EVOLUTION OF EGG RETENTION IN THE *SCELOPORUS SPINOSUS* GROUP: EXPLORING THE ROLE OF PHYSIOLOGICAL, ENVIRONMENTAL, AND PHYLOGENETIC FACTORS

M. L. Calderón-Espinosa[1,3], R. M. Andrews[2], and F. R. Méndez de la Cruz[1]

[1]*Laboratorio de Herpetología, Instituto de Biología, UNAM, CP 04510, Ciudad Universitaria, México D.F.*
[2]*Virginia Polytechnic Institute, Blacksburg, Virginia, USA*

Abstract: The evolution of viviparity in squamates has involved intermediate stages of egg retention. Reduction in the thickness of the eggshell, in relative clutch mass (RCM), and in clutch frequency would have facilitated the transition from oviparity to viviparity, while low temperatures are likely the ultimate selective force that promoted this evolutionary shift. We tested these ideas using the *Sceloporus spinosus* group. Because it is the sister clade of the viviparous *Sceloporus formosus* group, we predicted that members of the *S. spinosus* group would exhibit extended egg retention and other features associated with the evolution of viviparity. To test this idea, we examined the ability to retain eggs past the time of normal oviposition in the *Sceloporus spinosus* group and evaluated the association between egg retention and physiological and environmental factors in a historical context. Gravid females were collected from seven localities at a wide range of altitudes. We estimated the normal stage of embryos at oviposition and the stage at oviposition when females were induced to retain eggs under captive conditions. Stages of embryos varied within clutches; less developed embryos were usually dead and the most advanced embryos were usually alive. The maximum stage observed was therefore used as an index of egg retention for each clutch. The maximum embryonic stage at oviposition was contrasted with RCM, egg membrane thickness, and several climatic variables (temperature and precipitation) in a phylogenetic framework. Females exhibited the ability to retain eggs as predicted. Maximum stage at oviposition varied within same clutch, same locality, and among localities. Variation observed in the maximum stage at oviposition was not related to egg membrane thickness, RCM, or clutch frequency or to environmental temperature and precipitation. Instead, mapping the maximum stage at oviposition on a phylogeny of the *S. spinosus* group suggested that the invasion of high elevations was associated with an enhanced potential for longer periods of egg retention.

Key words: Egg retention; Evolution of viviparity; *Sceloporus spinosus* group.

THE EVOLUTION OF VIVIPARITY in squamate reptiles has long been interpreted as an adaptive strategy that enhances the survival of embryos (Shine, 1985; Tinkle and Gibbons, 1977). This evolutionary phenomenon has occurred more than 100 times in the Squamata (Blackburn, 1992, 2000; Shine, 1985) and reversal to oviparity is rare, if it occurs at all (Lee and Shine, 1998; Smith et al., 2001). Factors that could be detrimental to embryos in a nest (i.e. extreme temperatures, environmental unpredictability, dry environments, predation, and microbial attack) have been suggested as selective forces that stimulated the evolution of this reproductive mode (Shine, 1985; Tinkle and Gibbons, 1977). Of these environmental factors, the most widely accepted on the basis of comparative and experimental studies is low temperature (Shine, 1985, 2002). According to the cold climate model, low temperature promotes the evolution of viviparity because egg retention provides the embryos a warmer or more stable environment than they would experience in a nest (Shine, 1985, 2004). In contrast, predictions of an unpredictability hypothesis were falsified for some Australian oviparous scincid species suggesting that at least in that system, female inability to predict climatic environmental conditions did not promote the origin of viviparity (Shine, 2002).

Viviparity has evolved from oviparity (Lee and Shine, 1998). Observations on reproductively bimodal species like *Lacerta vivipara*, *Lerista bougainvilli*, and *Saiphos equalis* suggest that this involved a gradual increase in the length of egg retention (Andrews and Mathies, 2000; Heulin et al., 2002; Shine,

---

[3] Correspondence: e-mail, mlce@ibiologia.unam.mx

1985; Smith and Shine, 1997). The scarcity of species that lay eggs when embryos are at intermediate stages, (between stage 33 and 40), suggests that this transition occurs rapidly or that intermediate forms are not adaptive (Blackburn, 1995, 1998). Some oviparous populations of bimodal species lay eggs with embryos well beyond the modal stage at oviposition observed in squamates (stage 30, Andrews and Mathies, 2000). In *L. vivipara*, for example, females of oviparous populations closely related to one of the viviparous clades, lay their eggs in a greater range of stages (30–35) than those from the basal oviparous clade (stages 30–32) (Heulin et al., 2002). In reproductively bimodal species, extension of intrauterine development of embryos is negatively correlated with eggshell thickness (Heulin et al., 2002; Qualls, 1996; Qualls et al., 1995; Smith and Shine, 1997). Reduction in eggshell thickness, increased vascularization of the oviduct, and more extensive development of the chorioallantois membrane are suggested physiological and morphological modifications associated with the evolutionary transition from oviparity towards viviparity (Andrews, 1997; Guillette, 1993; Heulin et al., 2002).

To assess the phylogenetic context of the evolution of viviparity in reptiles, Lee and Shine (1998) mapped reproductive mode on reptile phylogeny. They concluded that the evolution of viviparity in this group has been under phylogenetic constraint, as it has evolved in some squamates but not in archosaurs, turtles and sphenodontid reptiles. The hypothesis that the evolution of viviparity is physiologically constrained was explored in the genus *Sceloporus* by contrasting a clade with both oviparous and viviparous species (*S. scalaris* group) and a clade in which all species are oviparous (*S. undulatus* group) (Andrews, 2002; Andrews and Mathies, 2000; Andrews and Rose, 1994). The capacity to support embryonic development *in utero* under conditions that inhibit oviposition was assessed for oviparous species of these species groups. The evolution of viviparity in the *S. undulatus* species group is constrained due to the inability of embryos of most species to continue development beyond the normal stage at oviposition (Andrews and Mathies,

2000). *Sceloporus virgatus*, however, is an exception; its embryos continue development until stage 37 (Andrews, 1997; Andrews and Rose, 1994). In contrast to most species in the *S. undulatus* group, however, *S. scalaris* and *S. aeneus*, close relatives of viviparous species, have the ability to support embryogenesis in utero to advanced stages—at least stage 36 in *S. aeneus* (Andrews, unpublished data), and 39.5 in *S. scalaris*, (Mathies and Andrews, 1996).

