

WHY DOES A TRAIT EVOLVE MULTIPLE TIMES WITHIN A CLADE? REPEATED EVOLUTION OF SNAKELIKE BODY FORM IN SQUAMATE REPTILES

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Abstract.—Why does a trait evolve repeatedly within a clade? When examining the evolution of a trait, evolutionary biologists typically focus on the selective advantages it may confer and the genetic and developmental mechanisms that allow it to vary. Although these factors may be necessary to explain why a trait evolves in a particular instance, they may not be sufficient to explain phylogenetic patterns of repeated evolution or conservatism. Instead, other factors may also be important, such as biogeography and competitive interactions. In squamate reptiles (lizards and snakes) a dramatic transition in body form has occurred repeatedly, from a fully limbed, lizardlike body form to a limb-reduced, elongate, snakelike body form. We analyze this trait in a phylogenetic and biogeographic context to address why this transition occurred so frequently. We included 261 species for which morphometric data and molecular phylogenetic information were available. Among the included species, snakelike body form has evolved about 25 times. Most lineages of snakelike squamates belong to one of two “ecomorphs,” either short-tailed burrowers or long-tailed surface dwellers. The repeated origins of snakelike squamates appear to be associated with the in situ evolution of these two ecomorphs on different continental regions (including multiple origins of the burrowing morph within most continents), with very little dispersal of most limb-reduced lineages between continental regions. Overall, the number of repeated origins of snakelike morphology seems to depend on large-scale biogeographic patterns and community ecology, in addition to more traditional explanations (e.g., selection, development).

Key words.—Biogeography, body form, character evolution, community ecology, homoplasy, macroevolution, phylogeny, reptiles.

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Why does a trait evolve multiple times over the course of the phylogenetic history of a clade? Why do some traits evolve repeatedly within a clade and some only once? Why might a trait evolve more than 10 times but less than 30? Given the burgeoning use of phylogenies to study character evolution, these may not seem like neglected questions in the field of evolutionary biology, but in many ways they are.

In examining the evolution of a trait, evolutionary biologists often focus on two levels of explanation. First, they address the potential advantages that this trait may confer in a given selective environment. This may be studied with a variety of approaches, including comparative methods (showing a correlation between the trait and a selective environment among taxa; e.g., Harvey and Pagel 1991; Martins 2000), studies of trait function and organismal performance (e.g., Arnold 1983; Wainwright and Reilly 1994), studies of natural selection in wild populations (e.g., Endler 1986; Grant 1999), or a combination of these approaches (e.g., McPeck et al. 1996). At another level, evolutionary biologists address the genetic and developmental mechanisms which actually create novel phenotypes (e.g., Wilkins 2002; Carroll et al. 2005). Although elucidating the developmental origin and selective advantage of a trait may be necessary to explain why that trait has evolved in a particular instance, they may not be sufficient to explain why the trait has evolved 10 times. To address this type of question, we need to incorporate additional levels of explanation.

At least two additional factors may be important in determining the number of origins of a phenotypic trait over the

history of a clade (Fig. 1). One is the biogeographic context of the selective environment. It is well known that convergent evolution may produce similar phenotypes in different geographically isolated regions (e.g., marsupial mammals in Australia versus placentals in the New World; Futuyma 1998). In general, the geographic separation of the selective environment should favor multiple origins of a trait. In other words, the trait may evolve wherever the selective environment is encountered, and the spread of a lineage to different geographically isolated regions containing this same selective environment may lead to multiple origins (Simpson 1953).

Another factor is competitive interactions. Even if a trait could confer a selective advantage in a given ecological context, the trait may not evolve because other species occupy that “niche” or “adaptive zone” in a given region. Many adaptive radiations are thought to be associated with diversification into previously vacant or underutilized niches (e.g., Futuyma 1998; Schluter 2000). Similarly, the evolution of the trait over the history of the clade may also affect how often it evolves. For example, the evolution of the trait in one lineage may “fill up” the available niche space, lessening the probability that the trait will evolve again within the clade. On the other hand, competition (intraspecific and/or interspecific) within the ancestral selective regime may also promote invasion of new adaptive zones or niches (reviewed by Schluter 2000).

Interactions between biogeography and competition may also be important. For example, evolution of the trait in a geographically isolated lineage cannot “pre-empt” the evo-

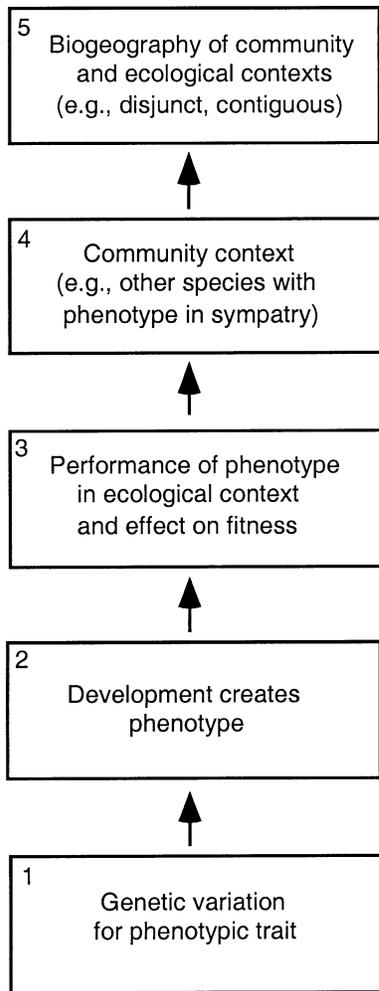


FIG. 1. Simplified conceptual diagram illustrating the general factors that may determine the number of times that a phenotypic trait evolves within a clade. Boxes 1 and 3 are the typical subjects of microevolutionary and comparative research, whereas Boxes 1 and 2 are studied in evolutionary developmental biology (“evo-devo”). However, Boxes 4 and 5 may also be important to explain the number of times that a trait evolves; whether to explain why the trait evolves so frequently (e.g., Box 5) or so rarely (e.g., Box 4). The diagram is greatly simplified; for example, Box 3 can influence Box 1. Furthermore, not all of these factors will be important in every case (e.g., characters may evolve through drift, competition may not always be important).

lution of the trait in other lineages outside that region. However, if the trait is associated with high dispersal ability or evolves in a geographically widespread lineage, then competitive interactions may limit additional origins of the trait. The selective environment and suites of competing species may vary considerably over time as well as space (e.g., climate change, mass extinctions), and these temporal dynamics may also lead to repeated origins of a trait (e.g., “incumbent replacement” when a group diversifies after the extinction of a competing lineage; Rosenzweig and McCord 1991).

The idea that biogeography and competition are important in explaining patterns of homoplasy may seem obvious. Yet these factors are largely neglected in the current literature, including an edited volume devoted exclusively to homoplasy

(Sanderson and Hufford 1996). Previous studies have also discussed general trends in character evolution (e.g., McNamara 1990; McShea 1994; Wagner 1996), and biased patterns of homoplasy (e.g., more changes in some clades than others or more losses than gains; Sanderson 1991; 1993), but with little discussion of the roles of biogeography and competition. Nevertheless, some phylogenetic studies have described cases where competition and/or biogeography may drive patterns of homoplasy. For example, Losos et al. (1998) postulated that interspecific interactions drive the repeated evolution of *Anolis* lizard ecomorphs on different islands in the Greater Antilles. Espinoza et al. (2004) hypothesized that the repeated origins of herbivory in liolaemid lizards are associated with a unique correlation between cool climates and herbivory in this clade and the geographic isolation of cool, high elevation habitats (i.e., the habitats where herbivory is favored are more geographically isolated when herbivory is correlated with cool climates, dramatically increasing the number of origins of herbivory).

In this paper, we explore the evolution of body form in squamate reptiles (lizards and snakes), with an emphasis on how biogeography and community context may influence the number of times that snakelike body form evolves. The transition from lizardlike body form (four well-developed limbs, nonelongate body) to snakelike body form (limbs reduced or absent, elongate body) seemingly has occurred numerous times in squamates, given that there are several major clades of limb-reduced squamates (e.g., snakes, amphisbaenians, dibamids), six families in which both body forms are present, and that both forms occur within several genera as well (e.g., *Brachymeles*, *Chalcides*, *Lerista*, *Scelotes*; Lande 1978). There has been some discussion of the selective factors that might drive this transition (e.g., Gans 1975; Walton et al. 1990; Wiens and Slingluff 2001) and of the developmental and genetic mechanisms that underlie it (e.g., Raynaud 1985; Lande 1978; Cohn and Tickle 1999; Wiens and Slingluff 2001; Shapiro 2002; Shapiro et al. 2003). Numerous authors have commented on how frequently this transition seems to have occurred (e.g., Gans 1975; Greer 1991; Zug et al. 2001; Pianka and Vitt 2003), but no studies have addressed the larger-scale factors that might explain its repeated occurrence.

MATERIALS AND METHODS

Phylogeny and Taxon Sampling

In general, squamate species were selected that were included in recent molecular phylogenetic studies and that were relevant to the evolution of snakelike body form. We generally avoided morphology-based phylogenies because of the potentially misleading effects of limb reduction (and associated traits) on squamate phylogenetics (e.g., Lee 1998). Nevertheless, we did consider taxonomic assignments based on morphological data in some cases.

A single squamate phylogenetic tree containing all of the species of interest does not yet exist. Instead, we combined results from several individual phylogenetic studies into a single “supertree” (i.e., Sanderson et al. 1998). However, it should be understood that we used a supertree approach merely to summarize results from different studies with limited

taxonomic overlap, rather than to resolve conflicts between studies over the phylogeny of individual groups (which is far more controversial). Although we generally prefer direct analysis of character data (i.e., the “supermatrix” approach), combining data from the different studies would have been extremely difficult given the limited overlap in the taxa and characters sampled by each one.

