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Evolution of viviparity: variation between two sceloporine lizards in the ability to extend egg retention

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(With 3 figures in the text)

The evolutionary transition between oviparity and viviparity in squamate reptiles presumably occurs via a gradual increase in the duration of egg retention, the production of thinner eggshells, and increases in the vascularity of maternal and embryonic tissues. The 'ease' of this transition may differ among taxa. For example, in the genus *Sceloporus*, the *scalaris* species group contains both oviparous and viviparous species, and female *Sceloporus scalaris* can extend egg retention facultatively in response to the absence of a suitable site for oviposition without impairing embryonic development. In contrast, the *undulatus* species group contains only oviparous species, and, while female *Sceloporus virgatus* can extend egg retention, doing so retards embryonic development. I tested several hypotheses that would explain the greater ability of *S. scalaris* than *S. virgatus* to extend egg retention. In this study, female *S. scalaris* retained eggs for 19 d without affecting the mortality of embryos, total developmental time, or dry mass of hatchlings. In contrast, when female *S. virgatus* retained eggs for 18 d, embryos had very high mortality and eggs took significantly longer to hatch than control (non-retained) eggs, although the dry mass of hatchlings was not affected. The ability of *S. scalaris* females to retain eggs with little negative effect on embryonic development was associated with relatively large chorioallantois, relatively thin eggshells, and relatively small clutch masses. These observations suggest that phylogenetic differences in the ability to extend egg retention may facilitate or constrain the evolution of viviparity in some lineages.

Introduction

Most squamate reptiles (lizards and snakes) are oviparous, that is, females produce eggs, and as much as three quarters of embryonic development takes place after oviposition (DeMarco, 1993). In contrast, a substantial minority of squamates, about one-fifth, are viviparous, that is, females produce fully-developed young. The evolutionary transition from oviparity to viviparity presumably occurs via increases in the period of egg retention, production of thinner eggshells, increases in the vascularity of maternal and embryonic tissues (placentation), and modifications of the hormonal system that support extended egg retention (Packard, Tracy & Roth, 1977; Shine, 1985; Shine & Guillette, 1988; Guillette, 1993).

Oviposition by oviparous squamates typically occurs at embryonic stages 29–31 [Blackburn (1995), staging according to Dufaure & Hubert (1961); hatching or birth is at stage 40]. However, many oviparous squamates exhibit some degree of facultative egg retention; that is, if conditions for nesting are not appropriate, egg retention may be prolonged beyond the time when oviposition normally takes place (Stamps, 1976; Cuellar, 1984). Presumably, some of the variation in the ability to retain eggs is genetic, and provides the raw material for evolution through natural selection of longer periods of egg retention and, ultimately, viviparity (Shine & Guillette, 1988). Thus, investigation into the

consequences of facultatively extended egg retention on embryonic development should provide novel insights into the transition between oviparity and viviparity.

The evolution of viviparity in squamates is associated with cold-climates (Shine, 1985). The most compelling support for this conclusion is provided by the geographic distributions of closely related taxa, one of which is oviparous and the other viviparous; in most cases, the viviparous taxon is found at a higher elevation or latitude than the oviparous taxon (Shine & Bull, 1979; Shine, 1985). Parallel support is provided by the positive association between the length of egg retention and elevation for lacertid lizards (Braña, Bea & Arrayago, 1991).

Independent of climate, however, observations on the reproductive biology of *Sceloporus* lizards indicate that selection for extended egg retention and the evolution of viviparity might be more efficacious in some lineages than others. For example, the *scalaris* species group contains both oviparous and viviparous species, and viviparity has had at least two independent origins within the group (Guillette *et al.*, 1980; Mink & Sites, 1996). *Sceloporus aeneus* and *Sceloporus bicanthalis* are closely related sister species of central Mexico; *S. aeneus* is oviparous and is found at moderate elevations while *S. bicanthalis* is viviparous and is found at high elevations (Guillette, 1982). The close relationship of these species, and their non-overlapping elevational distributions, suggest that this origin of viviparity is comparatively recent. Moreover, females of the oviparous *Sceloporus scalaris* from high elevation populations normally oviposit when embryos are at stages 35–37, while females from low elevation populations oviposit at stages 31–32 (Mathies & Andrews, 1995). However, facultative egg retention by females from the low elevation population does not negatively affect embryonic development, at least up to stages 38–39 (Mathies & Andrews, 1996). These observations on reproductive mode and intraspecific variation in the length and consequences of egg retention thus suggest that the transition to viviparity in the *scalaris* species group is 'easy'.

In contrast, all members in the *undulatus* species group are oviparous, even though the range of the species group includes montane habitats (Guillette *et al.*, 1980), and the ability of females to extend egg retention beyond stages 30–31 is limited. Female *Sceloporus undulatus* lay their eggs at approximately stage 30, whether or not suitable oviposition sites are available (Crenshaw, 1955; Sexton & Marion, 1974; Mathies & Andrews, unpubl. data). Female *S. virgatus* can extend egg retention facultatively, but embryonic development is retarded (Andrews & Rose, 1994). These observations suggest that selection for extended egg retention in this taxon would not be as efficacious as it would be in the *scalaris* group.

The objectives of this research were to investigate some of the proximate explanations for the differing abilities of *S. scalaris* and *S. virgatus* to retain eggs and support the development of embryos *in utero*. Observations by Andrews & Rose (1994) suggested that low water uptake by eggs might limit development of *S. virgatus* embryos *in utero*. I therefore tested the hypotheses that *S. scalaris* females have smaller clutch volumes than *S. virgatus* females, thus leaving more room for eggs to expand *in utero* as a result of water uptake, and that *S. scalaris* eggs take up more water than *S. virgatus* eggs while *in utero*.

I also tested two hypotheses that don't distinguish between water uptake and gas exchange as factors that could limit embryonic development *in utero*. One hypothesis was that the eggshells of *S. scalaris* are thinner than those of *S. virgatus*; thin eggshells would facilitate both water uptake and gas exchange. The other hypothesis was that the extraembryonic membranes associated with gas exchange of *S. scalaris* embryos have relatively larger surface areas than those of *S. virgatus* embryos. The rationale for the latter hypothesis is as follows. The chorioallantois, which is formed early in development by the fusion of the external surface of the allantois and the inner surface of the chorion, is highly vascularized and is apposed to the inner surface of the shell. This composite membrane

presumably facilitates the exchange of gases between foetal and maternal tissues (Yaron, 1985; Blackburn, 1993), and the allantois itself is an important reservoir of water for the embryo (Hoyt, 1979; Simkiss, 1980). Because previous observations indicated that the development of *S. scalaris* embryos is not retarded by extended egg retention, I predicted that their chorioallantois would have a greater surface area than those of *S. virgatus* at comparable stages of development.

To test these hypotheses, I manipulated the length of egg retention and compared the development and water uptake of embryos from control clutches (that were oviposited at the same time that oviposition occurred in the field) with the development and water uptake of embryos from experimental clutches (that were retained within females for 18–19 d beyond the time of normal oviposition). In addition, some experimental clutches were reduced in size by surgically removing one oviduct and the eggs that it contained. This latter treatment allowed me to determine the effect of reduced clutch volume (and hence, more space for egg expansion) on embryonic development and water uptake.

Materials and methods

Collection and maintenance of gravid females

Eighteen gravid *Sceloporus scalaris* and 24 gravid *Sceloporus virgatus* were collected in late June 1994 at the Appleton-Whittell Ranch Sanctuary (1460 m) and in the Chiricahua Mountains (1600–1800 m), Arizona, respectively, and shipped within a few days to Blacksburg, VA. Females were placed into 3 controlled light and temperature chambers (Percival model no. I-30BL with B1 option). Females were housed in small groups in plastic tubs and fed crickets and wax moth larvae on 6 days per week. Vegetation and rocks within the cages were sprayed with water twice a day to provide drinking water. The sand substratum of the cages was kept dry to inhibit oviposition, and thereby induce experimental females to retain their eggs until the selected sample date (see the next section). To minimize any chamber or position effects, tubs with females were rotated among chambers and among shelves in the chambers every 5 days in a fixed pattern so each tub spent equal time in all possible locations within and among chambers.

