Determinants of Embryonic Stage at Oviposition in the Lizard *Urosaurus ornatus*

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ABSTRACT

Relatively few squamate reptiles oviposit eggs with embryos at developmental stages greater than stage 30. To investigate potential proximate and ultimate bases of this phenomenon, we experimentally induced females of the lizard *Urosaurus ornatus* to retain their eggs past the normal time of oviposition (NTO). This procedure allowed us to determine whether the length of egg retention is fixed or facultative and to evaluate the effects of retention on embryos, hatchlings, and females. Females were able to retain eggs facultatively for at least 29 d past the NTO. However, retention resulted in arrested development of embryos; arrest occurred at stages 30–30.5, which is only slightly more advanced than that at the NTO (stage 29.5). Embryogenesis was reinitiated when eggs were removed from females and placed in incubation media. Hatching success of these eggs was high (87%), and incubation time was not affected by the number of days that development had been arrested. However, the snout-vent length and water content of hatchlings were negatively related to the length of retention, and they ran slower than hatchlings from control eggs obtained at the NTO. Retention of eggs past the NTO had no detectable effect on the body condition or running speeds of females. Developmental arrest and the adverse effects of retention on hatchling phenotype, if widespread among squamates, would account for the limited range of embryo stages at oviposition and act as major constraints on the evolution of viviparity.

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Introduction

One of the most interesting paradigms to emerge from studies of squamate reproductive biology is that oviposition typically occurs when embryos are at developmental stage 30 (Shine 1983*a*; DeMarco 1993; Blackburn 1995). Most species retain eggs within the oviducts until the embryos attain at least stage 26, but very few retain eggs past stage 33 (according to the staging system of Dufaure and Hubert [1961], where stage 40 is the stage at hatching). Thus, the amount of embryonic differentiation (65%–80% of the total) that occurs in utero and the length of time spent in utero (25%–40% of the total period of development, Shine 1983*a*; DeMarco 1993) is substantial.

The fact that most oviparous squamates deposit eggs when embryos are at or near stage 30 indicates that a minimal amount of development must occur in utero and that the maximal amount is limited. The minimum stage at oviposition is presumably determined by the time required to complete the eggshell. For example, most of the eggshell of the lizard *Sceloporus woodi* is produced within 8–10 d of ovulation, but deposition of the calcareous layer continues until the time of oviposition at stage 27 (Palmer et al. 1993). Thus, the reason so few species oviposit eggs with embryos at stages less than stage 27 may be that the time required to produce an eggshell is longer than the time required by embryos to attain that stage (DeMarco 1993). The maximum stage at oviposition may be limited by the completed eggshell if it restricts respiratory exchange and growth of the embryo (Packard et al. 1977; Guillette 1982). The stage at oviposition also depends on whether the timing of oviposition is fixed or under facultative control. In cases where the timing is fixed and oviposition occurs soon after ovulation, embryos would be relatively undeveloped, although further embryonic development in utero might be physiologically possible. In contrast, the stage at oviposition might vary considerably in those taxa where the timing of oviposition is facultative.

Stage at oviposition may also be influenced in more subtle, indirect ways. First, incubation conditions are known to profoundly influence hatchling phenotype (Gutzke and Packard 1987; Shine and Harlow 1996; Mathies and Andrews 1997; Elphick and Shine 1998) and performance ability (Van Damme et al. 1992) in a wide variety of squamates. Thus, conditions experienced by embryos in the oviducts could influence the stage at oviposition if such conditions reduce hatching success or result in poor-quality hatchlings. Second, gravidity can impair the locomotor performance of females both during gra-

vidity (Shine 1980; Bauwens and Thoen 1981) and for a period thereafter (Sinervo et al. 1991). Thus, if carrying the burden of the clutch imparts energetic costs and/or reduces female survival, selection may favor females that oviposit their eggs as soon as the eggshells are complete and at necessarily early stages of development.

Recent studies have examined the effects of experimentally extended egg retention on embryonic development and hatchling phenotype as a means of identifying possible physiological constraints on the evolution of viviparity (Andrews and Rose 1994; Mathies and Andrews 1996). These investigations focused on species known a priori to retain eggs past stage 30 and have therefore sought to identify constraints acting "midway" between the transition from "typical" egg retention (i.e., oviposition of stage 30 eggs) to viviparity. In this study, we use the technique of inducing females to retain eggs past the normal time of oviposition to address a more fundamental question: What are the constraints that prevent a typical squamate from retaining eggs beyond stage 30?

The objective of this study was to identify possible determinants of embryonic stage at oviposition for a (presumably) typical oviparous lizard, *Urosaurus ornatus* Baird and Girard. One of our objectives was to determine the length of time females can maintain gravidity (i.e., whether egg retention is fixed or facultative). Our other objective was to evaluate the effects of extended egg retention on embryogenesis in utero and the hatching success of eggs, hatchling morphology and locomotor performance, and female body condition and locomotor performance.

Material and Methods

Collection and Initial Maintenance of Lizards

Gravid *Urosaurus ornatus* ($n = 24$) were collected by hand or noose near Animas, Hidalgo County, New Mexico, on July 1, 1996. These females had presumably ovulated recently because all other similarly sized females examined on this date that did not contain eggs had large follicles (as determined by dissection or external palpation). Females were weighed to 0.1 g and their snout-vent lengths (SVL) were measured to the nearest 1 mm on the day they were collected. They were housed temporarily in terraria at the Southwestern Research Station, Portal, Arizona. On July 4, females were transported to our animal care facilities at Blacksburg, Virginia, where they were housed until they were placed under experimental conditions. Females were fed crickets and provided with water daily during this period.

