EVOLUTION

The macroevolutionary singularity of snakes

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Snakes and lizards (Squamata) represent a third of terrestrial vertebrates and exhibit spectacular innovations in locomotion, feeding, and sensory processing. However, the evolutionary drivers of this radiation remain poorly known. We infer potential causes and ultimate consequences of squamate macroevolution by combining individual-based natural history observations (>60,000 animals) with a comprehensive time-calibrated phylogeny that we anchored with genomic data (5400 loci) from 1018 species. Due to shifts in the dynamics of speciation and phenotypic evolution, snakes have transformed the trophic structure of animal communities through the recurrent origin and diversification of specialized predatory strategies. Squamate biodiversity reflects a legacy of singular events that occurred during the early history of snakes and reveals the impact of historical contingency on vertebrate biodiversity.

From a phylogenetic perspective, snakes are indisputably nested within lizards (*1*, *2*) and are simply one example of a particularly species-rich and cosmopolitan group of "scaled reptiles" (Squamata). Yet, unlike lizards, snakes engage with human emotions in a visceral manner unmatched by almost any other group of organisms and for this reason have played important cultural roles in human societies (*3*). Most readily known for their lack of limbs and unique prey-capture strategies, snakes exhibit an incredible degree of ecomorphological diversity and specialization. The ~4000 extant snake species include shovel-snouted burrowers that hunt desert scorpions, slender arboreal predators that prey on tree snails, and paddle-tailed marine forms that probe reef crevices for fish eggs and eels. However, more than 25 clades of lizards have independently evolved limblessness (*4*), and other lineages also evolved dietary specialization, venom, highly mobile skulls, and/or advanced chemoreception (*5*–*7*)—all attributes typically associated with snakes. This convergence raises fundamental questions about how and why particular traits have influenced squamate diversification more generally.

Viewed across multiple traits, snakes nonetheless appear distinct from lizards with respect to ecology, morphology, and biogeography (*8*–*10*). These observations suggest that evolutionary dynamics in snakes are qualitatively different from those in lizards. If so, the origin of snakes is potentially consistent with a Simpsonian view of macroevolution (*11*, *12*), whereby major biodiversity expansions occur through qualitative phase shifts into new adaptive zones. These phase shifts can be conceptualized as macroevolutionary "singularities": patterns of rapid change across multiple organismic and ecological axes that, when viewed retrospectively through the prism of geological time, are sufficiently clustered together so as to seem virtually instantaneous. The term "singular" also refers to the fact that these transformations typically appear unpredictable from prior character states and phylogenetic position alone; such transitions have been documented within birds (*13*, *14*), mammals (*15*), and other taxa (*16*). Here, we characterize the tempo and mode of ecological and morphological innovation across squamate reptiles to address the role of macroevolutionary singularities in generating large-scale patterns of lizard and snake biodiversity. In particular, we test the extent to which phenotypic shifts have predictable consequences for evolutionary diversification across squamates.

Approach

We constructed a genomic backbone phylogeny for 1018 species of squamates, sequencing an average of 4.4 Mb across 4945 loci per taxon (figs. S1 and S2 and data S1). This phylogeny was then used as a scaffold upon which we added additional species from GenBank, yielding a species-level, dated phylogeny containing 6885 of the 10,759 squamate taxa (fig. S3 and data S2). We then time-calibrated a phylogenomic tree subsampled to 134 tips that spanned 31 fossil calibrations (figs. S4 and S5 and data S1) and used the resulting node dates as secondary calibrations for our full tree (fig. S6).

With this tree, we quantified macroevolutionary dynamics across a range of ecological, morphological, and environmental traits. Traits were collated from a variety of sources and augmented with primary natural history data from our field- and museum-based research programs (*17*), including a dietary dataset (n = 68,547 records) from preserved stomach contents and field observations that spanned 1314 species of snakes and lizards. For each species, we then computed a simple statistical index of net innovation, Ψ, defined as the absolute difference in phenotype between the focal species and the inferred ancestral state for all extant squamates (*17*). We calculated indices for net trophic innovation (Ψ_{diet}), morphological innovation in skull shape (Ψ_{skull}), presacral vertebral count (Ψ_{vert}), body elongation (Ψ_{elong}), and chemosensory processing (Ψ_{chem}). For the same traits, we also computed an index of absolute, branch-specific change along individual branches (fig. S7).

