Evolution of viviparity in squamate reptiles (*Sceloporus* spp.): a variant of the cold-climate model

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Abstract

An alternative to the cold-climate model for the evolution of viviparity is that the impetus for the initial transition from oviparity to viviparity is not an increase in the duration of egg retention but a shift in the location of nests to more superficial and thus warmer locations in the soil profile as temperature declines with increasing altitude or latitude. Shallower nest placement, however, would lead to increased egg mortality as a result of physiological stress or predation, and enhanced egg mortality would thus provide the initial benefit of extended egg retention. To test this hypothesis, I examined the thermal biology of three species of *Sceloporus* lizards living at high altitudes: *S. virgatus* (1800 m), *S. aeneus* (2800 m), and *S. bicanthalis* (3200 m). The oviparous *S. virgatus* and *S. aeneus* females laid eggs at depths of 6 and 2 cm, and mean body/nest temperatures were 24.6/25.2 °C and 19.9/20.8 °C, respectively. Because the initial increment in the duration of egg retention is presumably short, females of these oviparous species cannot initially keep their eggs appreciably warmer than they would be in nests. In contrast, mean temperatures of simulated nests (17.6 °C) of the viviparous *S. bicanthalis* were similar to the 17 °C low temperature threshold for development, and temperatures in some nests fell below freezing at night and rose to lethal levels during the day. Mean body temperatures of *S. bicanthalis* females were 20.1 °C; eggs retained during the entire developmental period would hatch 17 days sooner than they would if laid in a nest. Extended egg retention at the highest elevation site would thus provide two benefits: reduced mortality of eggs and accelerated development relative to that in a nest. These benefits, however, accrue because of the initial shift of nests from deeper to more superficial depths in the soil profile.

Key words: *Sceloporus*, viviparity, thermobiology, embryonic development, nest predation

INTRODUCTION

The evolution of viviparity in the squamate reptiles is associated with high elevations and latitudes (Packard, Tracy & Roth, 1977; Tinkle & Gibbons, 1977; Shine & Bull, 1979; Shine, 1985). The cold-climate model, as outlined most explicitly by Shine (1985), currently provides a widely accepted explanation for this phenomenon. The model has three major features: (1) viviparity evolves from oviparity through gradual increases in the length of egg retention, that is, the length of time that eggs spend in the oviduct prior to oviposition; (2) because of the thermoregulatory capabilities of females, retained eggs are warmer than they would be in a nest. As a result, the rate of embryonic development, which is temperature dependent, is enhanced, and the eggs are not exposed to deleterious extreme temperatures; (3) the thermal benefit of egg retention increases as environmental temperatures decrease. In essence, the cold-climate model posits that the evolution of viviparity is

associated with the thermal biology of embryos, such that egg retention and viviparity can compensate for the effects of declining ambient temperature on the rate of embryonic development with increasing elevation or latitude. Such compensation ensures successful embryonic development and that eggs hatch at an appropriate time of year (Shine & Bull, 1979; Shine, 1985).

The cold-climate model is particularly compelling because it provides a benefit for the intermediate stages of egg retention that are associated with the evolution of viviparity. None the less, Shine & Bull (1979) point out that the 'correlation between cold climate and the origin of live-bearing does not indicate that we have anticipated the correct reason for this correlation' and go on to say that 'it could be that cold climate merely correlates with some other factor that is a more direct cause of live-bearing or that cold climate is indeed the cause but that we have misinterpreted how it selects for live-bearing'. Despite their caveats, however, environmental

factors other than temperature have been difficult to associate with selection for an increase in the length of egg retention. For example, the idea that very wet or very dry environments might be associated with the evolution of viviparity (Weekes, 1935; Neill, 1964) seems unlikely because short periods of retention would not enhance the survival of eggs, assuming that these extreme conditions are typical of the entire incubation period. Another hypothesis is that high mortality of eggs in nests could make viviparity beneficial (Neill, 1964). However, Shine & Bull (1979) and Shine (1985) point out that an increase in the length of egg retention would not increase the survival of eggs if most mortality occurs shortly after oviposition. An increase in the length of egg retention would be favoured only if mortality is proportional to the length of the incubation period.

The idea that viviparity evolves in response to high egg mortality has been discounted as a general explanation for the evolution of viviparity for several reasons. Firstly, for turtles, a group whose nesting ecology is comparatively well known, the highest mortality typically occurs at oviposition and shortly thereafter (e.g. Moll & Legler, 1971; Congdon et al., 1983). Secondly, the association between recent origins of viviparity and cold climates (high elevations and latitudes) is very strong (Shine, 1985). Thirdly, the classic cold-climate model provides such a plausible explanation for the evolution of viviparity that the need for critical tests of its assumptions has been largely overlooked. In this paper, I hypothesize that enhanced mortality of eggs in nests may be the most immediate selective agent for extended egg retention. Because this hypothesis would operate only in the broader context of the cold-model, it should be viewed as complementary to the more general model. The following three steps provide a mechanism by which enhanced mortality of eggs would lead to the evolution of viviparity.

