). In addition, the log-likelihood ($\ln L$), parameter values of each model (σ^2 and θ), and model parameters of EB (a) and OU (α) are shown.

diversification of lineages (Meyer and Kassen 2007; Losos and Ricklefs 2009). The role that predators may play as selective agents in diversification, however, remains ambiguous and has largely been overlooked in previous studies. The most widely accepted hypothesis is that coevolutionary diversification underlies morphological disparity (Ricklefs 2010; Althoff et al. 2014; Núñez-Farfán and Kariñho-Betancourt 2015). Although the diversification of cordyline lizards appears to coincide with the diversification of various predator clades present in southern Africa (e.g., mongooses: Patou et al. 2009; birds of prey: do Amaral et al. 2009), the results from the MCCR test indicate a diversity-dependent rate of diversification, thereby suggesting that the ecological niche-filling theory is a more likely explanation for the observed pattern than predator-prey coevolution. Moreover, trade-offs may constrain evolutionary responses and limit the escalation of defensive traits (Cacho et al. 2015). On the one hand, an increase in spine length reduces running speed (Losos et al. 2002) and might have major consequences for other behaviors (e.g., feeding). On the other hand, an increase in traits associated with running speed may negatively affect climbing, which might be important for rock-dwelling taxa (Vanhooydonck and Van Damme 2001; Revell et al. 2007b).

The results from the phylogenetic comparative analyses suggest early partitioning of niches or resources where body armor is important, followed by a decrease in diversification rate and morphological diversification as these niches/resources became filled, consistent with the ecological opportunity theory (Harmon et al. 2003, 2010). The most plausible explanation is that traits that affect resource exploitation (e.g., diet and habitat) might

have correlated effects on vulnerability to predation (Lima and Dill 1990; Lima 1998). Our predictions was that morphological traits associated with resource partitioning would follow an EB process of evolution, similar to that observed for defensive traits. In contrast to our expectations, the phylogenetic comparative analyses conducted on pPC2 (i.e., microhabitat specialization; Table 2 and Supporting Information Table S1) provide significant support for the BM model, whereas WN and OU models were a better fit for pPC3 (dietary specialization; Table 2 and Supporting Information Table S1). Thus, defensive trait diversification does not appear to be related to differences in predation risk associated with structural microhabitat or dietary resource partitioning.

The diversification of Cordylinae, however, appears to coincide with global cooling during the late Eocene-Oligocene (Zachos et al. 2001) and a shift from oviparity to viviparity in the cordyline ancestor (Mouton and Van Wyk 1997; Stanley et al. 2011). These events would have permitted the dispersal of an ancestral species into novel environments and allowed for thermal habitat partitioning along microclimate axes. Thermal habitat partitioning has been observed among ectothermic vertebrates (Magnuson et al. 1979) and could drive defensive trait diversification if it directly affects the vulnerability to different types of predators in cordyline lizards. Along a thermal gradient, the utilization of colder microclimates might have forced individuals to increase the time spent basking that, given the conservative nature of optimal body temperature in lizards in general (Grigg and Buckley 2013), would have resulted in an increased vulnerability to visually orientated predators (e.g., birds of prey; Carter et al. 2010). Consequently, an increase in speed (and reduction in

armor) would have been favored to reduce the likelihood of getting captured by aerial predators (Mouton and Flemming 2001; Losos et al. 2002). In contrast, the utilization of warmer microclimates might have reduced the time spent basking, which in the case of rock-dwelling sit-and-wait foragers such as cordyline lizards would have resulted in increased vulnerability to terrestrial predation. Here, an increase in body armor would have been favored to avoid extraction from the crevice by terrestrial predators, such as mongooses and snakes (Cooper et al. 2000; Broeckhoven and Mouton 2015). Further support is provided by the fact that species that differ in antipredator phenotype occur sympatrically, suggesting that ecological interactions, besides competition for food or microhabitat, might have influenced diversification. Broeckhoven and Mouton (2015) provide such an example of temporal thermal resource partitioning between *O. cataphractus* and *Karusasaurus polyzonus*.

In summary, the results from our phylogenetic comparative analyses provide evidence for an “EB” in defensive trait diversification in cordyline lizards, despite the rare occurrence of EB patterns in comparative data (Harmon et al. 2010). Moreover, our findings support the hypothesis that predation can play an important role during diversification. We suggest that diversification along a microclimate niche axis resulted in shifts in vulnerability to different types of predators that require opposing morphological demands (e.g., armor vs. speed), leading to rapid divergent evolution of defensive traits.