The genus *Sceloporus* exhibits four different independent origins of viviparity, one for the *S. grammicus*, *S. megalepidurus* and *S. torquatus* groups, one for the *S. formosus* group and two in the *S. scalaris* group (Méndez de la Cruz et al., 1998). We chose the oviparous *Sceloporus spinosus* group as a model to evaluate the evolution of egg retention. We evaluated the effect of egg retention on embryonic development and tested the general hypothesis that members of the *S. spinosus* group should exhibit a greater capacity to retain eggs beyond the normal time of oviposition than 'typical' oviparous *Sceloporus* (e. g. about stage 30) because the clade shares a recent common ancestor with its entirely viviparous sister clade, the *S. formosus* group (Smith, 2001). We also tested the hypotheses that: 1) the maximum stage at oviposition is negatively related to several factors associated with the evolution of viviparity in other studies [relative clutch mass (RCM), eggshell thickness, and environmental temperature], and 2) the maximum stage at oviposition is associated with the phylogenetic history of the *Sceloporus spinosus* group.

Materials and Methods

The *Sceloporus spinosus* group is monophyletic (Wiens and Reeder, 1997) and all populations involved in this study are closely related (see below). The group is distributed along the Pacific coast of Mexico from Guerrero to southern Sonora and Chihuahua, and from Oaxaca to southern Tamaulipas, Nuevo León, and Durango (Sites et al., 1992; Smith, 1939). Their altitudinal distribution extends from sea level to about 2400 m; xeric habitats are the most typical (Smith, 1939). Mean size of sexually mature females varies

among populations (69–106 mm SVL) in parallel with mean clutch size (7–25 eggs) (Calderón, unpublished). Courtship occurs in early spring and eggs are laid mainly during middle or late summer. Females of some populations lay a single clutch per season while females in other populations may lay more than one (Castro-Franco, 2002; Valdéz-Gonzáles and Ramirez-Bautista, 2002). Oviposition is asynchronous within populations as females at all stages of gravidity are found at any one time during the reproductive season.

Gravid females were captured between May 2001 and July 2004 from seven localities: La Preciosa, Puebla, 19° 22′ N 97° 23′ 2″ W, 2400 m, $n = 10$; Huahutla, Morelos, 18° 25′ 58″ N 99° 02′ 01″ W, 1040 m, $n = 11$; El Rodeo, Morelos, 18° 44′ 8″ N, 99° 20′ 06″ W, 1100 m, $n = 28$; Cerro Gordo, Mexico, 17° 0′ 45.66″ N, 96° 33′ 17.34″ W, 2000 m, $n = 14$; Cd. Oaxaca-Mitla, Oaxaca, 17° 0′ 44.76″ N, 96° 33′ 21.96″ W, 1665 m, $n = 17$; Chamela, Jalisco, 19° 23′ 42″ N, 104° 57′ 39.66″ W, 30–70 m, $n = 24$; and Xalitla, Guerrero, 18° 00′ 13.9″ N, 99° 32′ 28.8″ W, 538 m, $n = 8$. Females were transported to the laboratory and housed individually in plastic terraria (51 × 35 × 21 cm). Terraria were provided with a 40W light bulb at one end to allow females to thermoregulate during the day. Room temperature fluctuated between 25 and 30 C during the observation period. Vegetation, woody debris, and rocks were provided as shelter. Females were fed wax worms and *Tenebrio* larvae three times per week and water was sprinkled on the vegetation and terraria walls daily. Most females were maintained on a dry substrate to promote egg retention (Andrews and Mathies, 2000; Andrews and Rose, 1994; Mathies and Andrews, 1996). The dry substrate consisted of a 5–7 cm deep layer of dry soil, and we were careful not to let water drip on the soil when lizards were watered. In 2001 and 2002 some females from La Preciosa ($n = 2$), Huahutla ($n = 3$), El Rodeo ($n = 14$) and Chamela ($n = 9$), were also maintained under control conditions (wet substrate) to establish the stage of the embryo when eggs would normally be laid. The wet substrate consisted of a 5–7 cm deep layer of soil that was mixed with sufficient water to make it suitable as a successful oviposition site. The soil was checked daily and water was added when needed to keep it moist.

We inspected the terraria three times per day. When clutches were laid, we recorded egg mass (0.01 g). Relative clutch mass (RCM) was calculated by dividing the mass of the clutch by the non-gravid mass of the female. Non-gravid mass of females was estimated from snout-vent length using the formula Mass = $c(\text{Length})^d$, where $c = 3.5 \times 10^{-5}$, and $d = 3.01$ (based on data for *Sceloporus occidentalis*, Andrews, 1982), because females were not weighed after oviposition. Embryos were staged according to the normal table of development of Dufaure and Hubert (1961). Half stages were used to indicate intermediate stages (i.e., an embryo between 30 and 31 was described as 30.5). Embryos were scored as alive or dead. Live embryos exhibited a heart beat at the time of dissection. In those few cases where eggs desiccated because they were laid on the surface before they were recovered, we assumed that the embryo that exhibited the most advanced stage in a clutch was alive at oviposition. While the tissues of dead embryos had lost some integrity, staging was still possible. The relative position of eggs in oviducts and their corresponding embryonic stages were recorded for nine gravid females that died or were euthanized in the laboratory.

To estimate the length of egg retention, we contrasted the mean date of oviposition between females maintained under wet conditions and females maintained under dry conditions. This estimate was made only for two localities, La Preciosa and El Rodeo, because control and experimental females were collected during the same reproductive season only at these localities.