Thermal and light regimes

Environmental chambers were programmed to fluctuate between 19 and 33 °C (mean = 27 °C) on a diel cycle that represented body temperatures of gravid females (see fig. 1a in Andrews & Rose, 1994). This temperature regime was based on field observations of the body temperatures of free-ranging gravid females (Andrews & Rose, 1994; Mathies & Andrews, 1995). For *S. virgatus*, mean body temperatures of gravid females and mean temperatures of eggs in nests are 25 °C (Andrews & Rose, 1994). For *S. scalaris*, mean body temperatures of gravid females should average slightly higher than those of *S. virgatus*; mean body temperatures during activity are the same (34 °C) for both species, but night-time temperatures were higher at the *S. scalaris* than at the *S. virgatus* site (Mathies & Andrews, 1995; Rose, unpubl. data). Thus, females with eggs *in utero* (and eggs after oviposition or removal from females, see below) were incubated at temperatures that were similar to temperatures exhibited in the field.

The light cycle (L:D 14:10) in all chambers was the same as that in Arizona in mid-July.

Experimental manipulations: control versus experimental eggs

The experimental manipulations involved the length of time that eggs were incubated *in utero* and whether females with eggs *in utero* carried half or entire clutches. Within both species, females oviposited their entire clutch at the normal time (control), had half of their clutch removed (at the normal time of oviposition), and

retained the rest of the clutch for 18–19 d, or retained the entire clutch for 18–19 d. Oviposition by both species occurs with the onset of the summer monsoon rains (Andrews & Rose, 1994; Mathies & Andrews, 1995). In 1992 and 1993, oviposition occurred in the first week in July. Accordingly, oviposition for this study was induced on 5–6 and 7–8 July, respectively, for *S. scalaris* and *S. virgatus*. Within each treatment group, eggs (one or more per clutch) were randomly chosen to be sampled at 1 of the 3 times: normal oviposition (Time 1), 18–19 d later (Time 2), and at hatching. The data collected at each sampling date included the wet and dry masses of eggs and embryos, stages of embryos, and wet and dry masses of hatchlings. The specific sampling protocols for embryos are presented in the next section and protocols for each of the 3 treatments follow.

Gravid females of each species were randomly assigned to 1 of the 3 treatments (Fig. 1).

1) Females in the control treatment laid all of their eggs at approximately the same time that eggs were oviposited in the field (Time 1). Oviposition by 6 *S. scalaris* and 7 *S. virgatus* was induced with an injection of 0.1 cc oxytocin. The few females that did not lay their eggs in response to oxytocin were killed and their eggs surgically removed from the oviducts. Eggs from each of these clutches were randomly assigned to 1 of 3 groups: eggs be sampled at Time 1, Time 2, or at hatching.

2) Females (6 *S. scalaris* and 10 *S. virgatus*) in one experimental treatment (retained-half clutch) had their clutches reduced by approximately one-half, by surgically removing the entire left oviduct (methods given by Mathies, 1994), at the same time that control eggs were oviposited. Half of the eggs from the left oviduct were sampled and the remaining half were allowed to hatch. Females, with their remaining eggs, were returned to the environmental chambers until Time 2, when they were killed. At this time, half of the eggs from the right oviduct were sampled and the remaining half were allowed to hatch.

3) Females (6 *S. scalaris* and 7 *S. virgatus*) in the other experimental treatment (retained-entire clutch) retained their entire clutches until Time 2, when the females were killed and their entire clutches removed. Approximately one-half of the eggs from each clutch were sampled at time 2 and the remaining eggs were allowed to hatch.

Control and experimental eggs that were not sampled were marked individually, weighed, and placed randomly in plastic shoe boxes (2 boxes per species) half filled with vermiculite. Distilled water was added periodically to maintain the ratio of the mass of distilled water to the mass of dry vermiculite at 0.7 to 1.0 (approximately −230 to −200 kPa, Tracy, Packard & Packard, 1978; Packard *et al.*, 1987). This water potential was within the range of that measured at nests of *S. virgatus* in the field (−300 to >−100 kPa, Rose, unpubl. data). Boxes with eggs were placed in the environmental chambers alongside the tubs with gravid females and rotated as for the tubs that contained females. As a consequence, all embryos were exposed to essentially the same thermal regime.

Sample sizes for experimental groups 2 and 3 were reduced because 1 *S. scalaris* and 3 *S. virgatus* died shortly

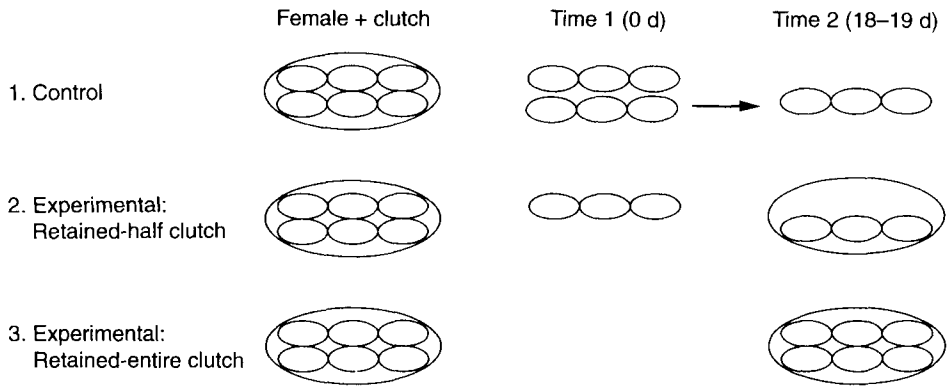

FIG. 1. Schematic diagram of the three treatments. Gravid females are represented by large ovals and eggs are represented by small ovals. Eggs are shown as free if they were oviposited or removed from females at Time 1 or within females if they were retained *in utero* to Time 2. The arrow indicates eggs that were oviposited at Time 1, but sampled at Time 2. Time is in days from the date of oviposition.

after surgery and because 2 *S. scalaris* and 2 *S. virgatus* females laid their entire clutches prior to Time 2 (Table IV provides final sample sizes for all groups). Experimental females whose clutches were reduced surgically, and survived surgery, remained healthy during the experimental period, and experimental females that laid their clutches early and were therefore not killed at Time 2 remained healthy for at least several months after the experimental period.

Sampling protocols

All eggs were weighed at oviposition or when removed from females. At least one embryo per clutch per sample period was staged according to photographs in Dufaure & Hubert (1961). Half stages were assigned if the embryo was intermediate in characteristics between the Dufaure & Hubert stages. When eggs were sampled, wet and dry masses of the shell, embryo and extraembryonic contents (extraembryonic membranes, the yolk and remaining fluid components) of each egg were determined. Components were dried to a constant mass for 24–48 h at 50–60 °C. Wet and dry masses were determined to the nearest 0.01 mg with an analytical balance. One or two eggs from each control clutch were preserved in formalin at Times 1 and 2 as were 1 or 2 eggs from each retained-entire clutch at Time 2.

