Phylogenetic Insights on Evolutionary Novelties in Lizards and Snakes: Sex, Birth, Bodies, Niches, and Venom

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Abstract

Squamate reptiles (lizards and snakes) are a diverse clade in which there appears to have been multiple origins of many remarkable traits, including: (*a*) parthenogenetic reproduction, (*b*) viviparity, (*c*) snake-like, limb-reduced body form, (*d*) herbivory, and (*e*) venom. These repeated transitions make squamates an outstanding/excellent system for addressing many fundamental questions in evolutionary biology. For example, they are the only vertebrate group with true parthenogenesis (with at least 40 separate origins), they have more origins of viviparity than any other group of vertebrates, and they have undergone dramatic changes in body form (lizard-like to snake-like) dozens of times. New molecular phylogenies for squamates have overturned many traditional hypotheses and taxonomies based on morphology and are now revealing exciting new insights into the evolution of many of these traits at both higher and lower taxonomic levels. In this review, we summarize many of these new insights and outline important areas for future research.

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INTRODUCTION

Squamata (lizards and snakes) includes 9,004 species in 61 families (**Figure 1**), of which 3,339 species are snakes (Serpentes) and 5,486 are lizards (Uetz et al. 2011; **http://www. reptile-database.org/**). Squamates display striking diversity in almost every aspect of their biology (e.g., morphology, ecology, behavior, physiology; Greene 1997, Pianka & Vitt 2003). Because of this incredible diversity, and because many species are relatively easy to study, various groups of squamates have been used as model systems for studies in many biological subdisciplines, but especially in evolution and ecology. Many of these studies have been conducted in a phylogenetic context (e.g., Espinoza et al. 2004, Kearney et al. 2009, Losos 2009, Lynch 2009, Vitt et al. 2003, Wiens et al. 2006), which makes it possible to rigorously trace the evolution of these organisms' traits and their diversity.
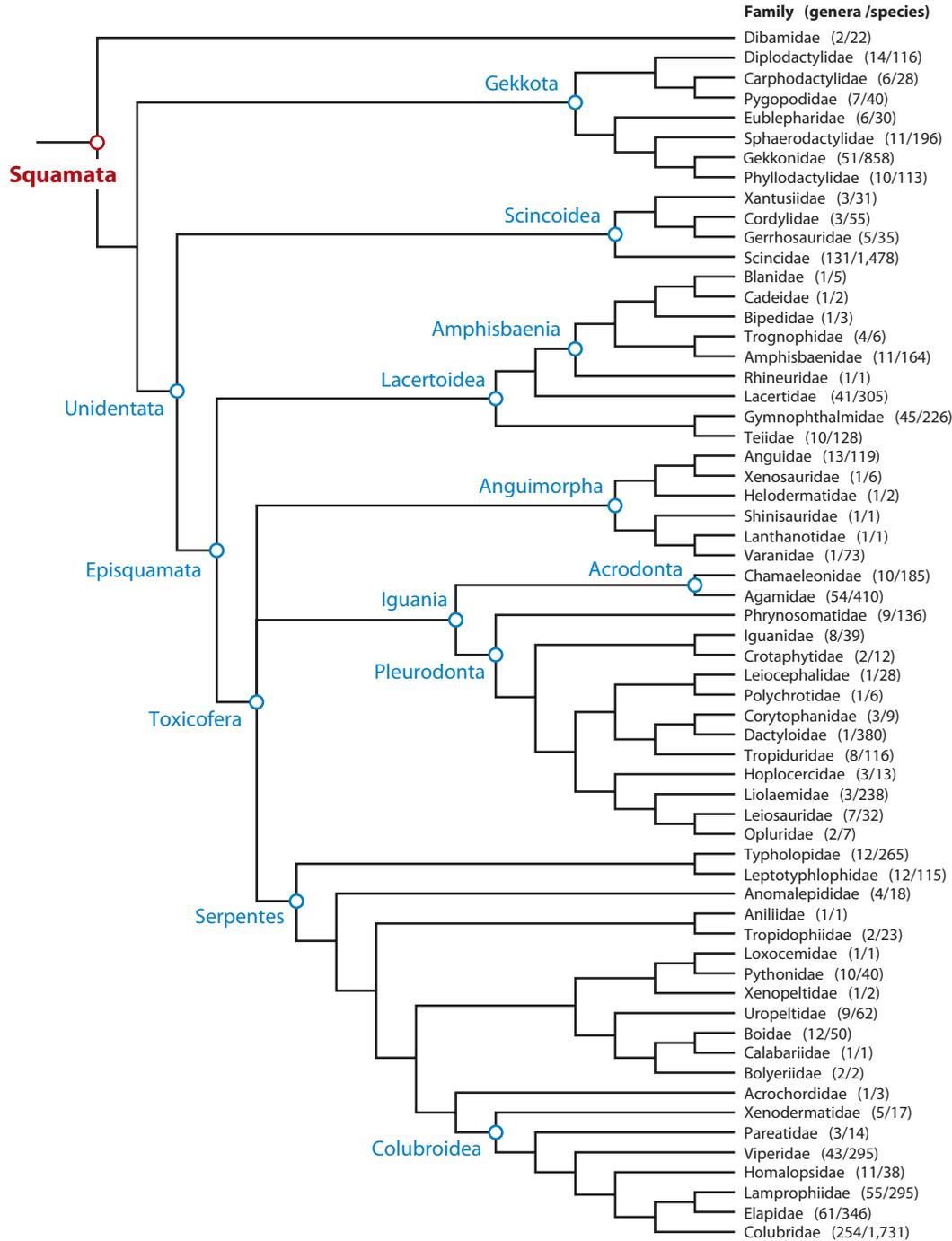
In the past decade, there has been an explosion of new phylogenies of squamates (at all taxonomic levels) and of comparative studies that use these phylogenies to address evolutionary questions. In many cases, these new molecular-based phylogenies have overturned many aspects of traditional morphology-based phylogenies and their corresponding classifications (e.g., Estes et al. 1988). These new phylogenies have also altered our perspectives on the evolution of many important traits. Here we review some of these new perspectives and show that squamates have much to teach us about many evolutionary questions of broad interest.