To estimate evolutionary rates, we developed a tip-rate metric for univariate and multivariate phenotypic data that is similar to the widely used "diversification rate" (DR) statistic for speciation rate (*18*); we refer to this metric as the "TR" statistic (*17*). We then used a variance-partitioning analysis to identify nodes that best account for species-level phenotypic variation [canonical phylogenetic ordination (CPO) (*19*)]. We also characterized heterogeneity in phenotypic macroevolution with multiprocess evolutionary models that allowed for shifts in both (trait) state and evolutionary rates, in which "state shifts" are defined as large jumps in phenotype on individual branches (*17*).

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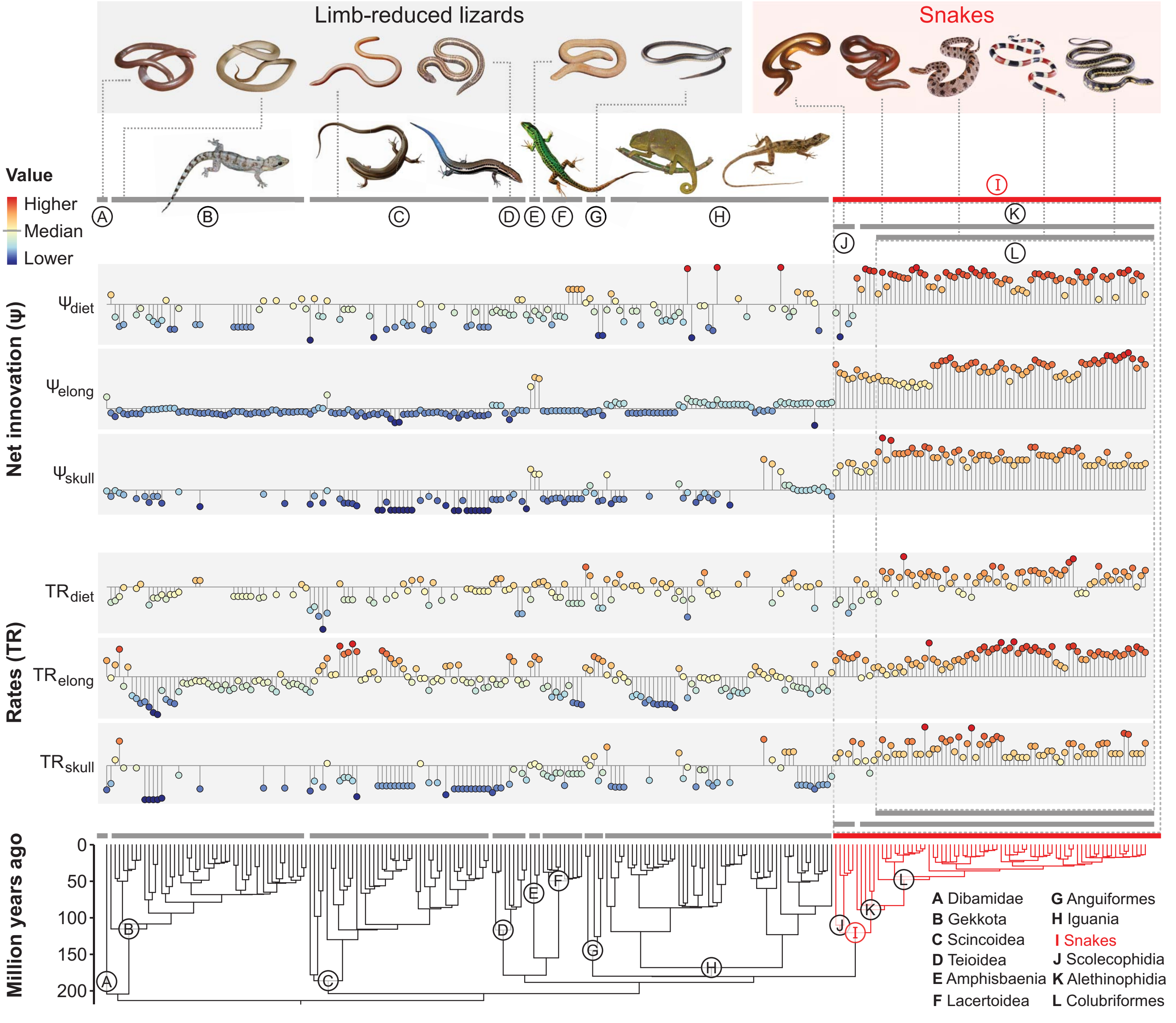