Eggs preserved in formalin were carefully dissected and the area of the chorioallantois was either measured or estimated. When the area was small (<30% of the inner surface area of the eggshell), the maximum and minimum diameters of the chorioallantois were measured using a dissecting microscope with an ocular micrometer. Similarly, when the area was large (>70% of the inner surface area of the eggshell), the maximum and minimum diameters of the section of the egg where the chorioallantois was not present were measured. Because these areas were relatively flat, surface areas were determined from the formula for an ellipse. Because of the curvature of the shell, however, chorioallantois areas of intermediate size were estimated to the nearest 5% of the surface area of the egg, and egg surface areas were estimated from egg mass using the formula, Area = $4.835\ \text{Mass}^{0.662}$ (Paganelli, Olszowka & Ar, 1974) where area is in cm^2 and mass is in grams. The size of the chorioallantois was expressed as absolute area and as a percentage of the surface area of the egg. Eggshell density (as an index of conductivity to gas and water) of control eggs at the time of normal oviposition was calculated as the dry mass of the eggshell divided by its surface area as calculated above.

Hatchlings were weighed to the nearest 0.1 mg within a few hours of hatching, and then killed by freezing. The carcasses were dried and weighed again. The incubation period was recorded as the time elapsed between Time 1, the presumed date of oviposition in the field, and the date when eggs hatched.

Relative clutch mass was determined as the mass of the clutch relative to the mass of the post-gravid female at Time 1. The wet and dry masses of each clutch were determined by multiplying the mean wet and dry egg masses, respectively, of eggs sampled from that clutch at Time 1, by the number of eggs in the clutch. For females whose clutch was halved in size, the number of eggs in the clutch was the sum of the eggs removed at Time 1 and at Time 2. Relative clutch mass was not determined for females from the retained-entire clutch treatment because their eggs were not sampled at Time 1.

Statistical analyses

To reduce pseudoreplication, analyses involving eggs, embryos and hatchlings were based on clutch means for each treatment and sample date. For example, if 2 embryos were staged at Time 2 from a clutch in the retained-entire treatment, then the stage for that clutch and time was the mean for those 2 individuals. Only comparisons of mortality during incubation were based on observations of individual eggs.

All parametric analyses were conducted using SAS software (SAS Institute Inc., 1985). Means are given ($\pm$S.E.). Analysis of covariance (ANCOVA) was performed only when slopes were judged homogeneous ($P > 0.2$ in all cases). Stage of embryonic development was used as the covariate for interspecific comparisons of chorioallantois area. I considered that stage could be used as a quantitative variable because it is linearly related to the age and mass of embryos over the ranges of stages observed in this study (Andrews & Rose, 1994; Mathies &

Andrews, 1996), and the relationship between chorioallantois area and stage was linear. Non-parametric analyses followed Siegel (1956). Fisher exact probability tests were used instead of χ^2 tests when the smallest expected frequency in a cell was four or less.

Results

Egg mortality, incubation period and mass of hatchlings

Mortality of eggs of *Sceloporus scalaris* and *Sceloporus virgatus* that were incubated from the time of normal oviposition, that is, eggs from control plus retained-half clutches incubated from Time 1, was low, averaging 19 and 9%, respectively, and did not differ between species ($\chi^2 = 0.7$, $P \gg 0.05$, Table I).

In contrast to the similar mortality of eggs incubated from the time of normal oviposition, eggs of the two species differed in their response to retention. The mortality of eggs of *S. scalaris* that were incubated after retention *in utero* was similar to that of eggs that were laid at the time of normal oviposition, and the overall mortality of eggs in all treatments was 13% (Table I). The mortality of eggs from control clutches and of eggs in entire clutches retained to Time 2 did not differ ($\chi^2 = 1.7$, $P > 0.10$). Moreover, mortality of eggs in retained-half clutches that were incubated from Time 1 and eggs from the same females that were retained to Time 2 and then incubated also did not differ ($P > 0.05$, Fisher exact test). In contrast, eggs from control clutches of *S. virgatus* had considerably lower mortality (10%) than eggs from retained-entire clutches (81%) ($\chi^2 = 18.1$, $P < 0.001$). Similarly, eggs in retained-half clutches that were incubated from Time 1 had lower mortality (8%) than eggs from the same females that were retained to Time 2 and then incubated (80%) ($P < 0.01$, Fisher exact test). Thus, hatching success of *S. scalaris* eggs was not affected by egg retention beyond the time of normal oviposition, while the hatching success of *S. virgatus* eggs that were retained was considerably decreased.

TABLE I

Mortality of eggs during incubation, mean length of the incubation period, and mean hatchling dry mass. Control T–1 = eggs oviposited at Time 1 and allowed to hatch, Ret.-half T–1 = eggs removed surgically from females at Time 1 and allowed to hatch. Ret.-half T–2 = eggs removed surgically from females at Time 2 and allowed to hatch, Ret.-entire T–2 = eggs from entire clutches removed surgically from females at Time 2 and allowed to hatch. Incubation period = number of days between Time 1 (normal oviposition) and hatching. (n) = number of clutches for which mean incubation period and hatchling dry mass were calculated (mortality during incubation reduced the original number of clutches). Means are given ± 1 S.E.

	(*n*)	No. of eggs that hatched/died	Mortality (%)	Incubation period (d)	Hatchling dry mass (mg)
S. scalaris					
Control T–1	(6)	17/5	23	39 (±1.1)	38 (±1.9)
Ret.-half T–1	(5)	12/2	14	39 (±1.6)	41 (+3.8)
Ret.-half T–2	(4)	9/1	10	41 (±1.0)	42 (±2.3)
Ret.-entire T–2	(4)	29/2	2	41 (±1.6)	44 (±0.8)
S. virgatus					
Control T–1	(7)	18/2	10	43 (±1.2)	68 (±3.1)
Ret.-half T–1	(4)	11/1	8	45 (±1.2)	61 (±4.9)
Ret.-half T–2	(2)	2/8	80	53 (±0.5)	67 (±1.0)
Ret.-entire T–2	(3)	4/17	81	52 (±2.0)	63 (±4.8)

The length of the incubation period for *S. scalaris* was not affected by egg retention beyond the time of normal oviposition, while the incubation period for *S. virgatus* was increased (Table I). For *S. scalaris*, the incubation period for control eggs did not differ from that of eggs that were retained to Time 2 and then incubated (39 versus 41 d, respectively, $F_{1,12} = 2.8$, $P = 0.123$, ANOVA). Eggs from retained-half clutches incubated from Time 1 were not included in this or the following comparisons of hatchling mass to maintain independence (eggs from the same females are in both groups of the retained-half clutch treatment). In contrast, for *S. virgatus*, the incubation period for control eggs was significantly shorter than for eggs that were retained to Time 2 and then incubated (43 vs. 52 days, respectively, $F_{1,10} = 25.1$, $P < 0.001$, ANOVA).

For *S. scalaris*, the dry mass of hatchlings from control eggs was similar to the dry mass of hatchlings from eggs that were retained to Time 2 and then incubated (38 versus 43 mg, respectively, $F_{1,12} = 5.8$, $P = 0.033$, ANOVA) (Table I). Similarly, for *S. virgatus*, the dry mass of hatchlings from control eggs did not differ from the dry mass of hatchlings from eggs that were retained to Time 2 and then incubated (68 vs. 65 mg, respectively, ($F_{1,10} = 0.4$, $P = 0.553$, ANOVA). Egg retention thus did not affect the size of hatchlings for either species.

Time 1: Females and clutches

Sceloporus scalaris females were smaller than *S. virgatus* females with mean gravid/post-gravid masses of 5.6/3.3 versus 9.2/5.7 g, respectively (Table II). *Sceloporus scalaris* also had smaller clutch masses (Table II), and lower relative clutch masses as well. I evaluated relative clutch masses with ANCOVA's where wet or dry clutch masses were the dependent variables, species was the class variable, and mass of the post-gravid female was the covariate. Relative to their body size, *S. scalaris* had lower wet and dry clutch masses than *S. virgatus* (wet mass: $F_{1,20} = 13.0$, $P = 0.002$; dry mass: $F_{1,20} = 28.1$, $P < 0.001$). At the grand covariate mean (post-gravid mass of females = 4.5 g), the adjusted wet clutch masses were 2.2 and 3.6 g and the adjusted dry clutch masses were 0.7 and 1.2 g, respectively. Relative clutch masses (wet clutch mass/post-gravid female mass) based on results of the ANCOVA were 0.5 and 0.8, respectively. Both *S. scalaris* and *S. virgatus* produced an average of 10 eggs per clutch. Thus, *S. scalaris* had smaller relative clutch masses than *S. virgatus*, although the number of eggs per clutch was the same.