EVOLUTION OF PARTHENOGENESIS

Among vertebrates, true parthenogenesis, production of viable offspring in the absence of sperm, has evolved only in squamates (Kearney et al. 2009). In contrast, unknown constraints limit or prevent the origin of unisexual reproduction in other vertebrates. For example, unisexual reproduction in fishes and amphibians is sperm-dependent (it requires mating with a closely related sexual species), whereas it is completely unknown in any form in crocodilians, turtles, and mammals (Fujita & Moritz 2009). Squamates occasionally reproduce by facultative parthenogenesis (Booth et al. 2011), but there are also dozens of well-documented cases of species that consist entirely of parthenogenetic clones (obligate parthenogens). The most recent review of squamate parthenogenesis documents 40 independent origins of obligate parthenogenesis, which are distributed among one clade of snakes (Typhlopidae) and seven major clades of lizards

Figure 1

Phylogeny of squamate reptiles to the family level (including numbers of genera and species) based on a summary of recent phylogenetic analyses using slow-evolving nuclear loci and model-based methods (branch lengths are arbitrary, given that no studies of divergence times include all families). Higher-level relationships follow Townsend et al. (2004, figure 1), Vidal & Hedges (2009, figure 2), Wiens et al. (2010, figure 4), and others. We place Dibamidae as the sister taxon to all other squamates following Townsend et al. (2004), Vidal & Hedges (2009), and our analyses of 25 nuclear loci (D.G. Mulcahy, B.P. Noonan, T. Moss, T.M. Townsend, T.W. Reeder, J.W. Sites, Jr., & J.J. Wiens, in review). Relationships and taxonomy within some clades follow additional sources (Amphisbaenia, Vidal et al. 2008; Gekkota, Gamble et al. 2011; Serpentes, Wiens et al. 2008, but using Lamprophiidae for Atractaspididae and Boodontidae). Relationships within Pleurodonta are based on a new analysis of 29 nuclear loci (Townsend et al. 2011); we also follow that paper in recognizing Dactyloidae as the family for *Anolis* (given the nonmonophyly of Polychrotidae when *Anolis* is included) Numbers of species and genera generally follow Uetz et al. (2011). This tree represents a summary of recent estimates of squamate phylogeny, but we recognize that not all studies agree on all aspects of this phylogeny and that some parts may change as new data are added (e.g., relationships among basal snake lineages, phylogeny of pleurodonts). The three species of *Anomochilus* (Anomochilidae fide Uetz et al. 2011) are considered here to belong to *Cylindrophis* (Uropeltidae), following Gower et al. (2005). Following Lawson et al. (2004), we consider *Xenophidion* to be a separate family (Xenophiidae) that is most likely the sister group of Bolyeriidae (rather than considering this genus part of Tropidophiidae, as in Uetz et al. 2011), but we have not shown this in the tree illustrated here.

Family (genera /species)

Dibamidae (2/22)
Diplodactylidae (14/116)
Carphodactylidae (6/28)
Pygopodidae (7/40)
Eublepharidae (6/30)
Sphaerodactylidae (11/196)
Gekkonidae (51/858)
Phyllodactylidae (10/113)
Xantusiidae (3/31)
Cordylidae (3/55)
Gerrhosauridae (5/35)
Scincidae (131/1,478)
Blanidae (1/5)
Cadeidae (1/2)
Bipedidae (1/3)
Trognophidae (4/6)
Amphisbaenidae (11/164)
Rhineuridae (1/1)
Lacertidae (41/305)
Gymnophthalmidae (45/226)
Teiidae (10/128)
Anguidae (13/119)
Xenosauridae (1/6)
Helodermatidae (1/2)
Shinisauridae (1/1)
Lanthanotidae (1/1)
Varanidae (1/73)
Chamaeleonidae (10/185)
Agamidae (54/410)
Phrynosomatidae (9/136)
Iguanidae (8/39)
Crotaphytidae (2/12)
Leiocephalidae (1/28)
Polychrotidae (1/6)
Corytophanidae (3/9)
Dactyloidae (1/380)
Tropiduridae (8/116)
Hoplocercidae (3/13)
Liolaemidae (3/238)
Leiosauridae (7/32)
Opluridae (2/7)
Typhlopidae (12/265)
Leptotyphlophidae (12/115)
Anomalepididae (4/18)
Aniliidae (1/1)
Tropidophiidae (2/23)
Loxocemidae (1/1)
Pythonidae (10/40)
Xenopeltidae (1/2)
Uropeltidae (9/62)
Boidae (12/50)
Calabariidae (1/1)
Bolyeriidae (2/2)
Acrochordidae (1/3)
Xenodermatidae (5/17)
Pareatidae (3/14)
Viperidae (43/295)
Homalopsidae (11/38)
Lamprophiidae (55/295)
Elapidae (61/346)
Colubridae (254/1,731)

Squamata
Gekkota
Scincoidea
Amphisbaenia
Lacertoidea
Unidentata
Anguimorpha
Acrodonta
Episquamata
Iguania
Pleurodonta
Toxicofera
Serpentes
Colubroidea

(Kearney et al. 2009, table 21.1). The number of these origins within clades is decidedly nonrandom with respect to their species richness; parthenogenesis has evolved more frequently than expected by chance in the species-poor Lacertidae, Teiidae, and Varanidae, whereas it is rare or unknown in the species-rich Colubridae, Iguania, and Scincidae (Kearney et al. 2009; **Figure 1**). These nonrandom origins are intriguing, but perhaps the central question about squamate parthenogenesis is: What factor(s) permitted squamates alone to evolve a completely sperm-independent mode of all-female reproduction?

Insights into this central question can be gained from studying the genetic and ecological attributes of these parthenogenetic lineages. First, the origin of parthenogenesis is usually associated with a historical hybridization event between closely related, sexual species: species A hybridizes with species B to produce a self-maintaining unisexual (female) parthenogen. In some cases, a second hybridization event between the parthenogen and another male produces a stabilized $3n$ parthenogen. The second hybridization event may involve a male from one of the same species involved in the original origin of the parthenogen, such that the offspring of this cross are triploid for two genomes of one ancestor and one of the other ($2n+n$). In other cases, a third sexual species may contribute another unique haploid genome to produce a $3n$ ($n+n+n$) species. No viable populations with more than $3n$ ploidy are known in nature (Kearney et al. 2009), but self-sustaining all-female $4n$ populations have now been synthesized in lab crosses between the obligate $3n$ parthenogen *Aspidoscelis exsanguis* and males of the sexual species *Aspidoscelis inornata* (Lutes et al. 2011). The only well-documented exception to the hybrid-origin model is found in the Central American genus *Lepidophyma* (Xantusiidae), in which multiple lines of evidence suggest two spontaneous origins of parthenogenesis without hybridization (Sinclair et al. 2010).