We used the Townsend et al. (2004) analysis of higher-level squamate relationships as the backbone for our tree (Bayesian analysis of combined data, their fig. 7B). To this backbone were added phylogenies from detailed studies within families and other higher taxa (snakes, amphisbaenians, iguanians). When more than one study was available for a given group (e.g., gymnophthalmids, snakes), we chose the study with the more extensive taxon sampling. When authors presented more than one analysis, we favored analyses based on the maximum amount of data (e.g., combined versus separate analyses of genes) and using explicit model-based methods (i.e., maximum likelihood or Bayesian methods rather than parsimony). Trees from model-based analyses were available in all studies but Frost et al. (2001) and Fu (2000); for the latter study, we used their weighted (2:1 transitions: transversions) parsimony tree. In summary, we used the following trees: Scincidae (Reeder 2003 [Australian *Sphenomorphus* group lygosomines, fig. 3]; Whiting et al. 2003 [*Scelotes* and relatives, fig. 1]; Schmitz et al. 2005 [Malagasy “scincines,” fig. 2]; Brandley et al. 2005 [higher-level scincid relationships, fig. 3]), Pygopodidae (Jennings et al. 2003, figs. 10 and 11), Gymnophthalmidae (Castoe et al. 2004, fig. 6), Cordylidae (Frost et al. 2001, fig. 2), Gerrhosauridae (Lamb et al. 2003, fig. 6), Anguillidae (data from Macey et al. 1999; maximum-likelihood reanalysis by Wiens and Slingluff 2001, fig. 1), Lacertidae (Fu 2000, fig. 2), iguanians (Schulte et al. 2003, fig. 5), amphisbaenians (reanalysis of original data from Kearney and Stuart 2004), and snakes (Slowinski and Lawson 2002 [for rooting, fig. 4]; Wilcox et al. 2002 [placement of *Trachyboa*, fig. 3]; Lawson et al. 2004 [overall snake relationships, fig. 1]). In a few cases, we used species thought to be closely related to those included in the molecular analyses but that were better represented in museum collections for morphometric analyses.

Not all readers may be convinced that the phylogenetic hypothesis of Townsend et al. (2004) for squamate families is correct. However, their phylogeny generally is supported by analyses of additional nuclear genes in ongoing studies by Reeder, Townsend, Wiens, and collaborators. Furthermore, given that most origins of snakelike body form occur within families, most of our results should be relatively insensitive to different hypotheses of relationships between families.

We did not include every species of squamate for which molecular phylogenetic information is available. Instead, we focused on trying to capture as many changes to snakelike body form as possible, by including limb-reduced forms and their close relatives. For example, we sampled relatively few iguanians, lacertids, teiids, and xantusiids; these are groups in which no limb-reduced forms are known (Zug et al. 2001). We also sampled a relatively limited number of species from clades invariant in lacking limbs. Nevertheless, we included

representatives of all families of amphisbaenians and almost all families of snakes.

Our taxon sampling was not comprehensive enough to capture every possible evolutionary transition between fully limbed and snakelike body form. However, it may include all or most of those outside of skinks (Scincidae). Even though we included 93 species of skinks, our sampling almost certainly underestimates the number of origins of limb-reduction within this group, given that there are many limb-reduced genera that we have not included (because of lack of phylogenetic information and/or scarcity in museum collections) and that there may be multiple cases of limb loss within some genera.

Morphometric Data

Morphometric data were obtained from preserved museum specimens for the species represented in the phylogenetic tree. Only specimens that seemingly were sexually mature and had unregenerated tails were used, to avoid biasing the results with potential ontogenetic shape changes or the reduced length of regenerated tails. These two criteria caused us to exclude many specimens; in several cases, the majority of available specimens for a given species were unusable. Thus, the sample sizes reported represent only those specimens from which useable data were obtained, and only a fraction of the total number of specimens examined overall. An average of 5.8 individuals per species was used, with sample sizes ranging from 1 to 40. Given that dissections are problematic for rare species, sexual maturity was inferred indirectly; in general we used only specimens that were within about 25% of the largest specimen sampled for that species. Unregenerated tails were identified based on homogeneity of coloration and scalation along the length of the tail and by comparing relative tail size to conspecifics.

Many species of squamates are difficult to sex externally, and sexing by direct examination of reproductive organs would have required destructive dissections of many rare specimens. In general, we consider sexual dimorphism in size and shape in squamates to be minor relative to the dramatic differences in body form that are the focus of the study. In cases where sex could be distinguished externally we tried to sample equal numbers of males and females. Similarly, we did not address intraspecific geographic variation, given our typically limited sample sizes. We are not aware of any major differences in body form among conspecific populations of squamates.

Six measurements were taken (to the nearest 0.01 mm) with digital calipers: snout-vent length (SVL), from the tip of the snout to the posteriormost extent of the cloacal opening; tail length (TL), from the posteriormost extent of the cloaca to the tip of the outstretched tail; head width (HW), the width of the head at the level of the posterior corner of the eye; head length (HL), from the tip of the snout to the posterior corner of the eye; forelimb length (FLL), from the posterior corner of the fully extended forelimb to the tip of the claw of the longest (outstretched) finger; hind-limb length (HLL), from the posterior corner of the fully extended hind limb to the tip of the claw of the longest (outstretched) toe. The number of fingers on the manus and number of toes on

the pes were also counted. Although other measurements could have been taken, many of these would be difficult to apply across taxa in which limbs and other obvious landmarks are lost (e.g., external ear openings). For some very large specimens, a meter stick was used for some measurements. Limb lengths (and limblessness) were assessed based on external data only, although internal limb remnants may be present in some taxa (these limb remnants presumably are not directly involved in locomotion). Similarly, the number of digits was counted based on external criteria only. In some taxa, a limb may be reduced to a stump or flap without obvious indication of separate digits, and these were considered to represent a single digit.

In general, data from nonscincid taxa were collected by J. J. Wiens, from nonlygosomine scincids by M. C. Bradley, and from lygosomines by T. W. Reeder. Most data on anguids and other anguimorphs (helodermatids, shinisaurids, varanids, xenosaurids) were from Wiens and Slingluff (2001), but new data for *Abronia graminea* replaced those for *Abronia oaxaca*. Although a single person collecting all data might have been preferable, measurements involving comparisons among closely related species generally were made by the same person. Furthermore, the measurements used are relatively standard and unambiguous.

Morphometric Analysis

We used principal components analysis (PCA) to summarize overall patterns of morphological variation. PCA was conducted using species as basic units, and the raw data were mean values for each species for each variable. Variables were natural-log transformed prior to analysis. Because the log of 0 is undefined (and because some species lack limbs and digits), 1 was added to all taxa for FLL, HLL, number of fingers, and number of toes. PCA was implemented in Statview (SAS Institute, Cary, NC) with varimax (orthogonal) rotation. Three PCs were initially retained; the third explained less than 10% of the total variance and additional PCs therefore were not considered. Loadings of individual variables on these PCs were plotted to visualize the morphometric differentiation of species.

We lacked data on HW for Australian skinks. Because HW and HL appear to be strongly correlated (J. J. Wiens, unpubl. data), we deleted HW so that these taxa could be included. Analyses including HW but excluding the Australian skinks gave similar results.

Ecological and Biogeographic Data

For the purposes of this study, we were primarily interested in whether a given species was surface dwelling (including grass swimmers) or burrowing, given that previous authors have suggested that limb-reduction is associated with subterranean microhabitat (e.g., Lee 1998; but see Wiens and Slingluff 2001). We use "surface-dwelling" to simply mean "not subterranean" and species in the surface-dwelling category also include those that are arboreal, aquatic, and saxicolous. Burrowing was defined as underground locomotion associated with digging (e.g., sand-swimming and making burrows for their own use) and was distinguished from being merely cryptic (e.g., hiding under rocks, logs, or leaf litter)

or using burrows made by other species. Nevertheless, we acknowledge that our definitions of burrowing and being cryptic may grade into each other in some cases. Furthermore, there are some species that are primarily active on the surface of the ground but which also burrow in some cases; these were considered surface dwellers. We culled data from the literature on the general microhabitat preferences of as many of the included species as possible. The quality of these data was quite variable, because many species are relatively rare. Importantly, inferences about ecology were based on ecology alone, and not on morphology.

We determined the limb-reduced squamate fauna of each major geographic region (regardless of whether or not species were included in our sampling). Literature sources included the following: Africa (Schleich et al. 1996; Branch 1998; Spawls et al. 2002), Asia (Zhao and Adler 1993; Manthey and Grossman 1997), Australia (Cogger 1992), Europe (Arnold and Burton 1978), Madagascar (Glaw and Vences 1994), North America (Stebbins 1985; Conant and Collins 1991), West Indies (Schwartz and Henderson 1991), and Middle and South America (Peters and Orejas-Miranda 1970; Cei 1993; Savage 2002). In some cases, the ranges of taxa were evaluated using the EMBL reptile database (<http://www.emblheidelberg.de/~uetz/LivingReptiles.html>), which includes generalized geographic information on all living squamate species.

Analysis of Character Evolution

We first mapped the evolution of body form onto the phylogeny by defining limb reduction as a discrete, binary trait. In the PCA, PC1 divided most squamates into those species with well-developed limbs versus those with elongate bodies, reduced limbs, and reduced numbers of digits (although a few species were intermediate). We arbitrarily considered those species with a score on PC1 less than 0.25 to be limb reduced, based on the general clustering of species in morphospace. This division generally made sense in terms of which taxa showed obvious signs of limb reduction (e.g., loss of limbs and digits). However, we also assigned two additional taxa to the limb-reduced category which had slightly higher scores on PC1 (both 0.36): *Sphenops sphenopsiformis* (two digits on forelimb, four on hind limb) and *Hemiergus peronii*. *Hemiergus peronii* is intraspecifically variable in the extent of digit reduction; the specimens we included had four digits on each limb, but other populations have only three digits on each limb (Choquenot and Greer 1989).

We also performed analyses in which limb-reduced forms were divided into long-tailed morphs and short-tailed morphs, based on their separation along PC3. Species with a score on PC3 greater than 1.0 (and a score on PC1 less than 0.25) were considered to belong to the long-tailed morph.

We first mapped these character states onto the phylogeny using parsimony with MacClade version 4.0 (Maddison and Maddison 2000). Two parsimony analyses were performed, one treating body form as a binary character (lizardlike or limb-reduced and snakelike) and the other treating the two limb-reduced morphs as separate, unordered character states. We then mapped these characters on the tree using maximum

likelihood (Schluter et al. 1997; Pagel 1999), implemented using Mesquite 1.5 (Maddison and Maddison 2004). As for parsimony, two analyses were performed, one treating body form as a binary trait and the other utilizing three character states (not limb-reduced; short-tailed limb-reduced morph; long-tailed limb-reduced morph). Likelihood analyses used the Markov k -state one parameter model (Mk1; Lewis 2001), assuming a single rate for all transitions between character states. To examine and summarize the results across the tree, the best estimate of the character state at each node was determined using the likelihood-ratio test. If the log-likelihoods of two states differed by 2.0 or more, then the state with the higher negative log-likelihood was rejected, and the alternate state was considered to be the best estimate for that branch (following Pagel 1999). If the difference in log-likelihoods was smaller (i.e., < 2.0), the reconstruction for that branch was considered ambiguous.

