

Natural History of Reptilian Development: Constraints on the Evolution of Viviparity

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Oviparity, or egg-laying, is the dominant mode of reproduction among vertebrates. Nevertheless, viviparity, the retention of the egg within the reproductive tract until embryonic development is complete, characterizes almost all mammals; it has also had at least 150 independent origins within the fishes, amphibians, and reptiles (Shine 1985, Blackburn 1992, Wourms and Lombardi 1992). These multiple origins suggest pervasive benefits to viviparity across a wide range of taxa, life histories, and habitats. In the squamate reptiles (lizards and snakes), for example, viviparity is the most common reproductive mode in cold climates, and recent origins of viviparity in this group are also associated with cold climates (Shine 1985). Gravid females in cold climates can thermoregulate to keep embryos warmer than they would be in a nest, thus enhancing development. Thermoregulation by the female may thus ensure that birth occurs at the appropriate season or even that reproduction is successful at all. Viviparity is also advantageous in very wet or dry habitats, for example, because it obviates the need for females to find suitable sites in which to lay their eggs.

In reptiles, viviparity is associated with a plethora of integrated morphological and physiological features that are not present in oviparous reptiles; these features are presumed necessary for successful embryonic development in the oviduct (Packard et al. 1977, Guillette 1993). Early insights into the evolution of these reproductive features were based on comparisons between typical oviparous and viviparous species. Some of the distinguishing features of the viviparous species examined were the major reduction or absence of an eggshell and the presence of some form of placentation (Weekes 1935 and included references). However, because the species used in these comparisons represented the extremes of a putative evolutionary sequence, their use as a model for elucidating the actual sequence or timing of the morphological and physiological changes attending the evolution of viviparity is limited. In fact, these observations are consistent with both a saltation model that posits that the characteristic features of viviparity arise suddenly and simultaneously, and a gradualist model that posits incremental evolution from one reproductive mode to the other (Blackburn 1992, 1995).

A relatively new and more powerful approach is clarifying-

A POWERFUL APPROACH IS CLARIFYING THE EVOLUTION OF VIVIPARITY: THIS APPROACH INVOLVES THE IDENTIFICATION AND CRITICAL EVALUATION OF CLOSELY RELATED TAXA THAT VARY IN REPRODUCTIVE MODE

ing the evolution of viviparity; this approach involves the identification and critical evaluation of closely related taxa that vary in reproductive mode (Guillette 1982, Heulin 1990, Mathies and Andrews 1995, Qualls 1996, Smith and Shine 1997, Méndez-de la Cruz et al. 1998). Of these studies, Qualls (1996) provides the best evidence that a gradual process describes the evolution of viviparity. His study on *Lerista bougainvillii*, an Australian skink, involved three conspecific populations: one oviparous, another viviparous, and a third that is morphologically and physiologically intermediate. An increase in the length of egg retention associated with reduction in eggshell thickness among these populations supports the hypothesis that viviparity evolves gradually from oviparity. The other studies presented data that are consistent with this interpretation—that is, conspecific populations or closely related species exhibited the expected grade of features intermediate between oviparity and viviparity.

In this article, we present a broad and unconventional perspective on the evolution of viviparity. Our goal as physiological ecologists is to determine “how animals are designed with reference to their natural environments and evolutionary histories” (Bennett 1987). We thus suggest that the key to understanding how viviparity evolves is to first understand why it has *not* evolved in particular taxa (see also Shine 1985, Packard et al. 1989). Our perspective

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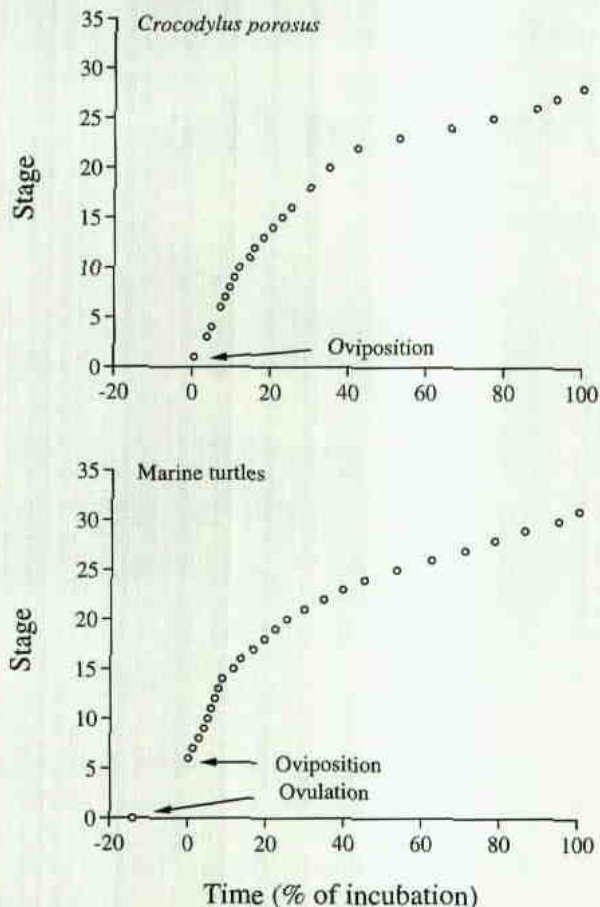


Figure 1. The relationship between embryonic stage and time since oviposition, with time expressed as percentage of the incubation period, in crocodylian and sea turtle embryos. (top) For *Crocodylus porosus* (Ferguson 1985), staging starts at oviposition, when the embryo is at the neurula stage (roughly equivalent to stage 14 for marine turtles). (bottom) For marine turtles (Miller 1985), staging starts at oviposition, when the embryo is at the gastrula stage. The time between ovulation and oviposition is estimated (Miller 1985) to be 8–9 days—equivalent to 14% of the time between oviposition and hatching (60 days). The shapes of the curves for both reptiles are similar; rapid development during the first half of incubation reflects tissue and organ differentiation, and slower development later in development reflects an increase in embryo size and biochemical and physiological changes that provide few new features for visual staging.

focuses on the consequences of extended egg retention on embryonic development in oviparous species and thus identifies potential constraints on the shift from oviparity to viviparity (more precisely, lecithotrophy, sensu Blackburn 1992). This approach lends itself to understanding the evolution of viviparity at high taxonomic levels (e.g., turtles versus squamates) as well as at low taxonomic levels (within genera and species).