Experimental Design

To assess the capacity of females to support embryogenesis in utero past the normal time of oviposition (NTO), we compared eggs that were retained past the NTO and the females that retained those eggs (experimental eggs and experimental females) with eggs that were obtained at the NTO and incubated under normal conditions and the females that laid those eggs (control eggs and control females). Gravid females were randomly assigned to treatments ($n = 12$, each treatment). Mean body size of control and experimental females (mass or SVL) did not differ (t-tests, $P > 0.05$).

Retention of eggs by experimental females was induced by keeping the substrates of their terraria dry. Such conditions have previously been shown to induce facultative egg retention in two other species of phrynosomatid lizard (Andrews and Rose 1994; Mathies and Andrews 1996).

The NTO for females in the population of *U. ornatus* we studied is unknown. However, the NTOs of other species in this region coincide with the onset of the first heavy summer rains that usually begin in the first 2 wk of July (Andrews and Rose 1994; Mathies and Andrews 1995). We therefore obtained clutches from the control females on July 16 by inducing oviposition with oxytocin. These females (now nongravid) were then placed under housing conditions identical to those described later for experimental females.

On the day control clutches were obtained, one egg from each clutch was dissected to determine the developmental stage of the embryo. Only one egg per clutch was staged because stage does not vary within clutches for a number of species of phrynosomatid lizards (DeMarco 1992), including *U. ornatus* (T. Mathies, unpublished data). Embryo stages were assigned according to the criteria of Dufaure and Hubert (1961) with the modification that half stages were assigned if the embryos had characteristics intermediate between the Dufaure and Hubert stages. The mean embryonic stage of the control eggs was 29.5 (SD = 0.43) and ranged from stages 29.0 to 30.0. Because females were randomly assigned to treatments, we assumed eggs within experimental females were at similar stages of development at this time.

Maintenance of Females

On the first day of the study (July 16), the control (nongravid) and experimental (gravid) females were transferred into individual terraria (12 × 27 × 15 cm) so that they could be monitored closely. Each terrarium contained pieces of roofing tile that provided perches and refugia; cage substrates consisted of dry sand. Wire screen over the top of each terrarium prevented escape and provided additional perch area. Light from room windows determined the photoperiod. Terraria were illuminated (0700–1800 hours, EST) by two fluorescent light bulbs (Vita-lite) suspended just above the top of each terrarium. A heating cable situated beneath one end of each terrarium provided a temperature gradient in which females could thermoregulate behaviorally. The heating cable was turned on and off at 0800 and 1600 hours EST, respectively.

Females were fed a variety of live insects (dusted with mineral and vitamin powder) once each day until satiated. To insure

that the capacity of experimental females to retain eggs was not simply an artifact of insufficient drinking water, we provided water once or twice each day by pooling small amounts on the flat surfaces of the roofing tile. Females readily drank water off these surfaces.

Egg Incubation

On July 16, control eggs were placed individually into 72-mL glass jars that contained moistened vermiculite and were sealed with plastic wrap secured with a rubber band. Eggs were buried completely in the vermiculite mixture. The initial ratio of distilled water to dry vermiculite was 0.7 : 1.0, with a water potential of -200 kPa (water potential determined by using a Wescor dew point microvoltmeter, Model HR 33T). Control eggs experienced temperatures that fluctuated between 24°C and 33°C ($\overline{X}$ = 27.2°C) each day (Fig. 1). This temperature regime was chosen to approximate the temperatures of eggs within experimental females. The temperature regime experienced by control eggs was produced by placing the jars into a constant temperature chamber (Percival Model 1-30 BL) each morning at 0800 hours. Each day at 1600 hours, the jars were removed from the constant temperature chamber and allowed to equilibrate with ambient room temperature. The temperature chamber was located in the same room as the terraria containing the experimental females. Temperatures within jars were periodically monitored by using a temperature probe placed within a sealed jar (containing a vermiculite and water mixture only), which was placed next to the jars with eggs. Probe temperatures were recorded every hour for 24 h and stored in a data logger. Jars and the temperature probe were rotated among shelves within the chamber at 3-d intervals to minimize position effects on embryonic development and to record temperatures at different positions within the chamber. Daily mean temperatures of control eggs did not vary over the course of the study except during the first 10 d, when daily means were approximately 2°C lower than at other times.

Temperatures of eggs within the experimental females were estimated for the periods each day when females were able, and unable, to thermoregulate. To estimate temperatures during the period when thermoregulation was possible, body temperatures (cloacal) of females were measured between 1300 and 1600 hours at approximately 7-d intervals by using a thermocouple thermometer. Each female was removed quickly from its terrarium (taking care not to disturb the female in the adjacent terrarium), had its cloacal temperature recorded, and then was returned to its terrarium. Body temperature of females averaged 35.4°C (SD = 1.3°C), which is similar to body temperatures observed for this species in the field (both sexes: $\overline{X}$ = 35.6°C; SD = 2.1°C; Pianka 1986). Thus, maximal temperatures of experimental eggs averaged (unintentionally) about 2°C higher than control eggs during the period each day when experimental females could thermoregulate (Fig. 1). Because the temperature chamber was located in the same room as the terraria containing the experimental females, eggs in both groups experienced similar temperatures during the period when females were not able to thermoregulate (approximately 1600–0800 hours). During these hours experimental females usually rested on the substrates of their terrariums, and measurements of substrate surface temperatures were similar to those of control eggs. Experimental eggs therefore experienced an overall mean incubation temperature that was about 1°C higher than that of control eggs.