Two other features characterize most parthenogenetic squamate lineages. One feature is a high level of genetic diversity. Hybrid origins result in high levels of intraindividual genetic heterozygosity (Kearney et al. 2009). Furthermore, for complexes with multiple origins (i.e., groups of similar clones that originated via several hybridization events), high between-clone genetic diversity exists. Complexes with multiple origins are well documented for many groups, including North American *Aspidoscelis* (Teiidae), Asian *Darevskia* (Lacertidae), and Australian *Heteronotia* (Gekkota) (Fujita & Moritz 2009). In these complexes, hybridization events between different ancestral lineages (different combinations of sexual species) seem to provide independent evolutionary "recombination experiments" and sometimes lead to multiple ploidy levels ($2n$ and $3n$).

The other feature is more ecological. Relative to their closest sexual relatives, parthenogenetic species are usually restricted to habitats that tend to be more disturbed, ecotonal (i.e., a transition between two habitat types), and/or drier and that occur at high latitudes/altitudes or on islands (Kearney 2005). Despite these ecological differences, coexistence between sexual and asexual relatives is common in several groups, and they may overlap on some important niche axes (e.g., diet, habitat use). Most intriguingly, whereas parthenogens may differ from their close relatives in physiology, endurance, thermal behavior, and parasite loads, these differences have no consistent direction (Kearney et al. 2009). Thus, on the basis of studies so far, squamates do not exhibit any obvious cost to asexuality (or conversely, any benefit to sexual reproduction). The absence of strong costs might also help to explain the widespread occurrence of asexuality in squamates as well as its persistence in some lineages.

With respect to the issue of constraints on the origins of unisexual reproduction, two are hypothesized to prevent the origin of true parthenogenesis in other vertebrates (constraints that squamates appear to have overcome). The first hypothesized constraint is the requirement for sperm to initiate egg development and embryogenesis. The second hypothesized constraint involves the meiotic processes that reduce ploidy levels prior to gametogenesis (Fujita & Moritz 2009, Kearney et al. 2009). Thus far, data are available only to evaluate the second

hypothesis in squamates. Modification of meiosis to produce unreduced ova may occur in two ways. First, a process of chromosomal duplication without cytokinesis (called premeiotic endomitosis or endoreplication) doubles the ploidy level (to $4n$ or $6n$). If this process is followed by meiosis, the somatic ploidy level ($2n$ or $3n$) is then restored (Cuellar 1971). Second, meiosis may proceed normally and then be followed by fusion of two haploid cells via many processes. Some of these processes may eliminate the "fixed heterozygosity" signature of a hybrid origin. For example, in theory, if crossing over between nonsister chromatids results in reciprocal exchange of alternative alleles and production of recombinant chromatids, then, over time, elimination of alleles in discarded polar bodies should erase the fixed heterozygosity signature of the hybrid origin (see details in Asher 1970, Neaves & Baumann 2011). Few of these processes have actually been documented, but Lutes et al. (2010) verified in *Aspidoscelis tesselata* (Teiidae) meiosis with twice the ploidy level and formation of bivalents with chiasmata between sister chromosomes, thereby providing a mechanism for the maintenance of a fixed ploidy level and fixed genetic heterozygosity.

Another unresolved issue in the evolution of squamate parthenogenesis is the association of parthenogenesis with historical hybridization. Specifically, was hybridization actually responsible for the origin of parthenogenesis, or did parthenogenesis evolve as a means to stabilize heterozygous hybrid genotypes that enhance fitness in selected environments (Kearney et al. 2009)? Whether hybridization actually causes parthenogenesis is presently unclear; if the two are not linked, then parthenogenesis must evolve simultaneously with the hybridization event, which seems unlikely. An alternative that does not require simultaneous origins hypothesizes that hybridization both induces parthenogenesis and directly confers traits on the new parthenogenetic lineage that aid in its establishment and maintenance. In this case, hybridization is unlikely to be the sole pathway to parthenogenesis (Sinclair et al. 2010) but instead the pathway in which the parthenogens are most likely to persist, given that hybridization leads to the fitness advantages of high within-clone heterozygosity, novel phenotypes, and the potential to generate among-clone diversity (Kearney et al. 2009). Thus, hybridization may offset the potential disadvantages of the loss of genetic variation that are typically associated with the loss of sex.

The hybrid nature of parthenogenetic genomes provide several advantages for genomics studies (Fujita & Moritz 2009), including: (*a*) fleeting glimpses of stages of tandem repeat evolution in mitochondrial genomes (some of which possess tandem duplications up to ∼10 kb in size) that are rarely seen in sexual systems, (*b*) intergenomic recombination via biased gene conversion (Hillis et al. 1991), and (*c*) germline ameiotic recombination that may eliminate heterozygosity (Asher 1970; but see Lutes et al. 2010). Many exciting issues remain understudied but are becoming accessible with emerging genomic technologies, including interlocus incompatibilities (hybrid genomes), restricted recombination and accumulation of deleterious alleles (Muller's ratchet; Muller 1964), intralocus effects (hybrid vigor versus hybrid dysgenesis), and reciprocal interactions between mitochondrial versus nuclear genomes in a mixed background. The influence of genetic (mutation) versus epigenetic (methylation) mechanisms on differential gene expression and phenotypic diversity may be most accessible to further study in these fixed heterozygous genotypes of hybrid origin (Fujita & Moritz 2009).

Parthenogenetic squamates may offer insights into many other important research areas. For example, the nonrandom phylogenetic distribution of parthenogenesis offers possibilities to look for shared similarities between sexual and asexual sister clades as well as differences between them that may have predisposed the latter to abandon sex. Further study of meiotic processes in the two species of *Lepidophyma* for which independent nonhybrid origins are inferred (Sinclair et al. 2010) should be especially rewarding with respect to hypothesized mechanisms of germline recombination and the elimination of heterozygosity (Neaves & Baumann 2011).

EVOLUTION OF VIVIPARITY

Viviparity (giving birth to live young instead of laying eggs) has been studied for more than a century in squamates (Blackburn 2006). In the 1970s, life history theory (Tinkle & Gibbons 1977, Tinkle et al. 1970) framed the question of why viviparity evolves in terms of selective costs and benefits, a major conceptual advance. More recently, phylogenetic studies have permitted estimates of the number, timing, and correlates of its origins (Lynch 2009, Lynch & Wagner 2010, Schulte & Moreno-Roark 2010, Shine 1985). Current research on viviparity in squamates now spans virtually all disciplines of biology, including anatomical and physiological aspects of maternal/fetal development, molecular details of fetal nutrition and maternal-fetal gas exchange, endocrinology of ovarian function and parturition, and studies of behavioral, ecophysiological, and evolutionary aspects of viviparity (Thompson & Blackburn 2006).

