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Scott L. Parker  
*Virginia Polytechnic Institute and State University, Blacksburg, VA*

Robin M. Andrews  
*Virginia Polytechnic Institute and State University, Blacksburg, VA*

Tom Mathies  
*National Wildlife Research Center*

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Embryonic responses to variation in oviductal oxygen in the lizard *Sceloporus undulatus* from New Jersey and South Carolina, USA

SCOTT L. PARKER1⁎, ROBIN M. ANDREWS1 and TOM MATHIES2

1Department of Biology, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061–0406, USA
2National Wildlife Research Center, Fort Collins, CO, 80521–2154, USA

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Viviparity in reptiles is hypothesized to evolve in cold climates at high latitudes and high elevations through selection for progressively longer periods of egg retention. Oxygen consumption of embryos increases during development and therefore longer periods of egg retention should be associated with maternal or embryonic features that enhance embryonic oxygen availability. We tested the hypotheses that embryos of the oviparous lizard *Sceloporus undulatus* from a high-latitude population in New Jersey are oviposited at more advanced developmental stages and have a higher growth rate at low oxygen partial pressures ($pO_2$) than embryos from a low-latitude population in South Carolina. These hypotheses were rejected; embryos from the two populations did not differ in embryonic stage at oviposition, survival, rate of differentiation or growth in mass when incubated under simulated *in utero* conditions at low oxygen concentrations. We also estimated the effective $pO_2$ experienced by lizard embryos *in utero*. At an effective $pO_2$ of 8.6 kPa (9% O$_2$), development of *S. undulatus* embryos is arrested at Dufaure and Hubert stage 30 and at a dry mass of 0.8 mg. Physiological and morphological features of gravid females, embryos, or both, that facilitate oxygen uptake for developing embryos appear to be a critical early step during the evolution of reptilian viviparity.


ADDITIONAL KEYWORDS: cold climate hypothesis – egg retention – evolution of viviparity – hypoxia.

INTRODUCTION

Reptilian viviparity is hypothesized to evolve in response to cold climates at high latitudes and high elevations through gradual increases in the retention of eggs within the oviduct (Packard, Tracy & Roth, 1977; Shine & Bull, 1979; Shine, 1985). The putative benefit to extending egg retention in cold climates is that embryonic development is faster inside the thermoregulating female than in a nest (Packard et al., 1977; Shine, 1983). According to the cold climate hypothesis, even relatively short increases in the duration of egg retention would enhance female reproductive fitness because embryos would be more advanced developmentally at oviposition and therefore hatch in a shorter period of time than non-retained eggs (Shine, 1985).

The widespread acceptance of the cold climate model is, however, based largely on indirect observations. For example, viviparous species are relatively more common at high than low elevations and latitudes. In contrast, relatively few studies test the cold climate model directly. One example is Mathies & Andrew's (1995a) demonstration that gravid female *Sceloporus scalaris* from a high-elevation population retained eggs longer and produced embryos that were more advanced developmentally compared with gravid females from a low-elevation population. In another example, Shine (2002) simulated short-term egg retention in the laboratory by subjecting eggs of the skink *Bassiana duperreyi* to a 2-week initial period of high incubation temperatures. Eggs subjected to simulated short-term egg retention at high
temperatures developed more rapidly, had greater survivorship and produced higher quality hatchlings than eggs incubated at lower temperatures (simulating conditions in a nest) for their entire incubation period.

The majority of oviparous lizards lay eggs when 25–40% of the total embryonic development time has been completed (Shine, 1983; DeMarco, 1993) and embryos have reached approximately stage 30 (Andrews & Mathies, 2000) of Dufaure & Hubert’s (1961) staging system, where stage 0 is fertilization and 40 is hatching. Relatively few oviparous lizard species retain eggs beyond stage 33, suggesting that the ability of females to retain eggs much past stage 30 is constrained (Mathies & Andrews, 1995a).

One of the constraints on egg retention past stage 30 is oxygen availability for embryonic development in utero (Andrews & Mathies, 2000; Andrews, 2002). Because of the growth of the embryo (Dmi’el, 1970; Vleck & Hoyt, 1991), the oviduct should become increasingly hypoxic during late development when the size and the metabolism of the embryo increases dramatically. Oviposition should thus occur when the demand for oxygen by the embryo exceeds the ability of the female to supply it. For example, in most species of Sceloporus lizards, embryonic development is retarded when gravid females are experimentally induced to retain eggs past the normal time of oviposition (Andrews, 1997; Mathies & Andrews, 1999; Andrews & Mathies, 2000). Extended gestation in the oviduct must therefore be associated with mechanisms that enhance oxygen availability in an environment that becomes increasingly hypoxic as development proceeds.