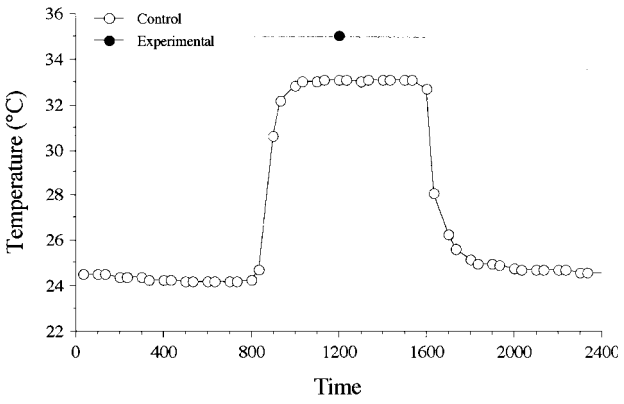

Figure 1. Incubation temperatures of eggs of *Urosaurus ornatus*. Open circles denote mean temperatures of control eggs (obtained at the normal time of oviposition and incubated in incubation media). The horizontal bar (*solid dot*, experimental temperature) denotes the mean temperature of experimental eggs (retained in utero past the normal time of oviposition) and the length of the period at this temperature. Temperatures of experimental eggs were similar to those of control eggs at all other times.

Sampling of Eggs

Control eggs were sampled by randomly selecting one egg from one randomly selected clutch at 2-d intervals, starting on the seventh day past the NTO. Experimental eggs were sampled similarly to control eggs and on the same days as control eggs. Clutches were sampled only once. Experimental eggs were obtained after determining the female's locomotor performance (using the methods described in "Egg Incubation"). Females were then killed by decapitation, and the eggs were immediately dissected out of the oviducts and weighed. Embryos were staged as above, and then the embryos and eggshells were dried at approximately 50°C for 24 h.

On the day each clutch of experimental eggs was obtained, one to four eggs from that clutch were placed individually into jars and incubated (see "Body Condition and Performance of Females") to evaluate the effect of retention on hatch-

ing success, hatchling phenotype, and hatchling performance. Eggs from some clutches of control eggs (one egg each from six clutches) were also incubated to hatching.

Hatching Success, Hatchling Phenotype, and Hatchling Performance

As the time of hatching approached, jars were checked twice each day for hatchlings. Hatchlings were weighed to the nearest 0.1 mg and their SVL measured to the nearest 0.5 mm within a few hours of hatching; SVL was measured again immediately after determination of locomotor performance. Hatchlings were held singly in terraria similar to those described for experimental females and were not given food or water.

The running speed of each hatchling was measured the day after it hatched in a walk-in controlled temperature chamber at a mean air temperature of 33.1°C (SD = 0.1°C). Hatchlings were given 0.5–1.0 h to equilibrate to the chamber temperature before their first run. We quantified the locomotor performance of each hatchling by allowing it to run down a "racetrack." The racetrack was 1 m long by 5 cm wide, with sand glued to its surface to increase traction. Speeds (m/s) were determined with a timing device connected to five pairs of infrared photocells positioned at 25-cm intervals along the racetrack that recorded the cumulative time taken for each hatchling to cross each successive infrared beam. If necessary, hatchlings were gently prodded with a small paintbrush to induce them to keep running. Each hatchling was run three times with only a brief pause between runs. For each run we calculated the mean speed over the 1 m of track, extracted the burst speed (defined as the fastest speed over any 25-cm segment of track), and recorded the number of times a hatchling stopped running. The measures of running speed used in the analyses were the fastest mean speed over any 1 m and the fastest burst speed from any run.

Hatchlings were killed (by freezing) immediately after quantifying their locomotor performance and then dried to a constant mass. The water content of each hatchling was calculated as its live body mass minus its dry body mass.

Body Condition and Performance of Females

Body condition and performance of control and experimental females were measured on the same day their eggs were sampled. Females were weighed to 0.1 g and had their SVL measured to the nearest 1 mm. The nongravid body mass of each experimental female was calculated as gravid body mass minus the total wet mass of the clutch. The physical condition of each female was assessed by its body condition (i.e., nongravid body mass adjusted for SVL and days past the NTO; see "Statistical Analysis"). Locomotor performance of females was measured similarly to that of hatchlings except that females were given between 1 and 2 h to equilibrate to room temperature before the first run and the number of times a female stopped running was not recorded.

Statistical Analyses

Analyses were conducted by using the statistical packages StatView 4.5 (Power PC Version Abacus Concepts), SuperANOVA, v1.11 (Abacus Concepts), and SAS (SAS Institute 1985). Analyses for components of eggs are based on one randomly chosen egg from each clutch. Analyses for hatchlings are based on the first hatchling to hatch from each clutch. Calculations of hatching success are based on individual eggs.