We begin by reviewing the general pattern of embryonic development of reptiles to set the framework for further

discussion. We then summarize the timing of oviposition in reptiles and how timing affects embryonic development in the oviducts. If, as we assume, the transition between oviparity and viviparity involves a progressive increase in the amount of development that takes place in the oviduct, then any factor that limits development in the oviduct also limits the potential for viviparity to evolve. We therefore discuss a number of reasons why oviposition occurs at particular stages of embryonic development. We next consider the consequences of extended egg retention in a clade of phrynosomatid lizards and how these consequences may restrain or enhance the evolution of viviparity in this group. Finally, we summarize evidence that hypoxia (oxygen deprivation) is the physiological factor that limits development of embryos in the oviducts.

Comparative embryology: developmental chronology

Embryonic development is a continuum from fertilization to hatching or birth. For comparative purposes, however, embryologists break development into a series of discrete stages that are demarcated by the appearance of distinct features, such as neurulation, torsion, pharyngeal clefts, limb buds, and so on. Ideally, a “normal table” of development would list these stages, extending from fertilization to hatching or birth, and would report the timing of events at some specific temperature. Such an ideal normal table is, however, not available for any reptile. For example, the most commonly used normal table for squamates starts at cleavage and has 40 stages, but these stages are not related to time (Dufaure and Hubert 1961). In contrast, whereas normal tables for crocodylians provide the timing of developmental events for particular incubation temperatures, staging does not start until oviposition (neurulation), and the normal table has only 28 stages (Ferguson 1985).

At a general level, however, patterns of development are comparable among reptilian taxa. First, differentiation is rapid initially but slows appreciably as the embryo approaches the end of development, with embryos passing through 80% or more of their recognized stages by approximately halfway through the developmental period. The relationship between developmental stage and time for crocodylian and sea turtle embryos illustrates this phenomenon (Figure 1). The finding that most stages are completed before mid-development, irrespective of taxon, reflects the fact that early development is characterized by tissue differentiation and organogenesis, processes that provide many distinct landmarks and thus result in the identification of many distinct stages. Later development is largely characterized by growth in size and by biochemical and physiological changes that provide few new features for visual staging. Indeed, by mid-development, embryos are small but recognizable lizards, snakes, crocodiles, or turtles.

A second general pattern of reptilian development is that growth in size is exponential (Figure 2). The magni-

tude of growth toward the end of development is considerably greater than depicted in Figure 2, for two reasons: size is indexed by length rather than mass, and the x-axis represents stage rather than time. That is, if the axes were mass and time, the curve would exhibit a much greater increase in size after mid-development than shown in Figure 2. The second half of development is thus characterized by a size increase of considerable magnitude.

Stage at oviposition

For oviparous reptiles, oviposition marks the transition between embryonic development within the mother and in the environment. The timing of this transition varies considerably among reptilian groups. Eggs of turtles, crocodylians, and sphenodontidans are invariably laid when embryos are in the earliest stages of development. In contrast, the eggs of most squamates are laid when embryos are approximately one-third through development, although oviposition can occur substantially earlier or later. As we discuss, several morphological and physiological factors may constrain the stage at oviposition and thus account for variation in the reproductive modes of reptiles.

Turtles, crocodylians, and sphenodontidans. Oviposition by turtles (Chelonia) and tuataras (Sphenodontida) occurs when the embryo is at the gastrula stage; hence, little development occurs in the oviduct (Ewert 1985, Mofat 1985). After reaching the gastrula stage, turtle embryos enter developmental arrest, resuming development only after oviposition. The amount of time that turtle embryos spend in developmental arrest varies. Turtle eggs remain in the oviduct for at least the 2-week period that is necessary for shell formation. However, the length of egg retention can vary considerably among females. The longest periods of egg retention for the North American slider turtle *Trachemys scripta* and the mud turtle *Kinosternon subrubrum* in South Carolina were 39 days and 50 days, respectively (Buhlmann et al. 1995). Some females of the chicken turtle, *Deirochelys reticularia*, retain their eggs over winter and lay them the following spring, presumably because conditions in the fall were not favorable for successful nesting. These eggs are thus retained by the female for 4–7 months before oviposition (Buhlmann et al. 1995) and hatch in the spring of the following year (Congdon et al. 1983). In contrast to all other reptiles, a long period of egg retention is characteristic of tuataras; eggs are in the oviduct for 6–8 months before oviposition (Cree et al. 1992). Development is presumably arrested while eggs are in the oviduct because embryos are at only the gastrula stage at oviposition.

Crocodylians oviposit when embryos are at the neurula stage and thus somewhat more advanced than those of turtles at the time of oviposition (Ferguson 1985). Unlike turtle embryos, however, crocodylian embryos do not undergo developmental arrest and therefore must be laid immediately. Nevertheless, oviposition at early develop-

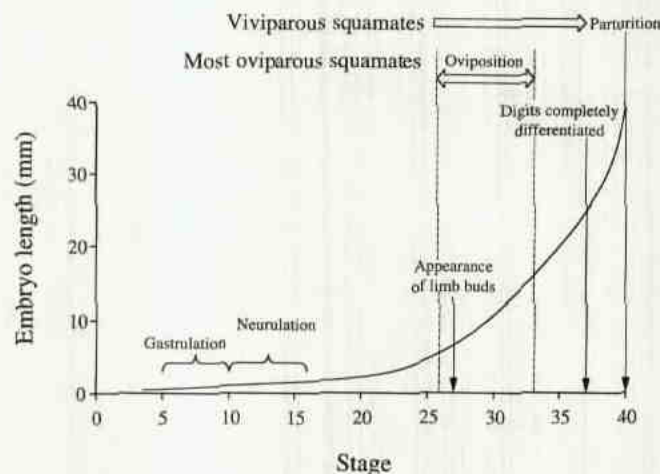


Figure 2. The relationship between embryo size and stage for *Lacerta vivipara*, a viviparous lizard. Embryo size is indexed by its length, which actually underestimates the actual size of the embryo (see text for details). Embryo stage follows Dufaure and Hubert (1961). Stages at which important developmental events occur (gastrulation, neurulation, appearance of limb buds, and digits completely differentiated), the range of stages over which oviposition occurs in the majority of oviparous squamates, and the stage of parturition in viviparous squamates are indicated. Figure modified from Xavier and Gavaud (1986).

mental stages appears to be characteristic of all turtles, crocodylians, and sphenodontidans. The fact that embryos of these taxa do not develop beyond an early developmental stage while in the oviduct has apparently limited the reproductive options open to these taxa—no turtle, crocodylian, or sphenodontidan is viviparous.