Viviparity appears to have evolved a minimum of 108 times in squamate reptiles (76% of the 141 estimated origins in vertebrates; Blackburn 2005), and viviparity occurs in ~20% of all squamate species. Most of these origins are in lizards, but up to 30 may occur in snakes (Greene 1997). The lizard family Scincidae seems to have the largest number of origins, at least on the basis of premolecular phylogenies and taxonomies (Shine 1985). Furthermore, in contrast to fishes and mammals, viviparity is variable at relatively low taxonomic levels in multiple clades (65 cases of variation in parity modes within genera, and several species are characterized by both parity modes) and appears to have evolved in relatively recent geological time (Schulte & Moreno-Roark 2010, Stewart et al. 2004).

Given the rarity of the transition to viviparity in other vertebrates, the frequency of this transition in squamates suggests that the genetic basis of the transition may be relatively simple and that similar selective pressures may be involved (Blackburn 2006). The physiological details of viviparity in squamates also suggest that this may be a relatively easy transition. The vast majority of viviparous squamates are characterized by simple (type I) placentation (lecithotrophic) in which shell-less eggs are retained in utero, and embryos are sustained largely or wholly by a large nutrient-rich yolk, with little placental nutrient exchange. Thompson & Speake (2006) noted that in lizards the evolution of viviparity requires: (*a*) a change in timing of expulsion of the conceptus, (*b*) a reduction of eggshell thickness to permit O_2 uptake, (*c*) a possible increase in vascular support of the uterus (permitting embryonic O_2 uptake), and (*d*) provisioning of water to the egg. Importantly, none of these steps requires new structures or processes, only upregulation (increased vascularity), downregulation (reduced eggshell), or change in timing (expulsion of conceptus) of existing processes.

However, more complex modes of placental development have now been documented in several species of skinks. Some skinks have tiny ovulated eggs that are virtually yolk-less (microlecithal), such that embryos require additional nutrients from the mother during development. For example, viviparous New World skinks of the genus *Mabuya* are characterized by extreme microlecithal eggs and novel morphological specializations associated with the evolution of complex placentas (Blackburn et al. 1984). Such placentas are unknown in other squamates but are convergent with those of mammals (Blackburn et al. 1984). These more complex types have been documented in only four or five lineages of lizards (all in the clade Scincidae, including South American species of the genus *Mabuya* and African species of the genera *Eumecia* and *Trachylepis*; Blackburn & Flemming 2009).

Understanding the repeated origins of viviparity in squamates requires understanding the selective pressures that favor its origin and maintenance. Many hypotheses for the evolution of viviparity focus on factors that kill eggs in the nest (e.g., cold climates, predation of clutch), but the same factors would presumably not be detrimental to eggs carried in utero. Natural

selection on factors to reduce egg mortality might also account for transitional stages, such as prolonged uterine retention of eggs by females, that presumably lead to viviparity (Shine 1985). Shine's (1985) early review examined alternative hypotheses and the empirical support for each hypothesis in terms of the presumed benefits of viviparity (increased offspring survivorship) versus the physiological costs to females associated with prolonged egg retention. Predictions were tested by identifying squamate clades in which viviparity had evolved and then looking for ecological correlates predicted by life-history theory.

Among the hypotheses for the origin of viviparity examined by Shine (1985), the "cold climate hypothesis" (CCH; Tinkle & Gibbons 1977) was the best supported and today remains the preferred working hypothesis for three reasons. First, uterine retention of eggs, coupled with female behavioral thermoregulation, accelerates embryonic development in cold climates because the female's body can be warmer than soil (reducing mortality from factors that kill eggs in the nest). Soil temperatures at high elevations and latitudes might be so low as to be lethal to eggs in a nest, or if not, may slow development such that hatchlings emerge so close to autumn frosts that survivorship is compromised (Shine 1985). This argument might also apply to very hot climates (uterine retention protects eggs from lethally high temperatures). Second, temperature clines are common (reflecting latitude or elevation), whereas variation in other variables (e.g., nest predation) may be more stochastic, so it is easier to envision selection favoring transitional stages along such temperature gradients. Third, the CCH hypothesis is consistent with the idea that intermediate stages of the oviparity-viviparity transition are adaptive (Blackburn 2006).

Shine (1995) emphasized that the CCH was a special case of a more general maternal manipulation (MM) hypothesis: females can enhance fitness-related phenotypic attributes in offspring by manipulating thermal conditions during embryogenesis. This claim applies to any environment in which viviparous females maintain body temperatures different from (not necessarily warmer than) those available in natural nests. For example, in tropical habitats females may maintain body temperatures more stable than those of the external environment (Shine 2004). Somewhat paradoxically, Tinkle & Gibbons (1977) noted that most viviparous squamates are tropical, and if the CCH explains the initial origins of viviparity, one must then ask if the spread of this mode into tropical regions was driven by the same factors as those favoring its origin or by different factors (e.g., exaptations).

Exaptationist explanations posit that viviparous species are "preadapted" to exploit new environments [e.g., marine (sea snakes), habitats where nest sites are rare] such that high numbers of viviparous species in the tropics might reflect selective advantages quite different from the factors that drove the origin of viviparity (Webb et al. 2006). Thus, hypotheses based on exaptation can be hard to test.

In contrast, the MM hypothesis makes several testable predictions (Shine 1995), including that: (*a*) gravid females should modify their thermoregulatory behavior to maintain more stable body temperatures than nongravid females, (*b*) these more stable body temperatures should modify phenotypic traits of the progeny, and (*c*) these phenotypic traits should enhance offspring fitness. Webb et al. (2006) tested these predictions in the tropical Australian elapid snake *Acanthophis praelongus* and showed that gravid females maintained the same mean body temperatures but less variable temperatures compared with nongravid snakes (thereby enhancing thermal precision in the former). Furthermore, neonates of gravid females maintained under less variable lab-controlled thermal gradients were born earlier and at larger body sizes than neonates born to females maintained under more variable maternal thermal regimes. This was the first study to show that differences in thermal variance during gestation can affect offspring phenotypes in viviparous reptiles. In addition, young snakes recaptured on study plots were larger than snakes not recaptured, possibly reflecting differences in survival associated with these maternal thermal

environments. The authors emphasize that although the magnitude of fitness advantages via uterine retention of eggs in cold climates (i.e., a large difference in thermal means/variances between retained versus oviposited eggs) may explain the origins of viviparity, similar thermal advantages extended to tropical environments could explain the radiations of viviparous groups in tropical environments.

