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Rates of Embryonic Development of *Sceloporus* Lizards: Do Cold Climates Favor the Evolution of Rapid Development?

ROBIN M. ANDREWS, TOM MATHIES, CARL P. QUALLS, AND FIONA J. QUALLS

Rapid embryonic development is a potential adaptation to cold climates. We tested this hypothesis for nine species of *Sceloporus* lizards from four species groups and one species of *Urosaurus*, an outgroup, using observations corrected for incubation temperature and hatchling size. Phylogenetically based comparisons indicated that relatively rapid development is characteristic of the *scalaris* species group and relatively slow development is characteristic of the *undulatus* species group. Comparisons within these lineages were therefore used to determine whether developmental rate was related to climate, as judged by elevation and latitude. Within the *scalaris* species group, developmental rates of cool climate populations of *S. aeneus* and *S. scalaris* were not faster than that of a warm climate population of *S. scalaris*. Within the *undulatus* species group, the developmental rate of cool climate populations or species were not faster than those of warm climate populations or species. In general, developmental rates of *Sceloporus* are lineage specific and do not appear to be adapted to local climates.

THE rate of embryonic development in reptiles is temperature dependent, such that warmer temperatures generally result in faster rates of development and thus shorter periods of incubation (e.g., Muth, 1980). For oviparous species, in particular, this means that incubation periods vary among microhabitats and along geographic gradients in parallel with environmental (soil) temperature (Packard and Packard, 1988). Such spatial variation in environmental temperature is an important component of models for the evolution of viviparity in squamate reptiles. Relatively low temperatures at high elevations and latitudes are thought to favor the evolution of an increase in the length of egg retention by females and, ultimately, of the evolution of viviparity (Packard et al., 1977; Tinkle and Gibbons, 1977; Shine, 1985). The selective basis for longer periods of egg retention is that gravid females are able to regulate temperatures that are more favorable to embryonic development than their embryos would experience in nests. While retained in utero, embryos will experience warmer temperatures (and develop more rapidly) than they would in nests, and they will be protected from extreme temperatures that are detrimental to normal development. However, an increase in the length of egg retention is not the only mechanism that would allow squamate embryos to cope with low ambient temperatures of nests. One alternative, or possibly complementary response, would be the physiological adaptation of embryos to cold.

Shine (1984) speculated that cold climates could select for tolerance to low temperatures by embryos and enhanced embryonic development at these low temperatures. However, few studies have focused on the thermal biology of squamate embryos, and the results are equivocal in this regard. Shine (1983a) found little variation in the length of incubation, or in the low temperature limits for development, among species of skinks at one site in Australia. Similarly, Andrews et al. (1997) found no variation in the effects of low temperature on survival or cold tolerance of embryos of *Sceloporus* lizards that could be attributed to climate. On the other hand, DeMarco (1992) reported more rapid embryonic development for high-elevation (HE) than low-elevation (LE) species of *Sceloporus* lizards when eggs were incubated at the same temperatures. Other studies that putatively document more rapid embryonic development in cool than warm climates (Rykena, 1988; Olsson et al., 1996) did not control for the degree of development at oviposition which thus confounds physiological adaptation with variation in developmental state at oviposition (Mathies and Andrews, 1995).

The objective of this research was to further evaluate the physiological adaptation hypothesis. We determined whether embryos from populations that live in cold climates develop faster, that is, have shorter lengths of incubation, than embryos from warm climates when incubated at the same temperature. To do so, we collected laboratory data on the length of incubation of *Sceloporus* lizards from a wide range of localities. We also included observations on *Urosaurus*, the sister group to *Sceloporus* (Reeder and Weins, 1996). For our incubation studies, we used fluctuating temperature regimes that simulated

temperatures of natural nests as well as constant temperature regimes. We supplemented these data with observations on incubation periods from the literature.