Studies demonstrating variation in egg retention along geographical climatic gradients have typically been conducted on species in lineages comprising both oviparous and viviparous populations (Guillette, 1982; Mathies & Andrews, 1995b; Smith & Shine, 1997; Qualls & Shine, 1998). In contrast, studies of species from entirely oviparous lineages also have the potential to provide support for the cold climate model. Demonstration that intermediate stages of egg retention are associated with low environmental temperatures independent of a phylogenetic history of viviparity would be particularly compelling. Moreover, intraspecific comparisons demonstrating variation in egg retention time along climatic gradients are unlikely to be confounded by species-specific adaptations unrelated to egg retention. Sceloporus undulatus (Latreille) is an appropriate species for studying the potential influence of climate on egg retention because it is a member of the entirely oviparous undulatus species group (Méndez-de la Cruz, Villagrán-Santa Cruz & Andrews, 1998) and it has a wide latitudinal distribution over much of the central and eastern United States (Stebbins, 1985). Moreover, many life history characters differ between populations (Tinkle & Ballinger, 1972; Ferguson & Brockman, 1980). For example, females from New Jersey (NJ) have a shorter reproductive period (Fig. 1) and produce two clutches of eggs per year at most (Angilletta, Winters & Dunham, 2000), whereas females from South Carolina (SC) produce three clutches per year (Tinkle & Ballinger, 1972). These differences between populations are associated with lower environmental temperatures in New Jersey than in South Carolina.

The first objective was to test two hypotheses related to the cold climate model. The first hypothesis was that gravid females from high-latitude populations retain eggs to more advanced stages of embryonic development than females from low-latitude populations. The second hypothesis was that embryos from high-latitude populations have a higher developmental rate while in utero than embryos from low-latitude populations. To test these two hypotheses, we contrasted egg retention and embryonic development in utero for a population of S. undulatus from near the northern latitudinal limit of the species distribution in New Jersey with those of a population from South Carolina.

The second objective was to estimate effective $pO_2$ experienced by lizard embryos in utero. To our knowledge, $pO_2$ experienced by lizard embryos in utero dur-
ing egg retention has not been quantified at any time during development. To meet this second objective we therefore first established a standard curve relating the developmental rate of embryos to \( pO_2 \) under simulated \textit{in utero} conditions. Data collected earlier (Mathies, 1998) provided data on the rate of development of embryos retained by females \textit{in utero} past the time of normal oviposition. \( pO_2 \) \textit{in utero} was then estimated from the standard curve (i.e. the growth rates of embryos under known \( pO_2 \)).

**MATERIAL AND METHODS**

**COLLECTION AND MAINTENANCE OF GRAVID FEMALES**

Gravid females of \textit{Sceloporus undulatus} were captured at Sumter National Forest, Edgefield County, SC, during 31 May – 4 June 2002 (\( N = 16 \)), and at Wharton State Forest, Burlington County, NJ, during 27 June – 30 June 2002 (\( N = 16 \)). After capture, gravid females from both populations were placed in cloth bags and transported within 5 days of capture to Virginia Polytechnic Institute and State University (Virginia Tech), Blacksburg, VA. Females were measured for snout–vent length (SVL) and weighed to the nearest 0.1 g before and after oviposition. Females were housed in plastic containers (73 \( \times \) 48 \( \times \) 22 cm, three females per container) in an animal room at Virginia Tech. Ambient light was provided from windows and lizards were also provided with fluorescent Vita-lites (08:00–18:00 h). A 100-W spotlight suspended at one end of each container (09:00–16:00 h) provided a temperature gradient that allowed females to thermoregulate. All containers were provided with boards and rocks for basking sites. Females were fed (crickets and mealworms dusted with vitamin-mineral supplement) and watered by misting daily.

**COLLECTION OF EGGS AND INITIAL SAMPLING OF EGGS AND EMBRYOS**

New Jersey and South Carolina females were initially assigned to one of two groups. Females in one group were provided with damp sand for oviposition. Females in the second group were provided only with dry sand in an attempt to induce them to retain eggs beyond the normal time of oviposition (Andrews & Rose, 1994). However, females did not retain eggs in response to the absence of a suitable nesting substrate; within populations, females that oviposited did so over the same period in the two substrate groups (NJ: 8 days; SC: 7 days) and same range of stages (NJ: 28–29.5; SC: 27–29.5). Therefore, we did not consider the initial oviposition substrates as part of the experimental design and eggs from both groups (i.e. damp and dry substrates) were pooled within each population for all subsequent analyses.