Time 1: Eggs and embryos

At Time 1, eggs and embryos obtained from control and retained-half clutches should have had

TABLE II

Mean body and clutch sizes (± 1 S.E.) for ***S. scalaris*** *and* ***S. virgatus*** *at Time 1 (control plus experimentally retained-half clutch treatments).* ***P*** *values are for one-way ANOVAs.* ***n*** *= number of females. Means are given ± 1 S.E.*

	S. scalaris ($n = 11$)	*S. virgatus* ($n = 12$)	$F_{1,21}$	P
Gravid female mass (g)	5.6 (±0.21)	9.2 (±0.34)	74.3	<0.001
Post-gravid female mass (g)	3.3 (±0.20)	5.7 (±0.30)	43.6	<0.001
Wet clutch mass (g)	2.4 (±0.16)	3.4 (±0.15)	23.2	<0.001
Dry clutch mass (g)	0.8 (±0.04)	1.2 (±0.03)	55.1	<0.001
Eggs per clutch	10.3 (±0.74)	9.7 (±0.28)	0.5	0.502

similar attributes, and this was true in general (Table III). However, for both species, water content of eggs was greater for the control than the retained-half clutch treatment, and this difference was statistically significant for *S. virgatus*. A possible explanation is that control eggs took up water between the time that females were injected with oxytocin and oviposition. Eggs from clutches that were surgically reduced in size would not have had this opportunity to take up additional water. The higher masses of eggs in control group than experimental retained-half clutch treatment reflect the relatively high water contents of control eggs, that is, the difference in egg mass of the two treatments is very similar to the difference in water contents. In addition, for *S. virgatus*, the dry mass of embryos from control clutches was greater than that of embryos from retained-half clutches, and this difference approached statistical significance.

Differences between the control and the retained-half clutch treatments at Time 1 (initial values) could confound comparisons between these treatments at Time 2. Moreover, if comparisons between these two treatments are confounded by their initial values at Time 1, then comparisons at Time 2 between these two treatments and the retained-entire clutch treatment are also problematical (at Time 1, retained-entire clutches were inside females and not sampled). To assess this potential problem, I conducted a further set of analyses.

To determine if the relatively low water contents of eggs from the retained-half clutches of *S. scalaris* and *S. virgatus* at Time 1 affected the results of comparisons at Time 2, I performed ANCOVAs with the water content of eggs at Time 2 as the dependent variable, water content at Time 1 as the covariate, and treatment (control versus retained-half clutch) as the class variable. For *S. scalaris* and *S. virgatus*, the water contents of eggs at Time 2 were related, albeit weakly, to water contents at Time 1 ($F_{1,8} = 7.4$, $P = 0.026$ and $F_{1,9} = 6.6$, $P = 0.030$, respectively), but treatment differences were highly significant ($F_{1,8} = 47.8$, $P < 0.001$ and $F_{1,9} = 18.0$, $P = 0.002$, respectively). Thus, egg retention affected the water content of eggs at Time 2 independently of their water content at Time 1.

To determine if the relatively low embryo masses of retained-half clutch eggs of *S. virgatus* at

TABLE III

Mean attributes of eggs and embryos for control and retained-half clutch (Ret.-half) treatments at Time 1. Ps are based on one-way ANOVAs. Water content of eggs is exclusive of the embryo. n = number of clutches. Means are given ± 1 S.E.

	Control ($n = 6$)	*Ret.-half* ($n = 5$)	*F*	*P*
S. scalaris				
Shell density (mg/cm^2)	3.0 (±0.16)	3.7 (±0.29)	4.7[1]	0.058
Egg mass (mg)	249 (±11.9)	226 (±12.9)	1.6[1]	0.237
Egg water (mg)	147 (±7.6)	120 (±4.1)	8.5[2]	0.017
Embryo dry mass (mg)	1.1 (±0.22)	1.9 (±0.41)	3.7[1]	0.085
Embryo water (mg)	16.9 (±4.7)	20.4 (±5.5)	0.2[2]	0.634
Embryo stage	30.7 (±0.33)	31.4 (±0.68)	1.1[1]	0.332
S. virgatus				
Shell density (mg/cm^2)	4.9 (±11)	5.5 (±0.32)	4.9[3]	0.052
Egg mass (mg)	391 (±14.5)	328 (±19.6)	7.0[3]	0.025
Egg water (mg)	211 (±12.2)	162 (±6.7)	10.4[4]	0.010
Embryo dry mass (mg)	4.8 (±0.51)	2.8 (±0.23)	9.5[3]	0.012
Embryo water (mg)	40.9 (±4.7)	37.7 (±5.9)	0.2[4]	0.677
Embryo stage	32.6 (±0.49)	32.3 (±0.44)	0.2[3]	0.704

[1] *d.f.* = 1, 9, [2] *d.f.* = 1, 8, [3] *d.f.* = 1, 10, [4] *d.f.* = 1, 9

Time 1 affected the results of comparisons at Time 2, I performed an ANCOVA with the dry mass of embryos at Time 2 as the dependent variable, their dry mass at Time 1 as the covariate, and treatment (control versus retained-half clutch) as the class variable. Embryo dry mass at Time 2 was not related to embryo dry mass at Time 1 ($F_{1,9} = 0$, $P = 0.999$), but treatments differed significantly ($F_{1,9} = 6.2$, $P = 0.034$). Thus, egg retention affected the mass of embryos at Time 2 independently of their mass at Time 1.

Time 2: Effect of treatment on eggs and embryos

In general, egg retention beyond the date of normal oviposition affected eggs and embryos of both *S. scalaris* and *S. virgatus*, and, for all comparisons in which significant treatment effects were detected, these effects were the result of differences between the control and the two experimental treatments; in no case did the two experimental treatments differ significantly ($P > 0.05$, Ryan–Einot–Gabriel–Welsh multiple range tests, Table IV). Control eggs were heavier and contained more water than experimentally retained eggs. Control embryos had greater dry masses than experimental embryos and had greater water contents. The developmental stage of embryos did not vary among treatments for *S. scalaris*, but did for *S. virgatus*.