Materials and Methods

Collection of eggs.—Gravid female *Sceloporus* were collected just prior to the time of natural oviposition, typically, in late June or early July. *Sceloporus aeneus* were collected near Milpa Alta, Estado de Mexico, Mexico. *Sceloporus scalaris* were collected at the Appleton-Whittell Research Ranch Sanctuary, Arizona. *Sceloporus virgatus* were collected at LE and HE localities near the Southwestern Research Station in the Chiricahua Mountains, Arizona. *Sceloporus occidentalis* were collected near Table Mountain, California (see also Adolph, 1990). *Sceloporus undulatus hyacinthinus* were collected near Blacksburg, Virginia. *Sceloporus undulatus consobrinus, Sceloporus clarki,* and *Urosaurus ornatus* were collected between Rodeo and Hachita, New Mexico. The year of observation and number of clutches used are given in Table 1. Within a few days of capture, gravid females or clutches of eggs were transported to the laboratory, and eggs were immediately placed under experimental conditions. Eggs were obtained from *S. aeneus, S. scalaris, S. clarki, S. virgatus,* and *U. ornatus* by using oxytocin to induce oviposition and females of other species were placed individually in terraria and allowed to oviposit naturally.

Thermal regimes for incubation of eggs.—Eggs were incubated in controlled temperature chambers (Percival model no. I-30BL with B1 option) representing three experimental temperature regimes in 1995, four experimental temperature regimes in 1996, two experimental temperature regimes in 1997, and six experimental temperature regimes in 1998 (Table 1, Fig. 1). In 1995, the diel ranges of temperatures were 20–30, 15–25, and 8–29 C, which were based on natural nest temperatures of *S. aeneus* and LE *S. virgatus* and on estimated nest temperatures of HE *S. virgatus* (Andrews et al., 1997). In 1996, temperature regimes of 14–24 and 21–31 C were similar to the 15–25 and 20–30 C regimes of 1995. Two additional temperature regimes in 1996 were 24–33 and 26–36 C, which provided slightly to considerably warmer temperatures, respectively, than were used in 1995. In 1997, eggs were incubated under two fluctuating temperature regimes, 22–32 and 22–33 C (not illustrated), that were similar in the pattern of diel variation to the 24–33 C regime in 1996 and also at two constant temperatures, 24 and 30 C. In 1998, incubation treatments were five constant temperatures: 23, 25, 28, 30, or 33 C and a fluctuating temperature regime: 8 h at 23 C, 4 h in which the temperature ramped linearly to 33 C, 8 h at 33, and 4 h in which the temperature ramped linearly back to 23 C (overall mean of 28 C).

Temperatures were monitored regularly during incubation with temperature probes placed in jars identical to those used to incubate eggs. One jar with a probe was placed toward the center and another at the periphery of each chamber. Overall, mean temperatures taken at different periods during the incubation periods never varied by more than 0.4 C.

Allocation of eggs to temperature regimes and experimental protocols.—At oviposition, eggs were marked individually and weighed. One or two eggs from each clutch were randomly selected for determination of developmental stage at oviposition. Embryos were staged according to criteria of Dufaure and Hubert (1961), except that half stages were assigned if embryos had features intermediate between two developmental stages. The remaining eggs from each clutch were randomly assigned to one or more temperature regimes for incubation or used in experiments not reported here.

In the majority of cases, eggs from each clutch were randomly distributed among temperature regimes such that each clutch was represented by one to three eggs per temperature regime. When the number of eggs per clutch or the number of clutches was limited, eggs were allocated to as many temperature regimes as possible. Andrews et al. (1997) provide a detailed explanation of the allocation of eggs to temperature regimes for data collected in 1995; similar protocols were followed in 1996, 1997, and 1998.