The effect of treatment (TRT) on the absolute running speeds (e.g., burst speed uncorrected for SVL) of hatchlings and adult females was investigated by using *t*-tests. For analyses of the relative running speeds of hatchlings and females and the body condition of females, we used regression models to test the main effect and each of the covariates to determine whether they influenced the response of the dependent variables. TRT was included as a dummy variable (control = 0, experimental = 1). Nonsignificant covariates ($P > 0.05$) were dropped from the analyses. TRT effects were computed only if the slopes were judged homogenous ($P > 0.05$).

Means or least squares means are given ± 1 SD. All analyses were tested for statistical significance at the $P < 0.05$ level.

Results

Capacity of Females to Retain Eggs and Support Development

None of the experimental females laid eggs over the course of the study, and the last female was sampled 29 d past the NTO. Thus, female *Urosaurus ornatus* exhibit a substantial capacity to retain eggs facultatively. Because experimental females were killed to obtain eggs, we do not know whether females could have oviposited naturally. However, all experimental females seemed healthy, a contention that is further supported by their body condition (see "Female Body Condition").

Mass of control eggs increased linearly over the course of the study (Fig. 2*A*; linear regression: $F_{1,10} = 80.76$, $P < 0.0001$), as did the dry mass of the embryos (Fig. 2*B*; linear regression: $F_{1,10} = 169.4$, $P < 0.0001$). In contrast, the mass of experimental eggs did not vary with the number of days past the NTO (linear regression: $F_{1,10} = 1.23$, $P = 0.29$). More important, the dry mass of embryos of experimental eggs was not related to the number of days past the NTO (linear regression: $F_{1,10} = 1.04$, $P = 0.33$). Water uptake and embryonic growth within experimental eggs were thus undetectable.

Stage of embryonic development within control eggs increased linearly over the sampling period (Fig. 2*C*; linear regression: $F_{1,10} = 255.7$, $P < 0.0001$), as did stage of embryonic development within experimental eggs (linear regression: $F_{1,10} = 7.45$, $P = 0.02$). However, the rate of embryonic differ-

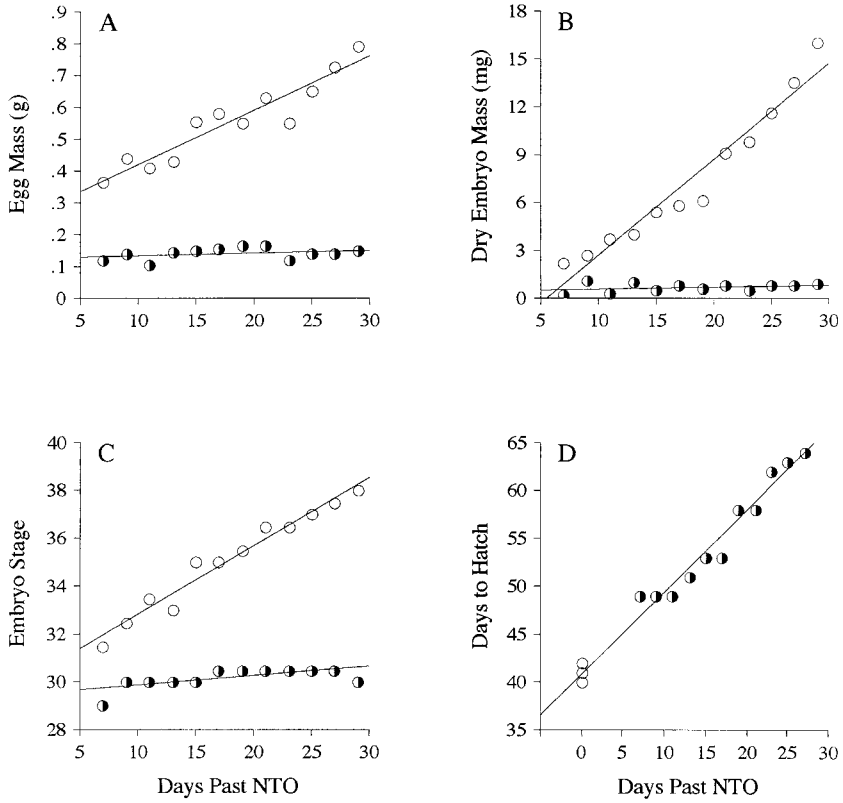

Figure 2. *A*, Relationship between the mass of eggs of *Urosaurus ornatus* and days past the normal time of oviposition (NTO) for control ($y = 0.17x + 0.25$, $R^2 = 0.89$) and experimental eggs. *B*, Relationship between dry mass of embryos and days past the NTO for control ($y = 0.6x - 3.29$, $R^2 = 0.94$) and experimental eggs ($y = 0.01x + 0.15$, $R^2 = 0.09$). *C*, Relationship between embryo stage and days past the NTO for control eggs and experimental eggs ($y = 0.04x + 29.44$, $R^2 = 0.43$). *D*, Relationship between days to hatch and days retained past the NTO for experimental eggs ($y = 0.86x + 40.76$, $R^2 = 0.95$; slope of the regression not significantly different from 1 [see "Results"]; data for control eggs not included in the regression calculation). In all panels, the open circles represent control eggs (obtained at the NTO and incubated in incubation media). The half-filled circles represent experimental eggs (retained in utero past the NTO).

entiation within experimental eggs was drastically slower than that in control eggs (heterogeneity of slopes test: $F_{1,20} = 112.43$, $P = 0.0001$). The extent of differentiation exhibited by embryos of experimental eggs was extremely limited; the mean stage of embryos of experimental eggs (30.2 ± 0.4) was similar to, but significantly higher than, that of control eggs (29.5 ± 0.4) sampled at the NTO ($t = 3.75$, df = 22, $P = 0.001$). Embryonic differentiation within experimental females reached a maximum of stage 30.5, and this maximum was reached within 17 d of the NTO. All but one of the embryos obtained from the remaining six females over the following 12 d were at stage 30.5 (Fig. 2*C*).