Fig. 1. Phenotypic innovation and evolutionary rates across squamates. Net innovation (Ψ) and evolutionary rate (TR) are shown across 246 squamate clades (bottom), subsampled from our full time-calibrated phylogeny of 6885 species (fig. S3). Snakes show greater phenotypic innovation and faster rates of evolution for innovation in skull shape (Ψ_{skull}), elongation (Ψ_{elong}), and diet composition (Ψ_{diet}); these results are even more striking for colubriform snakes (node L). Tip values are residuals from tree-wide median value; additional traits are shown in fig. S8.

Results

Phenotypic innovation across snakes and lizards

Snakes have undergone large transformations along multiple phenotypic axes (Figs. 1 and 2 and figs. S7 to S10) relative to other squamates. These shifts distinguish them both quantitatively and qualitatively from the ancestral squamate phenotype and from their closest living (nonsnake) relatives. Estimates of net innovation range from 1.6- to 3-fold higher in snakes relative to lizards (Fig. 1 and fig. S8). These results suggest that the origin or early diversification of snakes was associated with massive shifts in traits associated with feeding, locomotion, and sensory processing.

Rates for morphological and ecological traits generally increased early in the evolutionary history of snakes (Fig. 1). Snakes occupy distinct morphological space and exhibit elevated rates of morphological evolution relative to lizards, as evidenced by their greater body elongation (mean snake elongation index and rate >6× and >24× that of lizards; fig. S11), higher vertebral counts (mean count and rate for snakes >5× and >80× that of lizards; figs. S11 and S12), highly distinctive skull shape [mean snake rate >3× that of lizards; fig. S11 (*9*)], and greater rate of body mass evolution (snake rate >12× that of lizards; fig. S13). Patterns of morphological evolution are mirrored by ecological trait evolution: The rate of trophic niche evolution was 3.2-fold faster in snakes than in lizards, and snake diets have diverged much more from the ancestral squamate diet relative to lizards (Fig. 1).

Across a range of traits and rates, both CPO and multiprocess models reveal that much of the phenotypic variation across snakes and lizards can be explained by a single event that occurred early in the evolutionary history of snakes (Fig. 2 and figs. S14 and S15). Multiprocess phenotypic models generally recovered a phase shift in both rate and state associated with the ancestor of extant snakes or an associated early divergence within snakes (Fig. 2 and figs. S16 and S17), implying both decoupled