Containers were checked several times daily for eggs. When half the females had oviposited, the remainder were injected with oxytocin to induce oviposition: 14 June, five SC females; 5 July, six NJ females. The embryonic stage at oviposition for SC females injected with oxytocin did not differ from females that oviposited normally (\( t_s = 1.68, P = 0.130 \)). The embryonic stage at oviposition for NJ females injected with oxytocin did, however, differ from females that oviposited normally (\( t_{10} = 2.96, P = 0.022 \)). The difference in mean stage was relatively small (28.0 and 28.6, injected vs. normal oviposition, respectively), and both groups were therefore pooled in all subsequent analyses. Eleven clutches (four NJ, seven SC) could not be used in experiments because eggs desiccated or because females did not have shelled eggs.

Eggs were weighed within a few hours of oviposition and numbered consecutively within each clutch. The surfaces of the eggshells were kept moist until placed under experimental conditions. A single egg from each clutch was dissected and the embryo staged according to Dufaure & Hubert (1961). Half stages were assigned for embryos exhibiting intermediate suites of traits. After staging, embryos were dried to a constant mass at 40 °C and weighed. A single egg from each NJ clutch was used to measure the area of the chorioallantoic membrane (CAM). The extent of the CAM was visible through the eggshells at oviposition and therefore was easily measured. The major axis and minor axis of each egg and diameter of chorioallantois of each egg sampled were measured using a dial calipers. The surface area of the eggshell was calculated using the formula for area of a prolate spheroid and the CAM surface area was calculated using the formula for area of a circle. Relative CAM area was the ratio the surface area of the CAM to the total eggshell surface area.

**EXPERIMENTAL DESIGN AND MANIPULATION OF \( pO_2 \)**

Differentiation and growth of embryos were determined under simulated \textit{in utero} conditions. To simulate conditions in the oviduct, eggs were incubated under conditions such that channels in the shell remained fluid filled during incubation as they would be normally in the oviduct. Using this procedure, oxygen must diffuse through the fluid-filled eggshell before it is available to the embryo. This procedure may best simulate \textit{in utero} conditions because in the oviduct, eggs are pressed against the walls of the uterus and are therefore in close proximity to maternal blood supply. One or two eggs were placed in 70-mL specimen jars lined with Whatman filter paper moistened with physiological saline (pH 7.4). The filter paper was re-moistened with saline at least every 3 days to ensure that the eggshell channels remained open.
fluid filled during incubation (see also Seymour, Geiser & Bradford, 1991, for similar experimental approaches). We incubated eggs under a range of O₂ levels (target values: 4, 9, 15 and 21% O₂) (Table 1). Control eggs (simulating nest conditions) were placed in specimen jars with vermiculite moistened with distilled water (0.7 : 1.0 g H₂O-vermiculite) corresponding to a water potential of −200 kPa (21% O₂ only). The only difference thus between the experimental and control eggs was that the shells of the former were fluid filled and those of the latter were filled with air.

Eggs were placed, according to treatment, into one of four airtight metal boxes. The boxes were flushed regularly with the appropriate gas mixture (O₂ and N₂) using a Cameron Instruments, Model GF-3/MP gas mixing flow meter. Bubbling the gas mixture through distilled water saturated the air inside the boxes. Every time the boxes were flushed, the oxygen concentration inside the boxes was measured using an Applied Electrochemistry S-3 A/II oxygen analyser. Mean oxygen levels (in dry air) for the four oxygen treatments during the incubation period were 4.3, 9.5, 15.8 and 20.4%, respectively (Table 2). The actual values of pO₂ for the four oxygen treatments during the incubation period were 3.9, 8.6, 14.3 and 18.5 kPa, respectively, based upon a mean air pressure at Blacksburg (625 m) of 94.5 kPa (711 mmHg), a mean incubation temperature of 28 °C and a pH₂O of 3.8 kPa of water vapour in air.

The boxes were placed in a single environmental chamber and incubated for 10 days at a mean of 28 °C. Temperatures inside the environmental chamber varied linearly for 4 h between daily maximum and minimum temperatures (mean daily maximum chamber temperature: 32.9 °C, mean daily minimum: 22.9 °C, overall mean: 27.7 °C). The boxes were rotated within the chamber every 3–5 days to minimize position effects on embryonic development. After the experiment was completed, temperatures inside the boxes were measured over a 4-day period to determine the relationship between the temperatures within the boxes and temperatures within the environmental chamber. For simulated oviductal treatments, the temperature probe was placed at the bottom of the specimen jar in contact with the moistened filter paper. For simulated nest treatments, the temperature probe was placed in the centre of the specimen jar and covered with vermiculite. During these observations, the mean temperatures of the simulated oviduct and simulated nest treatments were 0.5 °C higher than the mean temperature inside the chamber (x<sub>experimental</sub> = 28.1 °C, x<sub>control</sub> = 28.1 °C, x<sub>chamber</sub> = 27.6 °C).