Step 1: Changes in the location of nests

Let us assume that an oviparous species is exposed to a relatively cool climate as a result of invasion of a high elevation or latitude. All other things being equal, the mean temperature of nests during the incubation period and the mean temperature of reproductive females will be lower in the new environment than in the old environment. The reason is that ambient temperature declines predictably with both increasing elevation and latitude. Nest temperature will decrease because soil temperature is a direct function of air temperature (Russell, 1973; Parton, 1984). Female body temperature will decrease as well, despite the ability of females to thermoregulate. Females may be able to compensate for low ambient temperature, while they are active, by increasing the time spent thermoregulating or by choosing relatively warm microhabitats or both (Hertz & Huey, 1981; Marquet et al., 1989; Adolph, 1990). However, body temperatures overall will decline with

elevation or latitude because the daily period when females can thermoregulate is a shorter portion of the diel cycle at high elevations and high latitudes than at low elevations and latitudes (Hertz & Huey, 1981; Marquet et al., 1989; Sinervo & Adolph, 1994; Mathies & Andrews, 1995). At high elevations and latitudes, mean body temperatures will thus become increasingly influenced by inactivity when body temperature reflects the relatively cool ambient temperature. This means that egg retention in the new environment will not necessarily ensure that retained embryos are even as warm as they were in nests in the old environment.

The importance of a suitable nest temperature is underscored by the migration of many squamates away from their home ranges to better insolated or warmer areas for nesting (Blair, 1960; Rand, 1972; Werner, 1983; Rose, 1993), or the selection of thermally favourable microhabitats within their home ranges (Sexton & Claypool, 1978; Burger & Zappalorti, 1986; Shine & Harlow, 1996). Nest site selection is an active process in which gravid females assess the suitability of potential sites before laying their eggs (Deslippe et al., 1990). These observations suggest that the first and most effective response to cool climates would be for females to select relatively warm microhabitats for their nests.

The three major variables that affect the temperatures of nests at any particular site are insolation, depth of the nest in the soil, and nest substrate. Squamates generally avoid shaded nest sites (Blair, 1960; Rand, 1972; Burger & Zappalorti, 1986), although exceptions include small species of the lowland tropics, e.g. Anolis (Andrews, 1988). At the same depth, nests in full sun have higher mean temperatures and greater variance in temperature than shaded nests (Packard & Packard, 1988). Both the mean and the range of nest temperature decrease with depth in the soil, and nests must be located closer to the surface in cool than hot environments to have the same mean temperature (although not the same range) (Packard & Packard, 1988). Nests of squamates in hot deserts are located well below the surface, while nests in cool habitats are located near the surface. For example, near Palm Desert, California, eggs of Dipsosaurus dorsalis must be placed at least 16 cm below the surface to avoid high lethal temperatures, and at least 20 cm for suitable moisture (Muth, 1980). At the other extreme, in northern Michigan, oviparous snakes place their eggs in well insolated sites and bury them only superficially; green snakes oviposit in rotting vegetation (Sexton & Claypool, 1978), and ringneck snakes place their eggs under the bark of rotting logs (F. N. Blanchard, 1936). Sexton & Claypool (1978) argue that one of the adaptations of oviparous snakes to life at high latitudes is use of such microhabitats for oviposition. Nest substrates also differ in their thermal properties. For example, some squamates place their nests in rotting vegetation or wood where microbial heat production would enhance temperature (Brown, 1956; Smith, 1969; Sexton & Claypool, 1978), although the ease of constructing nests in these substrates may contribute to their use for nesting.

As a consequence of the relatively low environmental temperatures at high elevations or latitudes, behavioural adjustments by female squamates should thus consist of a shift to shallower nests, irrespective of substrate type, in well-insolated microhabitats. Such a mechanism for enhancing embryonic temperatures could be an immediate or a rapidly evolved response because assessment of potential nest environments is a normal part of the female's behavioural repertoire. It would also be more effective than either a facultative or an evolved increase in the length of egg retention. The reason is that the temperature of the nest affects embryonic development from oviposition to hatching, whereas any temperature change accrued by an increase in the length of egg retention would be of shorter duration. The consequence of such changes in nesting behaviour is that embryos will be warmer in the new environment than they would have been if nesting behaviour had not changed.

Step 2: Increased mortality rates of eggs

Initially, changes in the location of nests might have little effect on the mortality of eggs. However, with increasingly low ambient temperatures and the corresponding shift to even more shallow locations for nests, eggs should have increased mortality. Eggs that are close to the surface of the soil would be exposed to large diel fluctuations in temperature and moisture, and temperature extremes are lethal to embryos (Muth, 1980; Packard & Packard, 1988; Overall, 1993). In addition, eggs that are located close to the surface of the soil, or placed superficially in loose substrates such as piles of rotting vegetation, may be more easily located by predators, or may be more easily exposed by rainfall, wind, or by accident, i.e. digging or trampling by large animals, than eggs that are located further below the soil surface. Thus, at some point on an elevational or latitudinal gradient, the effectiveness of behavioural compensation by nesting females for decreasing ambient temperature would be offset by the increased mortality of eggs.

Step 3: Selection for extended egg retention and viviparity

At this stage, an increase in the length of egg retention would be adaptive because it would reduce the time that eggs were at risk, and even short periods of egg retention would reduce the mortality of eggs. For example, risk that is proportional to the length of the incubation period has been observed for the eggs of *Anolis* lizards. Eggs are typically laid on the soil surface under leaf litter, and predation by ants occurs throughout the incubation period, although somewhat more rapidly at first (Andrews, 1982; Chalcraft & Andrews, 1999). Selection for increased periods of egg retention may thus be an immediate consequence of the increased risk

of mortality for eggs as a species invades increasingly higher elevations or latitudes.

One critical difference between the classic cold-climate model and the model presented here concerns the initial consequence of invasion of high latitudes or elevations by an oviparous squamate. The classic cold-climate model posits that an increase in the length of egg retention follows invasion of a cool climate; the selective benefit is the enhancement of embryonic development during the period of retention due to the thermoregulatory behaviour of females. Alternatively, my proposed variant of the cold-climate model posits that females shift to shallower nest locations; the selective benefit is the enhancement of embryonic development during the entire period of development as a result of more favourable nest temperatures nearer the soil surface. The opposing fundamental bases of these two models can thus be tested. To do so, I present a set of data on the thermal biology of three species of *Sceloporus* lizards at North American study sites ranging in elevation from 1800 to 3200 m.