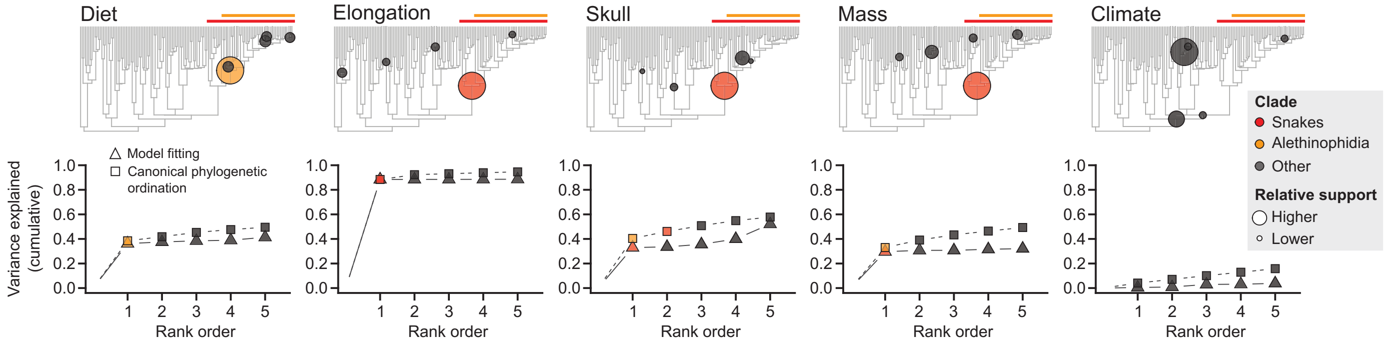

Fig. 2. Phase shifts associated with the early evolution of snakes explain trait variation across extant squamates. (Top row) Multiprocess models generally find strong support for major phase shifts in both trait state and rates associated with all snakes (red) or alethinophidian snakes (orange; 88% of snake diversity). (Bottom row) Cumulative trait variance across extant squamates explained by discrete phase shifts on single branches, as inferred from two distinct unsupervised learning methods. The total variance explained by five largest shift nodes (top row) is shown as triangles; results from stepwise canonical phylogenetic ordination are shown as squares. Individual node effects account for much less variance in climate (far right) relative to other traits.

rate dynamics in snakes relative to other squamates and also a major shift (jump) in phenotypic state. These findings are consistent with those of several recent studies that demonstrated phase shifts in the tempo and mode of cranial evolution associated with the origin and early radiation of snakes (*8*, *20*).

Trophic structure of squamate biodiversity

Snakes occupy much greater dietary space relative to lizards (Fig. 3A), and their diets are generally nonoverlapping owing to the disproportionate consumption of vertebrate prey by snakes (Fig. 3B and figs. S18 and S19). Snakes also use an aquatic and semiaquatic prey base that is largely untouched by lizards (separation along diet PC 2, Fig. 3, A and B, and fig. S18) (PC, principal component). Although snakes as a whole use a greater diversity of food resources than lizards, the diets of individual snake species are consistently narrower than those of lizards (Fig. 3D). Snakes have evolved a range of phenotypes to specialize on viscous prey (worms, slugs), slippery prey (eggs, eels, and caecilians), and noxious and dangerous prey (e.g., venomous snakes, mammals, centipedes, and scorpions), as well as snails and other "armored" resources (Fig. 3, A and B).

A small number of lizards (e.g., some monitor lizards, *Heloderma*, and *Lialis*) feed heavily on vertebrates, mainly exploiting prey already used by snakes. By contrast, finer partitioning of dietary categories among arthropod-feeding squamates (Fig. 3C) reveals that snakes and lizards consume largely nonoverlapping resources, with blindsnakes feeding almost exclusively on insect brood—larval and pupal ants and termites in particular—and with several other snake lineages specializing on spiders, centipedes, and scorpions. Conversely, arthropod-feeding lizards consume far more adult insects spanning multiple taxonomic categories, and lizard niche breadths are broader than those of arthropod-feeding snakes (Fig. 3C).

Previous analyses of trophic macroevolution across lizards (*7*) have formulated the "deep-history" hypothesis of squamate diets, in which resource use is deeply conserved, in contrast to dynamic patterns of trophic niche evolution observed in some vertebrate radiations (*21*–*23*). Our diet analyses recovered elements of this hypothesis (*7*, *24*) (fig. S20), but this legacy effect is dwarfed by the trophic shifts and expansions that have occurred in snakes throughout the Cenozoic (*25*). The origin of alethinophidian snakes (all snakes but blindsnakes; 88% of snake species; Fig. 2) accounts for nearly 40% of the variance in species-level diet state across squamates (fig. S14G). This shift, accompanied by an overall acceleration in the rate of trophic niche evolution in colubriform snakes (Fig. 1, TR_{diet}), led to a dramatic expansion of the squamate trophic universe. Overall, our results highlight the power of primary natural history data obtained from museum specimens and field observations to reveal major shifts in the ecological structure of the biota.