### ESTIMATION OF pO₂ IN UTERO

Data from Mathies (1998) were used to estimate actual pO₂ in utero for S. undulatus. Mathies (1998) provided one group of females collected near Blacksburg, VA, with a substrate of damp sand in which to oviposit (referred to as ‘control’ females). The normal time of oviposition (NTO) was defined as the time when approximately half of the control females laid eggs. A second group of gravid females was induced to retain eggs past the NTO by maintaining them on a dry substrate (referred to as ‘retained females’). A dry substrate simulates drought conditions and females may respond by facultatively retaining eggs (Andrews & Rose, 1994). Embryonic stage and dry mass were obtained from retained clutches that were sampled at regular intervals for as long as 27 days during the period of retention. Daytime body temperatures of the retaining females (08:00–16:00 h) averaged 32.6 °C and minimum body temperatures during inactivity (17:00–08:00 h) were approximately 22 °C. Overall mean daily body temperature of retained females thus averaged approximately 27 °C.

Effective pO₂ in utero was estimated by first determining the developmental rate of S. undulatus embryos after 10 days of retention in utero from previous studies (Mathies, 1998). A standard curve gen-

### Table 1. Allocation of eggs from each clutch to control and experimental treatments. Eggs (2–5) of treatments were incubated under simulated oviductal conditions and control eggs (6) were incubated under simulated nest conditions. When clutches had fewer than five eggs, no eggs were allocated to the control treatment

<table>
<thead>
<tr>
<th>Egg no.</th>
<th>% O₂ (treatment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>– (sampled at oviposition)</td>
</tr>
<tr>
<td>2</td>
<td>4 (simulated oviduct)</td>
</tr>
<tr>
<td>3</td>
<td>9 (simulated oviduct)</td>
</tr>
<tr>
<td>4</td>
<td>15 (simulated oviduct)</td>
</tr>
<tr>
<td>5</td>
<td>21 (simulated oviduct)</td>
</tr>
<tr>
<td>6</td>
<td>21 (control)</td>
</tr>
</tbody>
</table>

### Table 2. Mean oxygen concentration (%), standard error and range for the four oxygen treatments measured during the 10-day incubation period. Total number of oxygen concentration measurements made during the incubation period for each treatment is given in parentheses

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean oxygen concentration (N)</th>
<th>SE</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>21% O₂</td>
<td>20.4 (8)</td>
<td>0.06</td>
<td>20.1–20.6</td>
</tr>
<tr>
<td>15% O₂</td>
<td>15.8 (8)</td>
<td>0.13</td>
<td>15.4–16.4</td>
</tr>
<tr>
<td>9% O₂</td>
<td>9.5 (8)</td>
<td>0.29</td>
<td>8.2–10.4</td>
</tr>
<tr>
<td>4% O₂</td>
<td>4.3 (6)</td>
<td>0.37</td>
<td>2.9–5.6</td>
</tr>
</tbody>
</table>

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erased by the regression of embryonic developmental rate vs. \( pO_2 \) determined by the experimental manipulations of oxygen levels (described above) was then used to predict the \( pO_2 \) associated with the in utero rate of embryonic development.

DATA MANIPULATION AND STATISTICAL ANALYSES

The effect of oxygen treatment on egg survival was similar between populations and data were therefore pooled for all subsequent survivorship analyses. The effect of oxygen treatment on egg survival among the 4, 9, 15 and 21% treatments was analysed using a chi-squared test of independence (Freq Procedure, SAS Institute, 1996). The effects of oxygen treatment on survival between the 21% treatment and control (simulated nest) treatment were analysed using a Fisher's exact test (SAS Institute, 1996).

The effect of oxygen treatment and population on embryonic stage (differentiation) and dry mass (growth) at 10 days was evaluated using two-factor analysis of covariance (ANCOVA) with embryonic stage and embryo dry mass at oviposition as covariates (GLM procedure; SAS Institute, 1996). The 21% treatment and 21% control were contrasted using a one-way ANCOVA with initial stage or dry mass as the covariate. The effect of oxygen treatment on water uptake by eggs and growth of absolute or relative CAM area at 10 days was analysed using single-factor ANCOVA with egg mass and absolute or relative area of CAM at oviposition as covariates.

Observations of relative area of CAM were arcsine-square root transformed prior to analysis. Water uptake by eggs in each treatment was assessed as the difference in egg mass at 10 days and at oviposition. Post hoc pair-wise comparisons were analysed using a least significant difference test on least squared means. Probability values of less than 0.05 were considered significant. The assumption of homogeneity of slopes for all ANCOVAs was satisfied by testing for significance of the interaction of the covariate with treatment variables.

