

# Reconstructing an Adaptationist Scenario: What Selective Forces Favor the Evolution of Viviparity in Montane Reptiles?

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**ABSTRACT:** It is notoriously difficult to test hypotheses about the selective forces responsible for major phylogenetic transitions in life-history traits, but the evolution of viviparity (live bearing) in reptiles offers an ideal model system. Viviparity has arisen in many oviparous reptile lineages that have invaded colder climates. Thermal advantages (eggs retained within the mother's body will be warmer than those laid in the nest) are almost certainly important, but the actual selective pressures remain controversial. For example, the benefit to retention might involve faster development, protection against freezing, predation, or desiccation, or modification of hatchling phenotypes. I experimentally manipulated incubation regimes of a montane scincid lizard (*Bassiana duperreyi*, Scincidae) to test these ideas. Eggs maintained in cooler "nests" in the laboratory developed more slowly, were more likely to die before hatching, and produced inferior (small, slow) hatchlings. A 2-wk initial period of higher-temperature incubation (simulating uterine retention, an intermediate step toward viviparity) ameliorated these effects. In the field, I placed eggs in artificial nests at the upper elevational limit of natural nests and also extending a further 100 m higher on the mountain. The results mirrored those in the laboratory: retention at maternal body temperatures accelerated hatching, enhanced hatching success, and increased locomotor speeds of hatchlings. This selective advantage of uterine retention was greater at higher elevations and increased with longer retention. The causal process responsible was prolonged low-temperature incubation rather than freezing, desiccation, or predation, and both hatching success and hatchling viability were affected. Field experiments that directly re-create selective regimes may thus provide robust tests of adaptationist hypotheses.

**Keywords:** embryonic development, lizard, natural selection, nest temperatures, oviparity, phenotypic plasticity.

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Identifying the selective forces responsible for important events in phylogenetic history is a major goal of the adaptationist program (Williams 1966, 1975). Unfortunately, hypotheses about such forces are difficult to test in any rigorous way. Comparative analysis can discern patterns of correlated change, and we can attempt to infer process from pattern (Harvey and Pagel 1991). Nonetheless, even if two variables are tightly linked (organisms consistently evolve trait A when they encounter habitat X), the actual selective forces involved (i.e., the reasons that trait A enhances individual fitness) remain speculative. Thus, many people, including eminent philosophers of science, have criticized evolutionary hypotheses as nonfalsifiable (Gould and Lewontin 1979; see Endler 1986). Inevitably, however, some hypotheses are more amenable to rigorous falsification than are others. The best opportunity may come from hypotheses concerning the selective forces responsible for consistent, repeated patterns of evolutionary change and those that rely on factors likely to be still operating in present-day systems (i.e., they do not rely on specific events [such as meteor impacts] in past history). If modern-day systems can be used to explore these issues, we can directly examine the validity of assumptions underlying the adaptationist hypothesis.

Nonetheless, evaluating assumptions is still a long way short of demonstrating the validity of a putative selective force. How can we proceed to this extra step? We can try to identify present-day environments where such selective forces are at work, and find organisms that are in the process of such an evolutionary transition (i.e., display phenotypic traits intermediate between those at either side of the transition). Actually measuring selective pressures directly in such a system would be ideal, but this is usually not possible for logistic reasons. In particular, traits under strong selection usually exhibit low phenotypic variance, making it difficult to quantify fitness differentials (Endler 1986). The problem can be overcome by experimental manipulations that amplify the range of phenotypic variation and, hence, the intensity of selection (Sinervo et al.

1992; Sinervo 1994). One of the best such opportunities involves cases where an adaptationist hypothesis identifies a specific environmental factor as the selective agent acting on a specific trait. That is, we have two habitats that differ in the putative selective force; organisms with the ancestral state of the trait in question are restricted to one of those habitats. We can experimentally generate organisms that differ in the trait suggested to be adaptive to the other habitat, ideally by creating a phenotype intermediate between the ancestral and derived states of the trait under consideration (because most major transitions will require intermediate stages). Then, we can experimentally reproduce the selective force by placing both “normal” and manipulated organisms into the challenging environment. The adaptationist hypothesis predicts that the manipulation will enhance organismal fitness in the “challenging” environment more than in the usual habitat. Even better, if we can simulate successive intermediate stages in the evolutionary transition, we can test the hypothesis that progressive increments in trait values (as likely occur in most major transitions) do indeed enhance fitness. Such a procedure not only can potentially clarify whether or not the habitat does indeed impose the selective force but also can reveal the nature of that pressure. For many major transitions, there are multiple competing arguments about the exact process by which the novel environmental pressure modifies fitness differentially in organisms with and without the new “adaptive” trait.

### The Evolution of Reptilian Viviparity in Cold Climates

Robust tests of adaptationist hypotheses will be greatly facilitated by the choice of an appropriate study system. The transition from oviparity (egg laying) to viviparity (live bearing) in squamate reptiles offers an ideal model system with which to explore this approach. The advantages of this system are as follows.

First, comparative analyses have identified >100 independent transitions from oviparity to viviparity within lizards and snakes (Blackburn 1982, 1985; Shine 1985; Lee and Shine 1998).

Second, in virtually all cases where closely related oviparous and viviparous forms can be compared, live-bearers occur in cooler climates than their oviparous relatives (Shine and Bull 1979; Blackburn 1982, 1985; Shine 1985). This is true within as well as among species (Qualls et al. 1996; Smith and Shine 1997; Fairbairn et al. 1998; Heulin et al. 1999), strongly suggesting that invasion of cold climates has been the primary stimulus for the evolution of viviparity.

Third, the processes involved seem to have remained consistent over a long period, so that present-day studies are likely to be relevant to processes at work during pre-

vious evolutionary shifts. Two lines of evidence support this view: some phylogenetic origins of viviparity related to cold climate appear to be ancient whereas others are very recent (Shine 1985), and the same strong correlation between climate and reproductive mode is seen in present-day reptiles (Mell 1929; Sergeev 1940; Tinkle and Gibbons 1977).

Fourth, there are several alternative hypotheses about the actual selective forces involved—that is, the reasons why cooler nest sites reduce fitness for oviparous reptiles and why uterine retention of eggs ameliorates this penalty. For example, the benefits of retention might involve protection from freezing, from predation, from desiccation, from late hatching, or from suboptimal developmental pathways induced by low-temperature incubation (see table 1).

Fifth, intermediate stages between “normal” oviparity and viviparity are seen in a few transitional taxa (Blackburn 1995) and can potentially be simulated in the laboratory (Shine 1995; Andrews and Mathies 2000) or even in the field (present study). These intermediate stages involve retention of developing eggs in utero for a longer than normal period, thus resulting in oviposition of eggs with well-developed embryos and a consequent reduction in the subsequent period of incubation in the nest before hatching (Weekes 1933; Packard et al. 1977; Shine and Bull 1979).

Sixth, the consistent correlation between viviparity and cold climates (in both evolutionary transitions and present-day geographic distributions) suggests that the selective force for viviparity somehow involves incubation temperature, a physical factor that is easy to measure and manipulate experimentally. If we do this in the laboratory as well as in the field, any causal effects of temperature can be separated from those of other factors that might correlate with temperature (hydric environment, degree of exposure, etc.) in natural nests.