Climate and life-history evolution

Climatic niche and life history traits do not appear to be evolving along distinct evolutionary trajectories in snakes relative to lizards, thus departing from patterns observed for morphology and diet (Fig. 2). We find high lability of a key life-history trait (parity mode), and in climatic niche parameters (figs. S8 and S9). Despite some phylogenetic signal in climatic niche parameters (figs. S14 and S15), there is little evidence that such conservatism persists across large phylogenetic scales (figs. S21 and S22). Variation in climatic niche across squamates thus cannot be explained by deep divergences in their evolutionary past (Fig. 2 and figs. S14L and S15R).

Speciation rates in lizards and snakes

We quantified speciation rates across squamates, using phylogenetic imputation to infer placements for the 3872 squamate species for which genetic data were not available [fig. S23, (*17*)]. Snakes show substantially elevated rates of speciation relative to lizards [mean lizard $\lambda_{CLaDS} = 0.08$, $\lambda_{BAMM} = 0.09$; snake $\lambda_{CLaDS} = 0.18$, $\lambda_{BAMM} = 0.21$ (CLaDS, cladogenetic diversification rate shift model; BAMM, Bayesian analysis of macroevolutionary mixtures)] (Fig. 4 and figs. S24 and S25D), including other well-studied lizard clades thought to be the result of rapid radiation (e.g., *Anolis* and sphenomorphine skinks) (Fig. 4, C and D). Approximately 45% of the variance in tip-level speciation rates across squamates is explained by the node spanning colubriform snakes ($\lambda_{CLaDS} = 0.20$; $\lambda_{BAMM} = 0.23$; figs. S14 and S26). Outside of snakes, the most important rate shift involved the ancestor of the species-rich *Liolaemus* lizard clade from montane and arid South America (~260 species), which accounts for ~11% of the variance in tip-level rates. For higher taxa, speciation rate is correlated with species richness (n = 119 subfamilies; Spearman's r = 0.63) (Fig. 4C), but this positive correlation is driven by the presence of very small clades with low speciation rates. For clades with at least 50 species—collectively accounting for 96% of global squamate diversity—there is effectively no relationship between richness and rate ($n = 42$, $r = 0.03$). Similar decoupling between speciation rates and diversity patterns, both for taxonomic groups and geographic regions, has now been observed in a wide range of taxa (*18*, *26*, *27*).

We then tested whether previously proposed key innovations predict speciation rates across squamates more generally. We found that individual traits had minimal ability to explain speciation-rate variation (Fig. 4F and fig. S25E), even for those traits that have evolved multiple times across squamates [maximum coefficient of determination (R^2) = 0.01]. To the extent that these traits contributed to snake diversification, their influence is statistically confounded with a macroevolutionary singularity associated with