For turtles, crocodylians, and sphenodontidans, the apparent requirement for oviposition to occur early in development may reflect a suite of morphological and physiological constraints that were established early in the history of these taxa. The most immediate constraint on embryonic development may be the limited exchange of gases, particularly oxygen, in the oviduct. Gas diffusion is considerably slower in water than in air, and the pores and channels of the eggshell are filled with fluid when the egg is in the oviduct. Moreover, the heavily calcified shells of the eggs of crocodylians and most turtles (Packard and DeMarco 1991) may further exacerbate the problem of gas exchange. Indirect evidence to support this hypothesis for turtles includes the observations that the shell calcifies just before gastrulation (when development becomes arrested) and that, after oviposition, embryos of species with more permeable, flexible-shelled eggs develop faster than embryos of species with rigid calcareous shells (Ewert 1985).

Experimental studies on the northern snake-necked

turtle, *Chelodina rugosa*, in Australia provide direct evidence that hypoxia maintains developmental arrest in this species (Kennett et al. 1993). The rigid-shelled eggs are laid underwater in seasonal ponds, and the embryos remain in developmental arrest as long as the eggs are immersed in water. Embryos resume development only after the soil dries and oxygen tension rises. In an experiment in which eggs were shifted from water to an atmosphere of pure nitrogen, embryos remained in developmental arrest; they resumed development only when exposed to atmospheric air.

An alternative, or perhaps complementary, explanation for oviposition early in development by turtles and crocodilians concerns the adhesion of the embryo and the vitelline membrane to the shell (Ewert 1985, Ferguson 1985). Adhesion shortly after oviposition appears to have a respiratory function because the "chalking" (drying) of the shell that is associated with adhesion increases its conductance to gases (Thompson 1985). Because movement of an egg after oviposition prevents adhesion, retention of eggs in the oviduct, where they are inevitably jostled, would probably preclude adhesion and further development. This may be a moot point, however, because oviductal fluids would also prevent chalking as long as the egg remained in the oviduct.

Another potential constraint on extended egg retention for crocodilians and turtles is that much of the calcium used in development is mobilized from the shell (Packard and Packard 1984). If calcification of the shell of turtle and crocodilian embryos were reduced as a means to enhance gas exchange in the oviduct, then the later growth of the embryo would be limited. Calcium does not limit early development, however, and selection could presumably increase the amount of calcium stored in the yolk. Nevertheless, the suite of physiological and morphological features in turtles, crocodilians, and sphenodontidans may form a substantial barrier to appreciable embryonic development in the oviducts for these reptiles.

Squamates. Oviposition at early developmental stages may explain why no crocodilian, turtle, or sphenodontidan has evolved viviparity (Shine 1983). On the other hand, squamate reptiles exhibit nearly the entire gamut of possible embryonic stages at oviposition. At one extreme, approximately 20% of squamates are viviparous. Close to this extreme, some oviparous species lay eggs with late-stage embryos. For example, the North American green snake (*Liochlorophis vernalis*), the New Guinean skink (*Sphenomorphus fragilis*), and the Australian skink (*Saiphos equalis*) oviposit when embryos are within weeks, or even days, of hatching (Sexton and Claypool 1978, Guillette 1992, Smith and Shine 1997). At the other extreme, oviposition when embryos are at the gastrula stage occurs in *Chamaeleo chamaeleon* (Bons and Bons 1960) and at comparably early stages for varanid lizards (John Phillips, Zoological Society of San Diego, unpub-

lished data). A teiid, *Cnemidophorus uniparens*, oviposits at stages 21–22 (Billy 1988), and several other species of *Cnemidophorus* oviposit considerably earlier (Norma Manríquez, Universidad Nacional Autónoma de México, unpublished data). Reports of what appear to be extraordinarily long incubation periods after oviposition suggest that the eggs of some chameleons may parallel those of turtles, not only in the stage at oviposition (i.e., gastrulation) but also in the potential for embryos to remain in an arrested state even after oviposition (Bons and Bons 1960, Ewert 1985).

Despite the wide range of stages over which squamates oviposit, oviposition occurs much more frequently at some stages than at others (Figure 3). Stages at oviposition group tightly around a mode of stage 30, and the majority of oviparous squamate taxa oviposit at stages 26–33. This distribution suggests that the stage at oviposition is constrained. One possible constraint is on how early eggs can be laid. Perhaps oviposition does not occur much before stage 30 because of the time required to deposit the oviductal proteins (albumen) and the various layers of shell that surround the ovum. In chickens, for example, oviposition occurs 22 hours after ovulation, by which time the embryo has reached the gastrula stage. Because reptiles are ectothermic, their eggs are likely to require a longer period in the oviduct than those of birds—and their embryos will have attained more advanced embryonic stages—before the shell is laid down.

For example, female *Anolis carolinensis* and *Anolis limifrons* lay a sequence of one-egg clutches at minimum intervals of 6–7 days (Andrews 1979, 1985) and oviposit at stage 30 (Robin M. Andrews, unpublished data). Ova are released into the right and left oviduct alternately, such that females never have more than one egg per oviduct and few females have an egg in both oviducts. Thus, eggs have a transit time of approximately 6 days (assuming that no females have two oviductal eggs) to 9 days (assuming that 50% of the females have two oviductal eggs simultaneously). Similarly, in the laboratory, at a body temperature of 30 °C, *Sceloporus woodi* females secrete the proteinaceous fibers of the eggshell within the first 24 hours after ovulation; calcium deposition is maximal on days 3–9 but continues up to the time of oviposition, when embryos are at stage 27 and have been in the oviduct 12–14 days (DeMarco 1993, Palmer et al. 1993). These and other data (Miller 1985) on sea turtles suggest that reptile eggs have a necessary residence time in the oviduct of 1–2 weeks before the albumen and shell are deposited.

The critical question with respect to what causes oviposition to occur in the vicinity of stage 30 is, does oviposition by squamates occur as soon as the shell is complete? Or are eggs retained longer? Oviposition at stage 30 corresponds to retention in the oviduct for 25% (DeMarco 1993) to approximately 40% (Shine 1983) of total developmental time (time in utero plus time in nest). These percentages correspond to an egg retention time of 2–3