Both early (Shine 1985) and recent (Blackburn 2005) reviews have inferred the number of origins of viviparity under the assumptions that: (*a*) hypotheses of squamate phylogeny are not in serious error and (*b*) oviparity cannot re-evolve from viviparity (Dollo's law). However, one study (Lee & Shine 1998) suggested that reversals from viviparity to oviparity may be possible, but infrequent. A recent phylogenetic study (Lynch & Wagner 2010) provides the first unequivocal evidence for reversal of parity modes in squamates. A well-resolved phylogeny recovered the oviparous sand boa *Eryx jayakari* as deeply nested within an otherwise all-viviparous clade (boas and relatives) and suggested that this reversal occurred 60 million years after the origin of viviparity.

Other recent studies have combined analyses of the evolution of viviparity with time-calibrated phylogenies to make intriguing inferences about viviparity and past climate change. For example, Schulte & Moreno-Roark (2010) analyzed the timing of the evolution of viviparity across many lizard clades and found that viviparity typically evolved well before the glacial cycles and climatic cooling of the Pliocene period. Lynch (2009) evaluated the effect of parity mode on diversification rates in vipers (Viperidae), where diversification rate reflects the balance of speciation and extinction over time. Estimates of diversification rates revealed a dramatic (∼fourfold) decrease in rates in oviparous clades and a simultaneous increase (1.4-fold) in viviparous clades. This shift in rates seemingly occurred in the mid-Oligocene, a time considered to be a greenhouse-to-icehouse transition in global climate (Zanazzi et al. 2007). Taken together, these patterns suggest that the evolution of viviparity may have buffered vipers from the negative impacts of cooling climate.

EVOLUTION OF BODY FORM

Squamates have evolved an incredible diversity of body forms, and they offer an exciting and unusually tractable system for studying the origins of major changes in body plan, a key question in evolutionary biology. The diversity of squamate body forms may be most apparent when contrasting the typical four-limbed, pentadactyl, lizard-like morphology and the elongate, limbless, snake-like body form. This dramatic transition has occurred at least 25 times (Wiens et al. 2006), and in some cases it has occurred repeatedly among closely related species (e.g., the skink genus *Lerista*; Skinner et al. 2008). In contrast, outside of squamates, such dramatic changes in body form generally occur only rarely and in the distant past (e.g., turtles, whales). Here, we summarize recent phylogenetic studies on the evolution of snake-like body form in squamates and highlight areas in need of further study.

An important aspect of the evolution of snake-like body form is that there are actually two distinct snake-like ecomorphs (**Figure 2**; e.g., Wiens et al. 2006). One, the long-tailed surface dweller, often occurs in grassy habitats, and the majority of the total length consists of an elongate tail. This ecomorph appears to have evolved five times (in gekkonids, cordylids, gerrhosaurids, and anguids; **Figure 1**). In contrast, members of the short-tailed burrowing morph spend most of their lives underground, and their total length is dominated by the trunk. This ecomorph has evolved >20 times (Wiens et al. 2006), with most origins in Scincidae (but also including amphisbaenians, dibamids, and some anguids, pygopodids, and gymnophthalmids). However, this ecomorph may have evolved many more times, and accurately estimating this number will depend largely on acquiring more detailed phylogenies within scincids (e.g., Skinner et al. 2008). Intriguingly, most snakes resemble the short-tailed burrowing ecomorph even though most extant
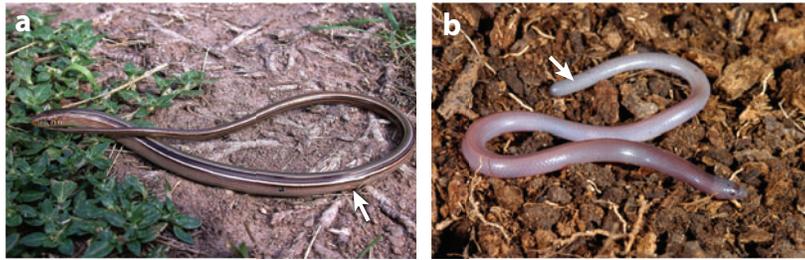
Figure 2

Representatives of the two ecomorphs of snake-like squamates; white arrows indicate the junction of tail and trunk. The two ecomorphs are superficially similar but differ in their body proportions and habitat (Wiens et al. 2006). (*a*) The long-tailed surface-dwelling ecomorph (*Ophiodes striatus*: Anguidae; from Argentina), in which the majority of the total length consists of the tail. (*b*) The short-tailed burrowing ecomorph (*Anelytropsis papillosus*: Dibamidae; from Mexico), in which the majority of the total length consists of the trunk. Photo credits: (*a*) Robert Espinoza, (*b*) Ted Townsend.

snakes are surface dwellers (Vitt & Caldwell 2009). Phylogenies within snakes (e.g., Slowinski & Lawson 2002, Wiens et al. 2008) suggest that many of the earliest snake lineages are burrowers (e.g., leptotyphlopids, typhlopids, anomalepidids, aniliids, loxocemids, uropeltids) (**Figure 1**; Vitt & Caldwell 2009). Thus, it appears that snakes were initially burrowers with the corresponding short-tailed morphology but subsequently radiated on the surface while retaining the same general morphology (Wiens et al. 2006).

Analyses of body-form evolution on time-calibrated phylogenies have revealed several intriguing results. First, a correlated set of changes is involved in the transition from the primitive to the snake-like morphology, including trunk elongation, reduction in limb size, and loss of digits (e.g., Brandley et al. 2008). Second, seemingly intermediate stages of body-form evolution may be retained for surprisingly long periods of time, from ∼9 to 63 million years (median = 27 million years; Brandley et al. 2008). These intermediate species have small limbs and reduced digit numbers but are not fully limbless. Third, this transition can occur quite rapidly. Recent studies of *Lerista* suggest that the transition from fully limbed to limbless body form (five to zero digits) may occur in only ∼3.6 million years (Skinner et al. 2008). Given that this transition can occur rapidly but typically does not, these results imply that selection favors the maintenance of intermediate body forms and that they are not merely brief transitory stages between the fully limbed and limbless forms. Fourth, recent analyses suggest that digits that are evolutionarily lost may be regained, seemingly in contradiction to Dollo's law (e.g., Brandley et al. 2008, Kohlsdorf & Wagner 2006, Kohlsdorf et al. 2010).