Eggs were placed individually in 65-ml glass jars containing moistened vermiculite. The jars were sealed with plastic kitchen wrap secured with rubber bands. The initial ratio of distilled water to dry vermiculite was 0.7:1.0 by mass: approximately −200 kPa (Tracy et al., 1978; Packard et al., 1987). The range of water potential observed in the field for *S. virgatus* nests is −300 to ≥100 kPa (B. R. Rose, unpubl.). No water was added to the jars thereafter except in 1998 when initial water potentials were restored monthly, and most eggs were not disturbed thereafter. In 1995, some eggs were weighed within a week of hatching to determine their water uptake. The ratios of final egg mass to initial egg mass for *S. virgatus* incubated under the 20–30 and 15–25 C temperature regimes av-

TABLE 1. MEAN INCUBATION PERIODS FOR *Sceloporus* AND *Urosaurus* LIZARDS AS A FUNCTION OF MEAN TEMPERATURE DURING INCUBATION, MEAN HATCHLING MASS, AND ELEVATION. Year and number of clutches in parentheses follow each species name. Means from constant temperature regimes are represented by a single value; means from fluctuating temperature regimes are followed by their ranges in parentheses. Because hatchling mass was not given by Christian et al., 1986, we used the mean hatchling mass from the other population of *S. consobrinus* in this study. All observations are from this study except as indicated: [a]DeMarco, 1992, 1993; [b]Sinervo, 1990; [c]Sexton and Marion, 1974; Marion et al., 1979; [d]Christian et al., 1986; [e]Overall 1994.

Species (year, n)	Incubation period (d)	Incubation temperature (°C)	Hatch. mass (mg)	Elevation (m)
scalaris species group				
S. aeneus (95, 10)	73	20.3 (8–29)	244	2800
S. aeneus (98, 11)	52	23	217	2800
S. aeneus (98, 12)	43	25	215	2800
S. aeneus (98, 12)	32	28	214	2800
S. aeneus (98, 11)	32	28 (23–33)	214	2800
S. aeneus (98, 10)	27	30	207	2800
S. aeneus (98, 14)	23	33	218	2800
S. scalaris (95, 8)	80	19.0 (8–29)	263	1460
S. scalaris (95, 7)	90	19.5 (15–25)	263	1460
S. scalaris (95, 14)	42	25.2 (20–30)	263	1460
S. scalaris (96, 12)	104	17.9 (14–24)	251	1460
S. scalaris (96, 13)	44	25.1 (21–31)	264	1460
S. scalaris (96, 14)	42	27.3 (24–33)	268	1460
S. scalaris (96, 12)	33	30.1 (26–36)	269	1460
S. scalaris[a]	30	30	350	2600
undulatus species group				
S. virgatus (95, 23)	106	19.5 (15–25)	435	1800
S. virgatus (95, 25)	54	25.2 (20–30)	435	1800
S. virgatus (95, 17)	106	19.5 (15–25)	448	2400
S. virgatus (95, 17	54	25.2 (20–30)	447	2400
S. virgatus (96, 10)	122	17.9 (14–24)	416	1800
S. virgatus (96, 12)	53	25.1 (21–31)	396	1800
S. virgatus (96, 15)	51	27.3 (24–33)	398	1800
S. virgatus (96, 13)	40	30.1 (26–36)	396	1800
S. virgatus[a]	36	30	420	1800
S. woodi[a]	40	30	400	24
S. occidentalis (96, 14)	56	25.1 (21–31)	643	2230
S. occidentalis (96, 13)	43	30.1 (26–36)	628	2230
S. occidentalis[b]	38	30	500	200
S. occidentalis[b]	39	30	590	750
S. occidentalis[b]	43	30	690	1330
S. occidentalis[b]	43	30	630	2230
S. hyacinthinus (95, 4)	60	25.2 (20–30)	488	750
S. hyacinthinus (97, 4)	75	24	617	750
S. hyacinthinus (97, 17)	62	26.2 (22–32)	653	750
S. hyacinthinus (98, 13)	85	23	556	750
S. hyacinthinus (98, 14)	66	25	554	750
S. hyacinthinus (98, 12)	48	28	555	750
S. hyacinthinus (98, 14)	47	28 (23–33)	548	750
S. hyacinthinus (98, 14)	39	30	536	750
S. hyacinthinus (98, 14)	35	33	547	750
S. hyacinthinus[c]	64	25	400	180
S. hyacinthinus[c]	40	30	410	180
S. consobrinus (97, 12)	57	26.2 (22–33)	477	1300
S. consobrinus[d]	40	30	477	2290
clarki species group				
S. clarki (97, 1)	61	26.0 (22–33)	940	1300