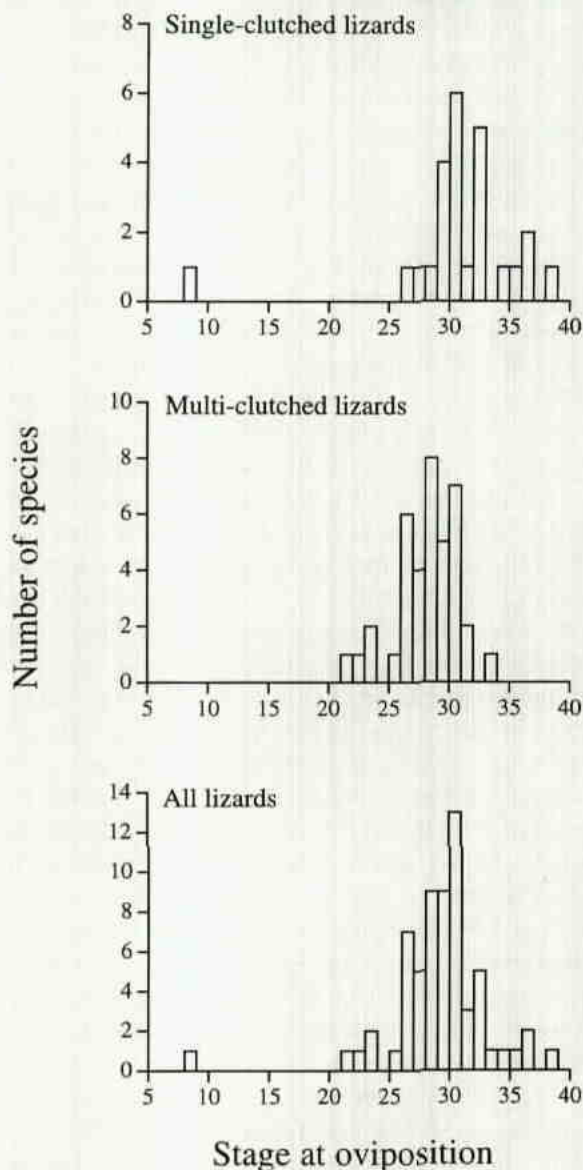
Figure 3. Embryonic stage at oviposition for lizards. (top) Lizards that lay a single clutch per reproductive season. (middle) Lizards that lay two or more clutches per reproductive season. (bottom) All lizards. The figures do not include varanids or the three species of *Cnemidophorus* for which oviposition occurs before any visible development of the embryo (see text for details). The data are from a literature review by Shine (1983) and an update to this data set by Blackburn (1995), with more recent data on stage at oviposition and clutch frequency from Robin M. Andrews (unpublished data).

weeks for small lizards that oviposit at stage 30 and that have a total developmental time of 50–60 days. Within a period of 2–3 weeks, embryos will have reached stage 30 during a period sufficient to completely shell the egg. Many squamates that oviposit at stage 30, however, have total developmental times that are longer than 50–60 days (Shine 1983, DeMarco 1993). If oviposition occurs at 25–40% of the total developmental period, eggs of such species would be in the oviduct for considerably longer than the 1–2 weeks required to completely shell the egg.

This line of reasoning suggests that, in species that oviposit earlier than stage 30, either oviposition occurs as soon as eggs are shelled or embryonic development was arrested before oviposition. That is, if development from ovulation to oviposition continued normally in the oviduct, then, after 2 weeks or more, well-developed embryos (i.e., at approximately stage 30) should be present, rather than the gastrula-stage embryos typical of varanids and some chameleons. For example, the period between ovulation and oviposition for varanid lizards is consistently approximately 1 month (Phillips and Millar 1998), despite oviposition at very early stages of development. By contrast, squamates that oviposit at stage 30 or later probably retain eggs for longer than the minimum period needed for deposition of the shell. Designation of stage 30 as the benchmark for judging how early eggs are laid relative to shell formation is, of course, a crude approximation; features of the shell or the body temperature of the female could alter the length of time required to shell eggs.

Another possible constraint that could cause oviposition to cluster around stage 30 is how late in development eggs can be laid. Such a constraint is particularly important because it directly limits the evolution of viviparity. Only a few oviparous species retain eggs well into the second half of development (Figure 3), which suggests that the shift from oviparity to viviparity does not occur often, is rapid, or both (Blackburn 1995). In any case, the paucity of species that oviposit at stages 34–39 suggests that such a strategy presents problems for embryos, the reproductive female, or both.

Embryos of oviparous species may experience a number of problems if they are retained past stages 32–33—even those of species that normally oviposit at these stages.



Large absolute increases in the mass of embryos in the second half of development are associated with greatly enhanced demands for gas exchange and water (Black et al. 1984, Birchard et al. 1995). If these demands are not met, development of retained embryos may be retarded (Andrews and Rose 1994). Further retention of eggs at this point would be beneficial only if it “bought time” for the female to locate a suitable oviposition site. Such a physiological constraint may, in addition to having specific consequences on embryonic development (see below), help to explain why so few species lay eggs with embryos beyond stages 32–33.

Females may also incur problems from extended egg retention. The burden of the clutch would not change appreciably during the first half of development, but it would during the second half, when embryos undergo large absolute increases in mass (Figure 2) as water is taken up by the extra-embryonic compartments of eggs and

the tissues of the embryo. Although retained eggs may take up less water than oviposited eggs (Mathies and Andrews 1996), a gravid female that retains eggs into the second half of development is faced not only with the problem of transporting a clutch that is increasing in mass, but also with the potential problem of passing enlarged eggs through the pelvic girdle during oviposition (Sinervo and Licht 1991). The rarity of oviposition after stage 34 may therefore be a direct consequence of increasing egg size.

Our concern in this article is largely with the proximate physiological factors that constrain the embryonic stages at which eggs are oviposited. However, it is also important to recognize that many ultimate factors affect the stage at oviposition. For example, extended egg retention may have a high survival cost for some species because of the burden that the clutch imposes on the female (Sinervo and Adolph 1991). Such a cost would favor oviposition as soon as the shell is formed (as long as suitable nest sites are available). Clutch frequency should also affect the stage at oviposition. Species that produce multiple clutches per season could minimize the interval between clutches by laying eggs at early stages of development, thus producing more clutches within a breeding season. On the other hand, for species that are limited to one clutch per season, the length of time that eggs spend in the oviduct would not reduce the reproductive output of the female. If embryos continue to develop in the oviduct in such species, then delayed oviposition would shorten the length of time that the eggs are in the nest and vulnerable to predators or other mortality agents. Single-clutched species would thus be more likely than multiple-clutched species to oviposit when embryos are at advanced stages.

This prediction is borne out by data on lizards (Figure 3); few multiple-clutched lizard species oviposit later than stage 30, whereas most single-clutched lizard species oviposit at stage 30 or later ($X^2 = 7.0$, $P < 0.01$, 2×2 contingency analysis, two-tailed test). Despite the association between clutch frequency and stage at oviposition, we believe that selection for oviposition to occur at very early or very late stages of embryonic development will seldom be effective because of the time necessary to complete the egg shell, on the one hand, and physiological constraints on embryonic development in utero, on the other.