These studies have revealed much about what has happened during the evolution of snake-like body form in squamates. However, much remains to be learned about why these transitions have occurred. Understanding the causes of these transitions requires consideration of several levels of explanation (Wiens et al. 2006, figure 1).

On one level, snake-like body form may evolve because an open niche exists in a region, especially when a region is geographically isolated (Wiens et al. 2006). A niche may be open because a given ecomorph has neither evolved there yet nor dispersed into the region from elsewhere. This level of explanation may be particularly important for understanding the number of transitions between ecomorphs. For example, the long-tailed ecomorph evolved once in the Northern Hemisphere and spread between North America, Europe, and Asia. This ecomorph also evolved independently in South America, Australia, and twice in Africa, with no dispersal between these

continents. However, this ecomorph is widely distributed within most continents. Thus, this ecomorph seems to disperse into regions that are geographically connected and evolve independently in regions that are not. In contrast, each origin of the short-tailed ecomorph tends to be geographically restricted within each continent, and multiple origins of this ecomorph have occurred on many continents. However, no statistical phylogenetic tests have been conducted to rigorously determine whether open niches and biogeographic isolation influence the evolution of these ecomorphs.

On another level, understanding these transitions will require understanding why these morphologies are favored in certain environments. This issue is complicated by the fact that superficially similar snake-like morphologies occur in quite different environments (grass versus underground). Presumably, the evolution of the short-tailed ecomorph facilitates burrowing, and the long-tailed ecomorph facilitates locomotion in grass. But the functional consequences of these different ecomorphs remain poorly understood. Bergmann & Irschick (2010) compared kinematics and body form among *Lerista* species differing in body shape and showed that more elongate species bend their bodies more than less elongate species, thus facilitating limbless locomotion via lateral undulation. Among fully limbed groups, body elongation allows for more flexibility and may increase maneuverability in less open habitats (e.g., Van Damme & Vanhooydonck 2002). Functional studies also suggest the possibility that intermediate forms (i.e., small limbs) are maintained because they allow individuals to shift between limbed and limbless locomotion under different circumstances (e.g., Renous et al. 1998).

On a third level, we need to know what genetic and developmental changes underlie these changes in morphology. At least three major changes require explanation: Why are digits and phalanges lost? What causes reduction in limb length? What causes the increase in vertebral number and the associated body elongation? Some excellent developmental studies now bear on these issues, but the surface has barely been scratched. For example, Shapiro et al. (2003) showed evidence that changes in the temporal expression pattern of the *Sonic hedgehog* gene were involved in digit loss in Australian skinks (*Hemiergis*). Gomez et al. (2008) addressed the mechanism by which vertebral numbers are increased in snakes and suggested that this occurs because the somite clock is much faster in snakes than the overall rate of development; the somite clock determines the number of somites that develop (and somites develop into vertebrae). Interestingly, the widespread origins of snake-like ecomorphs across squamates and in nearly all major clades (excepting Iguania) suggests that there may be few intrinsic genetic or developmental constraints on this transition.

Finally, the evolution of snake-like ecomorphs also has important consequences for phylogenetic analysis. Analyses of morphology alone tend to place all or most snake-like burrowers into a single clade (e.g., scincids, dibamids, amphisbaenians, snakes; Conrad 2008, Wiens et al. 2010). However, such a clade is overwhelmingly contradicted by molecular data and by combined molecular-morphological analyses (e.g., Wiens et al. 2010). These latter results strongly suggest that the "burrowing clade" is incorrect and that morphological analyses are misled by convergence/parallelism. Thus, homoplasy overcomes the phylogenetic signal from >200 million years of morphological divergence (Wiens et al. 2006). However, surprisingly, the burrowing clade is not associated with limb reduction and body elongation alone, given that limb-reduced, elongate species of the surface-dwelling ecomorph are not placed in this clade. Instead, the burrowing clade is united primarily by skull features (and the skull is used for digging; Vitt & Caldwell 2009). Other habitats seem to lack such a strong influence on phylogenetic analyses. For example, arboreal iguanians are placed with other iguanians rather than with arboreal gekkonids in phylogenetic analyses of morphology (e.g., Conrad 2008).

EVOLUTION OF ECOLOGICAL NICHES

The new phylogeny of squamates may also have many interesting implications for squamate ecology. In a prominent paper, Vitt et al. (2003) addressed many large-scale phylogenetic trends in squamate ecology using the traditional, morphology-based phylogeny. In this phylogeny, iguanians are the sister taxon of a clade that includes all other squamates (called Scleroglossa). Vitt et al. (2003) focused in particular on explaining the success of scleroglossans relative to iguanians in terms of ecological differences between these groups, given the greater number of scleroglossan species (~7,529 scleroglossans versus ~1,475 iguanians). However, new molecular phylogenies concur that Iguania is nested deep inside the traditionally recognized Scleroglossa (e.g., Townsend et al. 2004, Vidal & Hedges 2005, Wiens et al. 2010). Therefore, the disparity in their relative species diversities (both globally and in local and regional assemblages) may simply be a result of the younger age of iguanians. The new phylogenies suggest several other interesting reinterpretations of squamate ecology.

Vitt et al. (2003) discuss a major niche shift from diurnality to nocturnality in geckos. However, the extant sister group to squamates (*Sphenodon*) is actually nocturnal, at least as actively foraging adults (Vitt & Caldwell 2009). Furthermore, the sister group to all other squamates is Dibamidae, which are burrowers (Vitt & Caldwell 2009). Whether they are actually nocturnal is uncertain, but they clearly live in low-light environments. Given that the sister group to squamates is nocturnal and the two squamate clades closest to the root are either predominantly nocturnal (Gekkota) or effectively nocturnal (burrowing dibamids), these patterns suggest the possibility that there was actually a shift from nocturnality to diurnality early in the phylogenetic history of squamates (e.g., a parsimony reconstruction places nocturnality as the ancestral state in squamates, using the tree from Wiens et al. 2006; J.J. Wiens, unpublished results). A similar, alternate hypothesis is that the only surviving lineages in these clades are presently nocturnal or burrowing (e.g., if heliophilic species in these clades went extinct because of climate change). Thus, even though many lizards today are predominantly diurnal and sun-loving, the new phylogeny raises the possibility that this may not be the primitive condition in squamates. Furthermore, this ancient dichotomy between diurnal and nocturnal activity may actually be one of the more phylogenetically conserved aspects of squamate ecology [e.g., many presently nocturnal species may have evolved from an ancestor that evolved nocturnality >200 Mya, whereas diurnal species may trace this shift to >150 Mya, given the time-calibrated phylogenies in Wiens et al. (2006) and Vidal & Hedges (2009)]. However, these patterns are clearly in need of detailed analysis.