MATERIALS AND METHODS

Overview of the genus *Sceloporus*

The genus *Sceloporus* has approx. 70 species of which about 30 are viviparous (Sites *et al.*, 1992). Viviparity has evolved independently at least 4 times within the genus (Méndez-de la Cruz, Villagrán-Santa Cruz & Andrews, 1998), and the origin of viviparity is associated with high elevations at tropical latitudes (Guillette *et al.*, 1980). *Sceloporus* is thus a singularly appropriate taxon for an evaluation of the thermal environments associated with the evolution of extended egg retention and viviparity.

Species, study sites and observations on the thermal biology of females and their nests

To test the hypothesis that the first step in invasion of cool climates is a shift from deeper to shallower, and thus to warmer nest locations, I collected information on the thermal biology of gravid or pregnant female *Sceloporus* living at high elevations and on their nests. The focus is on temperature, although both temperature and moisture must be adequate for successful embryonic development (Muth, 1980; Packard & Packard, 1988). Nesting by *Sceloporus* is associated with times of year when soil moisture is suitable; I assume that within those periods, location of nests is related more to the thermal than to the moisture properties of nest sites.

Observations on *Sceloporus virgatus*, a member of the *undulatus* species group, were made at 1800 m in the Chiricahua Mountains of south-eastern Arizona, U.S.A. *Sceloporus virgatus* is terrestrial, and is associated with riparian habitats in scrub oak forest. I also made observations on *Sceloporus aeneus* at 2800 m and

Sceloporus bicanthalis at 3200 m in the transvolcanic axis of central Mexico. *Sceloporus aeneus* and *S. bicanthalis*, members of the *scalaris* species group, are sister species, but *S. aeneus* is oviparous and *S. bicanthalis* is viviparous (Guillette, 1982; Mink & Sites, 1996). Observations on *S. aeneus* were made at three localities: CICITEC (Estado de México), and Milpa Alta and Ajusco (Distrito Federal), all at 2800 m. These sites are near the upper elevational limit for oviparous *Sceloporus*; at elevations higher than 2900–3000 m, *S. aeneus* is replaced by its viviparous sister-species, *S. bicanthalis*. Observations on *S. bicanthalis* were made at Parque Nacional Zoquiapan (Estado de México) at an elevation of 3200 m. Both species are terrestrial and are associated with various species of bunch-grasses (*Festuca* and *Muhlenbergia*) that comprise the understory of montane pine forests.

Mean air temperature for the period from ovulation to hatching was used as an index of ambient temperature at each site. Data from the nearest weather station to each site were obtained from long-term records of the U.S. Weather Service and from the Servicio Meterológico Mexicano, Ciudad de México.

Monthly means of daily maximum temperature were used as an index of ambient temperatures most relevant to thermoregulating lizards. I compared the mean maximum temperatures and the diel range in temperatures (difference between monthly means of maximum and minimum temperature) at tropical and temperate latitudes as a function of elevation. Temperate latitudes were represented by July temperatures because they represent the annual maximum. Tropical latitudes were represented by the month with the highest mean maximum temperature and these typically occur in the spring. Records from Arizona and Oregon east of 121.5°N longitude were selected because they represent the maximum species diversity of *Sceloporus* in temperate regions and the northern part of the range of *Sceloporus*, respectively. Data from the first 5 stations within successive 500 m increments in elevation were selected from C. Blanchard (1985) for each state. Because of the paucity of data for high elevations, the data set for Arizona was supplemented with the highest station in New Mexico and the highest station in Colorado and the data set for Oregon was supplemented with the 5 highest stations in Idaho. Weather data for tropical latitudes within the range of *Sceloporus* were from Pearce & Smith (1984) and included all observations for Mexico ($n = 6$) and Central America as far south as Costa Rica ($n = 6$). Because few high elevation stations were represented in this data set, 2 further sources for weather records were used. One was the Servicio Meterologico Mexicano, Ciudad de Mexico, for Milpa Alta (2420 m), Ajusco (2839 m), Rio Frio (3000 m), and Nevado de Toluca (4140 m). These locations are located within 75 km of Mexico City. The other was the Annuario Meteorologico for 1965 and 1968 (Servicio Meteorologico y Sismologico Nacional, San Jose, Costa Rica) for Rancho Redondo (1780 m), Zarcero (1736 m), Sanatorio Durán (2337 m), and Villa Mills (3096 m).

The data reported here on the body temperatures of gravid *S. virgatus* and on the temperatures of nests were taken from Rose (1993) and Andrews & Rose (1994). The nesting biology of this species is well known. Females lay 1 clutch a year at the advent of monsoon rains in early July. Females leave their territories before oviposition, and dig nest burrows in exposed south-facing slopes. Eggs are placed at a depth of about 6 cm (Rose, 1993, pers. comm.). Oviposition typically occurs when embryos reach Dufaure & Hubert (1961) stages 31–32; eggs laid at stage 32 and incubated at simulated field temperatures hatch in 58 days (Andrews & Rose, 1994).