Seventh, nests of some high-elevation egg-laying reptiles can be found in large numbers, and eggs that have been translocated among nests will develop successfully (Shine et al. 1997). Thus, it should be feasible to move eggs to artificial nest sites at elevations immediately above those at which oviparous reproduction usually ceases. Quantifying the subsequent fitness of an (immobile) egg will be much easier than doing the same thing for other (mobile) phases in the life history.

In terms of the framework presented earlier in this article, the hypothesis of interest is the notion that oviparous reptiles invading colder climates are under strong selection to retain developing eggs in utero for progressively longer periods, eventually leading to viviparity. The two habitats to be compared will be those immediately above and below the usual upper elevational (=lower thermal) limit for

**Table 1:** Putative selective forces for the evolution of reptilian viviparity in cold climates

Life-history stage affected	Mediated via	Process causing differential viability	References <sup>a</sup>
Eggs	Low temperature per se	Eggs killed by freezing	Shine and Bull 1979
Eggs	Low temperature per se	Eggs killed by prolonged cool incubation	Shine 1983
Eggs	Prolongation of development	Eggs fail to hatch before winter	Sergeev 1940
Eggs	Prolongation of development	Eggs vulnerable to mortality for a longer period of time	Mell 1929
Eggs	Forced use of shallow nests	Eggs vulnerable to desiccation, predation	Andrews 2000
Hatchlings	Low temperatures per se	Cool incubation results in inferior hatchling phenotypes	Shine 1995
Hatchlings	Prolongation of development	Late hatching reduces fitness	Packard et al. 1977

Note: All of these alternative ideas begin with the premise that lower nest temperatures reduce offspring fitness and that retention at higher temperatures (inside the maternal oviduct) ameliorates this effect. However, they differ in terms of the life-history stage that is affected (egg vs. hatchling) and in whether low temperature per se causes the viability effect (vs. indirect consequences of low temperature). Some hypotheses attribute the fitness differential to mortality, but others invoke more subtle effects on viability.

<sup>a</sup> This column lists the first paper that clearly states the hypothesis; many of the same ideas have also been suggested by subsequent authors.

successful oviparous reproduction. Nest sites for the study species (see “Study Species and Area”) are simple, with no parental care, and standardized suitable “nests” can thus be provided in both habitats. The axis of phenotypic variation thought to be subject to selection is the duration of retention of developing eggs in utero, and we can generate “intermediate” stages along this axis simply by keeping new-laid eggs warm (rather than cool) during the early part of development. It is thus logistically feasible to simulate the situation in which selection is thought to favor the evolution of viviparity, both in the laboratory and in the field, and to measure the resultant selective forces.

### Predictions

The first step in testing these ideas is to generate specific, falsifiable predictions from the “cold-climate” hypothesis for the evolution of reptilian viviparity. I addressed the following predictions.

First, the upper elevational limit for egg layers in the field will correspond to the elevation above which there is a marked decrease in hatching success or hatchling viability. Second, different models (see table 1) predict different forms of this viability decrease above the elevational threshold for oviparous reproduction. For example, it might involve either increased mortality of eggs or decreased fitness of hatchlings (or both). Third, brief retention at higher temperatures (mimicking those experienced by eggs in utero) will enhance offspring viability via one or more of these mechanisms. Fourth, the viability increment due to “uterine” retention will be greater for eggs incubated for the rest of development at cooler rather than warmer temperatures (in the laboratory) and at higher rather than lower elevations (in the field). Fifth, progres-

sive increases in the duration of uterine retention will generate increasing benefits to offspring viability.

### Methods

#### *Study Species and Area*

Three-lined skinks (*Bassiana duperreyi*) are medium-sized (to 80 mm snout-vent length) insectivorous lizards that are abundant in cool-climate (high-latitude and/or high-elevation) regions of southeastern Australia (Cogger 1992). Each adult female lays a single clutch of three to nine eggs per year under rocks or logs in sunny positions (forest clearings; Pengilley 1972). The eggs are laid in early summer and hatch in autumn (Pengilley 1972; Shine and Harlow 1996). This species has been intensively studied in the Brindabella Range 40 km west of Canberra in the Australian Capital Territory. *Bassiana* are restricted to elevations <1,650 m above sea level, as are all other oviparous species in the same area (*Lampropholis delicata*, *Lampropholis guichenoti*, *Nannoscincus maccoyi*, *Tympanocryptis diemensis*; Pengilley 1972; Shine and Harlow 1996). In contrast, viviparous lizards extend to much higher elevations (*Egernia whitii*, *Eulamprus heatwolei*, *Eulamprus tymbanum*, *Pseudemoia entrecasteauxii*, all to >1,700 m; Pengilley 1972). All of these lizards except the fossorial *N. maccoyi* are diurnal shuttling heliotherms, with body temperatures typically maintained near 30°C throughout sunny weather during the lizards’ activity season (Shine 1983; Greer 1989).

In a finding that is critical to this study, Pengilley (1972) located one site at the extreme upper limit of oviparous reproduction. A ski run down the slopes of Mount Ginini (148°46’E, 35°32’S) provides a 50-m-wide clearing in the forest that begins at the peak (1,762 m) and runs all the

way down to 1,615 m. We have found >1,000 eggs of *Bassiana* at the lower end of the ski run, but none above 1,630 m. The habitat is superficially homogeneous along this elevational gradient, thus allowing me to examine potential nest sites both above and below the elevational threshold for oviparous reproduction.

#### *Experimental Design*

Data for this study were gained over two summers (1999–2000 and 2000–2001). In the first year, I incubated eggs in the laboratory to investigate the effects of low-temperature incubation and of brief retention at higher temperatures. I also conducted a pilot study wherein eggs were translocated to artificial nest sites above the usual elevational limit for reproduction. As in the laboratory study, I included a treatment whereby some eggs were maintained briefly at higher temperatures (to simulate uterine retention) before being deposited in the nest. Most eggs continued to develop after these translocations, so in the second summer, I conducted a more ambitious field experiment that involved differing durations of “uterine retention,” with more extensive controls.

#### *Laboratory Study, 1999–2000*

Previous work on *Bassiana* suggested that retention at high temperatures could modify phenotypes and increase hatching success but was based on a single “nest temperature” regime (Shine 1995). We now have much more extensive information on thermal regimes in natural nests of *Bassiana*, allowing more realistic simulation of the temperature conditions experienced by developing embryos and the attributes of potential nest sites above the usual elevational limit of distribution of this species (Shine and Harlow 1996; Shine et al. 1997, 2002; R. Shine, unpublished data).

Freshly laid eggs were collected from natural nests at Coree Flats (1,050 m; 148°48'E, 35°17'S) on December 14, 1999, and placed in individual glass vials containing moist vermiculite (water potential  $-200$  kPa), sealed with plastic food wrap to prevent evaporation (Shine and Harlow 1996). These vials were then transferred to cycling-temperature incubators with mean temperatures of 15°, 16°, 17°, or 18°C, with a diurnal cycle of 15°C in each case (i.e., mean temperature  $\pm 7.5^\circ\text{C}$ ). These regimes span the range seen in natural nests of *Bassiana* at high elevations in the Brindabellas (Shine and Harlow 1996). A fifth incubator (uterine retention treatment) was set up to mimic maternal body temperatures: 30°C from 0900 to 1700 hours but following the same schedule as the 18°C incubator outside of those “basking” hours (i.e., falling to 10.5°C at night, mean incubation temperature = 21°C).