We measured the thickness of the shell membrane of one egg per clutch for four to six clutches per locality. Shells were stored in 70% alcohol prior to processing for Scanning Electron Microscopy (SEM). Small pieces of eggshell were excised from the embryonic pole, submerged in dilute (1N) HCL overnight in order to remove the mineral layer, dried to the critical point, coated with gold-

paladium and observed with SEM model S-2460N Hitachi. Five measurements per shell were taken on printed images using a caliper (0.01 mm) and averaged to obtain mean shell membrane thickness. The mineral layer was removed from eggshells because its highly irregular distribution made obtaining consistent measures of thickness difficult. Clutch means were averaged for each site for subsequent correlation analyses.

Environmental data for each locality were obtained from Sistema Metereológico Nacional de México. Historical records (11–48 years) were used to calculate means for monthly precipitation, minimum and maximum temperature for the three months during which oviposition occurs (June–August). The difference between the monthly means of minimum and maximum temperature was used as an index of temperature fluctuation. For each variable, we averaged the three monthly means to obtain an annual value that was used in subsequent analyses. We also used the coefficient of variation (variance/mean) as an index of variability in rainfall for June, the first month of the egg-laying season.

All statistical analyses were conducted with Statistica vers. 4.5. We report mean values ± their standard errors. All analyses were tested for statistical significance at the $P < 0.05$ level. We evaluated the correlation of maximum stage at oviposition with eggshell membrane thickness, relative clutch mass, minimum temperature, fluctuation between minimum and maximum temperature and variation of minimum, maximum temperature and precipitation within a phylogenetic framework using site means for each variable. For this, we calculated independent contrasts with COMPARE software (Comparative Method Analysis, Martins, 2001). We evaluated correct standardization of branch lengths and then we used standardized contrasts to perform regression analysis with regression lines forced through the origin (Garland et al., 1992).

We conducted a phylogenetic analysis that included all localities involved in this study. We sampled one or two individuals per locality (Appendix I) and obtained approximately 1637 base pairs of mitochondrial genes

ND4 and associated tRNA[Hist], tRNA[Ser] and tRNA[Leu] as well as partial gene sequence of the 12S rRNA. Amplification cycles followed this PCR protocol: denaturation temperature: 94°/3 and 1 minute, annealing at 50–53 C/30 s, and extension at 72 C/1:00 and 2:30 min., respectively (Arévalo et al., 1994; Leache and Reeder, 2002). Amplification products were cleaned with PEG/NaCl precipitation. Sequences of primers employed are given in Appendix II. *Sceloporus formosus*, *Sceloporus olivaceus* and *Sceloporus torquatus* were employed as outgroups. Sequences were aligned by eye. We performed maximum parsimony and bayesian analyses in PAUP v. 4.01b10 (Swofford, 1998) and Mr. Bayes vr. 3.0 (Huelsenbeck and Ronquist, 2001). For maximum parsimony analysis we implemented branch and bound searches of unordered characters with equal weights, addition sequence "as is" and tree bisection reconnection options. Clade support was evaluated with 1000 replicates of non-parametric bootstrap analyses. A nucleotid substitution model was estimated with Modeltest vr. 3.06 (Posada and Crandall, 1998). Bayesian analysis was run for five million generations.

RESULTS

Oviposition and Egg Retention Under Control (wet) and Experimental (dry) Conditions

Most females maintained under wet conditions excavated nests in the substrate and laid their entire clutch. All embryos in these eggs were alive. Their stages ranged from 29.5 to 30.5 (mode 30). In contrast, females maintained under dry conditions retained eggs past the time of normal oviposition and they usually oviposited on the surface of the substrate and produced eggs over a period of several days. Egg retention was stressful to females; individuals that retained eggs for long periods of time were emaciated and less active than females that retained for short periods of time. Eight retaining individuals died prior to oviposition and three were euthanized because they were in such poor health. Control females from La Preciosa oviposited from 6 June until 21 June 2001, and experimental females from 22 June until 21 July 2001 while

control females from El Rodeo oviposited from 6 until 7 June 2002, and experimental females from 21 June until 24 July 2002. Modal lengths of egg retention were thus 21 and 30 days, respectively, for females from La Preciosa and El Rodeo.

Within Clutch Variation in Development of Embryos from Retained Eggs

Clutches laid by females maintained under dry conditions typically contained embryos that ranged widely in stage. For example, clutches with advanced embryos (>30.5) usually contained early embryos (≤30.5) as well (Table 1). Variance in embryo stage within the same clutch was the result of death of embryos at different times during retention. Live embryos were always those at the maximum stage found in a clutch, while dead embryos were usually at earlier stages. Only a few embryos per clutch reached late stages at most localities, and clutches with most advanced stages observed per locality were found at low frequencies at all localities (Fig. 1). However, at Chamela, Oaxaca, and Xalitla, some clutches contained embryos all at the same advanced stage and most of these embryos were all alive at oviposition.

Variation in embryonic stage within clutches was not related to the relative position of eggs in the oviduct; early and late embryos were intermixed in sequence in nine clutches of females that died and were necropsied. Similarly, the physical crowding of eggs in the oviduct as indexed by RCM was not related to either the degree of variation in stage (as assessed by the coefficient of variation) or to the maximum stage observed within each clutch at any locality ($P's > 0.05$, Spearman correlation analyses).

Within Locality Variation in Development of Embryos from Retained Eggs

The maximum stage per clutch varied considerably among females at each locality (Fig. 1). Maximum stage per clutch was not related to crowding in the oviduct as judged by female mass after oviposition or to relative clutch mass at any locality (all $P's > 0.05$, regression analyses). Moreover, the proportion of embryos within clutches that reached the maximum stage was not related to RCM at any locality ($P's > 0.05$, regression analyses).