Given that the molecular datasets used to reconstruct the trees within squamate families have limited overlap between families, there was no single molecular dataset with comparable branch lengths for all taxa. We dealt with this problem in two ways. First, we performed a set of analyses that assumed equal branch lengths. Given that most transitions to snakelike body form seem to occur among relatively closely related species (e.g., within families; see Results), this assumption seems unlikely to be problematic for our study. Second, we developed comparable branch lengths across the phylogeny for almost all taxa by combining penalized likelihood estimates of divergence dates between and within families. This new approach is described after the section on estimates of divergence times. Both methods gave similar results.

Initially, all analyses were performed on a fully resolved tree. To incorporate uncertainty in the phylogeny, we performed a set of analyses in which branches of the supertree were collapsed into polytomies and then randomly resolved (following Wiens 1999; see also Housworth and Martins 2001). We first inspected the original trees used to build the supertree, and collapsed branches on the supertree that (in the source trees) had Bayesian posterior probabilities (Pp) less than 0.95 (e.g., Alfaro et al. 2003; Erixon et al. 2003; Huelsenbeck and Rannala 2004), or maximum likelihood or parsimony bootstrap values less than 70% (Hillis and Bull 1993). For a given group, we only collapsed branches considering the support values from one phylogenetic method (i.e., the method used to estimate the tree used for that group). When the collapsed supertree was completed, we generated 1000 random resolutions of the polytomies (assuming all resolutions are equally likely), repeated the parsimony reconstructions on each tree, and averaged results across the 1000 topologies (using MacClade). Given that the parsimony and likelihood reconstructions yielded similar results for the fully resolved trees (and given that the random resolutions gave very similar results to those for the fully resolved tree), this analysis was performed only using parsimony.

Correlation between Morphology and Habitat

Previous studies (e.g., Camp 1923; Wiens and Slingluff 2001) have suggested that there are two general ‘‘eco-

morphs’’ of limb-reduced squamates: long-tailed surface dwellers (many of which are considered to be ‘‘grass swimmers’’) and short-tailed burrowers. In support of this hypothesis, our PCA separated limb-reduced species based on tail length, and these differences in tail length generally appeared to correspond to habitat use (see Results). To test the association between these limb-reduced morphs and habitat more explicitly, we used the maximum-likelihood method of Pagel (1994) to test for correlated evolution between morphotypes and habitat usage. First, we divided the limb-reduced squamates (those with scores on PC1 < 0.25) into those with relatively long tails (PC3 > 1.0) and those with relatively short tails. We then assigned all species (whether limb-reduced or not) to one of two habitat types, either primarily surface-dwelling or primarily subterranean. Pagel’s method was implemented in the program Discrete, version 4.0 (M. Pagel, 1998–2000; available at: <http://www.ams.rdg.ac.uk/zoology/pagel/mppubs.html>). This method only allows for tests of correlation between binary characters (note that Pagel’s program Multistate also does not analyze correlation between multistate characters). We first tested for an association between the short-tailed, limb-reduced morph and burrowing habitat. For morphology, the short-tailed morph was coded as state 1 and the long-tailed and fully limbed morphologies were coded as state 0. Similarly, primarily burrowing lifestyle was coded as state 1 and all other types of habitat use were coded as state 0. We then tested the relationship between the long-tailed, limb-reduced morph and surface-dwelling microhabitat use, coding the long-tailed morph as 1 and all other morphotypes (short-tailed limb-reduced and non-limb-reduced) as 0. Obviously, our coding of diverse morphologies and ecologies with a limited number of character states involves considerable oversimplification.

We used Discrete to obtain the log-likelihood for the model of evolution in which these two characters evolve independently and that in which they evolve dependently (i.e., the rate at which one character changes between each state when the other character has a given state). The difference between the two likelihoods was compared using the likelihood-ratio test statistic ($-2\log_e[H_0/H_1]$), where H_0 represents the null model (independent evolution of the characters) and H_1 the alternative model (i.e., dependent evolution). Simulations (Pagel 1998) have shown that for this type of analysis the likelihood-ratio test statistic generally follows a chi-square distribution with four degrees of freedom (corresponding to the difference in the number of parameters between the independent and dependent models), particularly for large phylogenies with extensive character change. Analyses were performed using both equal and estimated branch lengths (see below). Species lacking ecological data were excluded from these analyses. Ecological data were available for 248 of the 261 species, and both branch length estimates and ecological data were available for 245 species.

Estimating Divergence Times

We estimated absolute ages of clades to better interpret results in terms of biogeography and potential interactions between lineages. Dates were estimated using penalized likelihood (Sanderson 2002), a modified molecular clock method

that does not assume rate homogeneity among lineages. Eleven fossil calibration points were utilized. To avoid problems associated with the rapid rate of evolution in the mitochondrial genome, we used the nuclear RAG-1 data of Townsend et al. (2004). Although Townsend et al. (2004) used two nuclear loci (c-mos and RAG-1), RAG-1 includes a much larger sample of characters (~2750 base pairs versus ~360) and many more taxa. We did not use the combined mitochondrial and nuclear DNA data for branch lengths because of the difficulty in applying appropriate gene-specific models using the likelihood criterion (Yang 1996; Brandley et al. 2005). We modified some relationships in the Townsend et al. RAG-1 tree to conform to their combined-data tree ('modified RAG-1 tree' hereafter). With one exception (the placement of acontines as the basal clade in Scincidae; Brandley et al. 2005), these relationships were poorly supported in Townsend et al.'s RAG-1 tree. Most weakly supported branches were very short, strongly suggesting that overall divergence dates would be very similar across different possible resolutions of weakly supported branches.

We used the modified RAG-1 tree and its estimated branch lengths in the program r8s (Sanderson 2003) to estimate divergence times using penalized likelihood, and we calculated 95% confidence intervals for these estimated ages using non-parametric bootstrapping. All r8s analyses utilized the truncated Newton (TN) algorithm and the additive rate penalty function. All analyses were reoptimized 10 times (set.num.restarts = 10) to avoid entrapment on a local solution optimum. The optimal smoothing parameter (230) was estimated using cross-validation. For the bootstrap analyses, we created 500 replicate datasets from the Townsend et al. RAG-1 data using PHYLIP 3.6b (Felsenstein 2004); positions excluded by Townsend et al. were excluded prior to bootstrapping. These datasets were imported into PAUP* version 4.0b10 (Swofford 2003) and branch lengths for the modified RAG-1 tree were reestimated for each bootstrap replicate using the general time reversible model with parameters for invariable sites and among-site rate variation for variable sites (GTR + I + Γ ; selected based on Townsend et al. 2004). These trees and branch lengths were then analyzed using r8s with the same parameters as the original analysis, and standard deviations of the estimated ages were calculated from the distribution of ages by using the 'profile' command in r8s. The standard deviations were then doubled to calculate 95% confidence intervals for the age distribution.

The age of the root node was fixed (one node must be fixed when using r8s), but all other calibration points were treated as minimum age constraints. We chose the following 11 calibration points, which correspond to fossils that can be unambiguously assigned to extant clades: (1) The most recent common ancestor (MRCA) of Rhynchocephalia (*Sphenodon*) and Squamata was fixed at 227 million years ago (Mya; latest date for Middle Triassic) corresponding to the earliest identified rhynchocephalian (Sues and Olson 1990). (2) 144 Mya (latest date for Upper Jurassic) for the MRCA of Iguania and Anguimorpha based on the fossil anguimorph *Parviraptor estesi* (Rieppel 1994). (3) 99 Mya (Albian–Cenomanian boundary) for the MRCA of Helodermatidae, Anguidae, and *Xenosaurus* based on the fossil helodermatid, *Primaderma* (Nydam 2000). (4) 99 Mya (Albian–Cenomanian boundary)

for the MRCA of Teiidae + Gymnophthalmidae based on the fossil teiid, *Bicuspidon* (Nydam and Cifelli 2002). (5) 98 Mya (Middle Cretaceous) for the MRCA of Amphisbaenia and Lacertidae based on the fossil amphisbaenian, *Hodzhakulia* (Gao 1997). (6) 93.5 Mya (latest date for the Cenomanian) for the MRCA of the snakes, *Agkistrodon* (Viperidae), *Cylindrophis* (Uropeltidae), and *Dinodon* (Colubridae) based on the fossil pythonid, *Pachyrachis* (Zaher and Rieppel 2002). (7) 65 Mya (latest date for Upper Cretaceous) for the MRCA of Scincidae, Cordylidae, and Xantusiidae based on various fossil scincid genera (Carroll 1988). (8) 65 Mya (latest date for Upper Cretaceous) for the MRCA of Varanidae + *Lanthanotus* based on the fossil varanid, *Palaeosaniwa* (Balsai 2001). (9) 65 Mya (latest date for Upper Cretaceous) for the MRCA of Anguidae and *Xenosaurus* based on the fossil anguid *Odaxosaurus* (Gilmore 1928). (10) 60.5 Mya (minimum age of Torrejonian) for the MRCA of Rhineuridae and remaining amphisbaenians based on the fossil rhineurid, *Plesiorhineura* (Sullivan 1985). (11) 33.7 Mya (latest date for Eocene) for the MRCA of *Agkistrodon* (Viperidae) and *Dinodon* (Colubridae) based on the earliest fossil colubrid (Rage et al. 1992).

Estimating Branch Lengths for Comparative Analyses

Comparative methods depend on accurate estimates of branch lengths as well as tree topology (e.g., Martins and Garland 1991; Pagel 1994). Estimated branch lengths from molecular datasets are often used to infer branch lengths for comparative analyses, given the assumption that these branch lengths reflect the relative ages of different clades and the expected amount of change on each branch for all characters (given a stochastic model of character change; Felsenstein 1985). In our study, many phylogenetic estimates within different clades and families were based on different genes, such that combining the molecular datasets themselves to estimate branch lengths would be problematic. As a partial solution to this problem, we used the estimated ages of families and other major clades (based on the penalized-likelihood analysis of RAG-1 described above) to calibrate a penalized-likelihood analysis within each clade. Thus, the branch lengths for our comparative analyses were based on estimates of the absolute age of each lineage, and time provided a common currency allowing us to combine branch length estimates across diverse molecular datasets.