Eggs were randomly allocated to one of nine groups (20 eggs per group). Twenty eggs were incubated throughout development in each of the five incubators, whereas another 80 were kept for 2 wk in the uterine retention treatment and then transferred to one of the other incubators (20 to each of the nest simulations). Eggs were regularly rotated among shelves to avoid biases due to thermal stratification within incubators. I did not have enough incubators to replicate units at each temperature regime within the same year, but previous work has shown that phenotypic variation induced by among-incubator differences is very small relative to thermal effects (Flatt et al. 2001).

Lizards were measured (snout-vent length, tail length) as soon as they hatched and then housed individually in cages (22 cm  $\times$  13 cm  $\times$  7 cm) in a constant-temperature room set at 20°C. Under-cage heating allowed the hatchlings to select body temperatures between 20° and 38°C during daylight hours, with the heating switching off at dusk. The young lizards were fed mealworms and crickets dusted with reptile vitamins; water was available ad lib. At 1 wk of age, hatchlings were remeasured and had their locomotor capacity tested in a 1-m raceway maintained at 25°C (mean of two runs per lizard; see Shine et al. 1997 for details of methods). For analysis, I examined mean speeds (over 1 m) and sprint speeds (over the fastest 25 cm). The following week, the hatchling was released at the site from which it had been collected as an egg.

#### *Field Study, 1999–2000*

This work was conducted mostly to test the feasibility of translocating eggs to sites above the usual elevational limit of oviparous reproduction but ultimately provided useful data in its own right. Eighteen artificial nests (double-thickness concrete pavers [Boral Masonry], 30 cm  $\times$  30 cm, 10 cm thick) were set up in November 1999 along the ski run at Mount Ginini (see “Study Species and Area”). Two replicate pavers were set up at 10-m intervals over this range and grouped into low (1,620–1,650 m), middle (1,660–1,690 m), and high (1,700–1,730 m) sites for later analysis. Miniature temperature logger probes (Hobo-temp, Onset Computer, Pocasset, Mass.) were placed under all 18 nests to record incubation temperatures for eggs under the pavers. Exposure to sunlight (total incident radiation) of these artificial nests was similar to that recorded for natural nests (using gap light analysis techniques; Shine et al. 2002).

On December 14 and 15, 1999, eggs were collected from nests at Picadilly Circus (1,240 m above sea level; 148°50'E, 35°21'S) and put into bags constructed of 10-cm lengths of nylon stocking, tied at the ends to prevent ingress by predators and to prevent dispersal after hatching ( $n = 4$  or 5 eggs per bag; see Shine et al. 1997 for detailed meth-

ods). One hundred eighteen of these eggs (=25 stockings) were immediately placed under pavers at low (1,620–1,640 m), middle (1,660–1,680 m), and high (1,700–1,720 m) elevations on Mount Ginini. The other 76 eggs were returned to the laboratory and kept in the uterine retention incubator for 2 wk. On February 1, 2000, these “retained” eggs (in 16 stocking bags) were placed under the same pavers as above.

Every week from mid-February until mid-May 2000, the stockings were checked for hatchlings. If any hatchlings were present, the entire bag was returned to the University of Sydney. Eggs that had died (i.e., were collapsed or moldy) were dissected to determine the cause and embryonic stage of death. Any unhatched eggs from the stockings were placed in the  $18^{\circ} \pm 7.5^{\circ}\text{C}$  incubator until hatching occurred.

#### *Field Study, 2000–2001*

I expanded the fieldwork to increase the sample size, provide replication across years, and incorporate additional treatments and controls. The basic design was similar to that in 1999–2000, with the following exceptions. First, eggs were retained in the laboratory at two regimes: one simulated maternal body temperatures (as before), but the other ( $15^{\circ} \pm 7.5^{\circ}\text{C}$ ) simulated a natural (cool) nest. I added this treatment to control for any effects of translocation and laboratory incubation per se, independent of thermal regimes. Second, eggs were maintained in these laboratory treatments for 1, 2, or 3 wk to explore the effects of differing durations of uterine retention. And, third, I included a fourth site at lower elevation (Picadilly Circus; 1,240 m) because even the lowest site at Ginini (1,630 m) was close to the upper elevational extreme for reproduction in *Bassiana*. Four pavers were used at each site, for a total of 16 pavers.

Freshly laid eggs were collected from natural nests at Picadilly Circus on December 16, 2000, and from lower Ginini on December 27, 2000 (egg laying is delayed at the higher-elevation site; R. Shine, unpublished data). On each of these days, a stocking containing four or five eggs was placed under each paver. The other eggs were brought back to the laboratory and maintained for 1, 2, or 3 wk in either cool ( $15^{\circ}\text{C}$  mean) or warm (uterine retention) incubation. After the appropriate period, stockings containing eggs from each treatment were returned to the field site and placed under each of the 16 pavers. On weekly trips thereafter, stockings were checked, and any that contained hatchlings were brought back to the laboratory where they were processed in the same way as in the previous season (see “Field Study, 1999–2000”). Responses to the experimental treatments were very similar for eggs from the two

locations (Picadilly and Ginini), and thus data for these two locations have been combined for analysis.