Embryos of all experimental eggs that we examined were nonetheless alive, as indicated by regular contractions of the heart. Some embryos (one embryo each from four clutches) exhibited one or more deformities (i.e., disproportionately elongated trunk, protrusion of the mesencephalon, incomplete closure of the cranial region of the neural crest, and disruption of bilateral symmetry of the head). However, only one of the hatchlings resulting from these clutches was deformed.

The dampening effect of egg retention on the development of experimental eggs was not caused by continued deposition of material on eggshells (additional material could reduce the gas exchange capabilities of the eggshell and thus slow development). The mean dry mass of eggshells did not differ between control and experimental eggs (one-factor ANCOVA: intercepts test, $F_{1,20} = 0.16$, $P = 0.69$), and the covariate (days past the NTO) was not significant ($F_{1,20} = 2.57$, $P = 0.12$). The least squares means for dry shell mass of control and experimental eggs were 2.86 ± 0.38 mg and 2.84 ± 0.38 mg, respectively. These comparisons also indicated that females in both groups had completed the process of producing eggshells before the outset of this study and that control eggs were obtained at the appropriate time.

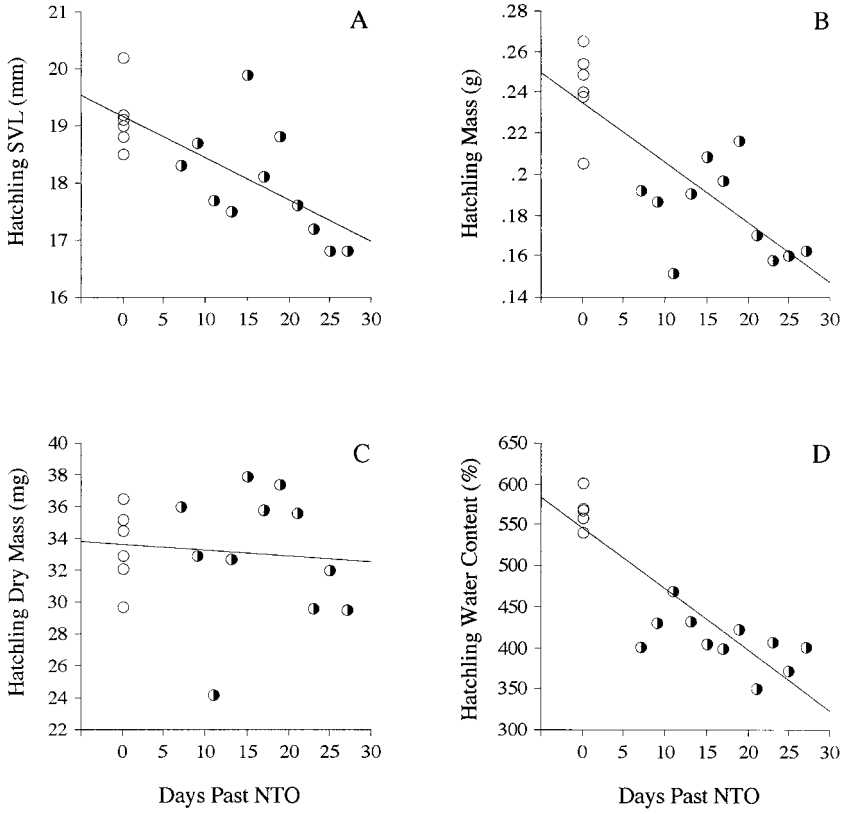

Figure 3. *A*, Relationship between SVL of *Urosaurus ornatus* hatchlings and the number of days eggs were retained past the normal time of oviposition (NTO; $y = -0.73x + 19.16$, $R^2 = 0.52$). *B*, Relationship between live mass of hatchlings and the number of days eggs were retained past the NTO ($y = -2.92x + 234.83$, $R^2 = 0.63$). *C*, Relationship between dry mass of hatchlings and the number of days eggs were retained past the NTO. *D*, Relationship between body water content of hatchlings (expressed here as the percentage of dry body mass) and the number of days eggs were retained past the NTO. In all panels, the open circles represent hatchlings from control eggs (obtained at the NTO and incubated in incubation media). The half-filled circles represent hatchlings from experimental eggs (retained in utero past the NTO). In each analysis, data for both groups of hatchlings are combined.

Hatching Success and Incubation Time

Hatching success was high for control ($n = 6$; 100%) and experimental eggs ($n = 30$; 86.7%). Control eggs hatched between 40 and 42 d ($\overline{X} = 41.33 \pm 0.82$) after the NTO. The only experimental egg that failed to hatch was that obtained from the last female to be sampled (i.e., 29 d after the NTO, embryos at stage 30). Dissection of this egg revealed that the embryo had died at stage 40 (i.e., the stage at hatching). Incubation time of experimental eggs (in incubation media) was slightly reduced relative to that of control eggs; incubation time scaled less than proportional ($b = 0.86$) to the number of days eggs were retained past the NTO (Fig. 2*D*; linear regression: $F_{1,9} = 164.11$, $P < 0.0001$). However, the slope of this regression did not differ from 1 ($t = 2.10$, df = 15, $P > 0.05$).