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DATA ARCHIVING

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LITERATURE CITED

- Abrams, P. A. 2000. Character shifts of prey species that share predators. *Am. Nat.* 156:S45–S61.
- Ackerly, D. D., D. W. Schwilk, and C. O. Webb. 2006. Niche evolution and adaptive radiation: testing the order of trait divergence. *Ecology* 87:50–61.
- Agrawal, A. A. 2007. Macroevolution of plant defense strategies. *Trends Ecol. Evol.* 22:103–109.
- Algar, A. C., and D. L. Mahler. 2016. Area, climate heterogeneity, and the response of climate niches to ecological opportunity in island radiations of *Anolis* lizards. *Glob. Ecol. Biogeogr.* 25:781–791.
- Althoff, D. M., K. A. Segraves, and M. T. Johnson. 2014. Testing for coevolutionary diversification: linking pattern with process. *Trends Ecol. Evol.* 29:82–89.
- Blomberg, S. P., T. Garland, and A. R. Ives. 2003. Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution* 57:717–745.
- Boettiger, C., G. Coop, and P. Ralph. 2012. Is your phylogeny informative? Measuring the power of comparative methods. *Evolution* 66:2240–2251.
- Bouckaert, R., J. Heled, D. Kühnert, T. Vaughan, C. Wu, D. Xie, M. A. Suchard, A. Rambaut, and A. J. Drummond. 2014. BEAST 2: a software platform for Bayesian evolutionary analysis. *PLoS Comput. Biol.* 10:e1003537.
- Broeckhoven, C., and P. le. F. N. Mouton. 2014. Under pressure: morphological and ecological correlates of bite force in the rock-dwelling lizards *Ouroborus cataphractus* and *Karusasaurus polyzonus* (Squamata: Cordylidae). *Biol. J. Linn. Soc. Lond.* 111:823–833.
- . 2015. Some like it hot: camera traps unravel the effects of weather conditions and predator presence on the activity levels of two lizards. *PLoS One* 10:e0137428.
- Broeckhoven, C., G. Diedericks, and P. le. F. N. Mouton. 2015. What doesn't kill you might make you stronger: functional basis for variation in body armour. *J. Anim. Ecol.* 84:1213–1221.
- Burbrink, F. T., and R. A. Pyron. 2010. How does ecological opportunity influence rates of speciation, extinction, and morphological diversification in New World ratsnakes (tribe Lampropeltini)? *Evolution* 64:934–943.
- Butler, M. A., and A. A. King. 2004. Phylogenetic comparative analysis: a modelling approach for adaptive evolution. *Am. Nat.* 164:683–695.
- Cacho, N. I., D. J. Kliebenstein, and S. Y. Strauss. 2015. Macroevolutionary patterns of glucosinolate defense and tests of defense-escalation and resource availability hypotheses. *New Phytol.* 208:915–927.
- Carter, A. J., A. W. Goldizen, and S. A. Tromp. 2010. Agamas exhibit behavioral syndromes: bolder males bask and feed more but may suffer higher predation. *Behav. Ecol.* 21:655–661.
- Colombo, M., M. Damerau, R. Hanel, W. Salzburger, and M. Matschner. 2015. Diversity and disparity through time in the adaptive radiation of Antarctic nototheniid fishes. *J. Evol. Biol.* 28:376–394.
- Cooper, W. E. Jr., J. H. Van Wyk, P. le. F. N. Mouton, A. M. Al-Johany, J. A. Lemos-Espinal, M. A. Paulissen, and M. Flowers. 2000. Lizard antipredatory behaviors preventing extraction from crevices. *Herpetologica* 56:394–401.
- Cooper, N., G. H. Thomas, C. Venditti, A. Meade, and R. P. Freckleton. 2016. A cautionary note on the use of Ornstein Uhlenbeck models in macroevolutionary studies. *Biol. J. Linn. Soc. Lond.* 118:64–77.
- Derryberry, E. P., S. Claramunt, G. Derryberry, R. T. Chesser, J. Cracraft, A. Aleixo, J. Pérez-Emán, J. V. Remsen Jr, and R. T. Brumfield. 2011. Lineage diversification and morphological evolution in a large-scale continental radiation: the neotropical ovenbirds and woodcreepers (Aves: Furnariidae). *Evolution* 65:2973–2986.
- Do Amaral, F. R., F. H. Sheldon, A. Gamauf, E. Haring, M. Riesing, L. F. Silveira, and A. Wajntal. 2009. Patterns and processes of diversification in a widespread and ecologically diverse avian group, the buteonine hawks (Aves, Accipitridae). *Mol. Phylogenet. Evol.* 53:703–715.
- Du Plessis, A., S. G. le Roux, and A. Guelpa. 2016. The CT Scanner Facility at Stellenbosch University: an open access X-ray computed tomography laboratory. *Nucl. Instrum. Methods Phys. Res. B* 384:42–490.
- Ehrlich, P. R., and P. H. Raven. 1964. Butterflies and plants: a study in coevolution. *Evolution* 18:586–608.
- Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39:783–791.
- Freckleton, R. P., and P. H. Harvey. 2006. Detecting non-Brownian trait evolution in adaptive radiations. *PLoS Biol.* 4:e373.
- Gavrilets, S., and J. B. Losos. 2009. Adaptive radiation: contrasting theory with data. *Science* 323:732–737.