## Results

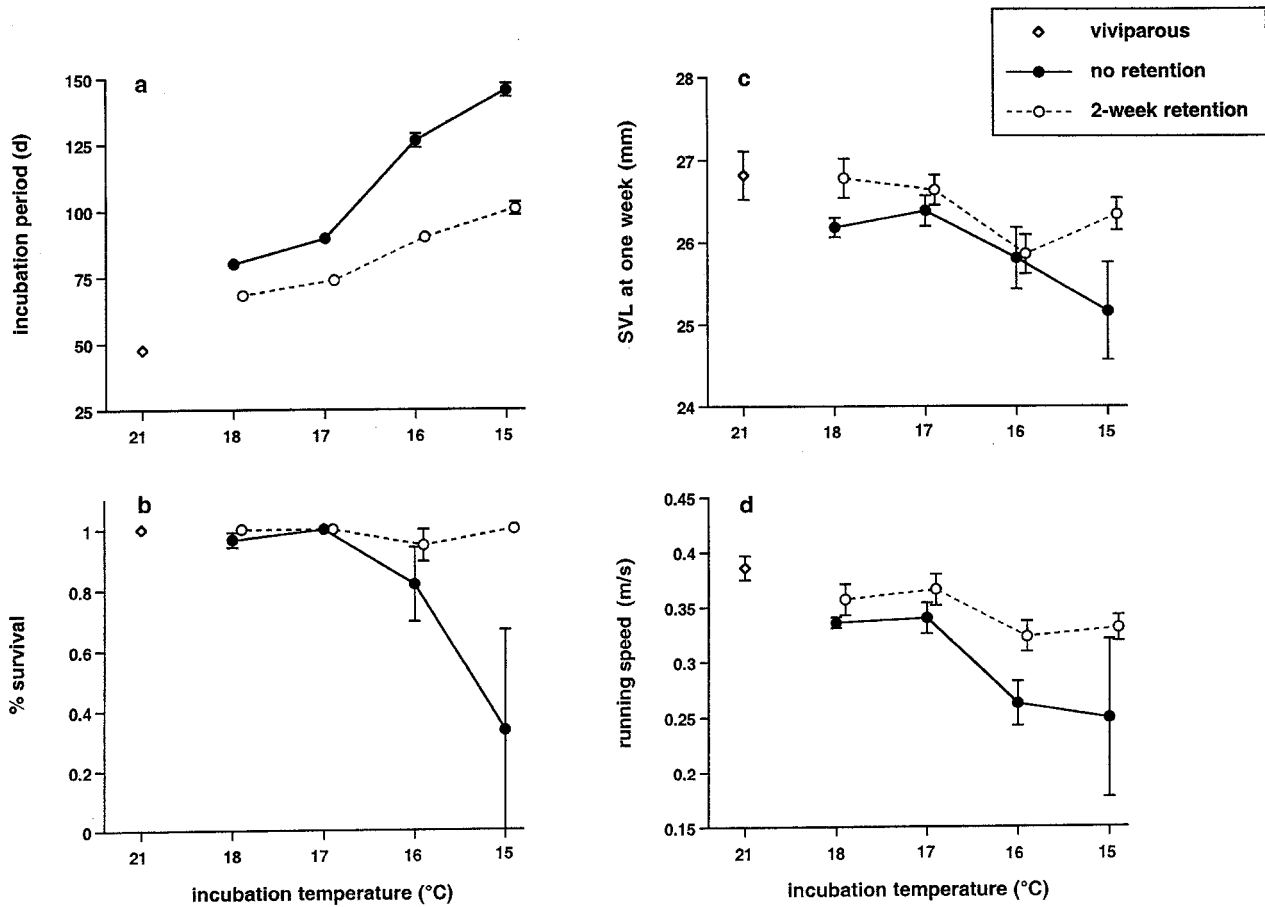
### *Laboratory Study, 1999–2000*

Thermal regimes during incubation affected several traits (fig. 1). Not surprisingly, eggs incubated at higher mean temperatures completed development sooner (ANOVA on incubation period,  $F = 391.91$ ,  $df = 3, 190$ ,  $P < .0001$ ), and a 2-wk period of retention at even higher temperatures further accelerated hatching ( $F = 514.87$ ,  $df = 1, 190$ ,  $P < .0001$ ). Importantly, however, there was a significant interaction between these two factors; “uterine retention” accelerated hatching to a greater degree in eggs that were then transferred to lower rather than higher mean incubation temperatures ( $F = 46.43$ ,  $df = 3, 190$ ,  $P < .0001$ ). Thus, 2 wk of “maternal” temperatures shortened the incubation period by 44 d when subsequent “nest” temperatures averaged  $15^{\circ}\text{C}$ , but by only 12 d when the nest averaged  $18^{\circ}\text{C}$  (fig. 1a).

The same general pattern, and the same kind of interaction, was evident for survival rate to hatching (fig. 1b). Eggs incubated at lower mean temperatures had much lower hatching success than did eggs kept warmer, but this effect was eliminated by an initial 2-wk period of “uterine retention” (interaction  $F = 10.11$ ,  $df = 3, 159$ ,  $P < .0001$ ). Dissection of dead eggs showed that mortality occurred over a broad range of embryonic stages.

The snout-vent length (SVL) of hatchlings was significantly affected by their incubation treatment ( $F = 2.82$ ,  $df = 3, 168$ ,  $P < .05$ ) but not by retention ( $F = 0.02$ ,  $df = 1, 168$ ,  $P = .97$ ; interaction  $F = 1.87$ ,  $df = 3, 168$ ,  $P = .14$ ). Growth rates in the first week after hatching were strongly affected by both of these factors, however, so the hatchling’s SVL at 1 wk of age was greater if the egg had been retained at higher temperatures immediately after laying ( $F = 7.13$ ,  $df = 1, 159$ ,  $P < .01$ ; see fig. 1c) and if it had subsequently developed at higher temperatures ( $F = 4.84$ ,  $df = 3, 159$ ,  $P < .005$ ; Fisher’s PLSD tests show that eggs from  $17^{\circ}$  and  $18^{\circ}\text{C}$  incubation produced larger hatchlings than those kept at  $16^{\circ}\text{C}$ ; interaction term  $F = 1.22$ ,  $df = 3, 159$ ,  $P = .30$ ).

Finally, higher-temperature incubation enhanced locomotor speeds of hatchlings, and retention at uterine temperatures accelerated them still further (fig. 1d; for speed over 1 m, effect of mean temperature,  $F = 9.95$ ,  $df = 3, 159$ ,  $P < .00001$ ; effect of 2-wk retention,  $F = 16.68$ ,  $df = 1, 159$ ,  $P < .0001$ ; interaction  $F = 1.52$ ,  $df = 3, 159$ ,  $P = .21$ ; for speed over 25 cm, effect of mean temperature,  $F = 3.80$ ,  $df = 3, 161$ ,  $P < .02$ ; effect of 2-wk retention,  $F = 4.10$ ,  $df = 1, 161$ ,  $P < .05$ ; interaction  $F = 2.21$ ,



**Figure 1:** Incubation periods, survival rates of eggs (% hatching), body sizes (snout-vent lengths), and running speeds (m/s, over 1 m distance) of hatchling skinks (*Bassiana duperreyi*) at 1 wk of age from a laboratory study mimicking natural nests at eight thermal regimes (mean temperatures of 15°, 16°, 17°, or 18°C, each with or without an initial 2-wk period of retention at higher temperatures). A ninth (viviparous) treatment involved retaining eggs at maternal body-temperature regimes throughout incubation. Mean values and associated standard errors are shown. See text for statistical analyses of these data.

df = 3, 161,  $P = .09$ ). This locomotor enhancement was not an indirect effect of the larger body sizes of lizards from higher-temperature incubation; there was no significant correlation between body size and running speeds in my data ( $P > .70$ ). Thus, the general pattern was that higher-temperature incubation produced “better” hatchlings (hatching early and alive, growing quickly, running fast). In keeping with this pattern, eggs maintained throughout development at maternal body temperatures (viviparous treatment in fig. 1) showed higher mean values for all of these traits than did their siblings that were incubated at more realistic nest temperatures.

*Field Study, 1999–2000*

Fortuitously, the two years of this study bracketed the entire range of weather conditions (and thus, nest tem-