Hatchling Phenotype

The six hatchlings representing the six control clutches seemed normal in terms of morphology and locomotor behavior, further indicating that the temperature regime we used for control eggs was appropriate for this species. Hatchlings from experimental eggs also seemed normal except for a few (six hatchlings from five different clutches) that exhibited one or more of the following abnormalities: bent tail, small body size relative to head, and/or one eye underdeveloped or absent. However, hatchling SVL was negatively related to the time eggs were retained past the NTO (Fig. 3*A*; linear regression: $F_{1,15} = 15.94$, $P = 0.0012$), as was the live mass of hatchlings (Fig. 3*B*; linear regression: $F_{1,15} = 25.08$, $P = 0.0002$). The dry mass of hatchlings was not related to days past the NTO (Fig. 3*C*; linear regression: $F_{1,15} = 0.16$, $P = 0.70$), and the mean dry mass of

hatchlings from the control (33.50 ± 2.45 mg) and experimental (33.06 ± 4.10 mg) groups did not differ ($t = 0.24$, df = 15, $P = 0.82$). Thus, the negative relationship between live hatchling mass and days past the NTO was due to a systematic reduction in the body water content of hatchlings (Fig. 3*D*).

Hatchling Locomotor Performance

Hatchlings from control eggs averaged a faster absolute mean speed ($t = 2.60$, df = 15, $P = 0.02$), a faster absolute burst speed ($t = 2.31$, df = 15, $P = 0.035$), and fewer stops per run than hatchlings from experimental eggs ($t = 3.73$, df = 15, $P = 0.002$). The means for absolute mean speed, absolute burst speed, and stops per run for hatchlings from the control and experimental eggs were 0.52 ± 0.08 m/s versus 0.39 ± 0.10 m/s, 0.79 ± 0.22 m/s versus 0.57 ± 0.17 m/s , and 2.8 ± 0.41 versus 4.7 ± 1.20, respectively.

Regression models were used to investigate possible causal bases for these differences in treatment effects. The initial regression models used mean or burst speed as the dependent variable, TRT as the independent variable, SVL and number of stops (STOPS) during a run as covariates, and SVL * TRT and STOPS * TRT as interaction terms. The slopes of the relationship between mean speed and stops and between mean speed and SVL were homogenous ($P > 0.05$), and these interaction terms were thus dropped from the analyses. Mean speed was not related to TRT ($F_{1, 13} = 2.4$, $P = 0.14$), although mean speed increased with SVL ($F_{1, 13} = 23.4$, $P = 0.0003$) and decreased with STOPS ($F_{1, 13} = 12.2$, $P = 0.0039$). In the analyses for burst speed, the covariate STOPS was not significant ($P > 0.05$), and it was dropped from the analyses. The interaction between TRT and SVL was significant in the reduced model ($F_{1, 13} = 4.9$, $P = 0.045$), and treatment effects are thus not directly comparable. However, the slope of the relationship between burst speed and SVL for control hatchlings was higher than that of experimental hatchlings, which suggests that retention of eggs past the NTO reduced the dependence of burst speed on hatchling size. Hatchlings from experimental eggs therefore exhibited low running speeds because they were smaller than the controls and because they stopped running more often than the controls.

Female Body Condition

The effect of retention of eggs past the NTO on the physical condition of females was assessed assuming that those females in good condition would weigh relatively more than females in poor condition. Female mass is a function of SVL, and mass may vary with the time past the NTO. The initial regression model thus used body mass as the dependent variable, TRT as the independent variable, SVL and days past the NTO as covariates, and SVL * TRT and days past the NTO * TRT as interaction terms. Only SVL contributed to variance in mass ($F_{1, 18} = 14.1$, $P = 0.0015$), and days past the NTO was therefore dropped from the analysis. Slopes of the relationship between mass and SVL for the two treatments were homogenous ($F_{1, 20} = 0.01$, $P = 0.92$), and this interaction term was dropped from the analysis. Females with longer SVLs had greater masses, as expected ($F_{1, 21} = 36.9$, $P < 0.001$), but body mass was not related to treatment ($F_{1, 21} = 1.6$, $P = 0.22$). The least squares means for nongravid body mass of control and experimental females were 3.3 ± 0.46 g and 3.1 ± 0.44 g, respectively, at the grand covariate mean (SVL = 47.4 mm).

Female Locomotor Performance

Control females averaged a faster absolute mean speed ($t = 3.47$, df = 22, $P = 0.002$) and a faster absolute burst speed ($t = 2.35$, df = 22, $P = 0.03$) than did experimental females. The means for absolute mean speed and absolute burst speed for control and experimental females were 1.28 ± 0.30 m/s versus 0.87 ± 0.08 m/s and 2.06 ± 0.99 m/s versus 1.29 ± 0.54 m/s, respectively.

Regression models were used to investigate possible causal bases for these differences in treatment effects. The initial regression models used mean or burst speed as the dependent variable, TRT as the independent variable, SVL and days past the NTO as covariates, and SVL * TRT and days past the NTO * TRT as interaction terms. No covariate was significant in either analysis ($P > 0.05$). Thus, experimental females ran slower than control females, but retaining eggs past the NTO did not cause a further decline in locomotor performance.