Inter Locality Variation in Development of Embryos from Retained Clutches

Mean maximum stage at oviposition did not vary among localities (K-W, $H_{6,82} = 8.47$, $P = 0.21$). This result may reflect high variability of maximum stage at oviposition and the relatively small number of clutches at each locality (Fig. 1). For example, no clutch from Chamela contained embryos beyond stage 32.5 while at least a few clutches from all other localities had embryos at stages up to 34–35. We therefore related mean locality values of maximum embryonic stage per clutch to other variables using analyses of standardized contrasts to examine biological associations of this variable.

Standardized contrasts of mean maximum stage at oviposition were not related to mean shell membrane thickness ($P = 0.21$, $F_{1,5} = 2.11$, $R = -0.54$) or to mean RCM ($P = 0.84$, $F_{1,5} = 0.04$, $R = 0.09$). Mean shell membrane thickness varied among localities ($P = 0.03$, $H_{6,25} = 13.9$), however, with Chamela and Oaxaca having the highest and the lowest values, respectively. Shell membrane thickness variation was negatively related to precipitation during egg laying season ($P = 0.04$, $F_{1,4} = 7.85$, $R = -0.81$). Mean relative clutch mass did not vary among localities ($P = 0.11$, $F_{6,61} = 1.83$) (Table 2).

Standardized contrasts of mean maximum stage at oviposition were not related to mean minimum or maximum temperature during the egg laying season ($P = 0.7$, $F_{1,5} = 0.39$, $R = -0.24$ and $P = 0.98$, $F_{1,5} = 0.5$, $R = 0.008$) or to temperature fluctuation (difference between maximum and minimum temperature) ($P = 0.19$, $F_{1,5} = 2.22$, $R = 0.55$). Similarly, mean maximum stage at oviposition was not related to variation in maximum and minimum temperature or precipitation during June, as assessed by the coefficient of variation ($P > 0.05$, $F_{1,5} = 0.95$, $R = -0.4$; $P > 0.05$, $F_{1,5} = 0.37$, $R = 0.26$ and $P > 0.05$, $F_{1,5} = 0.17$, $R = 0.18$ respectively). None of these environmental variables were associated with the altitude of the study localities ($P's > 0.05$ in all cases).

TABLE 1.—Data collected on retained clutches. The range of embryonic stages is the minimum and maximum stage of development observed within each clutch, within clutch variation in embryonic stage was measured as the coefficient of variation (C. V.), and the fate of embryos in each clutch is indicated by the numbers of live and dead embryos. X indicates data that are missing because eggs were desiccated when found. * indicates six clutches from Huahutla and La Preciosa that were not staged but were incubated at 30–33 C. Hatchlings document the presence of living embryos from these clutches.

Locality	Clutch no. clutch size (n)	Range of embryonic stages	Within clutch variation (C. V.)	Embryos live, dead
Cerro Gordo	1 (16)	30	0	X
	2 (17)	30–32.5	2.68	0,17
	3 (14)	30–31	0.99	0,14
	4 (23)	32.5–34	1.23	0,23
	5 (20)	30–33.5	3.94	0,20
	6 (14)	30–34	5.20	2,12
	7 (23)	31–34	3.07	3,20
	8 (17)	30–33.5	3.24	0,17
	9 (17)	30–31	1.34	0,17
	10 (13)	31–34	2.8	X
	11 (18)	29.5–32.5	2.38	0,18
	12 (14)	31.5–33	1.77	1,13
	13 (11)	30–31	1.17	0,11
	14 (15)	30–31	1.80	1,14
Chamela	1 (10)	30.5–31	0.84	10,0
	2 (11)	32	0	7,4
	3 (7)	30–32	3.72	2,5
	4 (13)	31–32.5	1.40	8,5
	5 (10)	31	0	X
	6 (11)	32	0	X
	7 (11)	30–32	2.08	X
	8 (9)	30–32	2.86	X
	9 (9)	32–32.5	0.68	0,9
	10 (8)	31–32	1.32	3,5
	11 (8)	32	0	8,0
	12 (8)	32	0	8,0
	13 (7)	31.5	0	X
	14 (9)	30	0	9,0
	15 (7)	30	0	X
El Rodeo	1 (9)	30	0	X
	2 (17)	30	0	1,16
	3 (23)	30–32.5	2.17	X
	4 (22)	30–32.5	2.6	X
	5 (11)	30–32	2.44	0,11
	6 (18)	30–32	3.14	13,5
	7 (21)	32–33	1.46	X
	8 (14)	28–30	2.78	0,14
	9 (17)	32–33.5	1.33	2,15
	10 (14)	30–32.5	2.27	0,14
	11 (18)	33–34	0.80	X
	12 (16)	31–33.5	3.13	1,15
	13 (12)	30–31	1.43	0,12
	14 (11)	30.5–33.5	1.43	0,11
	15 (20)	30–33.5	3.19	0,20
	16 (20)	31–33	1.95	0,20
Huahutla	1 (17)	32.5 (staged 2)	No data	1,16*
	2 (18)	28.5–30.5 (staged 2)	No data	0,18
	3 (11)	31–32 (staged 2)	No data	3,8*
	4 (10)	35 (staged 6)	No data	3,7*
	5 (14)	29.5–30 (staged 6)	No data	X
	6 (9)	32.5–34 (staged 3)	No data	0,9
La Preciosa	1 (10)	32–34 (staged 4)	No data	0,10
	2 (10)	29–30 (staged 2)	No data	1,9*
	3 (10)	34 (staged 2)	No data	1,9*

TABLE 1.—Continued.