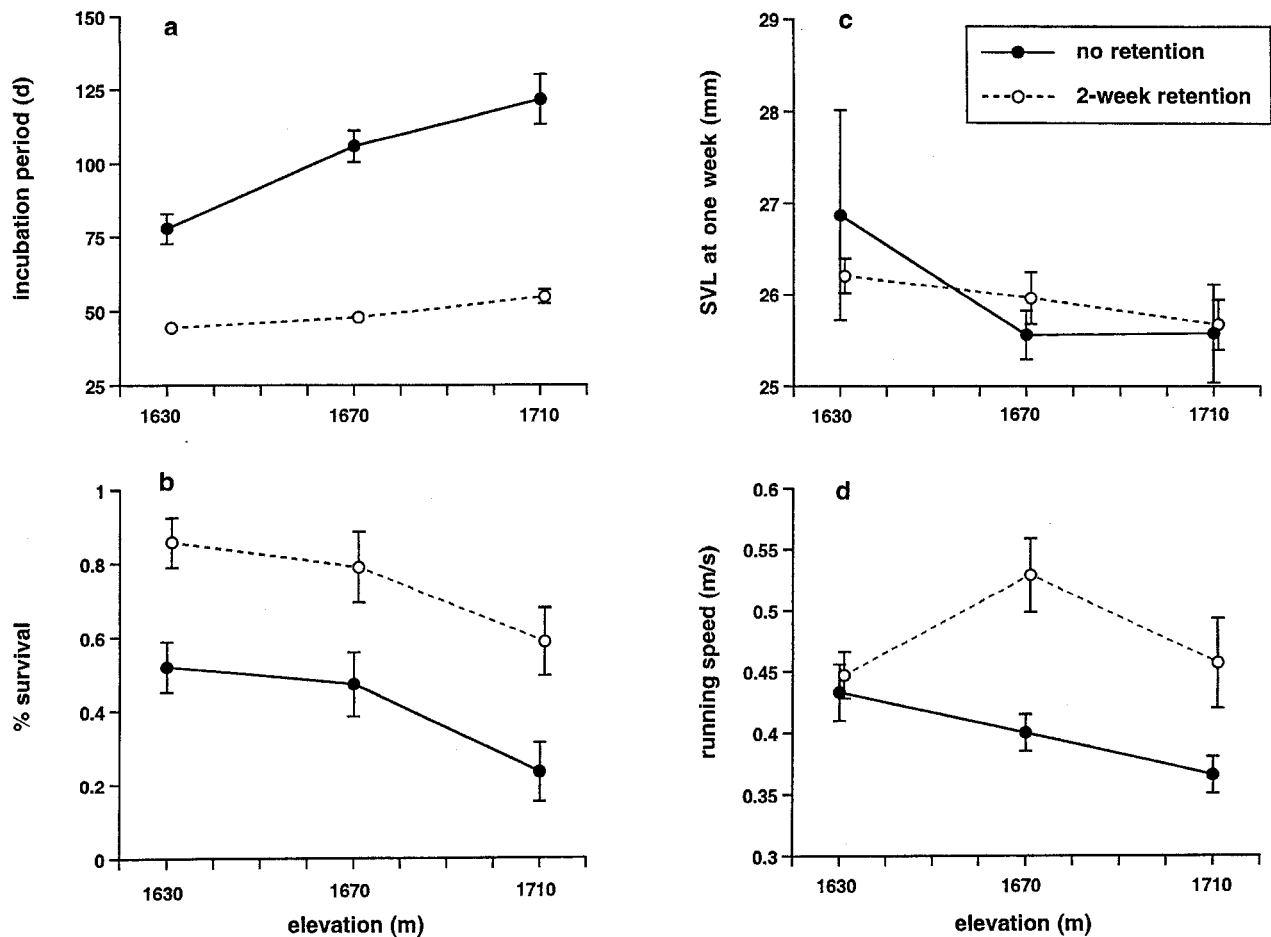
peratures) that we have recorded over seven years of studies in the Brindabella Range. The summer of 1999–2000 was the coldest on record over this period, with mean nest temperatures at different elevations on Mount Ginini averaging 13.6°–14.7°C. In contrast, the summer of 2000–2001 was the hottest that we have recorded, with mean nest temperatures per elevation ranging from 17.8° to 22.1°C. Thus, the laboratory incubation treatments (15°–18°C means) should provide a realistic simulation of normal conditions in field nests. Despite this variation in thermal conditions between years, the overall benefits to eggs of incubation at lower elevations were evident (and broadly similar) in both years of the study. Because of differences in experimental design among years, I analyzed data separately for 1999–2000 and 2000–2001.

For 1999–2000, a two-factor ANOVA (with elevation

and presence or absence of “uterine retention” as factors) gave similar results as for the laboratory study. For simplicity, this analysis did not include data from unmanipulated eggs (those that spent the entire incubation period in a nest), but data for these animals are shown in the figures. Total incubation periods were longer in higher-elevation nests ( $F = 19.44$ ,  $df = 2, 99$ ,  $P < .0001$ ) and in eggs that were not kept initially at uterine temperatures ( $F = 195.18$ ,  $df = 1, 99$ ,  $P < .0001$ ), with a strong interaction between these factors ( $F = 8.40$ ,  $df = 2, 99$ ,  $P < .0005$ ). Thus, a 2-wk retention period shortened incubation by 67 d for eggs at the top of the mountain but by only 33 d for eggs at the base of Mount Ginini (fig. 2a). Thus, elevation of the nest site strongly influenced incubation periods for “unretained” eggs (from 88 to 122 d) but had much less effect on “retained” eggs (44–55 d; fig.

2a). The hatching success of eggs also varied as a function both of the elevation of the nest ( $F = 6.17$ ,  $df = 2, 188$ ,  $P < .003$ ) and whether or not the egg had been retained for 2 wk at high (maternal) temperatures ( $F = 22.97$ ,  $df = 1, 188$ ,  $P < .0001$ ; interaction  $F = 0.02$ ,  $df = 2, 188$ ,  $P = .98$ ). Higher elevations reduced hatching success, and the effect was ameliorated by initial retention at maternal body temperatures (fig. 2b). Dissection showed that the embryos died at a wide range of developmental stages; eggs from high-elevation nests frequently displayed deformities (especially of the head and spine) similar to those seen in animals from low-temperature incubation in the laboratory study.

Nest elevation and retention at warmer temperatures had less effect on the body sizes of hatchlings (fig. 2c). Snout-vent length at hatching and at 1 wk of age did not



**Figure 2:** Incubation periods, survival rates of eggs (% hatching), body sizes (snout-vent lengths), and running speeds (m/s, over 25 cm distance) of hatchling skinks (*Bassiana duperreyi*) at 1 wk of age from field studies in the summer of 1999–2000. Some eggs were placed in artificial nests at various elevations as soon as they were found in natural nests. Other eggs (2-wk retention) were maintained in the laboratory for 2 wk at temperature regimes mimicking maternal body (=uterine) temperatures and then placed in the same artificial nests as the nonretained eggs. Mean values and associated standard errors are shown. See text for statistical analyses of these data.

differ among hatchlings from nests at different elevations or between those from eggs that were or were not retained at warmer regimes after laying (all  $P > .38$  in ANOVAs; see fig. 2c). Running speeds over 1 m were similarly unaffected (all  $P > .11$ ), but maximal speeds over 25 cm were enhanced by retention at warmer temperatures ( $F = 10.56$ ,  $df = 1, 99$ ,  $P < .002$ ; see fig. 2d). Elevation did not affect this trait ( $F = 1.35$ ,  $df = 2, 99$ ,  $P = .26$ ), but the interaction between elevation and retention showed the same pattern as for other variables (i.e., retention had the greatest effect on eggs in high-elevation nests; see fig. 2d) and was close to statistical significance ( $F = 2.83$ ,  $df = 2, 99$ ,  $P = .065$ ).

Field Study, 2000–2001

Analyses were more complex because of the additional treatments. A three-factor ANOVA on incubation periods (with elevation, number of weeks retention, and laboratory incubation regime as factors) produced a significant three-way interaction term ( $F = 3.34$ ,  $df = 6, 778$ ,  $P < .003$ ). Incubation periods increased at higher elevations, and developmental rates of eggs retained at cool temperatures in the laboratory were similar to (but slower than) those of eggs in nests (fig. 3a). Retention at warmer (uterine) thermal regimes accelerated development, especially at higher elevations (fig. 3a).

Survival rates of eggs also varied as a function of nest-site elevation and initial retention and the interaction between them. A three-factor ANOVA revealed a significant interaction between nest elevation and the thermal regime during an egg's initial retention period ( $F = 4.73$ ,  $df = 3, 779$ ,  $P < .003$ ). This interaction reflects the fact that the temperature at which an egg was retained had little effect on its hatching success if the egg was then incubated in a low-elevation nest (because hatching success was high for most such eggs; fig. 3b). However, retention at higher rather than lower temperatures dramatically enhanced hatching success for eggs incubated at higher elevations (fig. 3b). The longer the duration of initial retention at high temperatures, the greater the effect (fig. 3b). As in the previous year, deformities were commonly observed in embryos from dead eggs in high-elevation nests.