Mean body temperatures of gravid or pregnant female *S. aeneus* and *S. bicanthalis* were determined from measurements of their body temperatures during the normal activity period (Andrews, Méndez-de la Cruz, Villagrán-Santa Cruz and Rodríguez-Romero, 1999) and from estimates of their body temperatures during the period of inactivity. Body temperatures of active lizards were measured with a Schultheis quick-reading thermometer ($\pm 0.2\,°C$) or with a thermocouple thermometer ($\pm 0.5\,°C$) immediately after capture. To determine the body temperatures of non-active lizards, I assumed that they would be the same as the ambient temperature in retreat sites. Probes of Hobotemp data loggers were placed in the bases of bunch grasses where lizards took shelter, and these retreat temperatures were recorded hourly. Observations were made on 5 days between 10 and 18 July 1993 at the 3 *S. aeneus* localities (4 retreat sites) and on 2 days between 20 and 28 March 1994 at the *S. bicanthalis* locality (2 retreat sites), respectively. Overall mean body temperatures were determined from the hourly means of temperatures during activity and during inactivity.

Reproduction by *S. aeneus* is seasonal. Eggs are laid in mid- to late July. Oviposition occurs as early as stage 30 (Manríquez-Moran, 1995), but females may retain eggs longer (Guillette & Lara-Gongara, 1986). Eggs are laid in the freshly pushed-up mounds of pocket gophers *Pappogeomys merriami* (Gillette & Lara-Gongora, 1986). Eggs in 12 nests at Milpa Alta in 1993 and 1996 were located 0.5–2.5 cm below the soil surface in gopher mounds that were unshaded by vegetation. In contrast, reproduction by *S. bicanthalis* is considerably less seasonal. Pregnant *S. bicanthalis* are found from January to September, although the highest proportion of pregnant females occurs during March–June (Manríquez-Moran, 1995).

To determine nest temperatures of *S. aeneus*, nests and potential nest sites were monitored with Hobotemp data loggers. On 18 July 1996, a nest at the Milpa Alta site was carefully excavated from the side and a probe from a Hobotemp data logger was placed in the middle of the clutch at 2 cm below the surface. To determine the temperature lower in the soil profile at the same depth as *S. virgatus* nests, a second probe was placed 6 cm below the soil surface directly below the clutch. Probes were placed at 2 and 6 cm depth at 2 similar sites that did not contain eggs. Hourly readings were made

Table 1. Summary of ambient temperature, nest temperature, and female body temperature of three species of *Sceloporus*. Ambient temperature is the mean air temperature during the incubation/gestation period at each of the three locations; June–September for *S. virgatus*, March–September for *S. aeneus*, and the entire year for *S. bicanthalis*. Female body temperatures are the overall means of temperatures during activity and when inactive. Corrected temperature means represent effective temperatures for development (see Materials and methods). Nest depths were 6 cm for *S. virgatus* and 2 cm for *S. aeneus* and *S. bicanthalis*. Incubation periods are calculated from embryonic stage 30 to hatching or birth. Temperatures and associated incubation periods for nests at 6 cm for *S. aeneus* are given in parentheses

	Species		
	Sceloporus virgatus (1800 m)	*Sceloporus aeneus* (2800 m)	*Sceloporus bicanthalis* (3200 m)
Temperature (°C)			
Ambient	20.7	17.1	10.2
Nest/female	24.6/25.2	16.3/15.9 (16.4)	11.7/11.8
Nest/female (corrected)	24.6/25.2	19.9/20.8 (18.8)	17.6/20.1
Incubation period (days)			
Nest/female (corrected)	69/66	71/65 (78)	87/70

from 18 July to 1 August. On 1 August, data were transferred to a computer, and temperature recording was continued at the initial nest. At this time, probes were placed at a depth of 2 cm in an additional nest with eggs and at 2 other similar sites without eggs. Observations were continued to 27 September, about the time when hatching begins.

Because *S. bicanthalis* is viviparous, the temperatures at actual nest sites could not be monitored. To determine the nest temperature of a hypothetical oviparous *S. bicanthalis*, I assumed that nests would be placed in the same microhabitats as those used by *S. aeneus*. In March 1994, I recorded temperatures hourly for 24 h in 2 different gopher mounds at a depth of 2 cm. In 1996, 1 potential nest site was monitored from 21 July to 11 September.

Incubation period and temperature

Data on incubation length of *S. aeneus* and *S. undulatus* (a close relative of *S. virgatus*) at constant temperatures (Andrews, Mathies, Qualls & Qualls, 1999) were used to predict incubation periods at female and nest temperatures observed in the field. These data represent the length of incubation for embryos that are at stage 30 at the time of oviposition. To correct for the exponential relationship between the length of incubation (IP, days) and incubation temperature (T, °C), observations of the length of the incubation period were natural log transformed before regression analysis. Those relationships were:

$$S.\ aeneus \quad \ln IP = 6.0834 - 0.0917T \ \text{(eq. 1)}$$
$$(F_{1,4} = 229, P < 0.0001, R^2 = 0.98), \text{ and}$$
$$S.\ undulatus \quad \ln IP = 6.483 - 0.0913T \ \text{(eq. 2)}$$
$$(F_{1,3} = 77, P < 0.001, R^2 = 0.95).$$

The incubation period of *Sceloporus* lizards is the same when incubation occurs at a given constant tem-perature and at a symmetrically fluctuating temperature with the same mean as long as temperature does not fluctuate below the threshold for embryonic development (Andrews, Mathies, *et al.*, 1999). This condition was met for *S. virgatus* but not for *S. aeneus* and *S. bicanthalis*. Female and nest temperatures of these species fell below the 17 °C low temperature threshold for development of *Sceloporus* (Andrews, Qualls & Rose, 1997) during the diel cycle. To determine incubation periods from incubation temperature for these species, I substituted 17 °C for each hour that eggs were below this threshold, calculated a 'corrected' mean temperature, and then applied equations 1 and 2. These corrected means represent the effective temperature for development.