RESULTS

LIFE HISTORY DATA

Female SVL and mass (post-oviposition) did not differ between New Jersey and South Carolina (Table 3). Whereas egg mass of NJ females was greater than that of SC females, relative clutch mass (RCM) and clutch size did not differ between populations. Moreover, embryonic stage at oviposition did not differ between populations. Although some females from each population were injected with oxytocin (NJ: 6/12; SC: 5/10, injected/non-injected), the difference in mean embryonic stage between oxytocin-injected females and females that oviposited normally was small (< 0.6 stages) for both populations (see Material and Methods). It is unlikely therefore that injection with oxytocin biased the comparison of embryonic stage at oviposition between the two populations.

Egg mass (mg)  416
Clutch size 7.8
Body mass (g) 7.83
SVL (mm) 64.4

Table 3. Summary statistics: comparison of snout–vent length (SVL), post-oviposition body mass, relative clutch mass (RCM), clutch size, egg mass and embryonic stage at oviposition for female Sceloporus undulatus. Values represent means ± standard errors. Sample size is given in parentheses. NJ, New Jersey; SC, South Carolina

<table>
<thead>
<tr>
<th>Variable</th>
<th>SC</th>
<th>NJ</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>SVL (mm)</td>
<td>64.4 ± 1.05 (16)</td>
<td>65.5 ± 0.85 (16)</td>
<td>-0.139</td>
<td>0.890</td>
</tr>
<tr>
<td>Body mass (g)</td>
<td>7.83 ± 0.877 (10)</td>
<td>8.79 ± 0.409 (15)</td>
<td>-1.089</td>
<td>0.287</td>
</tr>
<tr>
<td>RCM</td>
<td>0.45 ± 0.058 (9)</td>
<td>0.34 ± 0.026 (16)</td>
<td>1.948</td>
<td>0.065</td>
</tr>
<tr>
<td>Clutch size</td>
<td>7.8 ± 0.56 (13)</td>
<td>6.6 ± 0.23 (14)</td>
<td>1.677</td>
<td>0.105</td>
</tr>
<tr>
<td>Egg mass (mg)</td>
<td>416 ± 17.5 (12)</td>
<td>470 ± 13.7 (12)</td>
<td>-2.405</td>
<td>0.025</td>
</tr>
<tr>
<td>Stage at oviposition</td>
<td>28.8 ± 0.25 (10)</td>
<td>28.4 ± 0.17 (12)</td>
<td>1.390</td>
<td>0.179</td>
</tr>
</tbody>
</table>

sponds to an average increase of one stage over the 10-day incubation period. In contrast, embryos incubated at 21% O₂ reached a mean stage of 30.5, corresponding to an average increase of almost two stages. Differentiation, however, at 21% under simulated oviductal conditions was lower than in the 21% control under simulated nest conditions ($F_{1,28} = 48.1$, $P < 0.001$, ANCOVA). Control embryos reached a mean stage of 32.1, corresponding to an increase of 3.5 stages over the incubation period.

Embryonic growth in mass was reduced at 9% O₂ and highest at 21% O₂ under simulated oviductal conditions (Fig. 2). Embryos incubated at 9% O₂ reached a mean dry mass of 0.84 mg after 10 days of incubation, corresponding to an average increase of 0.4 mg. Embryos incubated at 21% O₂ reached a mean dry mass of 1.9 mg, corresponding to an average increase of 1.36 mg. Growth at 21% O₂ under simulated oviductal conditions was lower than in the 21% control under simulated nest conditions ($F_{1,26} = 18.9$, $P < 0.001$, ANCOVA). Control embryos reached a mean dry mass of 3.7 mg, corresponding to an average increase of 3.2 mg over the incubation period.

EGG SIZE AND CAM

For NJ, the size of eggs at 10 days did not differ among treatments; water uptake was independent of oxygen treatment (Table 6). Water uptake at 21% O₂ under simulated oviductal conditions was lower than in the 21% control ($F_{1,17} = 19.3$, $P < 0.001$, ANCOVA). Eggs incubated at 21% O₂ under simulated oviductal conditions reached a mean wet mass of 588 mg, corresponding to an average increase of 149 mg over the incubation period. Control eggs reached a mean wet mass of 737 mg, corresponding to an average increase of 267 mg.

Oxygen treatment affected both the absolute area and the relative area of the CAM (Table 6) with larger areas associated with higher oxygen levels (Table 6). Pair-wise comparisons between treatments indicated that absolute and relative CAM area was larger in the 21% simulated oviduct than in the 9% and 15% oxygen treatments. In the 9% O₂ treatment, only 27% of the inner eggshell surface was covered by the CAM after 10 days of incubation, whereas the CAM covered more than 50% of the inner surface of the eggshell in control eggs.