The body sizes of hatchlings were less strongly affected by incubation conditions. In a three-factor ANOVA (same design as above), the SVL of offspring (both at hatching and at 1 wk of age) showed no significant effects (all  $P > .40$ ) except that eggs incubated at lower elevations produced larger offspring (fig. 4a; for SVL at hatching,  $F = 5.28$ ,  $df = 3, 464$ ,  $P < .002$ ; for SVL at 1 wk,  $F = 6.12$ ,  $df = 3, 427$ ,  $P < .0005$ ; Fisher's PLSD tests show that Picadilly = Ginini bottom > middle > top). Running speeds over 1 m showed no consistent effects of incubation

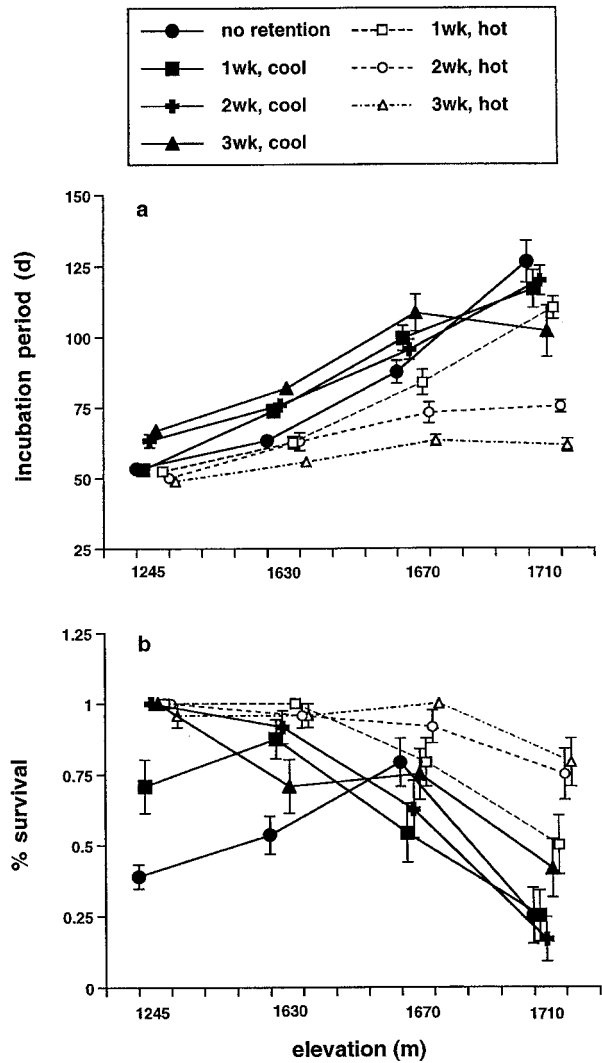


Figure 3: Incubation periods and survival rates (% hatching) of skink eggs (*Bassiana duperreyi*) from field studies in the summer of 2000–2001. Some eggs were placed in artificial nests at various elevations as soon as they were found in natural nests. Other eggs were maintained in the laboratory for 1, 2, or 3 wk either at (cool) nest temperatures or at warmer regimes mimicking maternal (= uterine) body temperatures and then placed in the same artificial nests as the nonretained eggs. Mean values and associated standard errors are shown. See text for statistical analyses of these data.

regime (all  $P > .09$ ), but higher-elevation nests produced hatchlings that were slower when tested over a shorter distance of 25 cm ( $F = 2.64$ ,  $df = 3, 385$ ,  $P < .05$ ; all other effects and interactions have  $P > .08$ ; see fig. 4b).

Calculation of Selection Differentials

From the above data, we can calculate the intensity of directional selection on a hypothetical mutant gene for

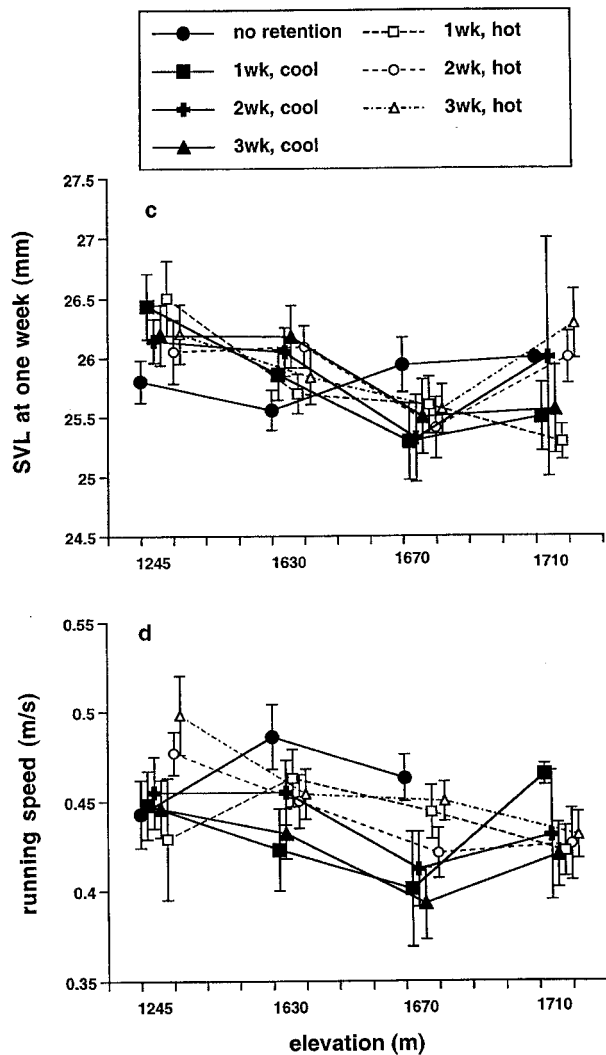


Figure 4: Body sizes (snout-vent lengths) and running speeds (m/s, over 25 cm distance) of hatchling skinks (*Bassiana duperreyi*) at 1 wk of age from field studies in the summer of 2000–2001. Some eggs were placed in artificial nests at various elevations as soon as they were found in natural nests. Other eggs were maintained in the laboratory for 1, 2, or 3 wk either at (cool) nest temperatures or at warmer regimes mimicking maternal (=uterine) body temperatures and then placed in the same artificial nests as the nonretained eggs. Mean values and associated standard errors are shown. See text for statistical analyses of these data.

prolongation of uterine retention of eggs at various elevations. The calculation necessarily ignores other factors; for example, some lizard species are better able to retain developing offspring in utero than are others (Andrews and Mathies 2000). However, I will proceed with the assumption that such obstacles do not exist. The simplest case involves hatching success because the link to fitness is clear. Using logistic regression with hatched or died as

the dependent variable and the duration of high-temperature (uterine) retention as a continuous independent variable, I conducted separate analyses for each elevation. All eggs retained at cool temperatures were scored as zero retention. To calculate relative fitness, the logistic regression coefficients were multiplied by the inverse of the mean fitness (survival rate) of eggs at each elevation (Janzen and Stern 1998). In the first (cooler) year of the study, directional selection on the duration of retention was significant at all three sites (lower Ginini, likelihood ratio from logistic regression  $\chi^2 = 9.95$ , 1 df,  $P < .002$ ; middle Ginini  $\chi^2 = 5.37$ , 1 df,  $P < .03$ ; upper Ginini  $\chi^2 = 7.80$ , 1 df,  $P < .006$ ). Data for the second (warmer) year of the study are more extensive and include a wider range of retention durations. Selection for prolongation of uterine retention was not statistically significant at the lowest elevation (Picadilly,  $\chi^2 = 2.99$ , 1 df,  $P = .08$ ), was marginally significant at the upper elevational limit of normal oviparous reproduction (bottom of Ginini  $\chi^2 = 5.72$ , 1 df,  $P < .02$ ), and was highly significant at both of the higher elevations (middle Ginini  $\chi^2 = 20.96$ , 1 df,  $P < .0001$ ; upper Ginini  $\chi^2 = 28.62$ , 1 df,  $P < .0001$ ). Overall, I detected statisti-