RESULTS

As predicted, temperature declined with increasing elevation; mean ambient temperatures at the 1800, 2800 and 3200 m sites were 20.7, 17.1 and 10.2 °C, respectively. The decline in ambient temperature with elevation was paralleled by a decline in both body and nest temperature (Table 1). Mean female and nest temperatures were virtually identical for all species before correction for the 17 °C low threshold for development. The diel pattern of temperature variation differed mainly in the greater amplitude of fluctuations of female than nest temperature (Figs 1 & 2).
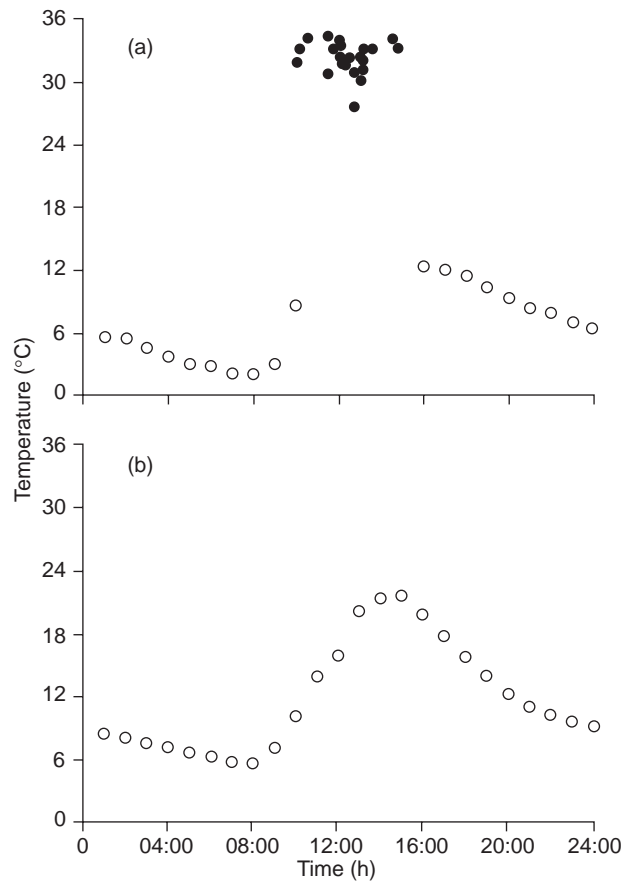
Body temperatures of females increased relative to nest temperatures for both for *S. aeneus* and *S. bicanthalis* after correction to reflect effective developmental temperature. The reason is that during the diel cycle of these species, female body temperatures were higher than nest temperatures during the day and temperatures less than 17 °C were discounted; this situation was exaggerated for *S. bicanthalis* because nest temperatures above 17 °C were so low relative to female
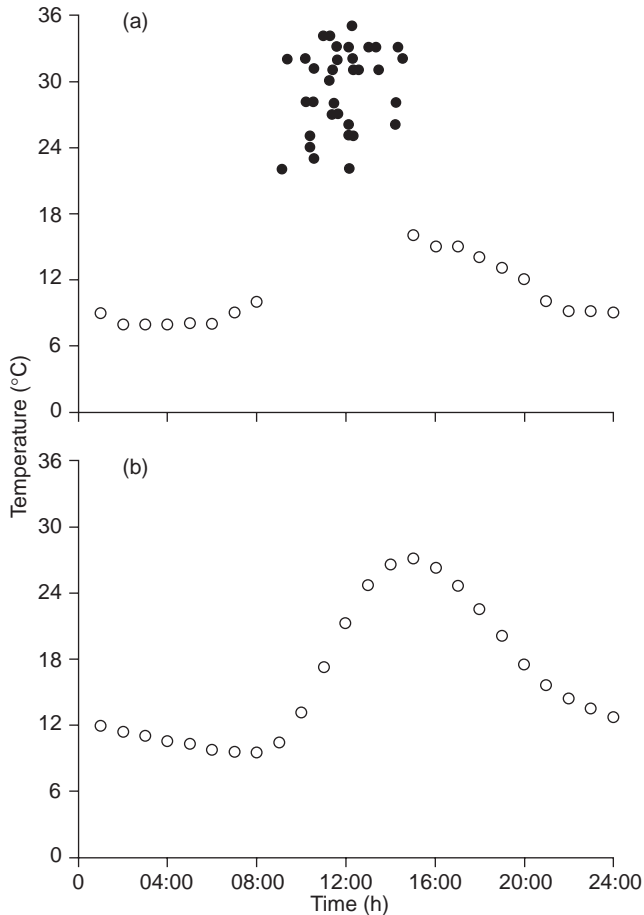
Fig. 1. (a) Body temperatures of gravid female *Sceloporus aeneus* during their activity period (●) from April to July 1993 and hourly means of temperatures in retreats when inactive (○) during 10–15 July 1993. (b) Hourly mean temperatures of a representative *Sceloporus aeneus* nest (MA2 at 2 cm, Table 2) from 18 July–27 September 1996.

Fig. 2. (a) Body temperatures of pregnant female *Sceloporus bicanthalis* during their activity period (●) in March 1994 and hourly means of temperatures in retreats when inactive (○) in March 1994. (b) Hourly mean temperatures of a simulated *Sceloporus bicanthalis* nest from 21 July–11 September 1996.

body temperatures above 17 °C (Table 2, Figs 1 & 2). While corrected female temperatures were higher than nest temperatures, the difference was not large, 0.6, 0.9 and 2.5 °C for *S. virgatus*, *S. aeneus* and *S. bicanthalis*, respectively. The reason for the similarity of mean body and nest temperatures at all sites is that both means are dominated by ambient temperature. The parallel between nest temperature and ambient temperature is not surprising: nests are in the ground, and soil temperatures reflect ambient temperature. But, why were the mean body temperatures of females so similar to nest temperature? After all, thermoregulation at relatively high body temperatures is a characteristic of the genus *Sceloporus* (Andrews, 1998).