Discussion

An implicit assumption of squamate reproductive biology is that the stage of embryonic development at oviposition is proximately determined by the timing of oviposition. In accord, eggs of *Urosaurus ornatus* obtained at the NTO contained embryos at approximately stage 30, the modal stage at oviposition in squamates. However, our more detailed observations reveal a novel proximate determinant of stage at oviposition that operates independently of the time eggs are retained. In the following section, we discuss the effects of egg retention on embryos, hatchlings, and females, and how these effects may proximately or ultimately influence the stage at oviposition. We then consider the importance of these effects with regards to the evolution of viviparity in squamates.

Determinants of Stage at Oviposition

Effects of Retention on Embryos. The effect of egg retention past the NTO on *U. ornatus* embryos was drastic; development was arrested at stages 30.0–30.5 (Fig. 2*C*). At 29 d past the NTO (i.e., 70% of the normal incubation time at 27°C), embryos within experimental females had advanced only one stage unit beyond that at the NTO and exhibited no detectable increase

in dry mass. If retention had not inhibited development, embryos would have attained at least stage 38 and undergone a 436% increase in dry mass during this period, as judged by the stage and mass of embryos of control eggs.

Retention of eggs past the NTO and the associated developmental arrest had no obvious effects on developmental rate or hatching success once eggs were removed from the oviducts. Experimental eggs immediately resumed development, their incubation time did not differ from that of control eggs, and their hatching success was high (87%). The ability to resume development was present even in eggs retained as long as 29 d past the NTO.

The proximate mechanism that initiates and maintains developmental arrest in *U. ornatus* is unknown, but hypoxia is one likely candidate. The oviducts of oviparous species may be incapable of meeting the oxygen needs of poststage 30 embryos (Packard et al. 1977; Guillette 1982; Shine 1983*a*), or perhaps the eggshell, during its formation, increasingly restricts the availability of oxygen to the embryo. It is clear that arrest did not result from the deposition of abnormal amounts of material onto eggshells (because of a longer-than-normal period of retention). The dry mass of eggshells of experimental eggs did not increase with longer periods of egg retention or differ from that of control eggs. A number of other mechanisms are unlikely or can be ruled out. First, the uterus of *U. ornatus* may secrete some inhibitory substance that maintains developmental arrest as in some mammals (Mead 1993), but no evidence for such a complex control system exists for squamates. Second, developmental arrest was probably not caused by the relatively high temperatures that embryos experienced when experimental females were active; oviposited eggs of *U. ornatus* develop normally and have high hatching success when exposed to even higher incubation temperatures than those used in this study (36°C for 6 h/d; Andrews et al. 1999). Third, normal development by embryos beyond stage 30 may require substantial water uptake by the egg (Shine 1983*a*; Packard and Packard 1988, Shadrix et al. 1994; Fig. 2*A*: note the rapid increase in egg mass between the NTO and the seventh day past the NTO). In contrast, eggs within experimental females did not take up additional water (Fig. 2*A*). However, eggs of two other phrynosomatid lizards continue to develop in utero when retained past their NTOs despite extremely limited water uptake (Mathies and Andrews 1996; Andrews 1997). Thus, water uptake by eggs in utero is not an absolute precondition to embryogenesis.

Effects of Retention on Hatchlings. Retention had a number of potentially deleterious effects on *U. ornatus* hatchlings, and the severity of these effects increased with longer periods of retention. Retention apparently interfered with embryogenesis such that longer durations of retention resulted in hatchlings that were smaller in SVL and live mass (but not dry mass), less hydrated, and slower runners than hatchlings from control eggs (Fig. 3*A*, 3*B*, 3*C*). No factors other than those experienced in the oviducts could have caused these effects because the incubation conditions that experimental eggs experienced after they were obtained from females (i.e., initial water content of eggs, incubation temperature, water potential of incubation media, and length of incubation) did not differ from those of control eggs. During the time they were in the oviducts, however, experimental eggs experienced slightly higher temperatures (~1°C difference; Fig. 1) and a different hydric environment than control eggs. Nonetheless, because experimental females regulated body temperatures similar to those of *U. ornatus* in the field, these effects are physiologically and ecologically relevant although their proximate causes are unknown.

The negative effects of retention on *U. ornatus* hatchlings may influence stage at oviposition by countering selection for a more advanced stage at oviposition. This assertion relies on two assumptions. The first is that a smaller and slower hatchling is "worse" than a larger and faster hatchling (i.e., selection favors larger, faster individuals). The second is that the transition from complete arrest to limited development in utero occurs gradually and that hatchlings incur at least some of the phenotypic effects we observed.

Effects of Retention on Females. Retention of eggs past the NTO had no apparent adverse effects on females despite the lengthy period of retention (29 d past the NTO). The body condition of experimental females did not decline or differ from that of control females. Running speeds of experimental females also did not decline during this period, although they ran slower than control females (presumably because of their greater mass and the bulk of the clutch). Retaining eggs past the NTO therefore does not seem to place any additional physiological demands on females and thus no impetus to curtail the length of egg retention. Normal embryonic development and water uptake of eggs in utero, if possible, would likely have adverse effects on females, at least at some point. However, we are concerned with an explanation for the observed stage at oviposition (i.e., ~stage 30), and it seems unlikely that retaining embryos to slightly more advanced stages than we observed would be deleterious to females. Thus, the effect of egg retention on female physiology is probably not an important selective influence on stage at oviposition in *U. ornatus*.