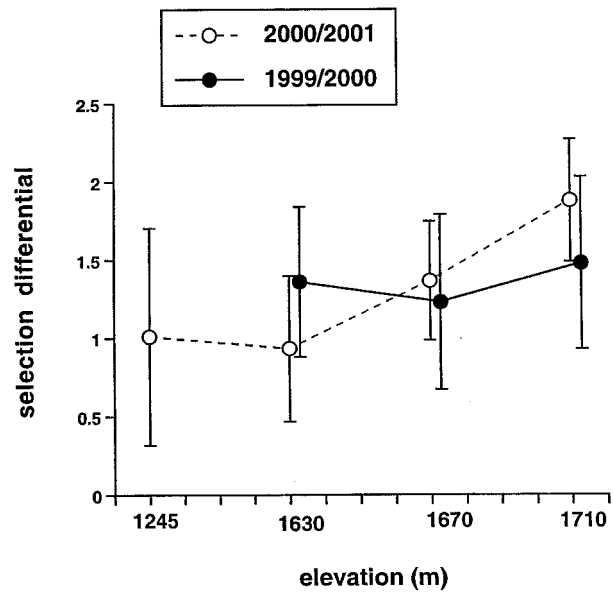


Figure 5: Selection differentials for the prolonged retention of eggs at high temperatures (mimicking uterine retention) as a function of elevation of the nests where eggs were then incubated in the field. The selection differentials were calculated from logistic regression coefficients and corrected to relative fitness using the methods of Janzen and Stern (1998). The dependent variable for these regressions was whether the egg hatched or died; thus, these selection differentials do not take into account sublethal effects of low-temperature incubation. Mean values and associated standard errors are shown for data from the summers of 1999–2000 and 2000–2001. See text for statistical analyses of these data.

cally significant selection for prolonged uterine retention of eggs at all three high-elevation sites in both years of the study (fig. 5). The calculated differentials (based on hatching success only) probably underestimate the real situation because most of the phenotypic traits that I measured also suggested that uterine retention enhanced offspring viability.

### Discussion

This study provides the most direct information yet available on selective forces operating on the uterine retention of eggs (and, thus, on the evolution of viviparity) in montane reptiles. Several aspects of this topic facilitated the study, enabling a much more robust examination of selective forces than would be possible for virtually any other major life-history transition. Similar advantages have stimulated previous studies also, but such work has generally been laboratory based and has often relied on (unrealistic) constant-temperature incubation. Field data on natural nests have often been lacking, so that the relevance of laboratory thermal regimes remains unknown. Even where field data have been available or actual field experiments have been conducted (Dumas 1964; Qualls 1997), the sites to be compared have been widely separated. This study was conducted on a single population of lizards, and the crucial comparisons spanned <100 m elevation in continuous habitat: a distance that a skink might well cover within a week's foraging activity (Pengilley 1972).

My data strongly support all of the following predictions from the "cold-climate" hypothesis for the evolution of reptilian viviparity. First, the upper elevational limit for egg layers in the field corresponded to the elevation above which there was a marked decrease in hatching success and hatchling viability. Second, this viability decrease at higher elevations comprised both a reduction in hatching success and a decrease in mean values for several hatchling traits (body size, growth rate, running speed) likely to affect organismal fitness (Andrews 2000). Third, brief retention at higher temperatures (mimicking those experienced by eggs in utero) enhanced both hatching success and offspring viability. Fourth, the viability increment due to "uterine" retention was greater for eggs incubated for the rest of development at cooler rather than warmer temperatures in the laboratory and at higher rather than lower elevations in the field. And, fifth, progressive increases in the duration of uterine retention generated increasing benefits to offspring viability. Thus, in the absence of constraints or opposing disadvantages, we would expect selection to favor a progressive increase in the duration of uterine retention of developing eggs at elevations above the usual upper limit for oviparous reproduction (fig. 5).

Both field and laboratory studies thus support the idea

that uterine retention of developing eggs can enhance hatching success and offspring viability in cold-climate reptiles. The similarity between results of the field and laboratory work suggests that the causal factor involved is nest temperature (the only factor manipulated in the lab) rather than some other correlated feature of high-elevation nests. Other studies on *Bassiana* show that hydric conditions in the nest have little or no effect on hatchling phenotypes, at least within the range recorded in the field (Flatt et al. 2001). Thus, my data support the hypothesis that reptilian viviparity evolves in cold climates because the thermal regimes of nest sites in such areas are less conducive to embryogenesis than are the thermal regimes experienced by eggs in utero. This hypothesis enjoys widespread support, but previous data have addressed it only indirectly (Tinkle and Gibbons 1977; Andrews 2000).

My study also addresses the applicability of alternative models about the selective forces involved in the transition from oviparity to viviparity in reptiles (table 1). The data show that prolonged incubation under cool conditions delays hatching, reduces hatching success, and generates "inferior" hatchling phenotypes and that uterine retention reduces all of these negative effects (table 1). The other putative selective forces listed in table 1 remain untested. Some were eliminated by the design of my experiments and so cannot have been responsible for the patterns in viability. For example, the increased mortality of eggs in nests at higher elevations was not due to freezing (nest temperatures were monitored and never fell below lethal levels; Shine 1983), desiccation (dead eggs were not shriveled), or predation (the nylon bags excluded predators). Also, a similar increase in mortality was seen in low-temperature incubation in the laboratory (fig. 1), where these mortality sources were excluded. For the same reason, the viability effects that I documented cannot be attributed to advantages of early hatching (either to avoid the onset of lethal conditions in winter or for more subtle reasons; table 1). We can thus conclude that none of these additional putative selective forces need to be invoked to explain why selection favors prolonged uterine retention of eggs in cold environments (fig. 5). Nonetheless, my study has not falsified these possibilities; such processes may well amplify the fitness disadvantages of cool nests and the consequent advantages of uterine retention. These ideas could feasibly be tested using the same kinds of methods as employed in this study.

The selection differentials calculated from logistic regression (fig. 5) reveal a progressive advantage to uterine retention of eggs at higher elevations, as predicted by the cold-climate hypothesis. However, about 20% of eggs hatched successfully even at the highest location that I tested, well above the elevational limit for oviparous reproduction in this area (figs. 2, 3). Why then are oviparous

lizards not found reproducing at these higher levels? The answer probably lies in sublethal effects of low-temperature incubation. Eggs that were kept at low temperatures either in the field or in the laboratory not only were less likely to hatch but also produced smaller, slower-running hatchlings (figs. 1, 2, 4). These phenotypic effects plausibly reduce hatchling fitness and so add to the disadvantages of high-elevation nesting.