Locality	Clutch no. clutch size (*n*)	Range of embryonic stages	Within clutch variation (C. V.)	Embryos live, dead
	4 (11)	34 (staged 1)	No data	1,10*
	5 (10)	30–33 (staged 4)	No data	0,10
	6 (9)	31.5–35 (staged 8)	No data	0,9
Oaxaca	1 (14)	29.5–33.5	4.92	0,14
	2 (17)	30–31	1.57	2,15
	3 (12)	33	0	X
	4 (19)	33–34.5	1.12	X
	5 (9)	32–34	1.75	0,9
	6 (10)	30–33	4.02	X
	7 (12)	32	0	0,12
	8 (12)	34.5	0	5,7
	9 (13)	33	0	0,13
	10 (9)	31–32	0.95	0,9
	11 (12)	33	0	3,9
	12 (13)	30–32.5	2.66	X
	13 (14)	33–34.5	1.60	4,10
	14 (14)	30–33	2.85	X
	15 (16)	30–32	2.43	0,16
	16 (14)	31–32	0.90	11,3
	17 (9)	32–33	1.50	4,5
Xalitla	1 (15)	32–32.5	0.80	4,11
	2 (13)	31.5	0	0,13
	3 (22)	32–34.5	1.97	1,21
	4 (10)	30.5	0	0,10
	5 (21)	32–32.5	0.79	0,21
	6 (17)	32	0	0,17
	7 (14)	31	0	1,13
	8 (13)	34.5	0	0,13

Phylogenetic Component of Patterns of Egg Retention

Phylogenetic relationships of the *S. spinosus* and *S. formosus* species groups were strongly supported judging by high bootstrap and posterior probability scores (Fig. 2).

FIG. 1.—Within and inter-locality variation in maximum stage at oviposition observed per clutch. Numbers represent the number of clutches.

Within the *S. spinosus* group, the first divergence is between Chamela and the other six localities. Chamela is a low elevation site and the maximum stage at oviposition was correspondingly low. In contrast, Chamela's sister clade was associated basally with high elevations and with advanced maximum stages at oviposition. Three low elevation sites (El Rodeo, Huahutla, and Xalitla) were nested with the 'high elevation' clade and presumably represent a reinvasion of low elevations without a corresponding reduction in the maximum stage at oviposition.
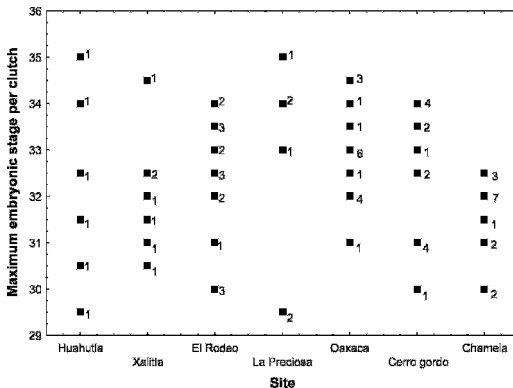
DISCUSSION

Females from the *Sceloporus spinosus* group exhibited the ability to retain eggs with intrauterine embryogenesis continuing beyond that of the normal stage at oviposition. However, the consequences of egg retention were quite different from those observed in other *Sceloporus* species. Moreover, the specific hypothesis of an association among biological and environmental variables was

TABLE 2.—Mean values of maximum embryonic stage at oviposition, eggshell membrane thickness, and relative clutch mass for each locality.

Locality	Maximum embryonic stage (X ± SD)	Eggshell membrane thickness in microns (X ± SD)	Relative clutch mass (X ± SD)
Chamela	31.7 ± 0.8	76.60 ± 9.14	0.31 ± 0.11
Cerro Gordo	32.5 ± 1.5	65.69 ± 7.19	0.32 ± 0.085
El Rodeo	32.3 ± 1.7	69.64 ± 7.92	0.30 ± 0.09
Huahutla	32.2 ± 2.8	72.42 ± 12.75	0.31 ± 0.12
La Preciosa	32.5 ± 2.5	77.23 ± 11.89	0.27 ± 0.12
Oaxaca	32.9 ± 1.6	57.04 ± 8.36	0.28 ± 0.06
Xalitla	32.4 ± 2.1	78.77 ± 4.5	0.31 ± 0.11

rejected. Our data did, however, support the hypothesis that the ability to retain eggs is associated with the evolutionary and geographic history of the group.

Within Clutch and Within Locality Variation in Embryonic Development

The high degree of variation in the stage at oviposition that we observed within clutches was unexpected. Such intra-clutch variation
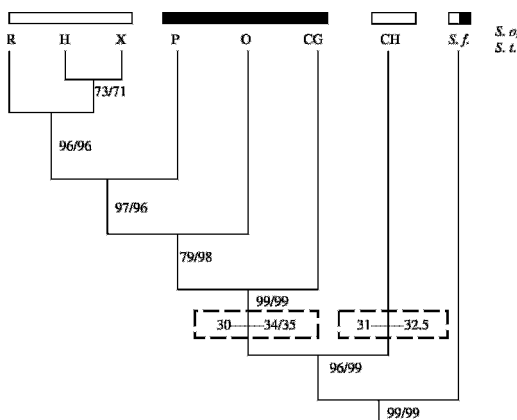

FIG. 2.—Phylogenetic pattern of maximum stage at oviposition during egg retention. Altitudinal distribution is indicated by open (low elevation) and black (high elevation) bars. The taxon with open and black bars occupies a broad altitudinal distribution. Range of stages at oviposition is shown at the base of clades. Letters identify different localities (R = El Rodeo, H = Huahutla, X = Xalitla, P = La Preciosa, O = Oaxaca, C = Cerro Gordo and CH = Chamela) and outgroups (*S.f.* = *Sceloporus formosus*, *S. o.* = *S. olivaceus* and *S. t.* = *S. torquatus*). The single most parsimonious tree (length = 539, C.I. = 0.7699, R. I. = 0.7459) and majority consensus tree obtained from Bayesian analyses of mtND4, RNAt [His, Ser, Leu] and 12S. Substitution model selected by LRTs criteria: TrN + I + Γ. Numbers below each clade represent clade support (bootstraps and posterior probabilities respectively).