For both species, eggs from the experimental retained-half clutches appeared to be heavier and contain more water than eggs from the experimental retained-entire clutches, although these differences were not statistically significant as judged by a posteriori comparisons. To determine if these comparisons were confounded by variability of crowding (number of eggs) in the abdominal cavity of females and variability in the size of eggs as indexed by their dry mass exclusive of the shell, I calculated residuals for the following two relationships for each species separately. First, I obtained the residual values for the regression of the dry weight of the clutch (to correct for variable water content) contained by experimental females at Time 2 and their post-gravid mass. Second, I obtained

TABLE IV

*Mean attributes of eggs and embryos for control and experimental (retained-half and retained-entire clutch) treatments at Time 2. **P** = significance level for comparisons among the three treatments (one-way ANOVAs). The two experimental treatments did not differ for any comparison for either species (Ps > 0.05, Ryan–Einot–Gabriel–Welsh Multiple Range Tests). Water content of eggs is exclusive of the embryo. **n** = number of clutches. Means are given ± 1 S.E.*

S. scalaris	Control ($n = 6$)	Ret.-half ($n = 5$)	Ret.-entire ($n = 4$)	$F_{2,12}$	P
Egg mass (mg)	778 (±5.0)	280 (±15.6)	235 (±9.1)	71.2	<0.001
Egg water (mg)	606 (±44.9)	134 (±8.9)	99 (±7.5)	81.9	<0.001
Embryo dry (mg)	11.1(±0.87)	6.9 (±0.55)	7.2 (±0.76)	9.9	0.003
Embryo water (mg)	92.3 (±8.0)	58.6 (±4.4)	57.2 (±5.1)	9.7	0.003
Embryo stage	36.7(±0.28)	36.2(±0.20)	36.4 (±0.24)	1.3	0.295
S. virgatus	Control ($n = 7$)	Ret.-half ($n = 5$)	Ret.-entire ($n = 7$)	$F_{2,16}$	P
Egg mass (mg)	699 (±28.1)	387 (±29.8)	346 (±12 .2)	70.4	<0.001
Egg water (mg)	444 (±26.0)	203 (±18.4)	157 (±10.6)	64.9	<0.001
Embryo dry (mg)	15.1(±0.20)	5.7 (±0.76)	6.2 (±0.32)	15.7	<0.001
Embryo water (mg)	120.2(±7.4)	54.9 (±6.5)	53.7(±2.6)	45.0	<0.001
Embryo stage	37.0(±0.29)	35.2(±0.20)	35.1 (±0.40)	10.7	0.001

the residual values for the regression of mean water content (extraembryonic plus embryonic) of eggs as a function of their mean dry mass (extraembryonic plus embryonic). These two sets of residuals should exhibit a negative relationship if the water content of eggs is related to the degree of crowding in the oviduct.

For both species, the water content of eggs (corrected for their dry mass) was negatively related to the dry mass of the clutch (corrected for female body size) (Fig. 2). For *S. scalaris*, water content of eggs was not significantly related to the dry mass of the clutch ($F_{1,6} = 0.9$, $P = 0.37$), and treatment was not significant ($F_{1,6} = 0.5$, $P = 0.518$, ANCOVA, water content was the dependent variable, the dry mass of the clutch was the covariate, and treatment was the class variable). However, for *S. virgatus*, water content of eggs was significantly related to the dry mass of the clutch ($F_{1,9} = 5.6$, $P = 0.042$), and treatment was not significant ($F_{1,9} = 0.4$, $P = 0.559$, ANCOVA, as for *S. scalaris*).

Time 2: Interspecific comparisons of eggs and embryos

The effect of egg retention on development and water uptake was less pronounced for *S. scalaris* than *S. virgatus*. First, despite the fact that *S. scalaris* embryos were smaller and less advanced in stage than *S. virgatus* embryos at Time 1, experimentally retained embryos of *S. scalaris* were larger and more advanced in stage than experimentally retained embryos of *S. virgatus* at Time 2. Second, at Time 2, the stages of experimentally retained embryos of *S. scalaris* did not differ from those of controls, while the stages of experimentally retained embryos of *S. virgatus* were significantly less advanced than those of controls. Thus, developmental rates of *S. scalaris* embryos were less retarded (based on both change in mass and in developmental stage) than *S. virgatus* embryos after the same length of extended egg retention.

For both species, control eggs took up considerable amounts of water between oviposition and Time 2; *S. scalaris* and *S. virgatus* eggs quadrupled and doubled their water contents, respectively. In contrast, eggs that were retained to Time 2 (Table IV) took up considerably smaller amounts of water, and the absolute amounts taken up by the two species were very similar. For example, at Time 1, eggs from *S. scalaris* had 120 + 20 = 140 mg of water (extraembryonic plus embryonic), while at Time 2, eggs from the retained-half and retained-entire clutches had 134 + 59 = 193 mg and 99 + 57 = 156 mg, respectively. At Time 1, eggs from retained-half clutches of *S. virgatus* had 162 + 38 = 200 mg of water, while at Time 2, eggs from the retained-half and retained-entire clutches had 203 + 55 = 258 mg and 157 + 54 = 211 mg, respectively. Thus, eggs from retained-half clutches took up about 50 mg of water and eggs from retained-entire clutches took up about 15 mg.

The density of eggshells of *S. scalaris* and *S. virgatus* were 3.0 versus 4.9 mg/cm^2, respectively (Table III, Control groups, $t_{11} = 10.1$, $P < 0.001$, *t*-test). Assuming that the structure of eggshells is similar between the species, the less dense shells of *S. scalaris* eggs should exhibit higher conductivity to water and gases than *S. virgatus* shells.

The chorioallantois

At the time of normal oviposition (Time 1), the chorioallantois covered 20–60% of the inner surface area of the control eggs in both species (Fig. 3). At Time 2, 18–19 days later, embryos from control eggs of both species were at stages 36–38 and their chorioallantois ($n = 4$, *S. scalaris*; $n = 4$, *S. virgatus*) covered the entire inner surface of the egg (not illustrated).

In contrast, egg retention retarded the development of the chorioallantois for both species, but especially so for *S. virgatus* (Fig. 3). At Time 2, the chorioallantois of all embryos from retained-entire clutches were smaller (Fig. 3a) than those of embryos from control clutches (coverage was 100%).

However, the chorioallantois of *S. scalaris* embryos were less retarded in development, i.e. closer to 100% coverage, than those of *S. virgatus* embryos whether expressed as a percentage of the surface area of the egg (Fig. 3a), or in terms of absolute size (Fig. 3b). The proportion of the surface area covered by the chorioallantois was significantly larger for *S. scalaris* than *S. virgatus* embryos

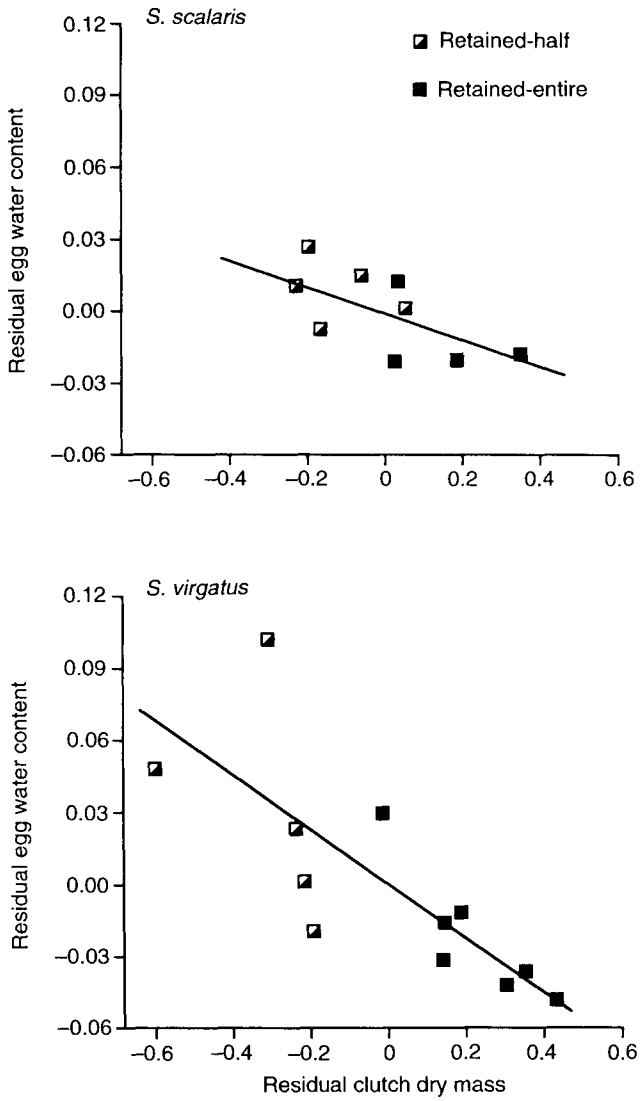

FIG. 2. The relationship between the residual water content of eggs (corrected for their dry mass) and the residual dry mass of the clutch (corrected for the body size of the female) for eggs from clutches that had been retained *in utero* for 18–19 d. The negative relationship between these residuals indicates that the amount of water in individual eggs is a function of the degree of crowding in the abdominal cavity. For neither *S. scalaris* nor *S. virgatus* were treatment effects significant. Least squares regression lines are shown for the pooled treatments. Half-filled squares represent the experimental clutches that had been reduced in size by surgically removing one oviduct and filled squares represent the experimental clutches that were entire.