For the penalized-likelihood analyses within each clade, we first obtained the original molecular datasets that were used to reconstruct the phylogeny within each clade. We then estimated branch lengths for each family-level tree using maximum likelihood (in PAUP*), utilizing the most general model (GTR + I + Γ ; generalized time reversible with parameters for invariant sites and a gamma distribution of rates among sites) with specific model parameters estimated separately for each dataset. In some cases, we made modifications to the original molecular datasets; for example, we combined the 12S and 16S sequence data within scincids from the studies of Reeder (2003), Whiting et al. (2003), Brandley et al. (2005), and Schmitz et al. (2005) to obtain comparable branch lengths across skinks. Molecular branch lengths were converted to a chronogram within each family using penal-

ized-likelihood analysis in r8s (see above). Optimal smoothing parameters were determined using cross-validation. Dates estimated for nodes in the squamate backbone analysis were used as fixed ages within families (e.g., age of Scincidae was fixed at 94.44 Mya), and other divergence dates within each family were estimated using penalized-likelihood. Estimated ages for families and other clades based on RAG-1 generally had 95% confidence intervals of ± 5 –12 million years (see Results), suggesting that these dates should be reasonable starting points for estimating ages of clades within families. In some cases, zero-length branches were estimated for a given clade, and these potential polytomies were resolved by adding a very small date estimate to the branch (10^{-6} million years), so that the relationships matched those in the original phylogenetic study. The chronograms from these individual analyses were then “pasted” onto the overall squamate chronogram to create a “superchronogram.” Although this superchronogram approach is not without potential problems, we consider it a useful alternative to simply assuming equal branch lengths. The superchronogram was also used to make crude comparisons of the ages of clades between families. Three taxa were excluded from the superchronogram because they were not included in within-clade analyses (*Trachyboa*, *Lepidophyma*, *Gonatodes*).

RESULTS

Morphometric Analyses

Morphometric and ecological data for the 261 sampled species are summarized in Appendix 1 (available online at <http://dx.doi.org/10.1554/05-328.1.s1>) and specimens examined are listed in Appendix 2 (available online at <http://dx.doi.org/10.1554/05-328.1.s2>). Scores for individual species on the three PCs are plotted in Figure 2 and listed in Appendix 1. The weights of each variable for each PC are shown in Table 1. PC1 contrasts variables associated with elongation (SVL, TL, with negative loadings) and variables associated with limb reduction (FLL, HLL, numbers of fingers and toes, with strong positive loadings). PC2 appears to reflect overall body size, with strong positive loadings for SVL and HL and smaller (negative and positive) loadings for other variables. PC3 primarily reflects relative tail length. PC1 explains 63.4% of the variance, PC2 explains 24.4% of the variance, and PC3 explains 8.6% of the variance.

Plotting PC1 against PC2 (Fig. 2A) shows that PC1 separates species into those with elongate, limb-reduced body form (snakelike) and those with well-developed limbs and more typical (lizardlike) body form. PC2 seems to separate species in both groups according to their overall body size. Several species (with relatively small body size) fall out as intermediate in terms of their body form on PC1. These include the bipedid amphisbaenians (which have elongate bodies, no hind limbs, but relatively well-developed forelimbs), as well as various skinks (e.g., *Plestiodon reynoldsi*) and gymnophthalmids (e.g., *Notobachia ablephara*, *Bachia*) with fore and hind limbs that are reduced but still present. Species with more negative loadings on PC1 include those that are completely lacking external limbs.

Plotting PC1 against PC3 (Fig. 2B) separates the limb-reduced species based mostly on their relative tail length.

Our survey of the ecological data (Appendix 1, available online) shows that sampled species with both high scores for PC3 (>1.0) and low scores for PC1 (<0.25) are all limb reduced but surface dwelling, including anguids (*Ophisaurus*, *Ophiodes*), pygopodids (*Delma*, *Pygopus*), gerrhosaurids (*Tetradactylus africanus* and *T. seps*), cordylids (*Chaemasaura anguina*), and one snake (*Epicrates striatus*). Thus, all of the long-tailed, limb-reduced species are surface dwellers. On average, the tail length is 2.3 times SVL in these species ($n = 24$), whereas tail length is 1.5 times the body length in species that are not limb reduced ($n = 146$).

Species with low scores for PC3 (<1.0) and PC1 (<0.25) have reduced limbs and shorter tails (average tail length is 0.5 SVL among the 91 species). Most of these species are burrowers (Appendix 1, available online), including all amphisbaenians, dibamids, some limb-reduced gymnophthalmids (*Bachia*, *Calyptommatus*, *Notobachia*), the pygopodid genus *Aprasia*, and numerous lineages of scincids (*Acontias*, *Acontophiops*, *Anomalopus*, some *Lerista*, *Melanoseps*, *Ophiomorus*, *Ophioscincus*, *Pygomeles*, some *Plestiodon*, *Scelotes*, *Sepsina*, *Typhlacontias*, *Typhlosaurus*, *Voeltzkowia*), and many snakes. However, not all of these shorter-tailed species are burrowers, including many snakes, an anguid (*Sauresia agasepsoides*), and some skinks (*Chalcides chalcides*, *Scelotes caffer*, *Feylinia*, *Paracontias*). Some of these nonburrowing lineages are only marginally limb reduced (i.e., *Sauresia agasepsoides*, *Chalcides chalcides*, *Scelotes caffer*) or not well characterized ecologically (*Feylinia*, *Paracontias*). In general, squamates with reduced limbs and short tails are burrowers, with the important exception of many snakes. However, even within snakes, the most short-tailed species (PC3 <-1.5) are burrowers (e.g., *Anilius*, *Cylindrophis*, *Leptotyphlops*, *Uropeltis*) and the most long-tailed species (PC3 > 0) are surface-dwellers (*Agkistrodon*, *Candoia*, *Casarea*, *Cereberus*, *Chondropython*, *Elaphe*, *Epicrates*, *Nerodia*, *Pareas*).

Phylogenetic Analyses

The phylogeny is summarized in Figures 3 and 4, along with estimates of divergence times (see Table 2 for confidence intervals on divergence date estimates). In general, likelihood and parsimony reconstructions give very similar results which are also similar for different branch length estimates (for likelihood) and different resolutions of weakly supported branches (for parsimony). We expect the most accurate reconstructions to be those based on maximum-likelihood analysis, treating the different ecomorphs as separate character states and using the estimated branch lengths. Ancestral state reconstructions using this combination of approaches are summarized in Figures 3 and 4.

Maximum likelihood.—Using the branch lengths estimated from the superchronogram and treating the two ecomorphs as separate character states shows 21–25 origins of the short-tailed morph from the fully limbed morph (with 1–4 reversals) and five origins of the long-tailed morph (Figs. 3, 4). The short-tailed morph gives rise to the long-tailed morph once (in snakes) and the long-tailed morph gives rise to the short-tailed morph once (in pygopodids). Treating body form

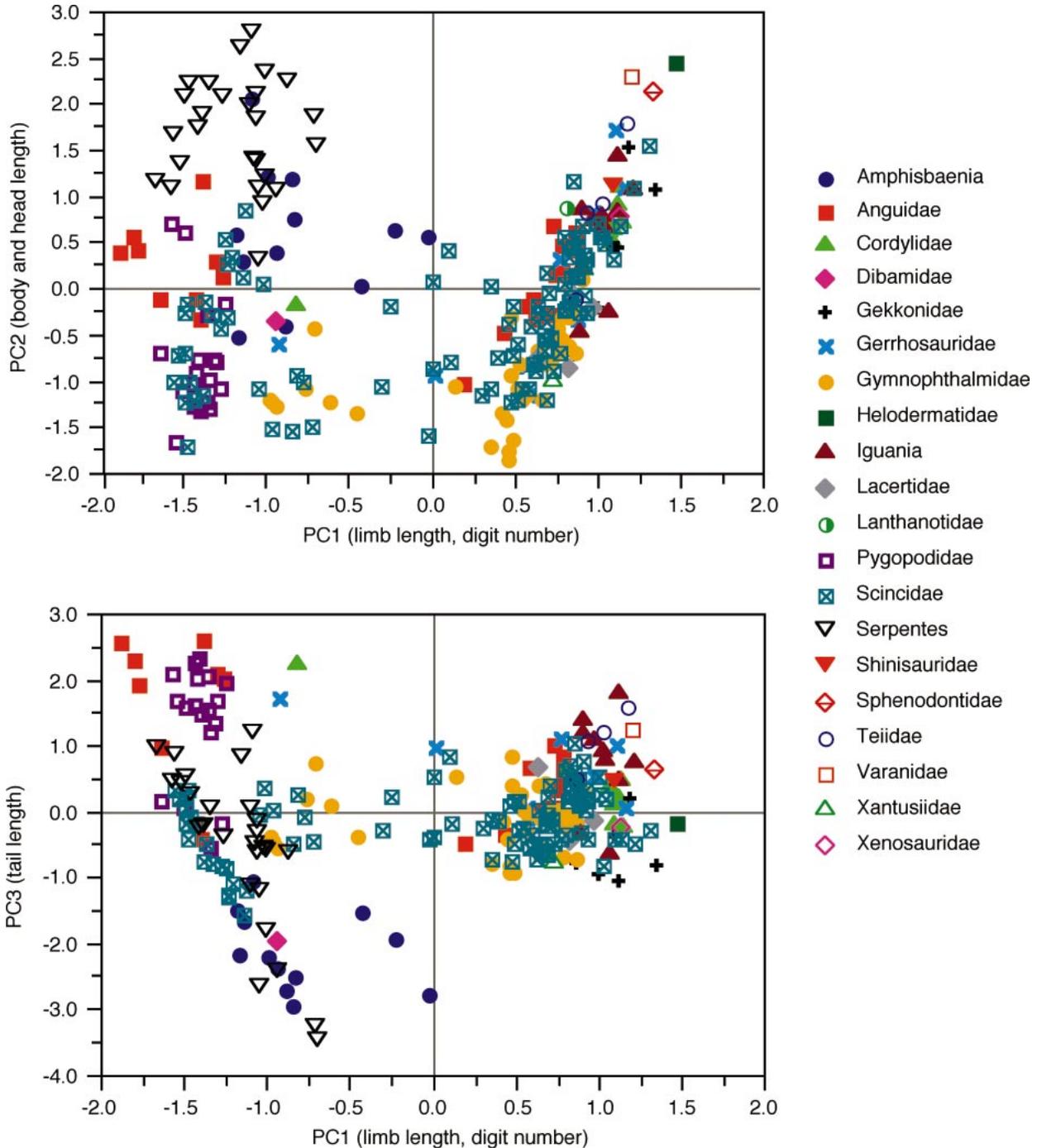


FIG. 2. Results of principal components analysis (PCA) of seven morphometric variables for 261 species of squamate reptiles, showing scores for individual species on (A) PC1 and PC2 and (B) PC1 and PC3. PC1 separates squamate species based largely on their degree of limb reduction, PC2 is related to overall body size, and PC3 reflects differences in relative tail length. The second graph (B) divides limb-reduced squamates into the short-tailed (mostly burrowing) and long-tailed (surface-dwelling) ecomorphs. Scores for individual species are listed in Appendix 1 (available online).

as binary shows 26–32 origins of the limb-reduced morph, with 1–6 reversals (ambiguous reconstructions for some nodes lead to ambiguity in the number of types of changes). Note that hypothesized reversals do not necessarily mean that limbs are reacquired, only that the morphology has become less elongated and/or limb reduced.