has not been reported for any other oviparous lizard species under natural conditions or under laboratory conditions where egg retention was induced by not providing a nesting substrate (Andrews, 1997; Andrews and Mathies, 2000; Andrews and Rose, 1994; Heulin et al., 2002). An even more unexpected observation was that the extent of embryogenesis varied within clutches as a result of embryonic death during the period of retention. Within clutch variation in the degree of embryogenesis in *S. spinosus* group generates a number of questions about possible physiological and morphological differences along oviducts or among eggs or embryos that influences their survival and development. We were able to determine that the position or degree of crowding in the oviduct was not related to embryo survival. Other possible factors related to variation in stage when embryos died could be heterogeneity in the degree of vascularization of the oviduct, the permeability of eggshells to oxygen, or that embryos may differ in their ability to develop or survive under low oxygen. Moreover, biochemical signals produced by embryos could differ. Embryos of nonmammalian viviparous vertebrates, like amphibians, secrete steroids and prostaglandins as pregnancy recognition systems (Guillette, 1989); these embryonic signals may influence development of maternal uterine tissue to benefit of the embryo (Crespi and Semeniuk, 2004). At this time, however, the reason why embryonic survival varied within clutches of *S. spinosus* is unknown. Nonetheless, the high mortality of retained embryos and gravid females suggests that egg retention reduce fitness and that selection would not normally favor this trait.

Maximum stage at oviposition also varied within localities when females were forced to retain eggs. Maximum stage ranged from 31–34 at Cerro Gordo, 31–32.5 at Chamela, 30–34 at El Rodeo, 30–35 at Huahutla, 29.5–34.5 at Oaxaca, 29–35 at La Preciosa, and 31–34.5 at Xalitla. Variation of stages of development under wet conditions was only 0.5–1.0 stages. While the stage at ovipostion varies little within clutches of lizards, variation among females also occurs among non-egg retainers in the genus *Scleoporus* (Andrews, unpublished data) as well as in species that retain eggs (Andrews, 1997; DeMarco, 1993; Mathies and Andrews, 1996; Smith and Shine, 1997). In both situations differences observed are of two to four or to five stages, respectively. For instance, females of *S. scalaris* from low and high elevations normally laid their eggs at stages 31–33.5 and 33–38, respectively, (Andrews, 1997; DeMarco, 1993; Mathies and Andrews, 1996). The variation in maximum stage at oviposition that we documented among females of *S. spinosus* group as a consequence of extended egg retention parallels variation in the stage at oviposition among females of other oviparous lizards under natural conditions.

Association Between Physiological and Demographic Traits and the Maximum Stage of Development During Egg Retention

In contrast to our prediction, differences in RCM among females at the same locality were small and did not account for differences in the maximum stage at oviposition. In fact, RCM exhibited little variation among females or among localities. This result suggests that changes in RCM are not related to the evolution of extended egg retention in *S. spinosus*, at least. The effect of RCM on embryonic development has only been evaluated for two other species. Half of the clutch (that is, one oviduct) of retaining females of *S. scalaris* and *S. virgatus* was removed; decreasing clutch volume increased water uptake by eggs *in utero*, but did not affect embryonic stage (Andrews, 1997).

Shell membrane thickness was also not related to the maximum stage at oviposition that occurred during egg retention in the *S.*

spinosus species group. It was, however, related to rainfall with thinner egg membranes associated with localities with higher rainfall and thicker membranes were associated with drier localities. Thicker shells may be adaptive in drier soils because they retard water loss (Andrews and Sexton, 1981). In parallel with our observations on *S. spinosus*, variation in the structure and thickness of eggshells of several other species of *Sceloporus* was not related to the capacity to retain eggs with developing embryos (Andrews and Mathies, 2000). The lack of association between embryonic development *in utero* and shell membrane or shell thickness in the *S. spinosus* and *S. undulatus* groups does not support the prediction that reduction in eggshell thickness is associated with extended egg retention. In contrast, eggshell thickness is reduced in populations with longer periods of egg retention in intra-specific comparisons of *Sceloporus scalaris* (Mathies and Andrews, 1995) and of reproductively bimodal species like *Lacerta vivipara* (Heulin et al., 2002, 2005), *Saiphos equalis* (Smith and Shine, 1997) and *Lerista bougainvilli* (Qualls, 1996; Qualls et al., 1995). These contrasting results imply that the evolution of egg retention and ultimately viviparity may involve different modifications that enhance embryonic development in utero, at least initially, among different taxa. Accordingly, a single species or clade cannot be used as a general model to explain evolution of viviparity (Blackburn, 2000).

Differences in clutch frequency also did not explain the variation in maximum stage at oviposition observed among females at different localities. Females that laid one clutch per season (La Preciosa, Cerro Gordo) exhibited the same ability to extend intrauterine embryogenesis as those that laid more that one clutch (El Rodeo, Huahutla, Xalitla). Clutch frequency had been suggested as a limiting factor of egg retention evolution in lizards (Andrews and Mathies, 2000; Shine, 1985); females that produce one clutch per year could retain eggs in utero, as this would not reduce their fitness (Shine, 1985). Our observations in the *S. spinosus* group suggest that extended egg retention could evolve in multiple clutch layers at first, but long periods

of egg retention would require a reduction in clutch frequency to one per season.