($F_{1,20} = 42.3$, $P < 0.001$, ANCOVA), with adjusted means of 72 and 42%, respectively. The absolute size of the chorioallantois was significantly greater for *S. scalaris* than *S. virgatus* embryos as well ($F_{1,20} = 9.2$, $P = 0.007$, ANCOVA), with adjusted means of 1.38 and 1.04 cm^2, respectively. These analyses were repeated using embryo dry mass as the covariate rather than stage, yielding identical statistical conclusions.

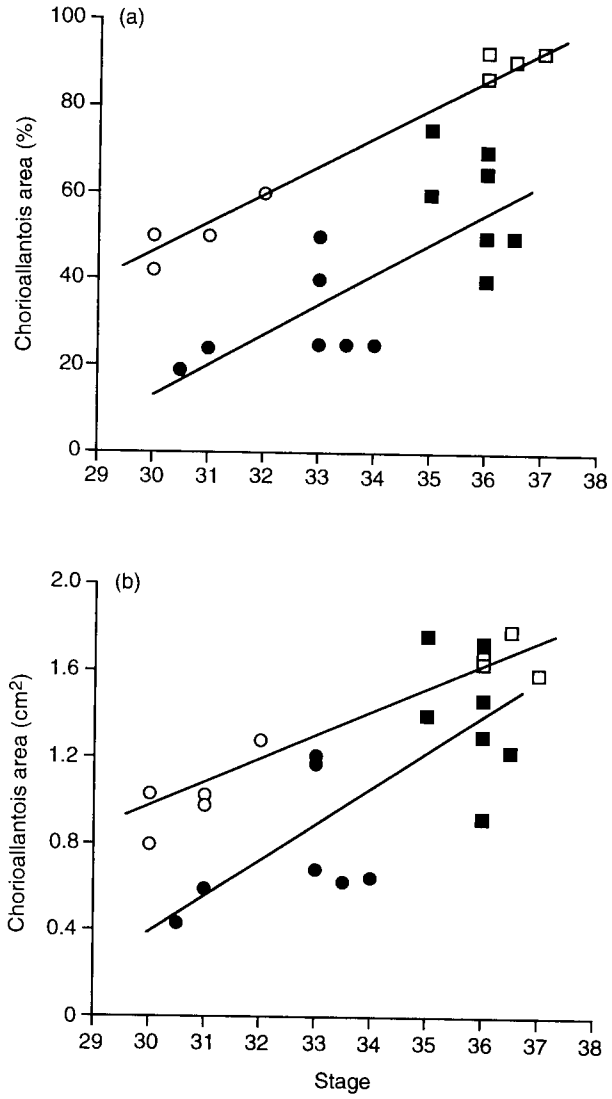

FIG. 3. (a) Relative area (%) of the chorioallantois as a function of embryo stage. (b) Area (cm^2) of the chorioallantois as a function of embryo stage. Open and closed symbols represent *S. scalaris* and *S. virgatus*, respectively, and circles represent control eggs at oviposition (Time 1) and squares represent eggs from experimentally retained-entire clutches after 18–19 days of retention (Time 2). Control eggs at Time 2 are not shown because their chorioallantoic area was 100% in all cases. Fitted regression lines for each species are shown.

Discussion

Interspecific differences in the effects of extended egg retention

Previous observations indicated that development of *Sceloporus scalaris* embryos was not affected by up to 30 d of extended egg retention, while a similar length of retention retarded the development of *Sceloporus virgatus* embryos (Andrews & Rose 1994; Mathies & Andrews 1996). In this study, the prediction that embryonic development of *S. scalaris* would be less affected by extended egg retention than that of *S. virgatus* was confirmed. For example, extended egg retention by *S. scalaris* did not delay hatching or increase egg mortality, while extended egg retention by *S. virgatus* delayed hatching by an average of eight days and the mortality of experimental eggs was considerably higher than that of control eggs. On the other hand, when eggs were *in utero*, embryonic development of both species was retarded, although less so for *S. scalaris* than *S. virgatus*. For example, at Time 2, experimental embryos of *S. scalaris* were 64% of the mass of control embryos, although the two groups did not differ in stage, while experimental embryos of *S. virgatus* were 40% of the mass of control embryos and two stages earlier in development.

In contrast to previous studies, however, embryonic development of *S. scalaris* while *in utero* was affected by extended egg retention, although the development of embryos from experimental eggs caught up with the development of embryos from control eggs by the time of hatching. Why was embryonic development of *S. scalaris* affected by extended egg retention in this study but not in the previous study? The two studies were identical in design except that respective incubation temperatures averaged 27 °C in this study and 26 °C in the study by Mathies & Andrews (1996). Thus, the relatively high incubation temperature, through its effects on the metabolic demands of the embryos, may have affected the ability of female *S. scalaris* to extend egg retention.

Embryonic development of *S. virgatus* is retarded by extended egg retention (Andrews & Rose, 1994), and this was true of this study as well. As expected, the mean higher temperatures (27 vs. 25 °C) used in this study resulted in faster developmental rates than observed by Andrews & Rose (1994), but, as judged by the low mortality of control eggs, the higher temperatures *per se* were not inimical to embryonic development. Only 9% of control eggs died during incubation in this study and only 12% in the study of Andrews & Rose (1994). Thus, incubation temperatures used in this study were within the range of temperatures at which successful development of *S. virgatus* occurs.

Tests of hypotheses

Why were embryos of *S. scalaris* less affected by egg retention than eggs of *S. virgatus*? I tested several hypotheses on proximate bases for differences between the species. One hypothesis was that *S. scalaris* would have a lower relative clutch mass at the normal time of oviposition than *S. virgatus*, and thus eggs of *S. scalaris* would have more room for expansion as a result of water uptake. This hypothesis was accepted as *S. scalaris* had a relative clutch mass of 0.5 while *S. virgatus* had a relatively clutch mass of 0.8. I also tested the hypothesis that eggs of *S. scalaris* would take up more water *in utero* than eggs of *S. virgatus*. This hypothesis was not supported. Experimental eggs of both species took up comparable amounts of water while *in utero*, and embryos of *S. scalaris* and *S. virgatus* were similar in dry mass (7 and 6 mg, respectively) when eggs were sampled at Time 2.

The quantity of water in eggs was negatively related to the crowding of eggs in the abdominal cavity for both species. These statistical relationships were weak, probably because of small sample sizes. Qualls & Andrews (unpubl. data) found a strong negative relationship between the quantity of water in

eggs and the crowding of eggs in the abdominal cavity for normal clutches of both species. Thus, hydrostatic pressure limits water uptake by eggs of both species.

Andrews & Rose (1994) suggested that water, rather than gas exchange, might limit embryonic growth *in utero*. However, despite the higher water contents of eggs from retained-half clutches than eggs from retained-entire clutches, the stages and dry masses of embryos did not differ between the two experimental treatments, nor did hatching success or hatchling dry mass. In a parallel study, maternal provisioning of water or minerals for viviparous snake embryos by females with normal clutches and with clutches that were surgically reduced in size did not differ (Sangha *et al.*, 1996). Thus, in this study, relatively high water contents of eggs from small clutches were either insufficient to promote normal embryonic development, development was not limited by the availability of water, or the experiment was not continued long enough to detect negative effects of water availability on embryonic development.