Table 4. Survival of *Sceloporus undulatus* embryos incubated at 4, 9, 15 and 21% O₂ simulated oviduct, and 21% O₂ control (simulated nest) treatments. S, survivors; NS, non-survivors; NJ, New Jersey; SC, South Carolina

<table>
<thead>
<tr>
<th>Treatment (%O₂)</th>
<th>4%</th>
<th>9%</th>
<th>15%</th>
<th>21%</th>
<th>21% Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>S (NJ/SC)</td>
<td>1/2</td>
<td>6/8</td>
<td>10/7</td>
<td>11/8</td>
<td>7/5</td>
</tr>
<tr>
<td>NS (NJ/SC)</td>
<td>12/6</td>
<td>5/1</td>
<td>3/0</td>
<td>2/0</td>
<td>1/0</td>
</tr>
<tr>
<td>% Survival (overall)</td>
<td>14.2</td>
<td>70</td>
<td>85</td>
<td>90.4</td>
<td>92.3</td>
</tr>
</tbody>
</table>

Table 5. Comparisons of *Sceloporus undulatus* eggs and embryos at 10 days of incubation. Means ± standard errors (N); NJ, New Jersey; SC, South Carolina; CAM, chorioallantoic membrane.

<table>
<thead>
<tr>
<th>Variable</th>
<th>SC</th>
<th>NJ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9%</td>
<td>29.8 ± 0.21 (7)</td>
<td>29.5 ± 0.16 (8)</td>
</tr>
<tr>
<td>15%</td>
<td>30.1 ± 0.14 (7)</td>
<td>30.0 ± 0.19 (10)</td>
</tr>
<tr>
<td>21%</td>
<td>30.6 ± 0.18 (8)</td>
<td>30.4 ± 0.20 (11)</td>
</tr>
<tr>
<td>Control</td>
<td>32.4 ± 0.19 (5)</td>
<td>31.8 ± 0.21 (6)</td>
</tr>
<tr>
<td>Embryo dry mass (mg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9%</td>
<td>0.93 ± 0.180 (7)</td>
<td>0.76 ± 0.150 (8)</td>
</tr>
<tr>
<td>15%</td>
<td>1.10 ± 0.235 (7)</td>
<td>1.10 ± 0.280 (10)</td>
</tr>
<tr>
<td>21%</td>
<td>2.20 ± 0.230 (8)</td>
<td>1.60 ± 0.260 (11)</td>
</tr>
<tr>
<td>Control</td>
<td>3.60 ± 0.890 (5)</td>
<td>3.70 ± 0.190 (6)</td>
</tr>
<tr>
<td>Egg wet mass (mg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9%</td>
<td>–</td>
<td>561 ± 23.5 (8)</td>
</tr>
<tr>
<td>15%</td>
<td>–</td>
<td>578 ± 14.0 (10)</td>
</tr>
<tr>
<td>21%</td>
<td>–</td>
<td>588 ± 16.2 (11)</td>
</tr>
<tr>
<td>Control</td>
<td>–</td>
<td>737 ± 38.4 (6)</td>
</tr>
<tr>
<td>Absolute area of CAM (mm²)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9%</td>
<td>–</td>
<td>99.3 ± 17.11 (8)</td>
</tr>
<tr>
<td>15%</td>
<td>–</td>
<td>153.9 ± 44.35 (7)</td>
</tr>
<tr>
<td>21%</td>
<td>–</td>
<td>202.2 ± 42.19 (8)</td>
</tr>
<tr>
<td>Relative area of CAM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9%</td>
<td>–</td>
<td>0.271 ± 0.045 (8)</td>
</tr>
<tr>
<td>15%</td>
<td>–</td>
<td>0.410 ± 0.104 (7)</td>
</tr>
<tr>
<td>21%</td>
<td>–</td>
<td>0.529 ± 0.103 (8)</td>
</tr>
</tbody>
</table>

DISCUSSION

TESTS OF HYPOTHESES

Results of the experiments do not support the hypothesis that *S. undulatus* females from high latitudes have a greater capacity to retain eggs than those at low latitudes or the hypothesis that *S. undulatus* embryos from high latitudes have a higher develop-
Table 6. Statistical tests of responses of Sceloporus undulatus eggs and embryos after 10 days of incubation in 9, 15 and 21% oxygen treatments. Statistical analyses were two-factor ANCOVAs comparing treatment and population (New Jersey (NJ) and South Carolina) except for analyses of egg wet mass and chorioallantoic membrane (CAM) which were one-factor ANCOVAs (NJ only).