Using equal branch lengths and treating the morphs separately reveals 20–23 origins of the short-tailed morph from the fully limbed morph (with 1–5 reversals), and five origins of the long-tailed morph. The short-tailed morph gives rise to the long-tailed morph once (in snakes) and the long-tailed morph gives rise to the short-tailed morph once (in pygo-

TABLE 1. Orthogonal score weights from PCA of morphometric data for 261 squamate species.

	PC1	PC2	PC3
SVL	-0.006	0.730	-0.222
TL	-0.250	-0.280	1.035
HL	0.134	0.547	0.055
FLL	0.302	0.173	-0.188
HLL	0.213	0.015	0.056
Fingers	0.372	0.041	-0.208
Toes	0.224	-0.099	0.007

podids). Treating body form as binary reveals 23–28 origins of the limb-reduced morph, with 1–15 reversals.

Parsimony.—Treating the two limb-reduced morphs as separate states shows that the short-tailed morph evolved 19–23 times from the fully limbed morph (ranges indicate multiple equally parsimonious reconstructions), whereas the long-tailed morph evolved five times. The short-tailed morph gave rise to the long-tailed morph once (in snakes) and the long-tailed morph gave rise to the short-tailed morph once (in pygopodids). There are 1–5 reversals from the short-tailed morph to the fully limbed morph. Treating body form as a binary character indicates that the limb-reduced morph evolved 24–28 times among the sampled species, with 1–5 reversals.

Uncertainty in the phylogeny.—After collapsing branches on the supertree that are weakly supported in the original studies (Figs. 3 and 4), randomly resolving the polytomies, and reconstructing character evolution on these trees using parsimony, we found 21.0–28.5 origins of the general limb-reduced morphology (mean = 25.400), with 1.5–8.0 reversals (mean = 4.418). Treating the morphs as separate states shows 16–23.5 origins of the short-tailed morph (mean = 20.335), and five for the long-tailed morph. Again, the short-tailed morph gives rise to the long-tailed morph once (in snakes) and the long-tailed morph gives rise to the short-tailed morph once (in pygopodids). There are 1.5–8.0 reversals from the short-tailed morph to the fully limbed morph (mean = 4.428).

Correlation between ecology and morphology.—Maximum-likelihood analysis of the relationship between the short-tailed, limb-reduced morph and subterranean habitat use shows a highly significant relationship ($P < 0.005$), using both estimated and equal branch lengths. Using the estimated branch lengths, the likelihood for the independent model is -246.3782 (matching values for equal branch lengths in parentheses; -199.4922) and for the dependent model is -210.1237 (-163.6477), with a likelihood-ratio test statistic of 72.5091 (71.6890) and four degrees of freedom. The relationship between the long-tailed, limb-reduced morph and surface-dwelling habitat use is also highly significant ($P < 0.01$), with a likelihood for the independent model of -186.0388 (-142.6493), the dependent model of -177.8595 (-135.4937), and a likelihood-ratio test statistic of 16.3586 (14.3111).

Biogeographic and Community Context

Here we list the limb-reduced lineages that are present in each region and evaluate whether they evolved limb reduction in situ or else evolved this morphology elsewhere and dis-

persed into the region (summarized in Fig. 5). We exclude snakes and amphisbaenians, because these two clades have spread to all (snakes) or most (amphisbaenians) major continental regions. We also include limb-reduced lineages known to be present in each region but not included in our phylogenetic analysis (i.e., species lacking molecular and/or morphometric data). We interpret limb-reduced lineages that are confined to a given region and which have their more fully limbed relatives confined to that region also as representing in situ evolution of the limb-reduced morph in that region (e.g., cordylids are confined to sub-Saharan Africa, so the origin of a limb-reduced cordylid represents in situ evolution in Africa). We treated Madagascar, the West Indies, and Middle America as separate regions. We have assumed that limb-reduced congeners do not represent multiple origins of limb reduction, but we acknowledge that this assumption may be proven wrong by future phylogenetic analyses.

Sub-Saharan Africa.—In our sample of species, there are three clades of the short-tailed morph in sub-Saharan Africa, all of which are scincids. One clade consists of the genera *Feylinia*, *Melanoseps*, and *Typhlacontias*. This clade presumably represents in situ evolution of this morph. The second clade contains the genera *Acontias*, *Acontophiops*, and *Typhlosaurus*. The putative sister group of the second clade is the limb-reduced *Ophiomorus*, which occurs in North Africa, the Middle East, and South Asia. However, this is only weakly supported in the phylogenetic analysis of Brandley et al. (2005). Furthermore, given that most species in this clade occur in southern Africa and most *Ophiomorus* occur in the Middle East we consider it likely that this clade represents in situ evolution of the short-tailed morph. The third clade includes the genera *Scelotes* and *Sepsina*. The short-tailed morph may have evolved twice in this clade and there may be a reversal from the snakelike morphology to the lizardlike morphology. This clade also represents in situ evolution of this morph in southern Africa. The long-tailed morph has evolved at least twice in sub-Saharan Africa, once in the cordylid genus *Chaemasaura* and again within the gerrhosaurid genus *Tetradactylus*.

Another lineage of skinks (Scincidae), the genus *Eumecia*, likely represents independent evolution of the short-tailed morph in sub-Saharan Africa, but was not included in our phylogenetic analysis. *Eumecia* have elongate bodies, reduced limbs (three digits per limb), moderately short tails (50–60% body length), and are surface-dwelling grass swimmers (Spawls et al. 2002). The genus is classified in Lygosominae, and is thought to be closely related to other African lygosomines (particularly *Mabuya* and its relatives) which are limbed (Greer 1977). The genus *Scolecoseps* was not included in our phylogenetic analysis, but seemingly is a short-tailed burrower that is similar to *Melanoseps* (Spawls et al. 2002), and may represent the same origin of this morph.

Madagascar.—Based on our sampling of taxa, Madagascar includes three genera of limb-reduced skinks (*Paracontias*, *Voeltzkowia*, *Pygomeles*). All three represent in situ evolution in Madagascar, but it is possible that *Voeltzkowia* and *Pygomeles* are the result of a single episode of limb reduction (assuming subsequent reversal in some *Amphiglossus*). Thus, there have been at least two (but possibly three) origins of the short-tailed morph. There are several other taxa of limb-

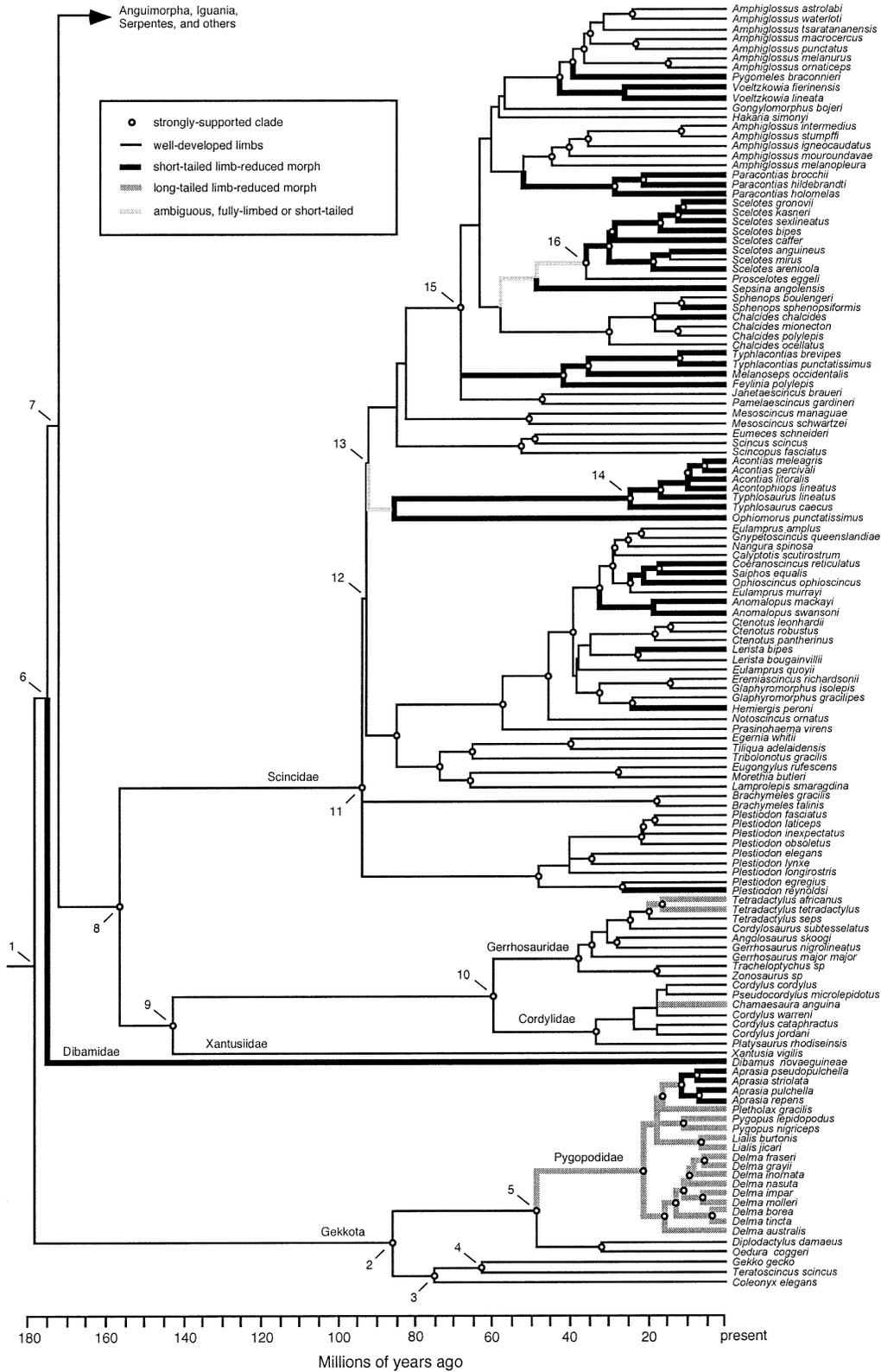


FIG. 3. Evolution of limb-reduced ecomorphs on a phylogenetic supertree for squamate reptiles. Character evolution is reconstructed using Pagel's (1999) maximum-likelihood method. A state was considered to be unambiguously reconstructed for a node if its log-likelihood was 2.0 units higher than the alternate state. Parsimony gives similar results, as does consideration of alternate resolutions of weakly-supported branches. Clades that are strongly supported in the original studies (Bayesian Pp ≥ 0.95; bootstrap ≥ 70%) are indicated

TABLE 2. Confidence intervals for ages of clades estimated from penalized-likelihood analysis of the RAG-1 gene. Numbered clades correspond to tree in Figures 3 and 4. All fossil calibration dates are constraints on the minimum age of a clade. An asterisk indicates that the confidence interval was truncated because the estimated date was younger than the fossil constraint.