Gravid or pregnant female *S. virgatus*, *S. aeneus* and *S. bicanthalis* had mean body temperatures of 34, 32, and 32 °C during the approx. 8, 6 and 5 h a day that they were active, respectively. For the other 16, 18 and 19 h a day their body temperatures would have approximated ambient temperature, and temperatures in presumed retreats fell as low as 19, 8 and 2 °C, respectively. The long period of inactivity each day results in overall mean

temperatures considerably lower than those temperatures 'selected' by females during activity. This factor is particularly important for the two Mexican species that had relatively low body temperatures during activity, and had relatively short activity periods. As a consequence of long periods of exposure to a common ambient temperature, mean body temperatures and nest temperatures at each site were similar.

The benefit of the shallow nests of *S. aeneus* was evaluated by comparing the temperature of nests with the temperature 4 cm lower in the soil profile. Temperature at 2 cm fluctuated both higher and lower than temperature at 6 cm but the overall mean temperatures at these two depths were virtually identical before correction (Table 2, Fig. 3). Corrected temperatures were 19.9 and 18.8 °C, respectively; the effective temperature of nests at 2 cm thus averaged about 1 °C higher than the temperature 4 cm deeper.

The biological importance of incubation temperature is best assessed from the lengths of incubation associated with body and nest temperatures (Table 1). For *S. virgatus*, eggs would have respective developmental

Table 2. Temperatures at nests of *Sceloporus aeneus* at Milpa Alta (MA) and at a potential nest site at Zoquiapan (ZO). MA2 and MA4 were nests with eggs, the remaining represented similar locations (see Materials and methods)

Nest no., Depth	Dates	Overall mean	Mean daily maximum	Mean daily minimum	Maximum	Minimum
MA1, 2 cm	18 Jul–1 Aug	16.9	29.9	8.4	37.8	4.9
MA1, 6 cm	18 Jul–1 Aug	16.8	24.9	10.7	33.4	7.8
MA2, 2 cm	18 Jul–1 Aug	16.3	28.0	8.5	33.7	5.0
MA2, 6 cm	18 Jul–1 Aug	16.4	24.6	10.3	30.5	7.4
MA3, 2 cm	18 Jul–1 Aug	16.3	28.5	9.3	37.1	6.0
MA3, 6 cm	18 Jul–1 Aug	16.6	23.8	11.7	36.5	9.1
MA2, 2 cm	1 Aug–27 Sep	16.2	28.2	9.2	36.7	5.6
MA2, 6 cm	1 Aug–27 Sep	16.5	23.4	11.7	30.2	9.2
MA4, 2 cm	1 Aug–27 Sep	16.2	28.3	9.0	35.7	5.3
MA5, 2 cm	1 Aug–27 Sep	16.3	28.0	9.2	37.3	5.2
MA6, 2 cm	1 Aug–27 Sep	16.6	28.6	9.9	36.1	6.4
ZO1, 2 cm	2 Jul–11 Sep	11.7	22.6	5.4	31.1	2.2
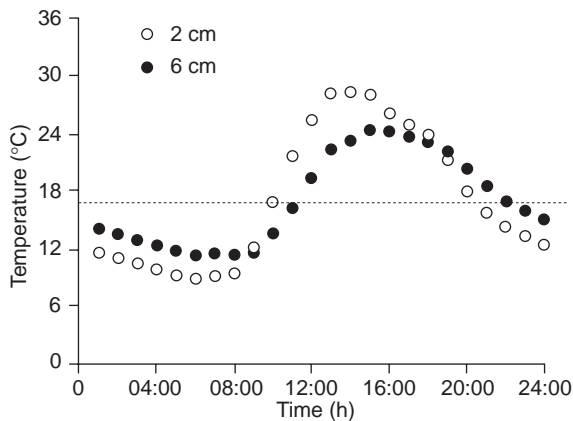
Fig. 3. Hourly mean temperatures of a representative nest of *Sceloporus aeneus* from 18 July–1 August 1996. Probes were placed in the nest at a depth of 2 cm and below the nest at a depth of 6 cm. The horizontal dashed line indicates the 17 °C threshold for embryonic development.

periods of 66 and 69 days if they were incubated at mean body and nest temperatures. For *S. aeneus*, eggs would have respective developmental periods of 65, 71 and 78 days if they were incubated at mean body temperature, nest temperature at 2 cm, and nest temperature at 6 cm. Eggs laid at 6 cm would thus take a week longer to hatch than eggs laid at 2 cm, and eggs laid at 2 cm would take a week longer to hatch than eggs retained by females during the entire incubation period. For *S. bicanthalis*, eggs would have respective developmental periods of 70 and 87 days if they were incuated at mean female and mean 'nest' temperatures. Eggs retained by the female during the entire incubation period would thus hatch more than 2 weeks earlier than eggs placed in a nest.