<table>
<thead>
<tr>
<th>Response</th>
<th>Treatment</th>
<th>Population</th>
<th>Results: treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage</td>
<td>$F_{2,16} = 11.4, P &lt; 0.001$</td>
<td>$F_{1,44} = 1.2, P = 0.276$</td>
<td>9% &lt; 15% &lt; 21%</td>
</tr>
<tr>
<td>Embryo dry mass</td>
<td>$F_{2,14} = 8.7, P &lt; 0.001$</td>
<td>$F_{1,44} = 2.5, P = 0.124$</td>
<td>9% &lt; 15% &lt; 21%</td>
</tr>
<tr>
<td>Egg wet mass</td>
<td>$F_{2,22} = 1.9, P = 0.175$</td>
<td>–</td>
<td>9% &lt; 15% &lt; 21%</td>
</tr>
<tr>
<td>Absolute area of CAM</td>
<td>$F_{2,10} = 3.7, P = 0.044$</td>
<td>–</td>
<td>9% &lt; 15% &lt; 21%</td>
</tr>
<tr>
<td>Relative area of CAM</td>
<td>$F_{2,10} = 3.5, P = 0.050$</td>
<td>–</td>
<td>9% &lt; 15% &lt; 21%</td>
</tr>
</tbody>
</table>

Figure 2. Embryonic responses (overall mean, SE) to incubation under 9, 15 and 21% $O_2$ (simulated oviduct) and 21% $O_2$ control (simulated nest). New Jersey and South Carolina populations were pooled for calculation of means. Filled circles represent embryonic stage, open diamonds represent embryonic dry mass. Results of statistical comparisons of treatment effects are presented in Table 6.

One explanation for the lack of divergence in egg retention and embryonic differentiation and growth between the two populations could be that overall body temperatures ($T_b$) of NJ and SC females do not differ. Despite the fact that average daytime air temperatures during May, June and July are 3–5 °C lower in New Jersey than in South Carolina (National Climate Data Center, Fig. 1), females from the two populations maintain very similar $T_b$ values when active (NJ: mean $T_b = 34.0$ °C, SC: mean $T_b = 33.1$ °C, Angelillets, 2001). Thus, NJ females are able to compensate for cooler air temperatures when active during the day by behavioral thermoregulation. In contrast, average minimum air temperatures during May, June and July are 4–6 °C cooler in New Jersey than in South Carolina. Because females cannot thermoregulate at night, night-time $T_b$ values of NJ females are likely to be lower than those of SC females. The overall mean incubation temperature of in utero embryos could thus be several degrees lower in New Jersey than in South Carolina.

Given that NJ females may have somewhat lower mean $T_b$ than SC females, why have the two populations not diverged in their reproductive biology as predicted? One reason may be that costs of retaining eggs are greater than the potential thermal benefits of egg retention for NJ females. The physical weight of the clutch and distension of the abdomen caused by eggs may result in decreased sprint speed and reduced locomotor performance, thus potentially increasing the female’s risk of predation (Shine & Bull, 1979; Serrvo, Hedges & Adolph, 1991; Miles, Serrvo & Frankino, 2000). Furthermore, the thermal benefit of egg retention is likely to be strongest when the difference between the mean temperature of eggs retained in utero and the mean temperature of eggs in nests is relatively large. Observations on the montane lizard Sceloporus virgatus, a close relative of S. undulatus, however, indicate that female $T_b$ and nest temperatures are nearly identical ($T_b = 24.6$ °C, nest = 25.2 °C) (Andrews & Rose, 1994). Thus, selection of thermally favourable nest sites and placement of nests at relatively shallow depths in the soil profile are alternative
Out of 22 species groups, viviparity has evolved four and growth may be due to phylogenetic constraint. Length of egg retention and embryonic differentiation season and reduce female fitness. ing a second clutch of eggs during a single reproductive retention could thus prevent NJ females from producing multiple clutches, prolonged egg retention would increase the interval between clutches. Prolonged egg retention could thus prevent NJ females from producing a second clutch of eggs during a single reproductive season and reduce female fitness. 

Finally, the lack of geographical variation in the length of egg retention and embryonic differentiation and growth may be due to phylogenetic constraint. Out of 22 species groups, viviparity has evolved four times in the genus Sceloporus and only one species group contains both oviparous and viviparous forms (Méndez-de la Cruz et al., 1998). The fact that viviparity only occurs in a few lineages suggests that live-bearing may evolve more readily in some lineages than in others (Andrews et al., 1999). The similarity of NJ and SC populations may thus reflect a general inability of members of the undulatus species group to support embryogenesis in utero during extended egg retention.