Consequences of extended egg retention for sceloporine lizards

Our research focuses on spiny lizards of the genus *Sceloporus* and tree lizards of the genus *Urosaurus*. *Sceloporus* and *Urosaurus* are sister taxa, that is, each other's closest relative. *Urosaurus* contains approximately 10 species, all of which are oviparous. *Sceloporus* contains approximately 70 species, almost half of which are viviparous (Sites et al. 1992). Viviparity has apparently arisen only four times in this genus: once in the ancestor of the *formosus* group; once in the ancestor of the *grammicus*, *megalapidurus*, and *torquatus* groups; and twice within the *scalaris* group

(Méndez-de la Cruz et al. 1998). The *scalaris* group is the only one to have both oviparous and viviparous species, and the close relationship of the oviparous and viviparous species suggests that viviparity is of recent origin in this group (Benabib et al. 1997). The few origins of viviparity and the large number of species groups (approximately 15) in which all members are oviparous suggests that the transition to viviparity may be difficult in *Sceloporus*.

Because oviposition at successively later stages of embryonic development is the putative transition to viviparity, the present-day consequences of extended egg retention on embryonic development provide insights into how easy it is for viviparity to evolve. We have therefore studied the consequences of the retention of eggs beyond the normal stage of oviposition. We initially focused on oviparous species known to retain eggs to advanced stages, but a more complete picture required that we also look at the consequences of egg retention by the more typical species—that is, those that oviposit when embryos reach approximately stage 30.

The experimental protocol common to all of our studies has been to keep gravid females of each species in the laboratory under conditions that inhibit oviposition. This approach allowed us to assess the capacity of females to support embryonic development in utero past the time of normal oviposition. To inhibit oviposition, we took advantage of a normal response to drought; because successful nesting in the field is dependent on adequate soil moisture, and rainfall is often unpredictable, females respond to drought by retaining their eggs (Andrews and Rose 1994). Experimental females were thus kept in cages with dry soil as a substrate, which simulated drought conditions and inhibited oviposition (Andrews and Rose 1994). Control females were housed under the same conditions as experimental females, except that the cage substrate was moist soil; most females oviposited normally under these conditions. Eggs from control females were incubated in a standard incubation medium and at the same temperature as eggs within experimental females.

One set of experiments examined egg retention by *Sceloporus scalaris*, an oviparous species that is a model for the transition from oviparity to viviparity (Figure 4). *S. scalaris*, a member of the *scalaris* species group, occurs in grasslands at many elevations in Arizona, New Mexico, and northern Mexico. Females from a low-elevation population are able to retain eggs for an unprecedented length of time, although they normally oviposit at stages 31–33 (Mathies and Andrews 1995, 1996, Thomas Mathies and Robin M. Andrews, unpublished data). Eggs retained as long as 1 month (to stage 38) and surgically removed from females develop at the same rate and have the same survival to hatching rate as control eggs. Eggs retained for a further 10–13 days advance to stage 40; these eggs produced viable hatchlings 1–3 days after they were surgically removed from females. When the same experiment was conducted at a slightly higher incubation temperature,

development was somewhat slower for retained embryos than for control embryos, presumably because the higher incubation temperature increased the oxygen requirements of embryos beyond that available in utero (Andrews 1997).

Nevertheless, among squamates, *S. scalaris* has an unusual ability to extend egg retention to within a few days of hatching and to do so with little or no impairment of embryonic development. The ability to support embryogenesis in utero to advanced stages is also found in another oviparous species in the *scalaris* group, *Sceloporus aeneus* (Robin M. Andrews, unpublished observation), the sister species of the viviparous *Sceloporus bicanthalis*. The ability of oviparous species in the *scalaris* group to extend egg retention to advanced embryonic stages has likely facilitated the evolution of viviparity in this group.

Sceloporus virgatus, an oviparous member of the *undulatus* species group, also normally lays eggs at stages 31–33. In contrast to *S. scalaris*, however, when eggs of *S. virgatus* are retained past the normal time of oviposition, as long as to stage 37, embryonic development is retarded (Figure 4; Andrews and Rose 1994, Andrews 1997). For example, the dry masses of embryos retained for 30 days past the normal time of oviposition were less than half those of controls, and retained embryos showed considerably lower survival to hatching than control eggs. Female *S. virgatus* that retain eggs beyond the normal time of oviposition thus have greatly reduced fitness. This assertion is not to imply that extended egg retention has no benefit in this species—females that do not retain eggs until the seasonal rains occur would have zero fitness for that reproductive season!

S. virgatus appears to be unusual, both within the *undulatus* species group and within the genus *Sceloporus*, in its ability to normally support embryonic development past stage 30 (Mathies 1998). No other species that we studied in the *undulatus* species group is able to retain eggs appreciably beyond stage 30. For example, females of *Sceloporus occidentalis*, another member of the *undulatus* group, oviposit when embryos reach stages 28–30, even if conditions are not suitable for nesting. This pattern is also characteristic of *Sceloporus graciosus* (a member of the *graciosus* species group). Females of two members of the *undulatus* species group—*Sceloporus undulatus consobrinus*, from Arizona, and *Sceloporus undulatus hyacinthinus*, from Virginia and Missouri—both typically oviposit when embryos are at stage 28–30 (Sexton and Marion 1974,

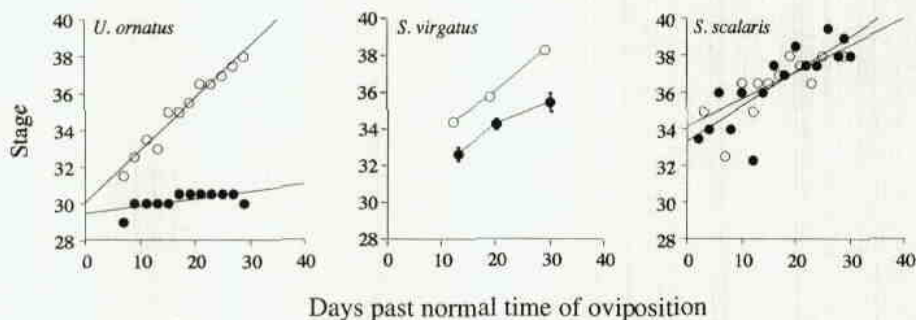


Figure 4. Embryo stage in three species of phrynosomatid lizards as a function of days elapsed since the normal time of oviposition. Eggs of control females (open circles) were oviposited at the normal time. Eggs of experimental females (solid circles) were retained in the oviducts for the number of days indicated. Oviposition by experimental females was inhibited by providing dry substrates for nesting. The development of the retained embryos was arrested for *Urosaurus ornatus* (left), was retarded for *Sceloporus virgatus* (middle), and proceeded normally for *Sceloporus scalaris* (right). Figure modified from Andrews and Rose (1994), Mathies and Andrews (1996), and Mathies (1998).