Clade	Estimated age (mya)	95% Confidence interval (mya)	Fossil calibration date (mya)
1	178.7	5.5	
2	86.5	8.7	
3	75.7	9.1	
4	63.7	7.7	
5	51.9	7.1	
6	176.4	5.3	
7	173.9	5.4	
8	157.6	9.0	65.0
9	143.8	11.8	54.0
10	60.3	10.4	
11	94.4	8.2	
12	94.1	7.6	
13	93.3	9.0	
14	24.6	6.0	
15	66.3	9.8	
16	36.8	8.3	
17	168.3	5.3	
18	161.6	6.1	
19	126.6	12.0	98.0
20	119.1	12.0	60.5
21	48.6	7.9	
22	32.9	6.4	
23	99.0	0.0*	99.0
24	163.9	5.6	
25	131.1	7.0	
26	93.5	0.0*	93.5
27	35.6	4.8*	33.7
28	162.2	5.6	144.0
29	146.4	7.7	
30	78.5	7.1	
31	74.5	6.5	
32	118.0	6.7	
33	65.0	0.0*	65.0
34	99.0	1.4*	99.0
35	69.0	7.7	
36	67.9	7.8	
37	38.2	7.8	

reduced skinks that were not included in the phylogenetic and/or morphometric analyses (e.g., *Amphiglossus stylus*, *Androngo*, *Cryptoscincus*, *Pseudacontias*; Glaw and Vences 1994; Andreone and Greer 2002) which may represent additional in situ origins of the short-tailed morph on Madagascar (Schmitz et al. 2005).

Europe.—We consider “Europe” to include North Africa (including the Saharan Desert) as well as the Middle East, corresponding to the western end of the traditional Palearctic zoogeographic realm (Brown and Lomolino 1998). Europe contains three clades of “scincine” skinks which correspond to the short-tailed morph, corresponding to one *Chalcides*

(i.e., *C. chalcides*), one *Sphenops* (*S. sphenopsiformis*), and the genus *Ophiomorus*. Limb-reduction in *C. chalcides* and *S. sphenopsiformis* may represent the same event. Furthermore, *Ophiomorus* may belong to the clade including *Acontias*, *Acontophiops*, and *Typhlosaurus* of southern Africa. Thus, Europe contains at least one independent origin of the short-tailed morph, but possibly three. It is also possible that there have been additional origins of limb-reduced body form within *Chalcides* involving species that are not included in this analysis.

There is one clade of the long-tailed morph in Europe, the anguid genus *Ophisaurus* (also including the genus *Anguis*). It seems likely that this clade evolved in Europe and subsequently spread to Asia and the New World, but the optimization of the ancestral geographic area is ambiguous (J. J. Wiens, unpubl. data).

Asia.—Our sampling of species includes one clade of the short-tailed morph, the dibamid genus *Dibamus*. *Dibamus* is thought to be the sister taxon of the limb-reduced Middle American genus *Anelytropsis* (Greer 1985; Estes et al. 1988), making it unclear whether *Dibamus* represents in situ evolution of this ecomorph. However, it seems likely that there have been several cases of in situ evolution of the short-tailed morph among Asian scincids which were not included in our tree. One of these is in the “scincine” genus *Brachymeles*. Although the representatives of this Philippine genus in our analysis belong to the fully limbed morph, other species have reduced limbs or lack limbs entirely (Brown 1956), suggesting one or more origins of the short-tailed morph in this genus. Based on the molecular phylogenetic analysis of Honda et al. (2000), the lygosomine genera *Isopachys* and *Lygosoma* (specifically, *L. quadrupes*) likely represent independent in situ evolution of the short-tailed morph in southeast Asia. There are several other Asian skinks with reduced limbs (and relatively short tails) that are of uncertain placement which may also represent independent, in situ evolution of this morph, including *Barkudia* and *Sepsophis* of India, *Davewakeum* of Thailand, *Larutia* of Malaysia, *Leptoseps* of Thailand and Vietnam, and *Chalcidoseps* and *Nessia* of Sri Lanka. However, *Leptoseps* and *Larutia* may be closely related to *Isopachys* (Greer 1997).

Asia also has one clade of the long-tailed morph (several species of *Ophisaurus*), but it is uncertain whether this morph evolved in Asia or evolved elsewhere and subsequently spread into Asia.

Australia.—In our sampling of species, Australia has five clades of short-tailed burrowers, the pygopodid genus *Aprasia* (which is derived from long-tailed grass swimmers) and four lineages of lygosomine skinks (which evolved from the fully limbed morph). The four lygosomine lineages are: (1) *Anomalopus*, (2) the clade consisting of *Ophioscincus-Coeranoscincus-Saiphos*, (3) some *Hemiergis*, and (4) some *Ler-*

←

with an open circle. Branch lengths indicate absolute time, as estimated using penalized likelihood. Numbered clades correspond to Table 2, which provides confidence intervals on the estimated ages of these clades based on penalized-likelihood analysis of the RAG-1 data for a limited set of taxa. Note that three taxa are missing from this tree (because they lacked branch length information), which are included in analyses of all 261 taxa. These are *Trachyboa* (sister taxon of *Tropidophis* on our tree), *Gonatodes* (sister of *Gekko*), and *Lepidophyma* (sister of *Xantusia*). The placement of all three taxa is strongly supported.

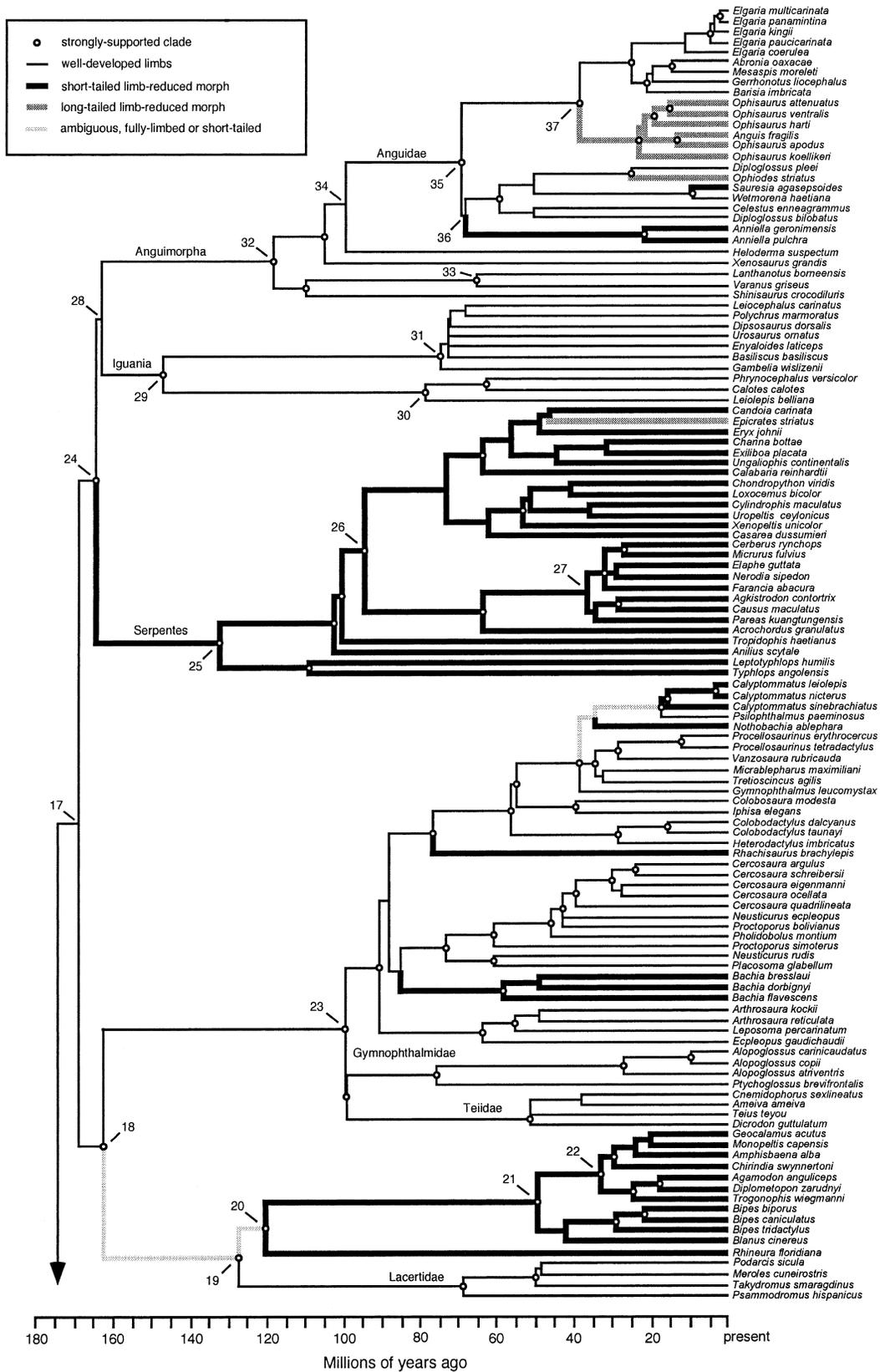


FIG. 4. Evolution of limb-reduced ecomorphs on a phylogenetic supertree for squamate reptiles and a continuation of Figure 3.