**IN UTERO pO2**

A fundamental assumption of the cold climate hypothesis for the evolution of viviparity is that there is a selective advantage for oviparous females to retain embryos in utero for progressively longer periods of time (Packard et al., 1977; Shine, 1985). In previous studies, experimentally induced egg retention in the lizard Urosaurus ornatus (sister genus of Sceloporus) for as long as 29 days past the normal time of oviposition resulted in embryonic development being arrested at stages 30–30.5 (Mathies & Andrews, 1999). In contrast, U. ornatus embryos laid at the normal time of oviposition were eight stages more advanced than retained embryos. In S. undulatus from Virginia, embryos retained for approximately 10 days past the normal time of oviposition had dry masses 4–8 times less than that of control embryos that were laid at the normal time of oviposition (Mathies, 1998). In these studies, egg retention inhibited embryonic development. Likewise, in the present study, hypoxia had a negative effect on embryonic development, water uptake and growth of CAM. Reduced growth of the embryo has also been observed in turtle (Kam, 1992), alligator (Deeming & Ferguson, 1991) and chick (Black & Snyder, 1980) embryos incubated under hypoxic conditions. The reduced rate of embryonic development of retained eggs in utero and of eggs incubated under simulated oviducal conditions at low pO2 supports the hypothesis that hypoxia inhibits embryonic development in utero.

What is the effective pO2 for in utero embryos? The question can be answered by comparing the stage and dry mass of embryos that were incubated at known pO2 under simulated in utero conditions (Fig. 2) with the stage and dry mass of embryos that were actually retained in utero for a similar length of time (Fig. 3; from Mathies, 1998). In Mathies’s (1998) study, embryos retained in utero for 10 days at an average temperature of 27 °C reached a mean stage of 29.5 and a mean dry mass of 0.8 mg. These values correspond most closely to those of eggs incubated under simulated oviducal conditions at 9% O2 and an average temperature of 28 °C, which reached a mean stage of 29.6 and a mean dry mass of 0.84 mg.

Studies of a variety of vertebrate taxa indicate that embryonic development normally occurs at relatively low pO2 (Ar & Mover, 1994). For example, pO2 measured within the uterine lumen of rabbits averaged 7.9 kPa (Mastroianni & Jones, 1965) and between 3.4 and 6.3 kPa in rats (Kaufman & Mitchell, 1990). Similarly, the pO2 of blood from 4-day-old chick embryos ranged between 6.0 and 10.8 kPa (Meuer & Baumann, 1987). The estimated pO2 for in utero S. undulatus embryos is therefore consistent with values observed in other vertebrate taxa.

Eggs incubated at 21% O2 under simulated oviducal conditions grew and differentiated more slowly than eggs incubated at 21% O2 under simulated nest conditions. Growth of embryos under simulated oviducal conditions was reduced by about half and differentiated by about two stages relative to controls. We assume that the major difference between treatments was whether eggshells were fluid or air filled. In this situation, the reduced rate of embryonic development in the 21% simulated oviducal treatment would be associated with a substantially lower rate of diffusion of oxygen in water than air (Wangensteen, Wilson & Rahn, 1970/71). Whereas fluid in the eggshell impedes the flow of oxygen, factors other than simple diffusion
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(e.g. O2 concentration gradient, shell membrane structure, etc.) affect the actual oxygen availability to embryos. For example, the hard-shelled eggs of the turtle *Emydura macquarii* that are fluid filled (at oviposition) have an O2 conductance roughly one-thirtieth of eggs in which the eggshells are air filled (Thompson, 1985). In our study, which involved parchment-shelled eggs, development was affected when eggshells were fluid filled but did not appear to have constituted a dramatic impediment to O2 availability.

HYPOXIA AND THE EVOLUTION OF EGG RETENTION

Results of this study suggest that retained *S. undulatus* embryos develop until embryonic oxygen consumption exceeds oviductal oxygen availability. Differentiation and growth of retained *S. undulatus* embryos is arrested at an embryo stage of approximately 30 and at a dry mass of approximately 0.8 mg. At this point in development, an effective pO2 of 9% is apparently sufficient to maintain embryonic metabolism, but not to support further differentiation or growth. In contrast, embryos of *Sceloporus scalaris* retained in utero for as long as 1 month developed at the same rate as control eggs incubated under simulated nest conditions (Mathies & Andrews, 1996). The capacity of *S. scalaris* to support prolonged embryonic development in utero with little or no detrimental effects suggests that this species possesses physiological or morphological features that enhance diffusion of respiratory gasses between maternal and embryonic circulation. The physiological features most obviously implicated are eggshell structure, vascularity of the oviduct and extra-embryonic membranes (Andrews & Mathies, 2000), and oxygen-binding affinity of embryonic blood (Ingermann, 1992). Of these features, eggshell structure does not appear to be associated with the capacity to support embryonic development in *Sceloporus* lizards (Mathies & Andrews, 2000). Comparative studies that isolate the contribution of maternal and embryonic physiological features associated with gas exchange would provide insight into the physiological and morphological changes that occur during the transition from oviparity to viviparity.

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REFERENCES


Figure 3. Embryo stage (A) and dry mass (B) of *Sceloporus undulatus* embryos in control (unfilled circles) and retained (filled circles) groups as a function of days past the normal time of oviposition (NTO) (Mathies, 1998).