Vitt et al. (2003) suggested that a major difference in habitat usage between scleroglossans and iguanians also developed at the root of squamate phylogeny. Specifically, more iguanian species use elevated perches, and more scleroglossans use terrestrial microhabitats (an important exception is gekkotans, which often use elevated microhabitats, but primarily at night). However, the new phylogeny suggests instead that iguanian domination of the diurnal, elevated niche actually occurred much later in the evolutionary history of squamates (~75 Mya, the timing of the earliest divergences within acrodonts and pleurodonts; Wiens et al. 2006). Interestingly, many noniguanian squamate clades contain lineages that are both diurnal and use elevated perches (e.g., some lacertids, cordylids, scincids, and geckoes; Vitt & Caldwell 2009), but none of these lineages appear to be older than the major clades of iguanians (using the chronogram of Wiens et al. 2006). Surprisingly, this obviously important niche does not appear to be strongly conserved beyond 75 Mya. For the arboreal niche, this might reflect the impact of the end-Cretaceous mass extinction event on plants and associated arboreal habitats.

Most squamates (particularly "lizards") feed on insects and other small arthropods (Vitt & Caldwell 2009). Nevertheless, there are trends in which lizard clades consume which

major arthropod clades (Vitt et al. 2003). Vitt & Pianka (2005) provided a statistical analysis of these trends. They emphasized the dichotomy between scleroglossans and iguanians with regard to diet, particularly the greater use of ants by iguanians. The new phylogeny, with its recent origin for iguanians, might be taken to suggest that ants were a strangely underutilized resource by squamates until the major diversification of iguanians ∼75 Mya (i.e., the timing of the earliest divergences within acrodonts and pleurodonts; Wiens et al. 2006). This would be a surprising pattern, given that ants are presently among the most numerically dominant groups of insects (e.g., ants can comprise 86% of estimated arthropod biomass in tropical rainforest canopies and 94% of arthropod individuals; Davidson et al. 2003). However, a new time-calibrated phylogeny for ants suggests that diversification of most major extant ant lineages occurred from 100 to 50 Mya (Moreau et al. 2006). Similarly, the fossil record suggests that ants were actually relatively rare in the Cretaceous and did not begin to dominate terrestrial ecosystems until the Eocene (Moreau et al. 2006). Thus, the high frequency of ants in the diets of iguanian lizards is relatively consistent with timing of diversification of both groups, given that iguanians are relatively recent within squamates (**Figure 1**).

The new phylogeny (**Figure 1**) also suggests that many other feeding-related traits that differ between iguanians and scleroglossans (i.e., noniguanians) are derived in iguanians rather than primitive for squamates. These include (iguanian trait first): sit-and-wait versus active foraging, visual versus chemical prey detection, and lingual versus jaw prehension of prey (see also Townsend et al. 2004, Vidal & Hedges 2009).

Apart from the scleroglossan-iguanian dichotomy, three other dietary trends in squamates are notable. First, a strong positive relationship exists between body size and prey size (e.g., Costa et al. 2008). Given that many squamate species consume many different types of arthropods, body size may actually be the most important variable in determining patterns of resource use and overlap among species.

Second, herbivory is present in squamates but is rare and confined entirely to lizards. Omnivory (10–90% plant matter in diet by volume) is more common and occurs in ∼12% of lizard species (Cooper & Vitt 2002), but it often involves eating fruits, flowers, and seeds rather than leaves. Consumption of >90% plant matter (i.e., herbivory) occurs in less than 1% of squamate species. These herbivorous species tend to feed on leaves, which may require a specialized gut flora to extract nutrients from plant cell walls. Across most of Squamata, herbivory appears to have evolved only 10–11 times (Espinoza et al. 2004). However, in the iguanian family Liolaemidae, herbivory has evolved at least 8 times, but ∼18 origins is more likely, depending on further resolution of liolaemid phylogeny. The causes of these trends are not clear. Outside liolaemids, herbivory seems to be associated with large body size, warm climate, and high body temperature (although rigorous tests are lacking), but in liolaemids, the evolution of herbivory is correlated with invasion of cooler climates and occurs in relatively small species (but that have high body temperatures). Somewhat paradoxically, many herbivorous lizards occur in relatively arid environments [e.g., liolaemids, most iguanids, *Uromastyx* (Agamidae), *Angolosaurus* (Gerrhosauridae)] where plants are less abundant and herbivory may be associated with a reduced insect fauna, but this has yet to be explicitly tested.

Third, even though most squamate species consume small arthropods, many snakes eat larger prey, including other vertebrates (e.g., Colston et al. 2010). Within snakes, the earliest split is between the primarily insectivorous scolecophidians and the alethinophidians (all snakes exclusive of leptotyphlopids, typhlopids, and anomalepidids), most of which consume other vertebrates (Colston et al. 2010). Snakes have many derived traits that may allow them to consume larger prey than other squamates (e.g., increased skull flexibility, ability to kill prey with venom or constriction prior to ingestion). Interestingly, no phylogenetic studies have addressed to what

extent these changes allow snakes to feed on larger prey items or whether the larger size of many alethinophidian snakes is more important. In fact, many large lizards also consume other vertebrates (e.g., varanids; Vitt & Caldwell 2009).

EVOLUTION OF VENOM DELIVERY SYSTEMS AND VENOMS

The evolutionary origin of squamate venom delivery systems and venoms has been a long-term interest of biologists (e.g., Kardong 1980, Kochva 1978), and venomous snakes are responsible for 20,000–94,000 human fatalities every year (Kasturiratne et al. 2008). Venom delivery systems (i.e., venom-conducting fangs and associated venom glands) have evolved within two extant squamate clades, helodermatid lizards and colubroid snakes (Vitt & Caldwell 2009), but the new squamate phylogeny (**Figure 1**) has profound implications for interpreting the evolution of squamate venom delivery systems and venoms.