DISCUSSION

Observations on the thermal biology of three species of *Sceloporus* suggest that there is little initial thermal benefit to extended egg retention as oviparous lizards invade high elevations. For example, female and nest temperatures of the montane *S. virgatus* at 1800 m were virtually identical, and even at 2800 m, female temperatures of *S. aeneus* were higher than nest temperature by only 1 °C. While 1 °C reflects acceleration of the developmental period by about 1 week over the entire period of development, because the initial increment in the duration of egg retention is presumably small, any enhancement of embryonic development as a result of higher temperatures *in utero* than in a nest would be of short duration relative to the remaining length of incubation in the nest. A shift from a nest depth typical of low elevation *Sceloporus* (6 cm below the soil surface) to 2 cm below the soil surface would also accelerate development by a week, but this acceleration would apply to the entire developmental period. Observations on *Sceloporus* thus suggest that the initial accommodation to a cold-climate involves a behavioural trait, that is, where females place their eggs. Relatively high nest temperature close to the soil surface explains why female *S. aeneus* placed their eggs in such shallow nests compared to those of *S. virgatus*.

While the higher mean temperature in nests close to the surface may initially enhance embryonic development relative to that in deeper nests, at even higher elevations, nests would have to be placed even closer to the surface to compensate for declining ambient temperature and would consequently suffer increased mortality. Observations in March 1994 on two simulated nests of *S. bicanthalis* at Zoquiapan illustrate this point (Fig. 4). The temperature in one such nest fell to −8 °C at night and rose above 50 °C during the day. Both of these temperature extremes are likely to be lethal to embryos. Embryos of *Sceloporus* do not develop at temperatures of 17 °C or lower (Andrews, Qualls *et al.*, 1997) and temperatures of 37 °C and higher are lethal, even for short-term exposure (Overall, 1993). Mean corrected nest temperature at this site was only 18 °C, and the mean daily maximum temperature, 23 °C, was only 6 °C above the threshold for development, and this exposure occurred during a short period
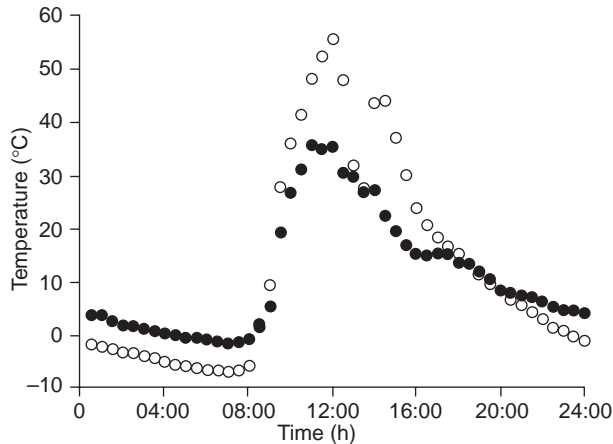
Fig. 4. Temperatures recorded at 30 min intervals over 24 h at two simulated nest sites of *Sceloporus bicanthalis* at Zoquiapan in March 1994.

during the day (Table 2, Fig. 2). These observations suggest that at locations such as Zoquiapan at 3200 m, oviparity is not a viable reproductive mode. Eggs placed close to the surface could be exposed to lethal temperatures, or enhanced predation, and eggs placed deeper in the soil would not be above 17 °C long enough for normal development to occur.

The ancestral *S. bicanthalis* presumably evolved viviparity because of these constraints. Enhanced mortality at high elevations as a result of both predation and physical causes would select for an increased duration in egg retention. Moreover, the longer the period of egg retention, the greater the thermal benefit from egg retention; the thermal benefit would be particularly strong at the highest elevations where the differential between nest temperature and female temperature is relatively large, and, because of the exponential relationship between the length of incubation and temperature, even a comparatively small temperature difference between females and nest sites, reflects a substantial increment in the rate of development (Table 1). For example, mean female temperature was only 2.5 °C higher than mean nest temperature, but eggs would hatch 17 days earlier if retained than if deposited in a nest. Such dual benefits would provide strong selection for longer periods of egg retention and, ultimately, viviparity.
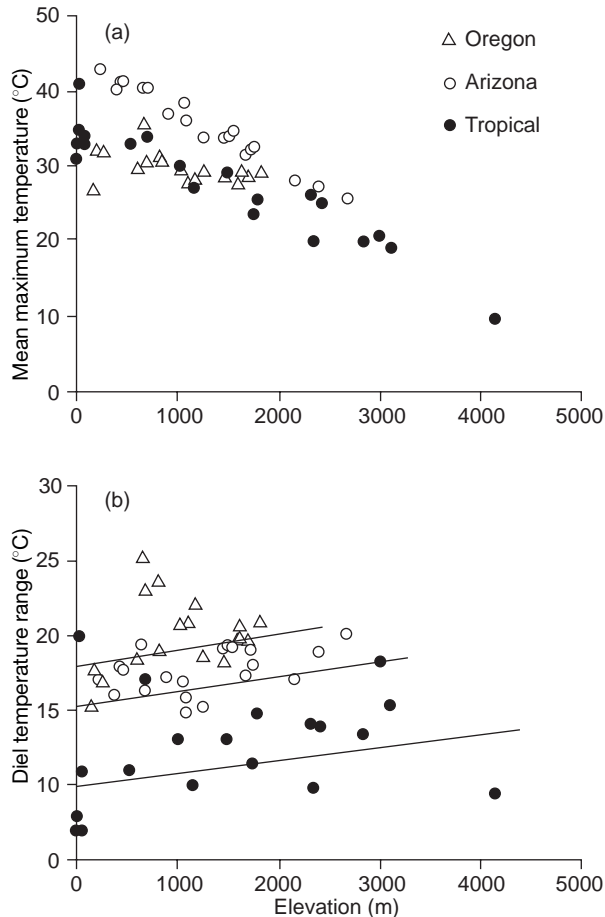
These observations further suggest that the thermal benefit of viviparity does not reside entirely in the ability of females to maintain higher mean temperatures than embryos would experience in a nest. The benefits of viviparity reside, in part, in the ability of gravid or pregnant females to control the variance in their body temperature. To do this, thermoregulating females use retreat sites that do not fall to detrimental levels at night and regulate temperatures during activity that do not rise high enough to be detrimental to embryos (Buechat, 1986; Andrews, Méndez-de la Cruz & Villagrán-Santa Cruz, 1997; Mathies & Andrews, 1997). None the less, at high elevations, the activity periods of lizards are