TABLE 1. CONTINUED

Species (year, n)	Incubation period (d)	Incubation temperature (°C)	Hatch. mass (mg)	Elevation (m)
S. clarki (97, 1)	45	30	890	1300
merriami species group				
S. merriami	37	31	370	800
S. merriami	34	31.4 (31–37)	440	800
Urosaurus				
U. ornatus (96, 8)	46	25.1 (21–31)	210	1500
U. ornatus (96, 6)	41	27.3 (24–33)	242	1500
U. ornatus (96, 8)	31	30.1 (26–36)	209	1500

eraged 2.8 (n = 8) and 3.1 (n = 4), respectively, and for *S. scalaris* in the 20–30 C temperature regime, the final ratio of egg mass to initial egg mass averaged 3.2 (n = 14). These eggs thus tripled their initial masses during incubation; this magnitude of water uptake is associated with high hatching success for parchment-shelled eggs in general (Packard, 1991), and for *Sceloporus* in particular (Tracy, 1980; Vleck, 1991). Hydric conditions in the jars, although they would not have been identical under all temperature regimes and incubation periods (Packard, 1991), produced eggs that had comparable ratios of water uptake during incubation.

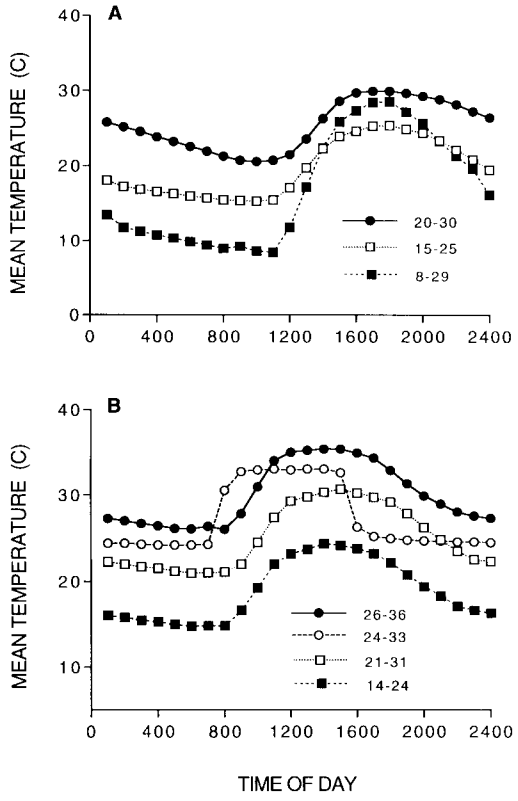

Fig. 1. Representative thermal profiles for incubation regimes. (A) Incubation temperatures used in 1995. (B) Incubation temperatures used in 1996. Values shown are hourly means.

Jars containing eggs were placed randomly within their assigned controlled temperature chambers. Within each chamber, jars were rotated within shelves, and shelves were rotated among positions within the chambers twice weekly to minimize position effects. Jars were checked for hatchlings each morning and evening, and hatchlings were immediately weighed to the nearest 0.1 mg.

Egg mortality was low overall, suggesting that none of the experimental protocols were unnaturally stressful to embryos. In 1995, respective egg mortality for *S. scalaris*, *S. virgatus*, *S. undulatus*, and *S. aeneus* across temperature regimes was 5, 5, 11, and 17%. In 1996, 1997, and 1998, the maximum mortality per species was 6%. Observations on incubation periods taken from the literature that were made at temperatures at which mortality during incubation was greater than 30% were not used; constant incubation temperatures higher than 31 C fell into this category (e.g., Sexton and Marion, 1974; Overall, 1994).