The remaining hypotheses that I tested concerned morphological features associated with both gas exchange and water uptake. The first of these features was the eggshell. In general, the evolution of extended egg retention and viviparity is associated with the production of relatively thin eggshells (Guillette & Jones, 1985; Heulin, 1990). With regard to their reproductive biology, both the *S. scalaris* and *S. virgatus* that I studied appear to be typical oviparous lizards; mean stage at oviposition is 32, and the incubation period is 6 weeks or more (Andrews & Rose, 1994; Mathies & Andrews, 1995) this study). But, despite these features, eggs of *S. scalaris* had significantly thinner (less dense) shells than eggs of *S. virgatus*. The lower density of *S. scalaris* eggshells could reflect thinner or less porous shells, either of which would lead to higher conductance.

The second morphological feature I considered was the chorioallantois. Because the chorioallantois is the putative surface of gas exchange for the embryo, I hypothesized that this structure should be larger for *S. scalaris* than *S. virgatus*. At the time of normal oviposition, embryos of *S. scalaris* had absolutely and relatively larger chorioallantois than embryos of *S. virgatus* (Fig. 3). At Time 2, the chorioallantois of *S. scalaris* embryos were still absolutely and relatively larger than those of *S. virgatus* embryos at the same stages of development (Fig. 3).

Thus, both hypotheses, that *S. scalaris* and *S. virgatus* would differ in the thickness of their eggshells and the size of the chorioallantois, were accepted. Both features would enhance gas exchange and water uptake by eggs of *S. scalaris* relative to those of *S. virgatus*. However, gas exchange is more likely to be critically facilitated by relatively thin eggshells and relatively large chorioallantois than is water uptake. First, gas exchange is likely to be limited by the thickness of the eggshell because the diffusion of O_2 and CO_2 is much slower in water than in air (Dejours, 1975), and the diffusion pathways through the shell are filled with water while the egg is in the oviduct (Thompson, 1985). In contrast, water uptake of eggs is unlikely to be related to the thickness of the eggshell *per se* because of the rapid diffusion of water through the porous eggshells of small lizards (Vleck, 1991). Second, the extensive vascularization of the chorioallantois, and its physical proximity to the most highly vascularized areas of the oviduct (Weekes, 1935; Masson & Guillette, 1987), presumably serve to facilitate gas exchange for the developing embryo. In contrast, water is taken up over the entire surface of the shell (for turtle eggs, at least) (Thompson, 1985), and the movement of water among compartments within the eggs is along osmotic gradients (Hoyt, 1979).

Water uptake and growth of the chorioallantois

The water reservoirs of the eggs of oviparous lizards function, in part, to buffer the embryo from changes of water availability in the environment, and eggs of small lizards take up large quantities of

water (Andrews & Sexton, 1981; Vleck, 1991; Shadrix *et al.*, 1994). Despite suggestions that the yolk sack and associated membranes are the site of water uptake by eggs (Weekes, 1935; Blackburn, 1993), my observations indicated that considerable water uptake occurred through the chorioallantois. The chorioallantois of control eggs of *S. scalaris* and *S. virgatus* covered the entire inside of the shell during half or more of the total incubation period. As water influx into eggs of both species continues throughout the incubation period (Andrews & Rose, 1994; Mathies & Andrews, 1996), much of the influx of water into eggs occurred through the chorioallantois.

Control eggs took up large quantities of water, and the largest fluid compartment of eggs was the allantois, as judged by the quantity of fluid immediately under the chorioallantois and surrounding the embryo and the yolk. My observations support the conclusion of Packard & Packard (1988) that the fluid compartment of squamate eggs that many authors (e.g. Badham, 1971) assumed to be albumen, was actually allantoic fluid. More recently, demonstration that proteins in eggs of *Anolis pulchellus* are not of oviductal origin (Cordero-López & Morales, 1995) suggests that some lizards, at least, do not have a true albumen layer.

My observations further suggested that water uptake was a more important proximate determinant of the size of the chorioallantois than the metabolic requirements of embryos. The allantois of control eggs expanded to accommodate a large influx of water, and expansion simultaneously increased the extent of the chorioallantoic membrane. In contrast, experimentally retained eggs took up relatively little water, and the extent of the chorioallantois at Time 2 was less than that of control eggs. The slower growth of the chorioallantois of the experimental than the control embryos was inexplicable in terms of embryonic requirements for water or for gas exchange, given that embryos in both control and retained eggs would have had the same requirements, at least initially. The size of the chorioallantois of experimental embryos thus seems to have been determined by water uptake *per se*. If this is correct, then an additional benefit of water uptake for the eggs of small squamates is to increase the surface area of the chorioallantois, the membrane responsible for gas exchange between the embryo and the external surface of the egg.

Implications for the evolution of viviparity

One implication of my results is that selection for extended egg retention and the ultimate evolution of viviparity would be more efficacious in some groups than others. Assuming that the population of *S. scalaris* that I studied is typical of the ancestral phenotype, pre-existing reproductive traits of this species would facilitate invasion of high elevations where extended egg retention, a putative transitional stage in the evolution of viviparity, is adaptive. Subsequent selection for further reproductive modifications could then readily occur. This scenario is supported by the wide distribution at moderate elevations of oviparous species in the *scalaris* species group and the more restricted and high elevation distributions of the viviparous taxa (Sites *et al.*, 1992). Moreover, females of *Sceloporus aeneus*, another high elevation oviparous member of the *scalaris* species group, can also extend egg retention well beyond the normal stages at oviposition (at least to stages 35–36, Andrews, unpubl. data). On the other hand, if the *S. scalaris* that I studied are descendants of high elevation populations, then the ability of *S. scalaris* from relatively low elevations to extend egg retention with little effect on embryonic development may simply reflect phylogenetic history rather than present-day ecological circumstances.

In contrast, *S. virgatus* is in the *undulatus* species group. All members of this group are oviparous, although representatives are found at high latitudes and in montane habitats, suggesting the presence of constraints to reproductive modifications that would enable the successful extension of egg

retention. Because embryonic development of *S. virgatus* is retarded by extended egg retention, successful invasion of high elevations is less likely to be successful, or would require greater simultaneous reproductive modifications, than for similar shifts in the range of *S. scalaris*.

At this time, my suggestion that the evolution of viviparity is more efficacious in some squamate groups than others, is best interpreted as a working hypothesis. It is, however, supported by the non-random distribution of the origins of viviparity among squamate taxa (Shine, 1985). A rigorous test of this hypothesis, however, requires a broader comparative analysis (see Garland & Adolph, 1994), contrasting the reproductive features of squamate lineages that include both oviparous and viviparous species to that of lineages that are wholly oviparous. The limiting factor for such an analysis is not the availability of appropriate taxa (Shine, 1985), but the data on the phylogenetic relationships and reproductive features of these taxa. Such a data set would contribute considerably to the understanding of the physiological basis for the evolution of viviparity.

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REFERENCES

Andrews, R. M. & Rose, B. R. (1994). Evolution of viviparity: constraints on egg retention. *Physiol. Zool.* **67:** 1006–1024.

Andrews, R. M. & Sexton, O. J. (1981). Water relations of the eggs of *Anolis auratus* and *Anolis limifrons*. *Ecology* **62:** 556–562.

Badham, J. A. (1971). Albumen formation in eggs of the agamid *Amphibolurus barbatus barbatus*. *Copeia* **1971:** 543–545.

Blackburn, D. G. (1993). Chorioallantoic placentation in squamate reptiles: structure, function, development, and evolution. *J. Exp. Zool.* **266:** 414–430.