A notable dichotomy is apparent in the placement of the venom delivery systems between the two extant squamate clades that have evolved them. The grooved fangs and venom glands of helodermatids are on the lower jaw, whereas in colubroids the venom delivery system is located on the maxilla bone of the upper jaw (either anteriorly or posteriorly). The dentition of these fangs traditionally has been described as being solid, grooved, or hollow (e.g., Kardong 1980). Elapids (cobras, mambas, sea snakes, and relatives), viperids, and the bizarre lamprophiid *Atractaspis* (sometimes called "mole vipers") have anteriorly located, hollow fangs (i.e., are front-fanged). Most other colubroids have some level of differentiation of posteriorly located maxillary teeth (i.e., are rear-fanged) that may be solid or variably grooved (Vidal 2002, Young & Kardong 1996).

Various competing hypotheses have been put forward regarding the evolutionary origins of venom-conducting fangs in snakes (e.g., Jackson 2003, Kardong 1980, Vidal 2002). Recent phylogenetic analyses of snakes (e.g., Pyron et al. 2011, Vidal & Hedges 2002, Wiens et al. 2008) now provide the necessary information to resolve many of these conflicting issues. First, because most colubroids possess differentiated maxillary dentition (e.g., Vidal 2002) and those that do not (e.g., constricting colubrines, snail/slug eating dipsadine colubrids; Fry et al. 2008) are relatively deeply nested in the colubroid phylogeny, the phylogeny suggests that fang-like maxillary dentition evolved early in colubroid evolution with subsequent secondary loss of fangs in some clades. Second, the front-fanged condition has evolved independently in *Atractaspis*, Elapidae, and Viperidae. Despite great strides in resolving colubroid phylogeny, our knowledge of their relationships is still quite incomplete (only 761 of ~2,736 species were included in the most comprehensive study so far; Pyron et al. 2011). Nonetheless, even though increased taxon sampling will undoubtedly refine these evolutionary hypotheses, the general conclusions stated above seem unlikely to change.

In addition to phylogenetic studies, recent studies on the development of snake fangs are also providing new insights on the evolution of these structures (e.g., Jackson 2007, Vonk et al. 2008, Zahradnicek et al. 2008). Phylogenetic reconstructions of adult morphology suggest that anteriorly placed fangs in viperids and elapids are not homologous. However, the recent developmental study of Vonk et al. (2008) suggests that this is not the case. These authors used the *Sonic hedgehog* gene (*shh*) as a molecular marker to visualize developmental events of the maxillary dental lamina (tooth-forming epithelium) in snake embryos. They discovered that the anterior and posterior subregions of the dental lamina are decoupled in nonfront-fanged colubroids (the presumed ancestral condition of Colubroidea), but the dental lamina is continuous in more basal noncolubroid snakes. This study also demonstrates that the front fangs of viperids and elapids (and the associated duct to the postorbital venom gland) actually develop from the posterior region of the maxilla (i.e., the posterior subregion of dental lamina) and that their development is

similar to that seen in the fangs of rear-fanged snakes. Given these results, the anterior location of fangs in viperids and elapids seems to occur through two processes: (*a*) loss of the anterior dental lamina (no *shh* expression) and (*b*) displacement of the posterior developing fang to an anterior position. Thus, the front fangs of viperids and elapids appear to be developmentally homologous (i.e., they are developmentally rear fangs). Although Vonk et al. (2008) provide a new model for the evolutionary origin and development of colubroid fangs, the generality of these findings must be confirmed with additional sampling. The third major front-fanged clade (i.e., *Atractaspis*) was not included, and its origin (homologous or convergent) will be of great interest. Also, the non-colubroid "outgroup" (i.e., *Liasis*) used is relatively distantly related to colubroids, and inclusion of the noncolubroid sister taxon to Colubroidea (*Acrochordus*; **Figure 1**) will help to more rigorously evaluate the ancestral colubroid condition.

One important discovery of recent molecular squamate phylogeny studies (**Figure 1**) has been the strong support for a novel clade containing snakes, anguimorphs, and iguanians (Toxicofera; Vidal & Hedges 2005). This particular phylogenetic result, coupled with the surprising finding of several known squamate venom toxins in lizard clades previously considered to be nonvenomous (i.e., varanids and iguanians; Fry et al. 2006), has profound implications for our understanding of the evolution of squamate venom systems (i.e., the glandular structures responsible for the production of venom toxins). The phylogenetic distribution of these shared venom toxins supports a single evolutionary origin of an ancestral toxicoferan venom system consisting of relatively simple mandibular and maxillary venom-secreting glands similar to those in iguanians. Within snakes and helodermatids, the venom systems independently became more complex, both in structure and in diversity of venom toxins produced; snakes eventually lost the mandibular venom glands, and helodermatids lost the maxillary venom glands. Associated with these elaborations of the venom systems was the independent evolution of venom delivery systems in snakes and anguimorphs (Fry et al. 2006, 2009). Such a scenario is contrary to the traditional view that venoms and venom delivery systems independently coevolved in snakes and helodermatids (Kochva 1978). As significant as this evolutionary hypothesis may be, additional squamate taxa need to be examined for the presence of venom glands and associated venom toxins. For example, only a single iguanian species was investigated, even though Iguania is composed of ~1,475 species (Uetz et al. 2011). Also, whereas the presence of venom toxins is now known from varanids and helodermatids, other distinctive anguimorph groups (i.e., anguids, shinisaurids, xenosaurids) need to be surveyed. On the basis of the phylogenetic information, some form of venom system is predicted to occur in these other toxicoferans.

Lastly, in addition to verifying the phylogenetic distribution of the production of venom toxins among the traditionally "nonvenomous" toxicoferans (i.e., those lacking relatively complex venom delivery systems), studies are needed to explore the ecological importance of these venom toxins in the nonvenomous toxicoferans. The original function of venom production may be to aid in prey digestion (Vitt & Caldwell 2009). It would be particularly interesting to confirm this role in iguanians and to address whether this evolutionary novelty may have played a role in the diversification of this speciose clade.

SUMMARY

New phylogenetic hypotheses that are emerging for squamates at all taxonomic levels are changing our ideas about many aspects of squamate biology, including diel behavior, feeding ecology, body-form evolution, and the evolution of venoms and venom delivery systems. Phylogeny-based research is also revealing much about ecological aspects of the origins and maintenance of parthenogenesis as well as finer details about the origins of several forms of viviparity

(including reversals to oviparity). As squamate phylogeny becomes more finely resolved and strongly supported, squamates may prove to be one of the best model systems for addressing the most fundamental questions in evolutionary biology, from the maintenance of sex to the origin of body plans.

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