Andrews (2000, p. 251) recently noted that “even after more than a century of speculation about the evolution of viviparity in reptiles, the selective basis for this phenomenon is still poorly understood.” Experimental studies like the present analysis, focusing on oviparous reptiles at the upper elevational limit of their distribution, may offer a useful approach to resolving some of these long-standing issues. We will certainly need studies on a wide range of taxa and in different places because many aspects of the biology of the study organism and of the habitat itself may well modify the operation of selective forces. For example, even closely related species may differ in their ability to retain developing eggs (Andrews and Mathies 2000) and in their physiological adaptations to low-temperature incubation (Shine 1999). One great advantage of studying the evolution of viviparity in cold-climate reptiles is the enormous potential for comparative study across disparate lineages and habitats. Indeed, this topic may provide an ideal model system for understanding the ways in which natural selection has operated during a major phylogenetic transition in modes of reproduction.

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#### Literature Cited

- Andrews, R. M. 2000. Evolution of viviparity in squamate reptiles (*Sceloporus* spp.): a variant of the cold-climate model. *Journal of Zoology* (London) 250:243–253.
- Andrews, R. M., and T. Mathies. 2000. Natural history of reptilian development: constraints on the evolution of viviparity. *BioScience* 50:227–238.
- Blackburn, D. G. 1982. Evolutionary origins of viviparity in the Reptilia. I. Sauria. *Amphibia-Reptilia* 3:185–205.
- . 1985. Evolutionary origins of viviparity in the Reptilia. II. Serpentes, Amphisbaenia, and Ichthyosauria. *Amphibia-Reptilia* 6:259–291.
- . 1995. Saltationist and punctuated equilibrium models for the evolution of viviparity and placentation. *Journal of Theoretical Biology* 174:199–216.
- Cogger, H. G. 1992. Reptiles and amphibians of Australia. 4th ed. Reed Books, Sydney.
- Dumas, P. C. 1964. Species-pair allopatry in the genera *Rana* and *Phrynosoma*. *Ecology* 45:178–181.
- Endler, J. A. 1986. Natural selection in the wild. Princeton University Press, Princeton, N.J.
- Fairbairn, J., R. Shine, C. Moritz, and M. Frommer. 1998. Phylogenetic relationships between oviparous and viviparous populations of an Australian lizard (*Lerista bougainvillii*, Scincidae). *Molecular Phylogenetics and Evolution* 10:95–103.
- Flatt, T., R. Shine, P. A. Borges-Landaez, and S. J. Downes. 2001. Phenotypic variation in an oviparous montane lizard (*Bassiana duperreyi*): the effects of thermal and hydric incubation environments. *Biological Journal of the Linnean Society* 74:339–350.
- Gould, S. J., and R. C. Lewontin. 1979. The spandrels of San Marco and the panglossian paradigm: a critique of the adaptationist programme. *Proceedings of the Royal Society of London B, Biological Sciences* 205:581–598.
- Greer, A. E. 1989. The biology and evolution of Australian lizards. Surrey Beatty, Sydney.
- Harvey, P. H., and M. D. Pagel. 1991. The comparative method in evolutionary biology. Oxford Studies in Ecology and Evolution. Oxford University Press, Oxford.
- Heulin, B., Y. Surget-Groba, A. Guiller, C. P. Guillaume, and J. Deunff. 1999. Comparisons of mitochondrial DNA (mtDNA) sequences (16 rRNA gene) between oviparous and viviparous strains of *Lacerta vivipara*: a preliminary study. *Molecular Ecology* 8:1627–1631.
- Janzen, F. J., and H. S. Stern. 1998. Logistic regression for empirical studies of multivariate selection. *Evolution* 52:1564–1571.
- Lee, M. S. Y., and R. Shine. 1998. Reptilian viviparity and Dollo's Law. *Evolution* 52:1441–1450.
- Mell, R. 1929. Beitrage zur Fauna Sinica. IV. Grundzuge einer Okologie der chinesischen Reptilien und einer herpetologischen Tiergeographie Chinas. Walter de Gruyter, Berlin.
- Packard, G. C., C. R. Tracy, and J. J. Roth. 1977. The physiological ecology of reptilian eggs and embryos, and the evolution of viviparity within the class Reptilia. *Biological Review* 52:71–105.
- Pengilley, R. 1972. Systematic relationships and ecology of some lygosomine lizards from southeastern Australia. Ph.D. diss. Australian National University, Canberra.
- Qualls, C., R. Shine, S. Donnellan, and M. Hutchinson. 1996. The evolution of viviparity within the Australian scincid lizard *Lerista bougainvillii*. *Journal of Zoology* (London) 237:13–26.
- Qualls, C. P. 1997. The effects of reproductive mode and climate on reproductive success in the Australian lizard, *Lerista bougainvillii*. *Journal of Herpetology* 31:60–65.

- Sergeev, A. M. 1940. Researches in the viviparity of reptiles. Pages 1–34 in Moscow Society of Naturalists (Jubilee Issue).
- Shine, R. 1983. Reptilian viviparity in cold climates: testing the assumptions of an evolutionary hypothesis. *Oecologia* (Berlin) 57:397–405.
- . 1985. The evolution of viviparity in reptiles: an ecological analysis. Pages 605–694 in C. Gans and F. Billett, eds. *Biology of the Reptilia*. Vol. 15. Wiley, New York.
- . 1995. A new hypothesis for the evolution of viviparity in reptiles. *American Naturalist* 145:809–823.
- . 1999. Egg-laying reptiles in cold climates: determinants and consequences of nest temperatures in montane lizards. *Journal of Evolutionary Biology* 12: 918–926.
- Shine, R., and J. J. Bull. 1979. The evolution of live-bearing in lizards and snakes. *American Naturalist* 113:905–923.
- Shine, R., and P. S. Harlow. 1996. Maternal manipulation of offspring phenotypes via nest-site selection in an oviparous reptile. *Ecology* 77:1808–1817.
- Shine, R., M. J. Elphick, and P. S. Harlow. 1997. The influence of natural incubation environments on the phenotypic traits of hatchling lizards. *Ecology* 78: 2559–2568.
- Shine, R., E. G. Barrott, and M. J. Elphick. 2002. Some like it hot: effects of forest clearing on nest temperatures of montane reptiles. *Ecology* (in press).
- Sinervo, B. 1994. Experimental tests of reproductive allocation paradigms. Pages 73–90 in L. J. Vitt and E. R. Pianka, eds. *Lizard ecology: historical and experimental perspectives*. Princeton University Press, Princeton, N.J.
- Sinervo, B., P. Doughty, R. B. Huey, and K. Zamudio. 1992. Allometric engineering: a causal analysis of natural selection on offspring size. *Science* (Washington, D.C.) 285:1927–1930.
- Smith, S., and R. Shine. 1997. Intraspecific variation in reproductive mode within the scincid lizard *Saiphos equalis*. *Australian Journal of Zoology* 45:435–445.
- Tinkle, D. W., and J. W. Gibbons. 1977. The distribution and evolution of viviparity in reptiles. *Miscellaneous Publications, Museum of Zoology, University of Michigan*, no. 154, pp. 1–55.
- Weekes, H. C. 1933. On the distribution, habitat and reproductive habits of certain European and Australian snakes and lizards, with particular regard to their adoption of viviparity. *Proceedings of the Linnean Society of New South Wales* 58:270–274.
- Williams, G. C. 1966. *Adaptation and natural selection*. Princeton University Press, Princeton, N.J.
- . 1975. *Sex and evolution*. Princeton University Press, Princeton, N.J.