Implications for the Evolution of Viviparity

The results of this study bear on several important issues regarding the evolution of viviparity in squamates. Most current models posit that viviparity evolves through selection for increasingly longer durations of egg retention in utero (Packard et al. 1977; Tinkle and Gibbons 1977; Shine 1985). When discussed in this context, the term "egg retention" comprises two different components: (1) the capacity to hold eggs within the oviducts (i.e., maintain gravidity), and (2) the capacity to support continued embryogenesis. The general assumption is that both components evolve concurrently (Weekes 1935; Packard

et al. 1977; Guillette et al. 1980; Shine and Guillette 1988; Shine 1991). Our observations for *U. ornatus*, however, indicate that increases in the length of egg retention can evolve independently of the capacity to support embryogenesis. Given that *U. ornatus* females can retain eggs for at least 29 d past the NTO, and incubation time for oviposited eggs is 42 d, females could theoretically support embryogenesis in utero for at least 70% of the postovipositional incubation time. Thus, it is clear from the dichotomous nature of egg retention exhibited by *U. ornatus* that the selective forces behind evolution of facultative egg retention and those for supporting continued embryogenesis in utero can be quite different.

While it is not possible to know the historic conditions that favored the evolution of facultative egg retention in *U. ornatus*, the ability to retain eggs is probably adaptive in its seasonally dry environment. For example, in years when the rains that permit nesting are delayed, females can facultatively retain eggs, oviposit them after the first major rain, and then immediately initiate vitellogenesis and produce another clutch. Under the same conditions, a species without facultative egg retention (i.e., timing of oviposition is obligate) would experience at least one bout of reproductive failure because eggs would desiccate after oviposition. Thus, facultative egg retention allows female *U. ornatus* to maximize their reproductive output (i.e., multiple clutches: up to six clutches annually; Tinkle and Dunham 1983) when the availability of favorable nesting conditions is discontinuous and unpredictable.

The observation of substantial facultative egg retention without continued embryonic development permits new insights into how viviparity might evolve. Consider an oviparous ancestral species that retains eggs facultatively to the extent exhibited by *U. ornatus*. Given the appropriate circumstances, viviparity might evolve relatively "easily" in such taxa because it is presumably easier to evolve one trait (e.g., the capacity to support embryogenesis in utero) rather than coordinate the concurrent evolution of two traits (e.g., facultative egg retention and the capacity to support embryogenesis). In taxa already possessing some capacity to support embryogenesis past the NTO, such an evolutionary transition would be easier still. In addition, unlike selection for an increased ability to support embryogenesis, which may tend to operate primarily in cold climates (Sergeev 1940; Tinkle and Gibbons 1977; Guillette et al. 1980; Shine 1983*b*), selection for facultative egg retention could operate in any environment where the onset of suitable nesting conditions is unpredictable. Data for *U. ornatus* suggest that it would not even be unreasonable if the duration of facultative retention exceeded that of the "normal" incubation time. Thus, the first component of retention (the capacity to maintain gravidity), after evolving in one geographic area (for reasons unrelated to the evolution of viviparity), might then allow this species to move into a previously unoccupied geographic area that favors the evolution of the second component of retention (the capacity to support uterine embryogenesis). The general applicability of such a scenario, however, awaits more information on the prevalence and extent of facultative retention among squamates. Perhaps the most important ramification of the effects of retention on *U. ornatus* embryos pertains to the ease and frequency with which viviparity is likely to evolve. Developmental arrest in utero may constitute a major constraint on the evolution of viviparity, particularly in lineages without facultative retention. In such lineages, the presumed intermediate stages of egg retention would require not only the modifications needed to support continued normal embryogenesis but also a concurrent increase in the capacity to retain eggs.

Shine (1983*a*) speculated that developmental arrest is the proximate mechanism that has precluded the evolution of viviparity in crocodilians and chelonians but noted that there was no evidence for a similar mechanism in squamates. Some species of chamaeleonid lizards apparently undergo developmental arrest at the gastrula stage (as in chelonians), though this phenomenon is not well documented (Ewert 1991 and references therein). Our observations on *U. ornatus* and recent observations on the lizard *Calotes versicolor* (Radder et al. 1998) represent the first unequivocal evidence for developmental arrest in squamates and raise the possibility that the absence of viviparity within distantly related lineages of reptiles is explicable by the same mechanism.

Developmental arrest may be common in squamates but has gone unnoticed simply because it is not readily apparent. In those species where egg retention has little or no facultative component, the time at which oviposition becomes physiologically inevitable might be timed to occur at, or shortly after, the time embryos undergo developmental arrest. Thus, part of the reason for the apparent scarcity of developmental arrest in squamates may be that unlike chelonians, which have a lengthy period of arrest (Ewert 1991), the period of arrest in many squamates may be relatively brief. Moreover, developmental arrest is not obvious even in species that retain eggs facultatively, such as *U. ornatus,* because oviposited eggs will always contain embryos at the proper stage, and they may hatch in the expected number of days. Clearly, additional studies on squamates in a diversity of lineages are needed to assess the frequency of developmental arrest and its influence on the evolution of viviparity.

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