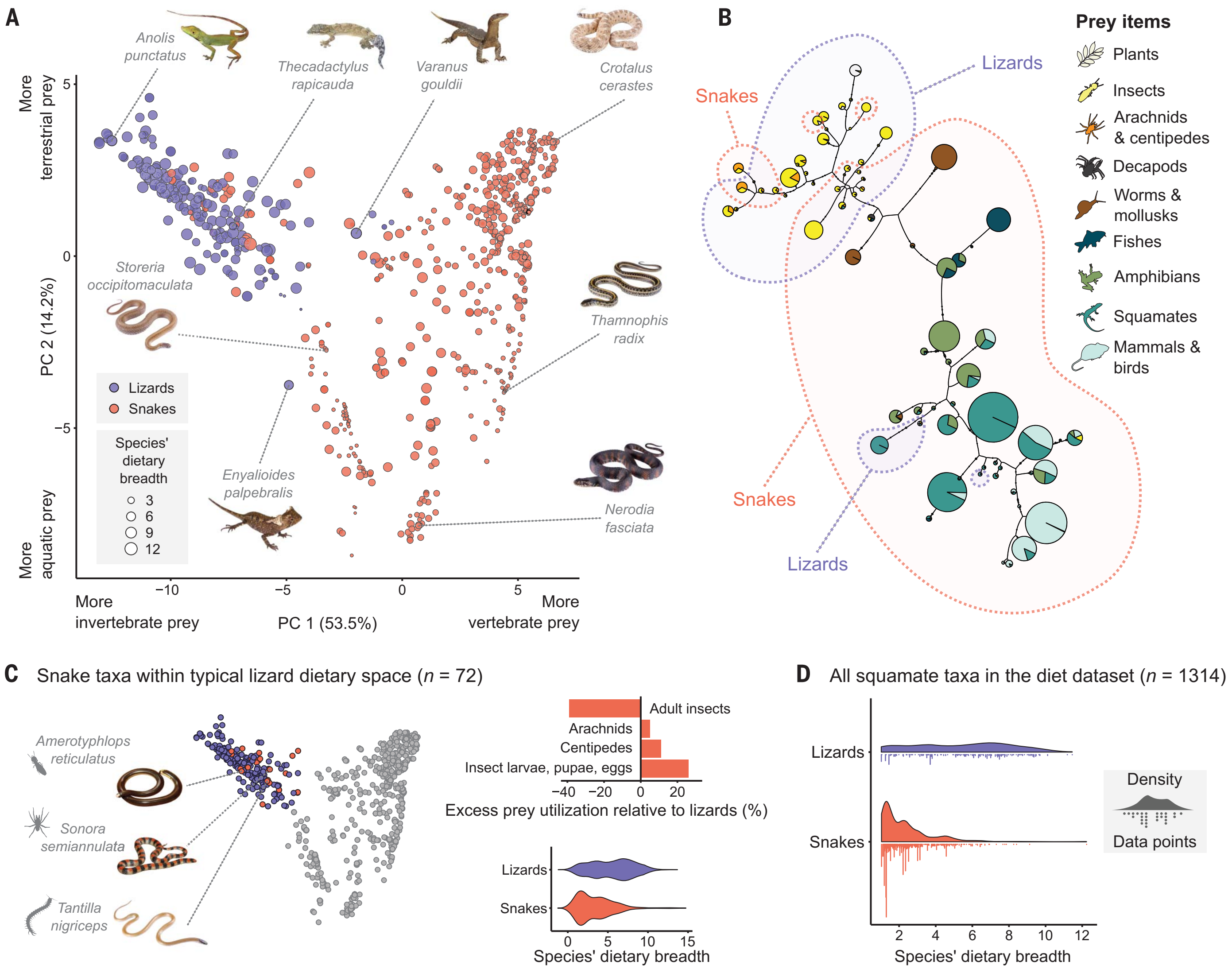

Fig. 3. Fundamental ecological differences between snakes and lizards. (**A**) Multidimensional trophic niche as inferred from 68,547 individual squamates (stomach contents, field observations). Individual points correspond to species (n = 1314); point size denotes dietary-niche breadth. (**B**) Similarity path through diet space as inferred by principal graph analysis, illustrating taxonomic structure of resource differences between snakes and lizards. Nodes (circles) are groupings of species that consume similar resources; colors represent fractional resource use of each inferred node, and node sizes are proportional to the number of species assigned to the group. (**C**) Apparent dietary overlap between some snakes and lizards (A) masks differences in the ways that these groups use invertebrate prey. Arthropod-feeding snakes tend to eat spiders, centipedes, and scorpions (e.g., *Sonora semiannulata* and *Tantilla nigriceps*), or soft-bodied larval insects (e.g., *Amerotyphlops reticulatus*), and they also consume far fewer adult insects relative to lizards. (**D**) Dietary-niche breadths are much greater for lizards than for snakes, even after accounting for intraspecific sampling variability.

the origin of snakes (Fig. 4E and fig. S27) (*28*). We also considered several biogeographic predictors of speciation rate, finding no evidence for associations with latitude (fig. S28) or biogeographic region (fig. S29). Similar to other vertebrate clades (*18*, *26*), the latitudinal gradient in squamate diversity appears largely decoupled from speciation rates, and geographic variability in speciation rate is largely a function of the relative fraction of snakes versus lizards in the biota.