- Givnish, T. J., K. C. Millam, A. R. Mast, T. B. Paterson, T. J. Theim, A. L. Hipp, J. M. Henss, J. F. Smith, K. R. Wood, and K. J. Sytsma. 2009. Origin, adaptive radiation and diversification of the Hawaiian lobeliads (Asterales: Campanulaceae). *Proc. R. Soc. B.* 276:407–416.
- Glor, R. E. 2010. Phylogenetic insights on adaptive radiation. *Annu. Rev. Ecol. Evol. S.* 41:251–270.
- Grigg, J. W., and L. B. Buckley. 2013. Conservatism of lizard thermal tolerances and body temperatures across evolutionary history and geography. *Biol. Lett.* 9:20121056.
- Harmon, L. J., J. A. Schulte, A. Larson, and J. B. Losos. 2003. Tempo and mode of evolutionary radiation in iguanian lizards. *Science* 301:961–964.
- Harmon, L. J., J. B. Losos, T. J. Davies, R. G. Gillespie, J. L. Gittleman, W. B. Jennings, K. H. Kozak, M. A. McPeck, F. Moreno-Roark, T. J. Near, et al. 2010. Early bursts of body size and shape evolution are rare in comparative data. *Evolution* 64:2385–2396.
- Ingram, T., L. J. Harmon, and J. B. Shurin. 2012. When should we expect early burst of trait evolution in comparative data? Predictions from an evolutionary food web model. *J. Evo. Biol.* 25:1902–1910.
- Janz, N. 2011. Ehrlich and Raven revisited: mechanisms underlying codiversification of plants and enemies. *Annu. Rev. Ecol. Evol. Syst.* 42:71–89.
- Joyce, D. A., D. H. Lunt, R. Bills, G. F. Turner, C. Katongo, N. Duftner, C. Sturmbauer, and O. Seehausen. 2005. An extant cichlid fish radiation emerged in an extinct Pleistocene lake. *Nature* 435:90–95.
- Lima, S. L. 1998. Nonlethal effects in the ecology of predator-prey interactions. *Bioscience* 48:25–34.
- Lima, S. L., and L. M. Dill. 1990. Behavioral decisions made under the risk of predation: a review and prospectus. *Can. J. Zool.* 68:619–640.
- López-Fernández, H., J. H. Arbour, K. Winemiller, and R. L. Honeycutt. 2013. Testing for ancient adaptive radiations in neotropical cichlid fishes. *Evolution* 67:1321–1337.
- Losos, J. B. 2010. Adaptive radiation, ecological opportunity, and evolutionary determinism. *Am. Nat.* 175:623–639.
- Losos, J. B., and D. L. Mahler. 2010. Adaptive radiation: the interaction of ecological opportunity, adaptation, and speciation. Pp. 381–420 in M. A. Bell, D. J. Futuyma, W. F. Eanes, and J. S. Levinton, eds. *Evolution since Darwin: the first 150 years*, Sinauer Assoc., Sunderland, MA.
- Losos, J. B., and R. E. Ricklefs. 2009. Adaptation and diversification on islands. *Nature* 457:830–836.
- Losos, J. B., and D. Schluter. 2000. Analysis of an evolutionary species-area relationship. *Nature* 408:847–850.
- Losos, J. B., P. le. F. N. Mouton, R. Bickel, I. Cornelius, and L. Ruddleck. 2002. The effect of body armature on escape behaviour in cordylid lizards. *Anim. Behav.* 64:313–321.
- Magnuson, J. J., L. B. Crowder and P. A. Medvick. 1979. Temperature as an ecological resource. *Amer. Zool.* 19:331–343.
- Mahler, D. L., L. J. Revell, R. E. Glor, and J. B. Losos. 2010. Ecological opportunity and the rate of morphological evolution in the diversification of Greater Antillean anoles. *Evolution* 64:2731–2745.
- Meyer, J. R., and R. Kassen. 2007. The effects of competition and predation on diversification in a model adaptive radiation. *Nature* 446:432–435.
- Moen, D., and H. Morlon. 2014. Why does diversification slow down? *Trends Ecol. Evol.* 29:190–197.
- Mouton, P. le. F. N., and A. F. Flemming. 2001. Osteoderm function in the lizard family Cordylidae. *Proceedings of the Sixth International Congress of Vertebrate Morphology, Jena, Germany, July 21–26, 2001. J. Morphol.* 248:264.