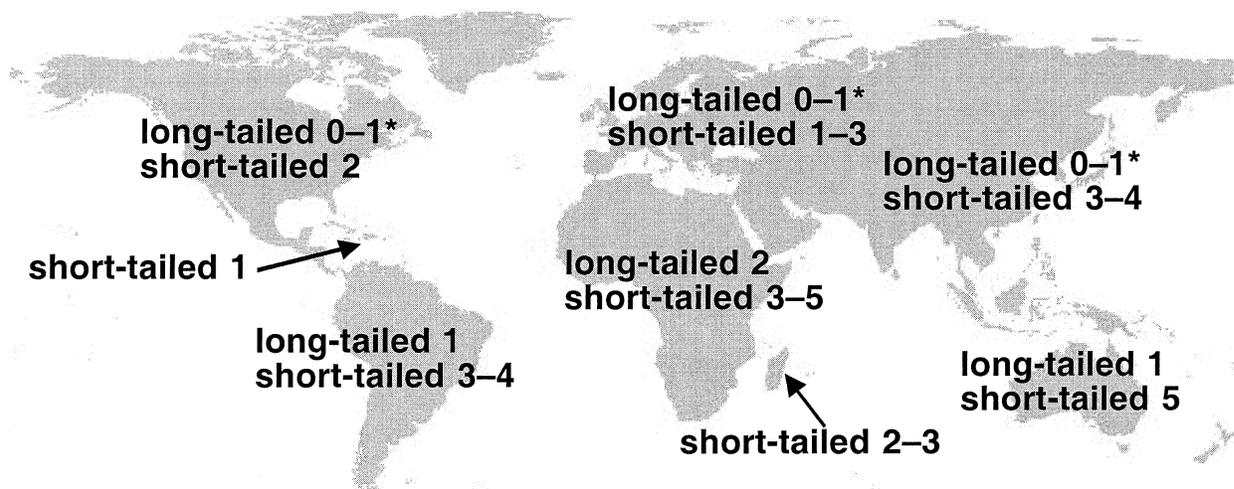


FIG. 5. Summary of the estimated number of origins of each of the ecomorphs of limb-reduced squamates in each major continental zoogeographic region (but also including Madagascar and the West Indies). Although the number of origins of each morph is similar on each continental region, different squamate clades evolve these morphs in different regions (e.g., all origins of the short-tailed burrowing morph occur within gymnophthalmids in South America but occur in lygosomine scincids and pygopodids in Australia). Ranges indicate uncertainty with respect to ancestral reconstructions and to estimates of the geographic origins of clades. The asterisk indicates that there is only a single lineage of the long-tailed morph (anguine anguids) which has dispersed among Asia, Europe, and North America (although it is unclear where this lineage originated). The number of origins of the short-tailed morph should be considered minimal estimates; these numbers may be considerably higher in some regions (e.g., Asia, Australia) as more detailed phylogenies for scincid lizards become available. Origins of the geographically widespread snakes and amphisbaenians are not included. Middle America seemingly lacks independent origins of either morph and is only questionably considered a separate biogeographic region, and is therefore not shown separately.

ista (includes full range from limbed to limbless body form). All five of these lineages appear to have evolved in situ. It is likely that additional origins of the short-tailed morph will be revealed as more densely sampled phylogenies of Australian skinks become available (T. W. Reeder, unpubl. data).

North America.—North America (continental U.S. and Canada) contains two clades of short-tailed burrowers, both of which evolved in situ. These are the ‘‘scincine’’ skink *Plestiodon reynoldsi* and the anguid *Anniella*. There is also one clade of long-tailed surface-dwellers (*Ophisaurus*), which is shared with Europe, Asia, and Middle America.

Middle America.—Middle America has two clades of short-tailed burrowers. One clade of short-tailed burrowers is the dibamid genus *Anelytropsis* which occurs in northeastern Mexico. Dibamids also occur in Asia. The other clade is the gymnophthalmid genus *Bachia* (*B. blairi*), which occurs only in Costa Rica and Panama. *Bachia* seemingly is derived from South America and did not evolve in situ. Middle America also contains one clade of long-tailed surface dwellers (the anguid genus *Ophisaurus*). This clade is shared with other continents and is likely derived from Eurasia. Thus, Middle America appears to have no lineages of limb-reduced squamates that clearly have evolved in situ. Furthermore, all three lineages of limb-reduced squamates have very restricted geographic ranges in Middle America.

West Indies.—The West Indies have one clade that is considered marginally limb-reduced, the anguid *Sauresia agaspsoides* of Hispaniola, which apparently became limb-reduced in situ on this island.

South America.—Our phylogeny suggest that there are at

least three clades of the short-tailed morph in South America, all of which represent in situ evolution and belong to the family Gymnophthalmidae: (1) *Bachia*; (2) *Calyptommatus-Notobachia*; and (3) *Rhachisaurus brachylepis*, which is considered to be marginally limb-reduced based on the PCA. *Calyptommatus* and *Notobachia* may represent separate origins of this morph, but there is some ambiguity in the ancestral-state reconstruction. South America has one clade of the long-tailed morph (the anguid genus *Ophiodes*) which represents in situ evolution.

Estimates of Divergence Times

The minimum divergence times for major clades of squamates (based on penalized likelihood analysis of RAG-1 data) are shown in Figures 3 and 4, and confidence intervals for the estimated dates are provided in Table 2. Although our divergence dates are only estimates of minimum age, the results suggest the possibility that most of the clades in which limb-reduction evolved arose after the breakup of Gondwanaland (completed ~100 Mya; Brown and Lomolino 1998), including the anguids (~70 Mya), pygopodids (~52 Mya), cordylids and gerrhosaurids (~61 Mya), gymnophthalmids (split of teiids and gymnophthalmids ~99 Mya), and scincids (~95 Mya). As expected, many of these lineages are isolated on single continents (pygopodids in Australia, gerrhosaurids and cordylids in Africa, gymnophthalmids in South America).

Although most limb-reduced squamates appear to have evolved in the last 100 million years, there are three exceptions; the amphisbaenians (~115 Mya), dibamids (stem orig-

inated ~165 Mya), and snakes (~125 Mya). Importantly, these three lineages are present on multiple continents, along with the anguine anguids, which apparently have more recently dispersed across the Northern Hemisphere.

DISCUSSION

Why Does Limb Reduction Evolve so Frequently in Squamate Reptiles?

Evolutionary biologists generally focus on how phenotypic traits originate (i.e., developmental and genetic mechanisms) and why they change in frequency within a population (i.e., the adaptive value of the trait). Although phylogenetic analyses can reveal how often a trait evolves within a clade, evolutionary biologists have generally not focused on trying to explain why a trait evolves a given number of times. In general, biogeographic isolation should be important in increasing the number of times a trait evolves, whereas competition may constrain the number of origins. In this paper we address why limb-reduced, snakelike morphologies have evolved so frequently in squamate reptiles. Synthesizing the results described above, we postulate that the repeated evolution of this trait is related to the combination of several key factors.

We found that there are two ecomorphs of limb-reduced squamates: long-tailed surface-dwellers and short-tailed burrowers (see also Camp 1923; Wiens and Slingluff 2001). However, there are exceptions to this general dichotomy, the most conspicuous being among snakes (see below). Both ecomorphs are present on all major continental regions. Thus, the “niche” or “adaptive zone” represented by these two ecomorphs is present in each region (we use “niche” in this ecomorphological context following Schluter 2000, p. 19). The potential adaptive value of the transition to snakelike morphology has not been well established, but it is generally assumed that snakelike body form facilitates locomotion underground and in dense grass (e.g., Gans 1975).

We postulate that a key reason why limb-reduced squamates have evolved so frequently is related to biogeographic isolation. Limb-reduced squamates have evolved independently in nearly every major continental region, and there has been relatively little dispersal of the limb-reduced morphs from one major continental region to another. Only four lineages of limb-reduced squamates (of ~27) are widespread on more than one continental region (amphisbaenians, anguine anguids, dibamids, snakes).

Furthermore, there appears to have been multiple origins of the burrowing morph within most regions (e.g., Africa, Asia, Australia, Europe, Madagascar, North America, South America). In many cases, this ecomorph seemingly evolved in allopatry on different parts of a given continent, further supporting the role of geographic isolation. For example, in North America, the burrowing ecotype has evolved twice, but these lineages are completely allopatric (*Anniella* occurs in California and Baja California and *Plestiodon reynoldsi* occurs in Florida). In South America, the three burrowing clades are also allopatric; *Bachia* occur mostly in northern South America south to central Brazil, the *Calyptommatus-Notobachia* clade is confined to the sand dunes of the São Francisco River in eastern Brazil, and *Rhachisaurus brachy-*

lepis occurs in the mountains of eastern (Minas Gerais) Brazil. In Europe (sensu stricto), there is no sympatry between the two lineages of the short-tailed ecomorph (*Chalcides* and *Ophiomorus*) and these lineages are generally allopatric throughout the region. On Madagascar, there are at least two limb-reduced lineages of skinks, and one of these (*Paracontias*) appears to be entirely allopatric with respect to the others (*Voeltzkowia*, *Pygomeles*; Glaw and Vences 1994). In Asia, some of the lineages of limb-reduced skinks are also allopatric, such as *Brachymeles* in the Philippines and *Isopachys* in Thailand and Myanmar (although *Lygosoma quadrupes* is widespread). Many other genera of limb-reduced Asian skinks also have restricted, allopatric geographic ranges even though their phylogenetic placement is uncertain, including *Larutia* in Malaysia, *Davewakeum* in Thailand, *Leptoseps* in northern Thailand and Vietnam, *Nessia* and *Chalcidoseps* on Sri Lanka, and *Barkudia* and *Sepsophis* in India. In Asia, Africa, and Australia, there may be some sympatry among limb-reduced burrowing lineages, and more detailed phylogenetic, biogeographic, and temporal information will be required to determine whether or not these morphs arose in allopatry or sympatry. However, Huey et al. (1974) commented that sympatry of fossorial lizard species is unusual, based on their studies in Australia and Africa.

Another factor that contributes to the multiple origins of limb reduction in squamates is that the two ecomorphs generally evolve independently of each other within a continent. In other words, the burrowing ecomorph generally evolves from fully limbed ancestors rather than from the surface-dwelling ecomorph, and the surface-dwelling ecomorph evolves from fully limbed species rather than burrowing ecomorph (the notable exception being pygopods, in which the surface-dwelling morph gives rise to the burrowing morph). In some ways, this is a very surprising result. One might assume that the easiest way to make a limb-reduced burrower is for a limb-reduced surface-dweller to simply undergo a shift in microhabitat usage, or vice versa. However, such changes are rare, relative to the number of independent origins of these morphs (i.e., only two changes between morphs, vs. ~20 origins of the burrowing ecomorph and five of the surface ecomorph). Thus, this dramatic change in body plan appears to be “easier” than a seemingly minor shift in microhabitat usage. However, the independent origins of the two ecomorphs in each region presumably is not the most important factor in explaining the repeated evolution of limb-reduced squamates, because there are so few origins of the surface dwelling ecomorph (although if the surface-dwelling ecomorph always gave rise to the burrowing ecomorph, there might be far fewer origins of limb reduction). Instead, biogeography seems to be the more important factor in driving repeated origins.