Conclusions

Global squamate biodiversity has been heavily shaped by a singularity in macroevolutionary dynamics that occurred in association with the origin of snakes and with their most successful constituent clade (Colubriformes, 3100 species) (Fig. 1), a radiation that shows no sign of slowing toward the present (*25*). Numerous hypotheses have been proposed to account for the dramatic evolutionary radiation of snakes, including shifts in feeding mechanisms, extreme cranial kinesis, body elongation, limb reduction, and venom (*8*, *25*, *29*, *30*). Furthermore, snakes show several genomic attributes, such as transposable element proliferation (*31*) and metabolic protein redesign (*32*), that might contribute to evolutionary versatility of the general snake body plan (*20*). However, our results highlight the challenge of linking specific innovations to the overall "success" of the snake phenotype. Replicated innovations in our dataset, including chemosensory innovation, limb loss, and body elongation, show no predictable effects on speciation rate (Fig. 4). Moreover, counterfactual experiments have occurred throughout squamate evolutionary history that support a decoupling between innovation per se and macroevolutionary regime shifts. For example, lizard (nonsnake) lineages that have evolved potent venom (*Heloderma*) have scarcely diversified, and perhaps the most extreme instance

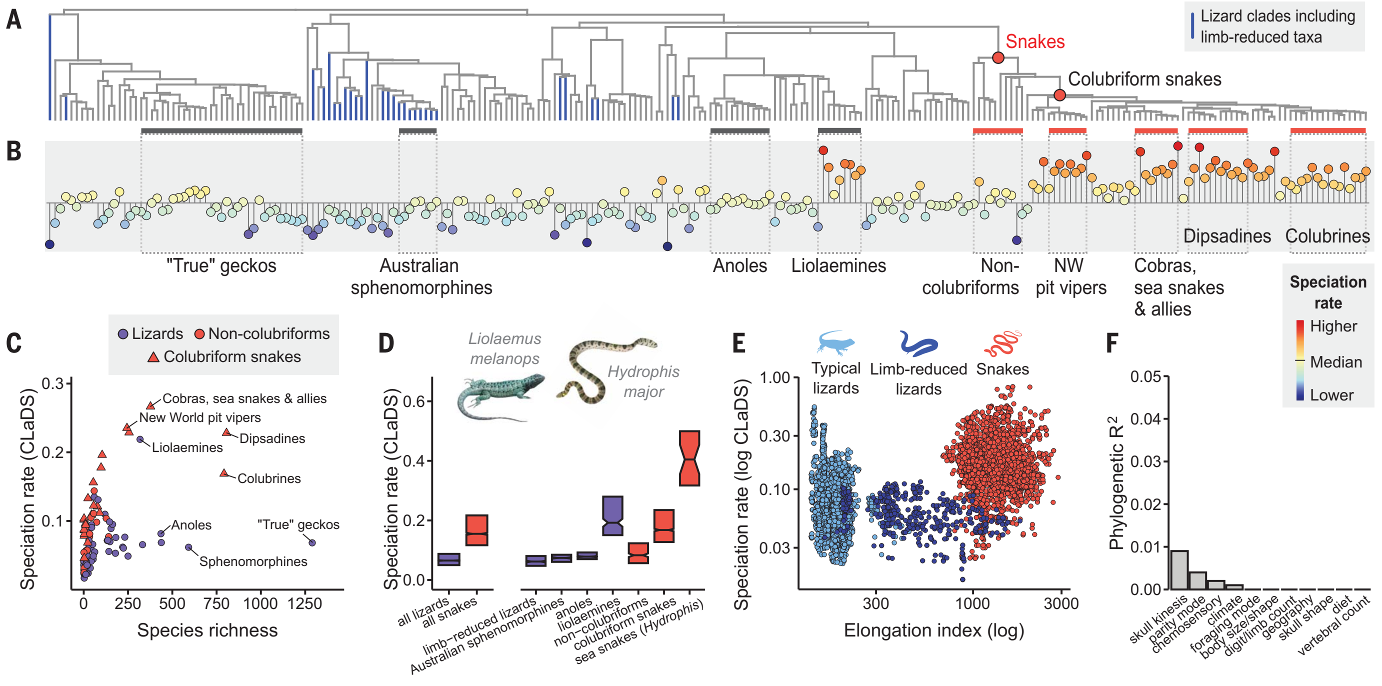

Fig. 4. Speciation rates in snakes are consistently elevated relative to lizards. (**A**) Subsampled phylogeny of 246 clades (as shown in Fig. 1) illustrating (**B**) speciation rates (CLaDS) relative to tree-wide median rate. Rates for each tip represent the mean rate for all species assigned to each terminal clade. (**C**) Species richness and tip-averaged speciation rate for major clades within squamates (subfamily level). (**D**) Median and interquartile range for tip speciation rates across all snakes and lizards and for several key groups. (**E**) Speciation rates for individual species as a function of body elongation. Snakes are elongate and have fast rates of speciation (D), but elongate and/or limb-reduced lizards show no trends toward faster rates. Phylogenetic distribution of limb reduction is indicated by blue branches in the phylogeny shown in (A). (**F**) Phylogenetic regression analysis reveals minimal effect of any phenotypic traits on speciation rates ($R^2 < 0.01$).

of cranial kinesis in a nonsnake squamate occurs in a species-poor group of legless geckos (*33*). Conversely, one lineage of snakes—the splitjaw snakes (Bolyeridae)—has further increased the flexibility of the alethinophidian trophic apparatus through dramatic skeletal innovations not seen in any other vertebrates (*34*). Yet this innovation was unaccompanied by any substantive ecological or species diversification, and the lineage is currently represented by a tiny clade of endangered or recently extinct island endemics (*Casarea dussumieri* and *Bolyeria multocarinata*).