- Mouton, P. le. F. N., and J. H. Van Wyk. 1997. Adaptive radiation in cordylid lizards: an overview. *Afr. J. Herpetol.* 46:78–88.
- Muschick, M., P. Nosil, M. Roesti, M. T. Dittmann, L. Harmon, and W. Salzburger. 2014. Testing the stages model in the adaptive radiation of cichlid fishes in East African Lake Tanganyika. *Proc. R. Soc. B.* 281:20140605.
- Nosil, P., and B. J. Crespi. 2006. Experimental evidence that predation promotes divergence in adaptive radiation. *PNAS* 103:9090–9095.
- Núñez-Farfán, J., and E. Kariñho-Betancourt. 2015. Is escalation of plant defence a common macroevolutionary outcome of plant-herbivore interactions? *New Phytol.* 208:635–637.
- Pagel, M. 1999. Inferring the historical patterns of biological evolution. *Nature* 401:877–884.
- Patou, M. L., P. A. Mclenachan, C. G. Morley, A. Couloux, A. P. Jennings, and G. Veron. 2009. Molecular phylogeny of the Herpestidae (Mammalia, Carnivora) with a special emphasis on the Asian Herpestes. *Mol. Phylogenet. Evol.* 53:69–80.
- Pennell, M. W., J. M. Eastman, G. J. Slater, J. W. Brown, J. C. Uyeda, R. G. FitzJohn, M. E. Alfaro, and L. J. Harmon. 2014. geiger v2.0: an expanded suite of methods for fitting macroevolutionary models to phylogenetic trees. *Bioinformatics* 15:2216–2218.
- Pennell, M. W., R. G. FitzJohn, W. K. Cornwell, and L. J. Harmon. 2015. Model adequacy and the macroevolution of angiosperm functional traits. *Am. Nat.* 186:E33–E50.
- Pincheira-Donoso, D., L. P. Harvey, and M. Ruta. 2015. What defines an adaptive radiation? Macroevolutionary diversification dynamics of an exceptionally species-rich continental lizard radiation. *BMC Evol. Biol.* 15:153.
- Posada, D. 2008. jModelTest: phylogenetic model averaging. *Mol. Biol. Evol.* 25:1253–1256.
- Pyron, R. A., F. T. Burbrink, and J. J. Wiens. 2013. A phylogeny and revised classification of Squamata, including 4161 species of lizards and snakes. *BMC Evol. Biol.* 13:93.
- Rabosky, D. L. 2006a. LASER: a maximum likelihood toolkit for detecting temporal shifts in diversification rates from molecular phylogenies. *Evol. Bioinform. Online* 2006:247–250.
- . 2006b. Likelihood methods for detecting temporal shifts in diversification rates. *Evolution* 60:1152–1164.
- Rabosky, D. L., and I. J. Lovette. 2008. Explosive evolutionary radiations: decreasing speciation or increasing extinction through time? *Evolution* 62:1866–1875.
- Rainey, P. B., and M. Travisano. 1998. Adaptive radiation in a heterogeneous environment. *Nature* 394:69–72.
- Rambaut, A. 2012. FigTree v1. 4. Available via tree.bio.ed.ac.uk/software/figtree/.
- Rambaut, A., M. A. Suchard, W. Xie, and A. J. Drummond. 2013. Tracer v1.6. Available via <http://tree.bio.ed.ac.uk/software/tracer/>.
- Revell, L. J. 2009. Size-correction and principal components for interspecific comparative studies. *Evolution* 63:3258–3268.
- . 2012. phytools: an R package for phylogenetic comparative biology (and other things). *Methods Ecol. Evol.* 3:217–223.
- Revell, L. J., L. J. Harmon, R. B. Langerhans, and J. J. Kolbe. 2007a. A phylogenetic approach to determining the importance of constraint on phenotypic evolution in the neotropical lizard *Anolis cristatellus*. *Evol. Ecol. Res.* 9:261–282.
- Revell, L. J., M. A. Johnson, J. A. Schulte, J. J. Kolbe, and J. B. Losos. 2007b. A phylogenetic test for adaptive convergence in rock-dwelling lizards. *Evolution* 61:2898–2912.
- Ricklefs, R. E. 2010. Evolutionary diversification, coevolution between populations and their antagonists, and the filling of niche space. *PNAS* 107:1265–1272.