Environmental and Historical Factors in the Evolution of Egg Retention

The origin of viviparity in the *S. spinosus* and *S. formosus* clade is a comparatively ancient event. By assuming a divergence rate of 2% per mya for our haplotype data set we calculated that these species groups diverged 5.7–7.1 mya and the two main clades within the *S. spinosus* group diverged 4.2–6.5 mya. In contrast, the most recent evolution of viviparity in *Sceloporus* is within the *S. scalaris* group (Benabib et al., 1997). Using haplotype data generated by these authors, we estimated that the oviparous *S. aeneus* and viviparous *S. bicanthalis* diverged 3.2–4.9 mya. The origins of viparous clades of *Lacerta vivipara* are even more recent with splits from oviparous clades during the Pleistocene (1.0–2.6 mya) (Surget-Groba et al., 2001). While members of the *S. spinosus* group appear to have had enough time to respond to selective pressures associated with changes in elevation, changes in the ability to retain eggs with developing embryos are limited in efficacy and appear to have been slow. For example, while embryos can be retained to stages as advanced as 34 or 35, retention is associated with high embryonic and female mortality. We observed a shift in the maximum stage at oviposition associated with the invasion of high elevations by the ancestor of the high elevation clade, that is, populations R, H, X, P, O, and CG (Fig. 2). Nonetheless, females from relatively low elevation localities (R, H, and X), which are nested within the high elevation clade, still are capable of retaining eggs to embryonic stages 34–35 which suggests that the maximum stage at oviposition was not modified when *S. spinosus* re-invaded low elevation localities 0.5 mya. Possible reasons to explain why more substantial changes in egg retention have not occurred in the *S. spinosus* group are physiological constraints on the ability to support embryonic development in utero, the absence of appropriate selective forces, or that oviparity may continue to have adaptive value in arid environments.

Literature Cited

Andrews, R. A. 1982. Patterns of growth in reptiles. *In* C. Gans and F. H. Pough (Eds.), Biology of the Reptilia. Vol. 13, Physiology D: Physiological ecology, Pp. 273–320. Academic Press, New York, New York, U.S.A.

———. 1997. Evolution of viviparity: variation between two sceloporine lizards in the ability to extend egg retention. Journal of Zoology (Lond) 243:579–595.

———. 2002. Low oxygen: a constraint on the evolution of viviparity in reptiles. Physiological and Biochemical Zoology 75:145–154.

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

Andrews, R. A., and B. R. Rose. 1994. Evolution of viviparity: constraints on egg retention. Physiological Zoology 67:1006–1024.

Andrews, R. A., and T. Mathies. 2000. Natural history of reptilian development: constraints on the evolution of viviparity. BioScience 50:227–238.

Arévalo, E., S. K. Davis, and J. W. Sites. 1994. Mitochondrial DNA sequence divergence and phylogenetic relationships among eight chromosome races of the *Sceloporus grammicus* complex (Phrynosomatidae) in Central Mexico. Systematic Biology 43:387–418.

Benabib, M., K. M. Kjer, and J. W. Sites. 1997. Mitochondrial DNA sequence based phylogeny and the evolution of viviparity in the *Sceloporus scalaris* group (Reptilia, Squamata). Evolution 51:1262–1275.

Blackburn, D. G. 1992. Evolutionary origins of viviparity in the Reptilia. I. Sauria. Amphibia-Reptilia 5:259–291.

———. 1995. Saltationist and punctuated equilibrium models for the evolution of viviparity and placentation. Journal of Theoretical Biology 174:199–216.

———. 1998. Reconstructing the evolution of viviparity and placentation. Journal of Theoretical Biology 192:183–190.

———. 2000. Reptilian viviparity: past research, future directions, and appropiate models. Comparative Biochemistry and Physiology A: Physiology 127:391–401.

Castro-Franco, R. 2002. Historia natural de lagartijas del estado de Morelos, México. M. Sc. Dissertation, Facultad de Ciencias, Universidad Nacional Autónoma de México.

CRESPI, B., AND C. SEMENIUK. 2004. Parent-offspring conflict in the evolution of vertebrate reproductive mode. The American Naturalist 163:635–653.

DEMARCO, V. 1993. Estimating egg retention times in sceloporine lizards. Journal of Herpetology 27:453–458.

DUFAURE, J. P., AND J. HUBERT. 1961. Table de développement du lézard vivipare: *Lacerta* (*Zootoca*) *vivipara* Jacquin. Archives DAnatomie Microscopique et de Morphologie Experimentale 50:309–328.

GARLAND, T., P. H. HARVEY, AND R. IVES. 1992. Procedures for the analysis of comparative data using phylogenetic independent contrasts. Systematic Biology 41:18–32.

GUILLETTE, L. J. JR. 1989. The evolution of vertebrate viviparity: morphological modifications and endocrine control. *In* D. B. Wake and G. Roth (Eds.), Complex organismal functions: integration and evolution in vertebrates, Pp. 219–233. Wiley, Chichester.

———. 1993. Evolution of viviparity in lizards. Bioscience 43:742–751.

HEULIN, B., S. GHIELMI, N. VOGRIN, Y. SURGET-GROBA, AND C. P. GUILLAUME. 2002. Variation in eggshell characteristics and in intrauterine egg retention between two oviparous clades of the lizards *Lacerta vivipara*: insight into the oviparity/viviparity continuum in Squamates. Journal of Morphology 252:255–262.

HEULIN, B., J. R. STEWART, Y. SURGET-GROBA, P. BELLAUD, F. JOUAN, G. LANCIEN, AND J. DEUNFF. 2005. Development of the uterine shell glands during the preovulatory and early gestation periods in oviparous and viviparous *Lacerta vivipara*. Journal of Morphology 226:80–93.

HUELSENBECK, J. P., AND F. RONQUIST. 2001. MRBAYES: Bayesian inference of phylogeny. Bioinformatics 17:754–755.

LEACHÉ, A. D., AND T. W. REEDER. 2002. Molecular systematics of the Eastern Fence lizard (*Sceloporus undulatus*): A comparison of parsimony, likelihood and bayesian approaches. Systematic Biology 51:44–68.

LEE, M. S., AND R. SHINE. 1998. Reptilian viviparity and Dollos law. Evolution 52:441–450.

MARTINS, E. P. 2001. COMPARE, version 4.4. Computer programs for the statistical analysis of comparative data. Available at: http://compare.bio.indiana.edu/. Department of Biology, Indiana University, Bloomington.

MATHIES, T., AND R. M. ANDREWS. 1995. Thermal and reproductive biology of high and low elevation populations of the lizard *Sceloporus scalaris*: implications for the evolution of viviparity. Oecologia 104:101–111.