Snakes thus appear to represent an ongoing adaptive radiation characterized by sustained ecological and species diversification that was triggered ultimately by one or more unknown and perhaps unknowable singular events. The principal challenge in resolving the causal basis of such singularities involves a lack of phylogenetic replication. By focusing on traits specific to squamates (e.g., cranial kinesis and venom), evolutionary biologists are necessarily limited to a small number of statistically independent data points or contrasts (*28*). However, some hypotheses, particularly those associated with genomic change or molecular evolutionary rates (*20*), could be tested across vastly greater phylogenetic scales (e.g., all vertebrates or metazoans). Likewise, the development of taxon-independent ontologies to describe key processes (e.g., sexual selection and trophic flexibility) might facilitate comparison of putative causal processes or agents across diverse branches of the Tree of Life. Nonetheless, the action of these and other innovations may be highly contingent on geographic and other circumstances peculiar to individual clades (*35*, *36*).

The view of phenotypic evolution emerging from squamates and other taxa (*15*, *20*, *37*) is that many of the most spectacular changes in the history of life have involved macroevolutionary singularities: rare, transformative events that are unpredictable from evolutionary time, phylogenetic position, and other factors. In this study, the recurrent evolution of snake-like traits in other groups of nonsnake lizards has failed to generate comparable evolutionary outcomes, suggesting that replicated patterns of adaptive radiation observed in many groups of organisms (*35*, *38*) may be superseded at deeper phylogenetic scales by systemic shifts that ultimately give rise to higher taxa (*11*). The extent to which such shifts are qualitatively distinct from those associated with young radiations is likely to remain a challenging problem. Perhaps most importantly, the pruning effect of extinction, through the selective elimination of ecologically and phenotypically intermediate clades, may exacerbate the distinctiveness of clades that comprise the "survivors" that we observe in the present day (*39*). Thus, transformation of the biota through time may be partly attributable to phenotypic changes that confer increased survivorship at the species or clade level (*40*, *41*). Determining why snakes and other groups—from bats to beetles, and from angiosperms to acanthomorph fishes—have experienced such dramatic shifts in evolutionary tempo and mode is, as Darwin put it, an "abominable mystery." Yet the major features of biological diversity may owe more to these revolutionary events than to the causal structures we infer by extrapolating contemporary microevolution through geological time.

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SUPPLEMENTARY MATERIALS

science.org/doi/10.1126/science.adh2449
Materials and Methods
Figs. S1 to S44
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MDAR Reproducibility Checklist
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