- Riesch, R., R. A. Martin, and R. B. Langerhans. 2013. Predation's role in life-history evolution of a livebearing fish and a test of the Trexler-DeAngelis model of maternal provisioning. *Am. Nat.* 181:78–93.
- Sallan, L. C., and M. Friedman. 2012. Heads or tails: staged diversification in vertebrate evolutionary radiations. *Proc. R. Soc. B.* 279:2025–2032.
- Schluter, D. 2000. *The ecology of adaptive radiation*. Oxford Univ. Press, Oxford, UK.
- Simpson, G. G. 1953. The Baldwin effect. *Evolution* 7:110–117.
- Slater, G. J., and M. W. Pennell. 2013. Robust regression and posterior predictive simulation increase power to detect early bursts of trait evolution. *Syst. Biol.* 63:293–308.
- Stanley, E. L., A. M. Bauer, T. R. Jackman, W. R. Branch, and P. le. F. N. Mouton. 2011. Between a rock and a hard polytomy: rapid radiation in the rupicolous girdled lizard (Squamata: Cordylidae). *Mol. Phylogenet. Evol.* 58:53–70.
- Tamura, K., G. Stecher, D. Peterson, A. Filipinski, and S. Kumar. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol.* 30:2725–2729.
- Thompson, J. N. 1994. *The coevolutionary process*. Univ. Chicago Press, Chicago.
- Tran, L. A. 2014. The role of ecological opportunity in shaping disparate diversification trajectories in a bicontinental primate radiation. *Proc. R. Soc. B.* 281:20131979.
- Uyeda, J. C., D. S. Caetano, and M. W. Pennell. 2015. Comparative analysis of principal components can be misleading. *Syst. Biol.* 64:677–689.
- Vamosi, S. M. 2005. On the role of enemies in divergence and diversification of prey: a review and synthesis. *Can. J. Zool.* 83:894–910.
- Vanhooydonck, B., and R. Van Damme. 2001. Evolutionary trade-offs in locomotor capacities in lacertid lizards: are splendid sprinters clumsy climbers? *J. Evol. Biol.* 14:46–54.
- Vermeij, G. J. 1987. *Evolution and escalation: an ecological history of life*. Princeton Univ. Press, New York.
- Wellborn, G. A., and R. B. Langerhans. 2015. Ecological opportunity and the adaptive diversification of lineages. *Ecol. Evol.* 5:176–195.
- Yoder, J. B., E. Clancey, S. Des Roches, J. M. Eastman, L. Gentry, W. Godsoe, T. J. Hagey, D. Jochimsen, B. P. Oswald, J. Robertson, et al. 2010. Ecological opportunity and the origin of adaptive radiations. *J. Evol. Biol.* 23:1581–1596.
- Zachos, J., M. Pagani, L. Sloan, E. Thomas, and K. Billups. 2001. Trends, rhythms, and aberrations in global climate 65 Ma to present. *Science* 292:686–693.
- Zheng, Y., and J. J. Wiens. 2016. Combining phylogenomic and supermatrix approaches, and a time-calibrated phylogeny for squamate reptiles (lizards and snakes) based on 52 genes and 4162 species. *Mol. Phylogenet. Evol.* 94:537–547.

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Supporting Information

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

Figure S1. Distributions of the likelihood ratios for three evolutionary model comparisons.

Figure S2. Distribution of test statistics of the EB model fit based on posterior predictive simulation.

Figure S3. Posterior predictive distributions of MDI and node height test slopes.

Figure S4. Plot of the absolute contrasts of body armour against node height.

Table S1. Loading scores of a phylogenetic principal components analysis performed on 20 size-corrected morphological traits.