Mathies 1998), as do female *Sceloporus clarki* (a member of the *clarki* species group). Moreover, embryos of these species do not develop further when eggs are retained beyond the normal time of oviposition (Mathies 1998). Initially, the similar stages at oviposition for *S. u. consobrinus* and *S. u. hyacinthinus* housed on moist and dry substrates would appear to be the result of the brief period, approximately 10 days, of facultative egg retention under drought conditions. However, during this 10-day period, embryos in control eggs had reached stage 32 and had dry masses four to eight times greater than those of the retained embryos. These observations indicate that development of retained embryos was arrested. Females in the *undulatus*, *graciosus*, and *clarki* species groups thus exhibit a limited ability to extend egg retention and to support embryogenesis in utero beyond stage 30.

In contrast to members of the *undulatus* group, a member of the sister genus to *Sceloporus*, *Urosaurus ornatus*, is able to retain eggs for at least a month past the time of normal oviposition (Mathies and Andrews 1999). As in most members of the *undulatus* group, however, development is arrested at approximately the time of normal oviposition. In this case, development is arrested at stage 30.5, whereas control embryos reach stage 38 after the same length of incubation (Figure 4). When retained eggs are laid, development is reinitiated and continues to hatching. Hatching success of retained eggs is as high as that of control eggs. Retention in *Urosaurus* thus has no obvious effect on embryonic development other than to delay hatching, and then only by the time spent in arrest. Retention does affect hatchlings, however. Hatchlings produced after long periods of retention are smaller and less hydrated than hatchlings produced after short periods of retention.

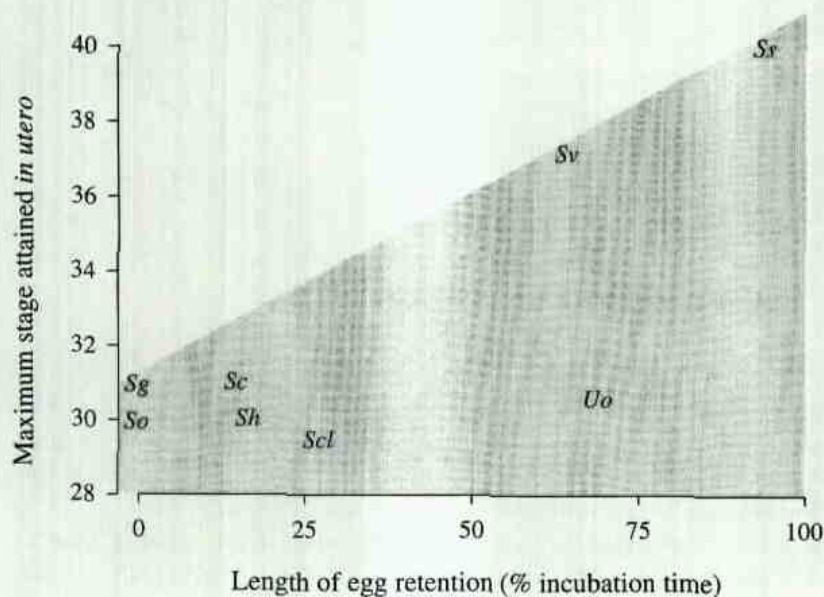


Figure 5. The maximum stage that embryos of *Sceloporus* and *Urosaurus* lizards reach while retained in utero as a function of the time that females are able to retain eggs. The time females are able to retain eggs is expressed as the percentage of the normal length of the incubation period, that is, from oviposition to hatching. The ability to support embryogenesis in utero can evolve along any trajectory within the shaded area proceeding from the bottom left to the upper right. The upper edge of the shaded area represents the necessary association between the length of egg retention and the stage of development for embryos that continue to develop while retained in utero. Abbreviations are as follows: *Sc*, *Sceloporus undulatus consobrinus*; *Scl*, *Sceloporus clarki*; *Sg*, *Sceloporus graciosus*; *Sh*, *Sceloporus undulatus hyacinthinus*; *So*, *Sceloporus occidentalis*; *Sv*, *Sceloporus virgatus*; *Ss*, *Sceloporus scalaris*; *Uo*, *Urosaurus ornatus*.

These observations on sceloporine lizards are the first reports of developmental arrest of well-differentiated reptilian embryos. By contrast, turtle and chameleon embryos arrest much earlier in development. The survival of well-developed embryos during developmental arrest suggests that gas exchange is sufficient to support the maintenance metabolism of these embryos but not their differentiation or growth. These observations also suggest that developmental arrest may actually be a common feature of the reproductive biology of oviparous squamates. It may not have been reported before simply because it is not detectable without experimental manipulation of the length of retention and the provision of an appropriate control. That is, a species in which development is arrested at stage 30 will always appear to lay eggs at the "right" stage.

A third notable finding from these studies is that extended egg retention actually encompasses two phenomena, both of which bear on the ease with which viviparity is likely to evolve (Figure 5). One phenomenon is the ability to maintain gravidity, that is, to facultatively retain eggs beyond the time when oviposition normally

occurs. This ability is related to the secretory activity of the corpora lutea; progesterone produced by this structure inhibits uterine contractions and maintains hyperemia and the depth of the oviductal mucosa (Guillette and Jones 1985, Shine and Guillette 1988). The second phenomenon is the ability to support embryogenesis in utero past the stage at which oviposition normally occurs. The evolution of these two phenomena is not necessarily linked. In some species, females are able to maintain gravidity as well as support embryogenesis (e.g., *S. scalaris*). In other species, females can maintain gravidity but embryonic development is arrested (e.g., *U. ornatus*).

Viviparity seems least likely to evolve for species in which females have a limited ability to maintain gravidity and embryonic development is arrested even after short periods of retention (lower left of Figure 5). In contrast, viviparity seems most likely to evolve when the ability to maintain gravidity is combined with continued embryogenesis in utero (upper right of Figure 5). In species that are able to maintain gravidity but in which embryonic development is arrested, the evolution of viviparity might seem to be limited (lower right of Figure 5). However, it could well be that, once the ability to maintain gravidity is in place, the further shift to embryonic development in utero, and ultimately to viviparity, might be easier than if selection had to act on both traits simultaneously.