There are two main ways in which the biogeography may contribute to the multiple origins of limb-reduced squamates. First, if the habitats occupied by an ecomorph are disjunct and the ecomorph has limited ability to disperse between patches of this habitat, then the biogeographic separation of these habitats should drive multiple origins of that ecomorph (i.e., there could be as many origins of the ecomorph as there are disjunct patches of habitat). Minimally, the habitats that these ecomorphs occupy are disjunct between continents.

Furthermore, although some lineages disperse well (e.g., long-tailed anguine anguids), many lineages of the burrowing ecotype have very limited geographic distributions within a continent. For example, many of these burrowing lineages are confined to geographically restricted sandy habitats (e.g., *Anniella*, *Calyptommatius*, *Plestiodon reynoldsi*). Thus, the biogeographic separation of suitable habitats may lead to a large number of independent origins of an ecomorph without requiring any role for competition.

Second, the biogeographic separation of these lineages may prevent the niches or adaptive zones that these ecomorphs occupy from filling up on each continent. Thus, the evolution of a given ecomorph in Africa has no chance to preempt the evolution of this ecomorph in Asia or the New World by filling this niche. This hypothesis assumes that competition might potentially limit the origins of these morphs if there were more widespread dispersal. Because the importance of competition can be controversial, we discuss the evidence for and against this idea more fully in the next section.

The Role of Competition in Limiting the Number of Changes

Does the presence of one or more limb-reduced ecomorphs in a region “preempt” other lineages from evolving this same ecomorph? Although we cannot support or reject this hypothesis with certainty, there are several cases in which a lineage that evolves a given ecomorph in one region fails to do so in another region, specifically in regions where that ecomorph is represented by a different clade. In many of these cases, our crude estimates of divergence times suggest that the lineage that evolved the limb-reduced ecomorph was present in the region before the lineage that failed to evolve this morph, a pattern which is consistent with the hypothesis that the evolution of the ecomorph in one lineage may have preempted the other lineage from evolving this morph in the region. (1) Lygosomine skinks evolve the short-tailed morph in central Africa, Australia, and Asia, but not in southeastern North America, southern Africa, and Madagascar (where other skink lineages evolve the burrowing morph instead), or South America (where gymnophthalmids repeatedly evolve this morph). Lygosomine skinks are considered to be relatively recent immigrants to the New World (i.e., 7–20 Mya; Honda et al. 2003). The limb-reduced skink lineage in southeastern North America (*Plestiodon reynoldsi*) appears to be at least 25 million years old (Fig. 3), although we cannot determine when exactly limb reduction evolved along this branch. We have estimated dates for the origins of limb-reduced skink clades in southern Africa and Madagascar, but it is uncertain when lygosomines first arrived in these regions. The gymnophthalmids have been in South America for at least 95 million years, and the limb-reduced lineages of gymnophthalmids also appear to be relatively old (*Bachia* ~55 Mya; *Rhachisaurus* ~70 Mya; *Notobachia-Calyptommatius* clade ~30 Mya). There are actually two lineages of lygosomine skinks which independently invaded the Americas (*Scincella* and *Sphenomorphus* of the *Sphenomorphus* group; *Mabuya* of the *Mabuya* group; Honda et al. 2003), both of which have limb-reduced members in some regions of the Old World but not in the New World. (2) *Plestiodon* skinks

evolved the burrowing morph in southeastern but not western North America, where the anguid *Anniella* does instead. Our analyses of RAG-1 data using penalized likelihood suggest that *Anniella* may be considerably older than the *Plestiodon* skinks of western North America (~70 Mya vs. ~25 Mya; although not shown in Fig. 3, RAG-1 data and divergence date estimates were available for *Plestiodon skiltonianus*). (3) Diplogossine anguids did not become burrowers in South America, possibly because this role is occupied by gymnophthalmids instead, even though South American diploglossines are clearly capable of evolving snakelike body form (i.e., *Ophiodes*) and other anguids have become burrowers in western North America (*Anniella*). Our estimates of divergence times support the idea that gymnophthalmids are older than diploglossines (~95 Mya vs. 55 Mya), and the South American diploglossines may be considerably younger (~25 Mya) than the limb-reduced gymnophthalmid lineages (see above). (4) Gekkotan lizards are present nearly worldwide, but only one lineage has become limb reduced (pygopods in Australia). In theory, geckoes could evolve the limb-reduced morph on any continent (and all major regions have ground-dwelling geckoes), and it is possible that they have not done so because other lineages occupy this niche on other continents besides Australia. On the other hand, geckoes are relatively old and widespread, and may have been present in many regions before other limb-reduced ecomorphs arose.

The long-tailed, surface-dwelling ecomorph is present in North America, Middle America, Europe, and Asia, but seems to have evolved only once across this entire region and then dispersed (the anguine anguids). In contrast, the surface-dwelling ecotype has evolved independently in South America, Africa, and Australia (in diverse squamate lineages), where anguine anguids are absent. The presence of anguine anguids may have preempted other origins of the surface-dwelling ecotype in different regions of the Northern Hemisphere. Furthermore, cordylids and gerrhosaurids include many widespread, fully limbed lineages in Africa (both families) and Madagascar (gerrhosaurids only), and each includes a clade of the limb-reduced, surface-dwelling ecomorph. However, they fail to evolve the burrowing ecotype, possibly because this role was occupied by various lineages of skinks in Africa and Madagascar. Our estimates of divergence times suggest that cordylids and gerrhosaurids are each less than 35 million years old, whereas each of the limb-reduced lineages of skinks in Africa and Madagascar is at least 35 million years old (Fig. 4).

Overall, it seems that even though limb-reduced squamates have evolved many times, they have not evolved as many times as they could, given that many lineages become limb reduced in some regions but not others. This observation raises a novel question: why are there so few origins of limb reduction in squamates? One potential explanation is that when a given ecomorph has evolved in a region, additional transitions to this same ecomorph by other lineages may become less favorable (i.e., because the relevant “niche” is less “open”). Although this hypothesis posits a role for competition in limiting the number of evolutionary transitions, it does not assume that species of the same ecomorph cannot coexist in sympatry.

To be fair, there is also substantial evidence against the

importance of competition. First, there are two lineages of limb-reduced squamates that (at present) are globally widespread: snakes and amphisbaenians. This observation begs the question: why does the presence of these two lineages not prevent other limb-reduced squamates from evolving?

A second major line of evidence against competition is that multiple ecotypes have evolved on the same continental region (see above), even when snakes and amphisbaenians are excluded. In many cases when two ecomorphs have evolved on the same continent they have done so in allopatry (e.g., North America, South America). Nevertheless, in other cases (e.g., Africa, Australia) there seems to be broad-scale sympatry among lineages of the burrowing ecomorph, and it is possible that different lineages of the burrowing ecomorph evolved in sympatry. Similarly, the long-tailed ecomorph arose twice in Africa, and there is some sympatry among the two clades (e.g., Branch 1998). More fine-scale phylogenetic and temporal information may help determine whether these ecomorphs arose in sympatry or have become sympatric secondarily.

We acknowledge that we have not provided explicit statistical tests for the role of biogeographical isolation and competition in increasing and limiting the number of origins of limb-reduced body form in squamates. To our knowledge, such tests do not yet exist, and application of standard statistical methods is problematic because of the limited number of continental regions and trait origins and the lack of appropriate null models. Development of new statistical methods tailored to this general problem should be an important area for future research.

Genetic and Developmental Constraints

An important part of the explanation for why limb-reduction evolves repeatedly in squamates is that there is sufficient lability in the genetic and developmental systems to allow this change to occur in many different clades (Fig. 1). Considering the squamate phylogeny of Townsend et al. (2004), there is only one major clade of squamates which lacks limb-reduced representatives, the Iguania. Given the ubiquity of limb reduction among squamate lineages, it seems unlikely that there are widespread intrinsic constraints on the evolution of this trait in squamates. Nevertheless, the argument that there are genetic and developmental constraints can always be applied to increasingly smaller phylogenetic scales to explain the lack of limb reduction in specific lineages (but at the risk of seeming ad hoc).

Snakes and Squamate Ecomorphs

Among the various lineages of limb-reduced squamates, snakes are clearly the most species rich, with nearly 3000 species currently described (Zug et al. 2001). Snakes generally have elongate bodies and relatively short tails, and thus appear to belong to the burrowing ecomorph. Yet, snakes occur in a variety of habitats (e.g., terrestrial, arboreal, aquatic, fossorial), and many (if not most) snake species are surface dwellers rather than burrowers (Zug et al. 2001). The phylogeny offers a resolution to this paradox. The two most basal lineages of snakes are the scolecophidians (anomalepidids, leptotyphlopids, and typhlopids) and the anniliids, which are

both burrowers (Zug et al. 2001). Many other basal snake lineages are also burrowers (e.g., anomochilids, cylindrophids, loxocemids, uropeltids, xenopeltids, the pythonid *Calabaria*, the boiid *Eryx*). We suggest that snakes initially evolved as short-tailed burrowers but have maintained the body form of this ecomorph after reinvading surface habitats (see also Wiens and Slingluff 2001). Among our sample of species these two basal snake lineages have relatively short tails and all other snakes have relatively longer tails (with the exception of the burrowing *Cylindrophis* and *Uropeltis*; based on the ratio of SVL to TL).

Conclusions and Prospects

Evolutionary biologists have generally embraced the use of phylogenies to make inferences about character evolution, especially using the repeated origins of a trait to infer adaptation. However, there has been relatively little attention paid to explaining the specific number of origins of a trait, a topic which may require consideration of other factors in addition to function and development. In this paper, we address the question of why a dramatic change in body form has occurred so frequently (or infrequently) in squamate reptiles. We found that there are two ecomorphs of limb-reduced squamates, one of which seems to have evolved repeatedly on nearly every major continental region. We postulate that biogeographic isolation may help explain why there have been dozens of origins of snakelike morphologies, and we speculate that competition may help explain why there have been only dozens of origins and not hundreds (i.e., given that there are >3,000 species of limbed squamates, and that many clades that evolve a limb-reduced ecomorph in some regions fail to do so in other regions where the same ecomorph is present). Although these larger-scale factors obviously are complex and difficult to address, we anticipate that they will become increasingly tractable in the next few years. For example, we have provided a crude example of how combining phylogenetic, biogeographic, and temporal information can allow us to make inferences about the community context in which ecomorphs evolved on each continent (i.e., determining which lineage was present in a region first). This general approach for reconstructing the ancestral "ecological theater" for character evolution (in groups with limited fossil records) could be greatly improved simply by obtaining data for all the relevant taxa for the same gene(s).

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