Blackburn, D. G. (1995). Saltationist and punctuated equilibrium models for the evolution of viviparity and placentation. *J. Theor. Biol.* **174:** 199–216.

Braña, F., Bea, A. & Arrayago, M. J. (1991). Egg retention in lacertid lizards: relationships with reproductive ecology and the evolution of viviparity. *Herpetologica* **47:** 218–226.

Cordero-López, N. & Morales, M. H. (1995). Lack of proteins of oviductal origin in the egg of a tropical anoline lizard. *Physiol. Zool.* **68:** 512–523.

Crenshaw, J. W., Jr (1955). The life history of the southern spiny lizard, *Sceloporus undulatus undulatus* Latreille. *Am. Midl. Nat.* **54:** 257–298.

Cuellar, O. (1984). Reproduction in a parthenogenetic lizard: with a discussion of optimal clutch size and a critique of the clutch weight/body weight ratio. *Am. Midl. Nat.* **11:** 242–258.

Dejours, P. (1975). *Principles of comparative respiratory physiology*. Amsterdam: North Holland Publishing.

DeMarco, V. (1993). Estimating egg retention times in sceloporine lizards. *J. Herpetol.* **27:** 453–458.

Dufaure, J. P. & Hubert, J. (1961). Table de développement du lézard vivipare: *Lacerta (Zootoca) vivipara* Jacquin. *Arch. Anat. Microsc. Morphol. Exp.* **50:** 309–328.

Garland, T. & Adolph, S. C. (1994). Why not to do two-species comparative studies: limitations on inferring adaptation. *Physiol. Zool.* **67:** 797–828.

Guillette, L. J., Jr (1982). The evolution of viviparity and placentation in the high elevation, Mexican lizard *Sceloporus aeneus*. *Herpetologica* **38:** 94–103.

Guillette, L. J., Jr (1993). The evolution of viviparity in lizards. *Bioscience* **43:** 742–751.

Guillette, L. J., Jr & Jones, R. E. (1985). Ovarian, oviductal, and placental morphology of the reproductively bimodal lizard, *Sceloporus aeneus*. *J. Morphol.* **184:** 85–98.

Guillette, L. J., Jr, Jones, R. E., Fitzgerald, K. T. & Smith, H. M. (1980). Evolution of viviparity in the lizard genus *Sceloporus*. *Herpetologica* **36:** 201–215.

Hoyt, D. F. (1979). Osmoregulation by avian embryos: the allantois functions like a toad's bladder. *Physiol. Zool.* **52:** 354–362.

Heulin, B. (1990). Étude comparative de la membrane coquillère chez les souches ovipare et vivipare du lézard *Lacerta vivipara*. *Can. J. Zool.* **68:** 1015–1019.

Masson, G. R. & Guillette, L. J., Jr (1987). Changes in oviducal vascularity during the reproductive cycle of three oviparous lizards (*Eumeces obsoletus*, *Sceloporus undulatus* and *Crotaphytus collaris*). *J. Reprod. Fertil.* **80:** 361–372.

Mathies, T. C. (1994). *Life history evolution of the lizard* ***Sceloporus scalaris****: comparisons of lowland and montane populations*. MSc thesis, Blacksburg: Virginia Polytechnic Institute and State University.

Mathies, T. & Andrews, R. M. (1995). Thermal and reproductive biology of high and low elevation populations of *Sceloporus scalaris*. *Oecologia* **104:** 101–111.

Mathies, T. & Andrews, R. M. (1996). Extended egg retention and its influence on embryonic development and egg water balance: implications for the evolution of viviparity. *Physiol. Zool.* **69:** 1021–1035.

Mink, D. G. & Sites, J. W., Jr (1996). Species limits, phylogenetic relationships, and origins of viviparity in the *scalaris* complex of the lizard genus *Sceloporus* (Phrynosomatidae: Sauria). *Herpetologica* **52:** 551–571.

Packard, G. C. & Packard, M. J. (1988). The physiological ecology of reptilian eggs and embryos. In *Biology of the Reptilia* **16B**, *Ecology*: 523–605. Gans, C. & Huey, R. B. (Eds). New York: Academic Press.

Packard, G. C., Packard, M. J., Miller, K. & Boardman, T. J. (1987). Influence of moisture, temperature, and substrate on snapping turtle eggs and embryos. *Ecology* **68:** 983–993.

Packard, G. C., Tracy, C. R. & Roth, J. J. (1977). The physiological ecology of reptilian eggs and embryos, and the evolution of viviparity within the class Reptilia. *Biol. Rev.* **52:** 71–105.

Paganelli, C. V., Olszowka, A. & Ar, A. (1974). The avian egg: surface area, volume, and density. *Condor* **76:** 319–325.

Sangha, S., Smola, M. A., McKinney, S. L., Crotzer, D. R., Shadrix, C. A. & Stewart, J. R. (1996). The effect of surgical removal of oviductal eggs on placental function and size in the viviparous snake, *Virginia striatula*. *Herpetologica* **52:** 32–36.

SAS Institute Inc. (1985). *SAS users guide: statistics*. Version 5 edn. Cary, NC: SAS Institute Inc.

Sexton, O. J. & Marion, K. R. (1974). Duration of incubation of *Sceloporus undulatus* eggs at constant temperature. *Physiol. Zool.* **47:** 91–98.

Shadrix, C. A., Crotzer, D. R., McKinney, S. L. & Stewart, J. R. (1994). Embryonic growth and calcium mobilization in oviposited eggs of the scincid lizard, *Eumeces fasciatus*. *Copeia* **1994:** 493–498.

Shine, R. (1985). The evolution of viviparity in reptiles: an ecological analysis. In *Biology of the Reptilia* **15:** 605–694. Gans, C. & Billett, F. (Eds). New York: John Wiley & Sons.

Shine, R. & Bull, J. J. (1979). The evolution of live-bearing in lizards and snakes. *Am. Nat.* **113:** 905–923.

Shine, R. & Guillette, L. J., Jr (1988). The evolution of viviparity in reptiles: a physiological model and its ecological consequences. *J. Theor. Biol.* **132:** 43–50.

Siegel, S. (1956). *Nonparametric statistics*. New York: McGraw-Hill Book Co.

Simkiss, K. (1980). Water and ionic fluxes inside the egg. *Am. Zool.* **20:** 385–393.

Sites, J. W., Jr, Archie, J. W., Coles, C. J. & Flores Villela, O. (1992). A review of phylogenetic hypotheses for lizards of the genus *Sceloporus* (Phrynosomatidae). *Bull. Am. Mus. Nat. Hist.* **213:** 1–110.

Stamps, J. A. (1976). Egg retention, rainfall and egg laying in a tropical lizard, *Anolis aeneus*. *Copeia* **1976:** 759–764.

Thompson, M. B. (1985). Functional significance of the opaque white patch in eggs of *Emydura macquarii*. In *Biology of Australian frogs and reptiles*: 387–395. Grigg, G., Shine, R. & Ehrmann, H. (Eds). Chipping Norton: Surrey Beatty & Sons.

Tracy, C. R., Packard, G. C. & Packard, M. J. (1978). Water relations of chelonian eggs. *Physiol. Zool.* **51:** 378–387.

Vleck, D. (1991). Water economy and solute regulation of reptilian and avian embryos. In *Egg incubation: its effects on embryonic development in birds and reptiles*: 245–259. Deeming, D. C. & Ferguson, M. W. J. (Eds). Cambridge: Cambridge University Press.

Weekes, H. C. (1935). A review of placentation among reptiles, with particular regard to the function and evolution of the placenta. *Proc. zool. Soc. Lond.* **2:** 625–645.

Yaron, Z. (1985). Reptile placentation and gestation: structure, function, and endocrine control. In *Biology of the Reptilia* **15:** 527–603. Gans, C. & Billett, F. (Eds). New York: John Wiley & Sons.