Physiological constraints on the evolution of viviparity

Comparisons among the major groups of reptiles suggests that, just as viviparity is more likely to evolve in some sceloporine lizard species than in others, it may also be more likely to evolve in some groups than in others. Viviparity has evolved at least 100 times within reptiles (Shine 1985). Viviparous species are, however, distributed unevenly at all taxonomic levels. Turtles, archosaurs (crocodilians and birds), and sphenodontidans are entirely oviparous, whereas viviparity has evolved numerous times among lizards and snakes. Within the squamates, some families are entirely oviparous and others are entirely viviparous. Even within families in which both modes of reproduction are represented, independent origins of viviparity are more common in some lineages than in others (Shine 1985). These observations suggest the existence of constraints at various levels (phylogenetic, physiological, and ecological) on the evolution of viviparity in reptiles.

Our research has emphasized physiological constraints

on embryonic development when embryos are retained within the oviducts. Determining the consequences of extended egg retention by oviparous species on embryonic development provides a way to evaluate the relative ease with which viviparity could evolve in different lineages (Figure 5). For example, viviparity has and could evolve readily in the *scalaris* species group. Extended egg retention has no obvious limits or disadvantages for embryonic development in this group; two oviparous members of this lineage can extend egg retention to stages 38–40 without compromising the rate of embryonic development or hatching success. In contrast, whereas females of *S. virgatus* in the *undulatus* species group can extend egg retention to stage 37 (albeit greatly retarding embryonic development), females of other species in this group are unable to support embryogenesis past stage 30, regardless of environmental circumstance. Moreover, because most species in the *undulatus* group exhibit a limited ability to maintain gravidity, continued development in utero, even if physiologically possible, would be precluded. These observations suggest that the evolution of viviparity in the *undulatus* group, although possible, is much less likely than in the *scalaris* group.

The arrest or retardation of embryonic development in utero implicates some form of physiological limitation. Because carbon dioxide diffuses more rapidly than oxygen in liquid, hypercapnia is less likely to limit embryonic development than hypoxia. Insufficient availability of water is also unlikely to be a general constraint on development in utero. Turtle and crocodilian eggs are provided with all the water the embryos will need for their entire development, yet development in the oviducts is very limited. In contrast, squamate eggs are provided with minimal amounts of water; substantial water uptake from the nest substrate is usually necessary for normal development. Nevertheless, embryos in the retained eggs of *S. scalaris* can develop normally almost to hatching with little additional water uptake (Mathies and Andrews 1996).

We therefore infer that hypoxia is the most likely limiting factor for embryonic development in utero; turtle and crocodilian embryos may simply experience hypoxia at earlier developmental stages than lizard embryos. The support for this assumption is, however, largely indirect. For example, one line of evidence is that viviparity in reptiles is always associated with the thinning or loss of the shell and the consequent proximity of the oviduct and the embryonic membranes, a feature that presumably enhances gas exchange. Another line of evidence is that embryos of turtles and crocodilians that develop in utero past the stage of normal oviposition die before hatching or are deformed (Ewert 1985, Ferguson 1985).

Three morphological features determine the availability of oxygen to embryos; these features, either alone or in combination, could account for differences in the capacity to support embryogenesis in utero between turtles and squamates and among species of phrynosomatid lizards.

One feature is the thickness and structure of the shell. Eggshells of reptiles differ enormously in the mineralization of the outer shell layer and in the density, organization, and size of the proteinaceous fibrils that constitute the inner shell layer (Packard and DeMarco 1991). Both layers act as barriers to gas diffusion, although the fibril layer may be the more effective (Feder et al. 1982). The second feature is the extent of vascularization of the extra-embryonic membranes that line the inner surface of the eggshell. These membranes are the yolk sac, which is the only vascularized extra-embryonic membrane early in development, and the chorioallantois, which becomes increasingly important for gas exchange as it grows over the inner surface of the shell during the latter part of development. In two species of *Sceloporus*, for example, the chorioallantois covers 20–30% of the inner surface of the shell by stage 30 and the entire inner surface by stages 36–38. Coverage by the chorioallantois is completed earlier in *S. scalaris* than in *S. virgatus*, which is consistent with their respective abilities to support embryonic development in utero (Andrews 1997). The third feature is the vascularity of the oviduct. Vascularity of the oviduct varies during the reproductive cycle (Masson and Guillette 1987) and between oviparous and viviparous sister species (Guillette and Jones 1985). The amount of oxygen available for embryonic metabolism is presumably affected by the proximity of the vascular membranes of the embryo to the oviduct, by the efficiency of oxygen transfer between maternal and fetal capillaries, and by the amount of capillary development.

To assess the importance of gas exchange as a limiting factor for embryonic development in the oviduct, we evaluated the hypothesis that differences in shell structure are associated with differences in the capacity of embryos to continue development in the oviduct. This hypothesis is attractive because viviparity is thought to require a major reduction of the shell. This hypothesis was, however, not supported, at least for the lizards that we studied (Figure 6; Mathies 1998). For example, two features of the shell that should enhance gas exchange—thickness and conductance to water vapor—are related not to the extent of embryonic development that can occur in utero but rather to the mass of the egg. It thus appears unlikely that some feature of the eggshell accounts for differences in the amount of embryonic development that can take place in the oviduct among species of *Sceloporus*. Hypoxia is still likely to be the limiting factor, however. The eggshell is but one of three morphological features that affect oxygen availability to embryos; the contributions of the two other features, the extra-embryonic membranes and the oviduct, to the diffusion of gases during development have yet to be determined for any reptile.

Our conclusion that viviparity is more apt to evolve in some groups than in others because of phylogenetic variation in a physiological constraint is based on a small subset of reptilian species, and we do not know whether the