———. 1996. Extended egg retention and its influence on embryonic development and egg water balance: implications for the evolution of viviparity. Physiological Zoology 69:1021–1035.

———. 2000. Does reduction of the eggshell occur concurrently with or subsequent to the evolution of viviparity in phrynosomatid lizards? Biological Journal of the Linnean Society 71:719–736.

MÉNDEZ DE LA CRUZ, F. R., M. VILLAGRÁN-SANTA CRUZ, AND R. M. ANDREWS. 1998. Evolution of viviparity in the lizard genus *Sceloporus*. Herpetologica 54:521–532.

POSADA, D., AND K. A. CRANDALL. 1998. Modeltest: Testing the model of DNA substitution. Bioinformatics 14:817–818.

QUALLS, C. P. 1996. Influence of the evolution of viviparity on eggshell morphology in the lizard, *Lerista bougainvillii*. Journal of Morphology 228:119–125.

QUALLS, C. P., R. SHINE, S. DONNELLAN, AND M. HUTCHINSON. 1995. The evolution of viviparity within the Australian scincid lizard *Lerista bougainvillii*. Journal of Zoology 237:13–26.

SHINE, R. 1985. The evolution of viviparity in reptiles: an ecological analysis. *In* C. Gans and F. Billet (Eds.), Biology of the Reptilia, Vol. 15, Development, Pp. 605–694. John Wiley and Sons, Inc., New York.

———. 2002. An empirical test of the "predictability" hypothesis for the evolution of viviparity in reptiles. Journal of Evolutionary Biology 15:553–560.

———. 2004. Does viviparity evolve in cold climate reptiles because pregnant females maintain stable (not high) body temperatures? Evolution 58:1809–1811.

SITES, J. W. JR., J. W. ARCHIE, C. J. COLE, AND O. FLORES-VILLELA. 1992. A review of phylogenetic hypotheses for lizards of the genus *Sceloporus* (Phrynosomatidae): Implications for ecological and evolutionary studies. Bulletin of the American Museum of Natural History 11.

SMITH, E. N. 2001. Species boundaries and evolutionary patterns of speciation among the malachite lizards (Formosus group) of the genus *Sceloporus* (Squamata: Phrynosomatidae). Ph. D. Dissertation, Faculty of the graduate school of the University of Texas at Arlington, USA.

SMITH, H. M. 1939. The Mexican and Central American lizards of the genus *Sceloporus*. Zoological series Field Museum of Natural History, Chicago 26:59–172.

SMITH, S. A., AND R. SHINE. 1997. Intraspecific variation in reproductive mode within the scincid lizard *Saiphos equalis*. Australian Journal of Zoology 45:435–445.

SMITH, S. A., C. C. AUSTIN, AND R. SHINE. 2001. A phylogenetic analysis of variation in reproductive mode within an Australian lizard (*Saiphos equalis*, Scincidae). Biological Journal of the Linnean Society 74:131–139.

SURGET-GROBA, Y., B. HEULIN, C. P. GUILLAUME, R. S. THORPE, L. KUPRIYANOVA, N. VOGRIN, R. MASLAK, S. MAZZOTTI, M. VENCZEL, I. GHIRA, G. ODIERNA, O. LEONTYEVA, J. C. MONNEY, AND N. SMITH. 2001. Intraspecific phylogeography of *Lacerta vivipara* and the evolution of viviparity. Molecular Phylogenetics and Evolution 18:449–459.

SWOFFORD, D. L. 1998. PAUP. Phylogenetic Analysis Using Parsimony. Sinauer Associates, Massachusetts, U.S.A.

TINKLE, D. W., AND J. W. GIBBONS. 1977. The distribution and evolution of viviparity in reptiles. Miscelaneus Publications of Museum of Zoology University of Michigan 154:1–55.

VALDEZ-GONZÁLES, M., AND A. RAMÍREZ-BAUTISTA. 2002. Reproductive characteristics of the spiny lizards, *Sceloporus horridus* and *Sceloporus spinosus* (Squamata: Phrynosomatidae) from México. Journal of Herpetology 36:36–43.

WIENS, J. J., AND T. W. REEDER. 1997. Phylogeny of the spiny lizards (*Sceloporus*) based on molecular and morphological evidence. Herpetological Monographs 11:1–101.

APPENDIX I

.Species used in the phylogenetic annalysis, their museum numbers, Gen Bank accession numbers and localities. Acronym: IBH = Instituto Biología-Herpetologia.

Taxon	Sample	ND4 and tRNAs	12S	Locality
Outgroup				
Sceloporus formosus		AF210346	L40455	
S. olivaceus		AF210361	AF440095	
S. torquatus		AF154244	AF154179	
Ingroup				
S. horridus albiventris	IBH17824	EF025743	EF025751	Chamela, Jalisco
	IBH17823	EF025744	EF025752	Chamela, Jalisco
S. h. horridus	IBH17825	EF025745	EF025753	El Rodeo, Morelos
	IBH17826	EF025746	—	Santa Inés, Morelos
	IBH17827	EF025747	EF025754	Xalitla, Guerrero
S. spinosus caeruleopunctatus	IBH17828	EF025748	EF025755	Cd. Oaxaca-Mitla, Oaxaca
S. s. spinosus	IBH17829	EF025749	EF025756	La Preciosa, Municipio Las Minas, Puebla
	IBH17831	EF025750	EF025757	La Preciosa, Municipio Las Minas, Puebla

APPENDIX II

Primers used in this study.

Primer name	Sequence (5′ → 3′)	Source
Nd4	TGACTACCAAAAGCTCATGTAGAAGC	Arévalo et al., 1994
Leu	TRCTTTTACTTGGATTTGCACCA	Arévalo et al., 1994
12e	GT(A/G)CGCTTACC(A/T)TGTTACGACT	Leache and Reeder, 2002
tPhe	AAAGCAC(A/G)GCACTGAAGATGC	Leache and Reeder, 2002