constrained and their body temperatures, even during activity, are low compared to congeners at lower elevations. Embryos have the same temperatures as their viviparous mothers, and the temperatures of females decline regularly with increasing elevation at tropical latitudes (Andrews, 1998). The long developmental periods of viviparous *Sceloporus* at high elevations are indicators of the strong thermal constraint on embryonic development. For example, at 3200-3400 m in Mexico, female *Sceloporus mucronatus* are pregnant for 6–7 months (Méndez-de la Cruz, Guillette & Villagrán-Santa Cruz, 1988), and this is typical of viviparous *Sceloporus* in these habitats (Méndez-de la Cruz, Villagrán-Santa Cruz & Andrews, 1998). The long gestation period at high elevations is presumably due to the limiting effect of low body temperatures of females on embryonic development.

The conclusion that the precursor for the evolution of viviparity is a behavioural shift by gravid females to increasingly superficial nest sites in cold climates rather than an immediate increase in the length of egg retention is supported by observations on *Sceloporus* on an elevational gradient. Can this conclusion be extended to latitudinal gradients? One difficulty with making this extrapolation is that temperature regimes on these gradients differ in several ways. At tropical latitudes, for example, seasonal variation in temperature is slight and lizards are active year round, while at temperate latitudes, seasonal variation in temperature is marked, and lizards are active only during the warmest part of the year. Moreover, while daily maximum temperatures at temperate latitudes during the summer are similar or higher than those at tropical latitudes, daily minimums are lower (Fig. 5). As a consequence, diel variation in ambient temperature is greater at temperate than tropical latitudes ($F_{2,54} = 46.8$, $P < 0.001$, ANCOVA). Would shorter activity seasons and lower minimum ambient temperatures at high than low latitudes favour egg retention rather than a shift in nest location as the most immediate response to declining ambient temperature? Unfortunately, the information required to address this issue does not exist.

Given the wide acceptance of the classic cold-climate model, it is surprising that its assumptions have been tested so infrequently. Shine (1983) examined the thermal and reproductive biology of oviparous and viviparous skinks at a 1040 m site in Australia. Both viviparous and oviparous females had mean body temperatures of 23–25 °C, and the mean nest temperature of the oviparous species was 17 °C. As a result of this 6–8 °C difference, the young of the viviparous species were born about 1 month earlier than young of the oviparous species. Similarly, the young of viviparous *Lacerta vivipara* are born before the eggs of oviparous conspecifics hatch when the two reproductive forms are housed together in outdoor enclosures (Heulin, Osenegg & Lebouvier, 1991). These observations support the classic-cold climate model because thermoregulation by viviparous females clearly enhances the development of their embryos relative to the embryos of oviparous

Fig. 5. (a) Monthly means of daily maximum temperatures at tropical and temperate latitude sites as a function of elevation; (b) the diel range in temperature (difference between monthly means of maximum and minimum temperature) at the same sites.

these sites thus provide a more direct evaluation of the actual mechanism for the selection for extended egg retention and viviparity.

Taxon specific differences in ecology and reproductive biology may also contribute to the apparently disparate conclusions of studies by Shine (1983) and Huelin *et al.* (1991) versus this study. For example, the skinks studied by Shine (1983) are associated with relatively mesic and cool habitats, have relatively low body temperatures while active, and nests are placed under logs and stones. In contrast, sceloporine lizards are characteristic of open and well-insolated habitats, have relatively high body temperatures while active, and eggs are placed in nests excavated in the ground in fully insolated sites. Given the differences in the thermal biology of these two groups of lizards, behavioural changes in nest location may be more efficacious in changing nest temperature in *Sceloporus* than in skinks.

Given that my study and those of Shine (1983) and Heulin *et al.* (1991) are the only tests of the assumptions of the cold-climate model, our conclusions are best viewed as complementary rather than conflicting. Shine (1995) has recently proposed a third hypothesis – one that connects the thermal environment of embryos with hatchling fitness in cold climates. While incubation temperature clearly affects the size, shape and performance of hatchlings (Shine & Harlow, 1996), any association between hatchling fitness, incubation temperature, and the transition between oviparity and viviparity has yet to be demonstrated. None the less, it is clear 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.

species. In contrast, my observations on *Sceloporus* indicate that viviparity does not provide substantially warmer mean temperatures to developing embryos than they would experience in a nest.

The explanation for the apparently conflicting conclusions of studies by Shine (1983) and Huelin *et al.* (1991) versus this study resides in their respective evolutionary and geographic perspectives. While the studies of Shine and Huelin *et al.* clearly support the thermal benefits of viviparity once it is established, these studies do not necessarily support thermal benefits to embryos as the selective mechanism by which extended egg retention and viviparity evolve. Observations were made at sites where oviparous and viviparous species are sympatric (experimental sympatry in the case of *Lacerta*) and where both reproductive modes are viable, that is, in Shine's (1983) study, viviparous species have presumably invaded habitats warmer than where viviparity evolved. My observations focus on the evolutionary transition to viviparity, and I made observations at sites that encompass the upper elevational limit at which oviparity is a viable reproductive mode. Thermal data at

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