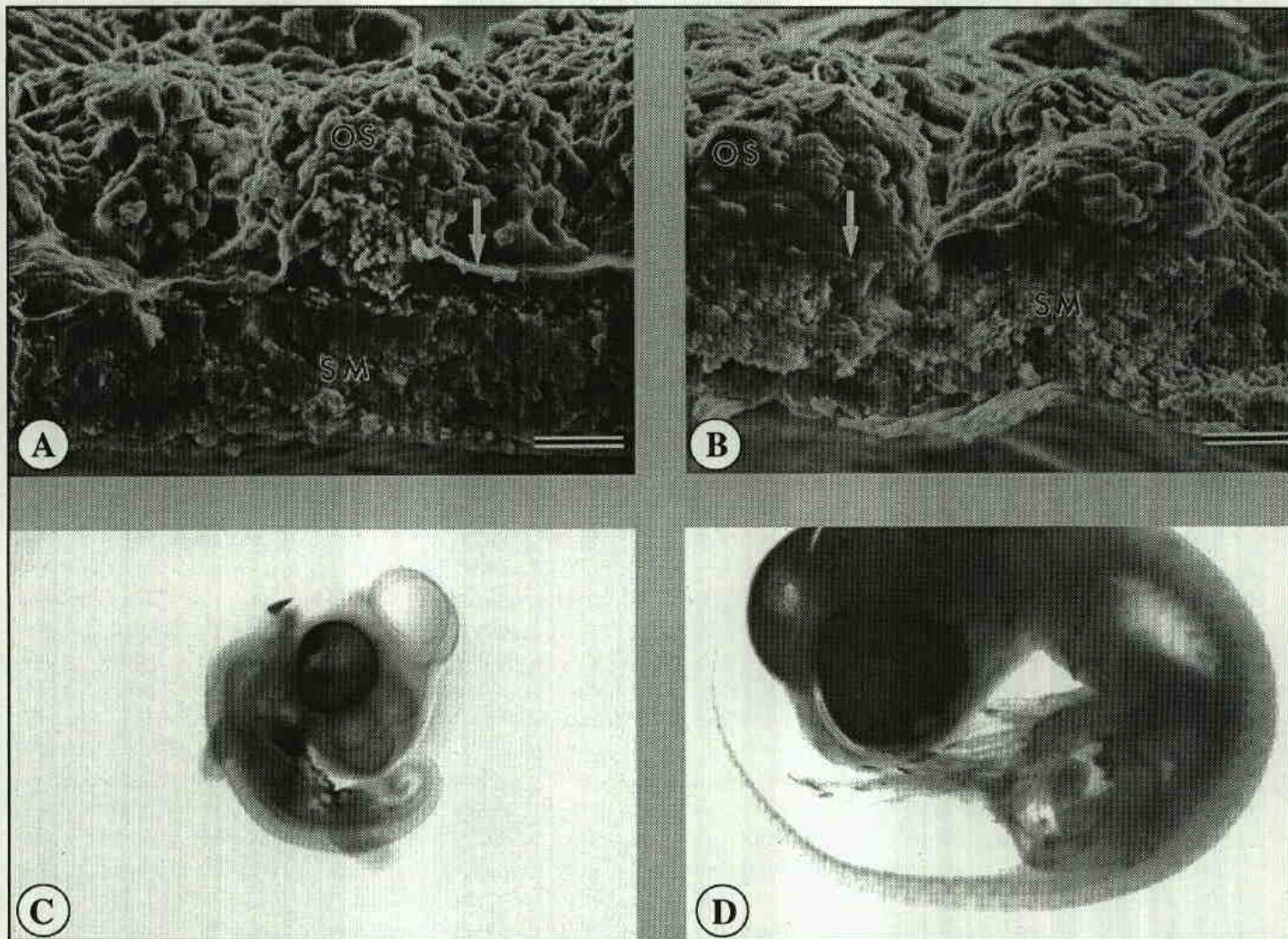


Figure 6. Lack of correlation between eggshell structure and the maximal embryo stage attained in utero in the sister species *Sceloporus undulatus consobrinus* and *Sceloporus virgatus*. (a) Edge view of an eggshell of *S. u. consobrinus*. (b) Edge view of an eggshell of *S. virgatus*. Eggshells, examined at the time of oviposition, consist of an outer calcareous layer (OS) and an inner layer of densely packed fibers, the shell membrane (SM). Arrows indicate a thin cuticle deposited on the outer surface of the shell. Scale bars = 30 μ m. The eggshells of the two species are indistinguishable in morphology and permeability to water vapor. (c and d) The relative sizes and degrees of differentiation that embryos of *S. u. consobrinus* and *S. virgatus* reach in utero. Because embryos of these species were not available, they are represented by embryos of *Sceloporus scalaris* at stage 31 (c) and stage 37 (d), respectively. At these stages, embryos of all *Sceloporus* are similar in size and appearance. The embryos in (c) and (d) are approximately 2.4 and 4.4 mm in maximum dimension, respectively. Despite the similarity of their eggshells, the maximal size and degree of differentiation reached in utero are considerably greater in *S. virgatus* than in *S. u. consobrinus*.

same constraint operates in other groups. The stage at oviposition is known for fewer than 100 of the approximately 5500 species of oviparous squamates, and in some families the stage at oviposition is not known for even one representative. Moreover, the consequences of experimentally extended egg retention have been determined for only a few species of phrynosomatid lizards. Nevertheless, what little information is available reveals a wide diversity of embryonic natural histories. Additional research on how long embryos are retained in the oviduct and how much development takes place during this time will surely provide important insights into the evolution of vivi-

parity; the information in this article is the tip of the iceberg with respect to the potential wealth of information that awaits discovery.

Further understanding of constraints on the evolution of viviparity would be greatly enhanced by systematic studies on the natural history of embryos. One specific research target is groups for which little to no information is available (e.g., Amphisbaenia, Anguidae, Cordylidae, Pygopodidae, Varanidae, and snakes in general). A second target is groups that show substantial variation in embryonic natural history (Chamaeleontidae, Tropicuridae, Scincidae, Colubridae). Comparative research on closely

related taxa with diverse embryonic natural histories within these families would be especially informative. A third target is those squamates that have atypical embryonic natural histories. For example, unlike most squamates, gekkonine and sphaerodactylid geckos lay rigid-shelled eggs and often place them in dry, exposed environments. These taxa thus have both atypical eggs and atypical oviposition sites. Are eggs of these geckos relatively impervious to gas exchange? Would their embryos therefore experience hypoxia at earlier stages than species of diplo-dactylid and eublepharine geckos, which produce flexible-shelled eggs? Are their eggs therefore laid when the embryos are at early developmental stages? Are exposed nests necessary to ensure adequate gas exchange for embryonic development? All of these questions would be easy to address, and their answers would provide general insights into constraints on egg retention.

We have argued that physiological constraints are the most important proximate factors affecting the potential for particular taxa to shift from oviparity to viviparity. Clearly, however, physiological factors are not the only constraints that have affected the evolution of viviparity. For example, the very early stage at oviposition that appears to be typical of varanid lizards has at least several possible explanations. Perhaps, as active predators, varanids lay their eggs as soon as possible to avoid detrimental burdening of the female. Alternatively, their particular choice of nest sites may favor sturdy eggs that are resistant to desiccation, predators, or pathogens but that as a consequence have poor gas exchange capabilities. Whatever the ultimate explanation or explanations for the oviparity of varanids in particular, the present-day reality is that obligate oviposition at early embryonic stages by some squamates, turtles, crocodylians, and sphenodontids, possibly associated with developmental arrest in utero, precludes viviparity in these taxa now and would have done so in the past.

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