Calculation of the length of incubation and mean temperature during incubation.—Variable amounts of development occur before oviposition among squamates (Shine, 1983b; Blackburn, 1995), which means that incubation period per se does not necessarily index the rate of development. To avoid this problem, we used the length of the incubation period from stage 30 for all comparisons because stage 30 is the modal stage of oviposition for squamates in general (Blackburn, 1995), and for *Sceloporus* in particular.

The mean stage at oviposition ranged from 29–30 for *S. undulatus hyacinthinus* (Sexton and Marion, 1974), *S. occidentalis, S. clarki, S. undulatus hyacinthinus,* and *S. undulatus consobrinus* (our obs.); the lengths of the incubation periods for these species were not adjusted for stage at oviposition. We assumed from Overall (1994) that oviposition by *Sceloporus merriami* Stejneger occurred at stage 30. In contrast, *S. aeneus, S. scalaris,* and *S. virgatus* exhibit delayed oviposition, and oviposition occurred over a range of developmental stages as well. Therefore, for these three species, we used regressions of incubation period on stage at oviposition to estimate the lengths of the incubation periods from stage 30 rather than from the actual time of oviposition.

For a given mean incubation temperature, we considered that the effect of a fluctuating versus a constant temperature regime was developmentally equivalent (see also Results). The reason is that, when temperatures fluctuate more or less symmetrically about their mean value, the enhancement of growth at temperatures above the mean is balanced by the relatively low developmental rates below the mean, and this is true as long as temperature does not fluctuate below the temperature threshold for embryonic development and the relationship between developmental rate and temperature is linear (Shine and Harlow, 1996). When temperature does fluctuate below the threshold for embryonic development, the enhancement of growth at high temperatures is not counterbalanced by correspondingly low growth at low temperatures; thus, development is faster than would be expected from the mean temperature. Because incubation temperatures fell below threshold temperatures in the 8–29 regime (mean = 16.5 C), we corrected this mean temperature by substituting the threshold temperatures for *S. aeneus* and *S. scalaris* (17 C and 14 C, respectively; Andrews et al., 1997) for each hour that eggs were incubated below their threshold temperature and calculated adjusted means of 20.3 and 19.0 C, respectively. These adjusted means reflect the effective incubation temperatures, that is, temperatures below the threshold are developmentally equivalent.

Analyses.—All statistical analyses were conducted with SAS software (vers. 5 ed., Statistical Analysis Systems, Inc., Cary, NC, 1985, unpubl.). Analyses were based on mean values for each clutch in each temperature regime.

Because the relationships within *Sceloporus* are well defined (Weins and Reeder, 1997), we evaluated the association between incubation period and climate within a phylogenetic framework. Named subspecies of *S. undulatus* were considered species in these analyses on the basis of Weins and Reeder's (1997) phylogeny; *S. u. hyacinthinus* and *S. u. consobrinus* are subsequently referred to as *S. hyacinthinus* and *S. consobrinus.*

Comparative analyses were based on the residuals of the multiple regression of the length of incubation (incubation period) as a function of mean incubation temperature and hatchling live mass. For analyses that utilized CAIC and McClade software, residuals were averaged to give a mean residual incubation period for each of the 10 species in our dataset. To avoid negative values in these analyses, the positive value of the lowest negative mean residual (a positive constant) was added to each of the mean residuals; residual incubation periods ranged from 0.0 (shortest) to 3.3 (longest). We tested the prediction that the residual incubation period is directly related to nest temperature using the method of independent contrasts (CAIC; Purvis and Rambaut, 1995). Mean air temperature (from Blanchard, 1985) during the incubation period at appropriate geographic locations was used as an index of nest temperature in this analysis. Representative sites for each species were selected on the basis of their proximity to the collecting localities. We assumed that mean air temperature or geographic location provide crude, but useful, indices of the temperatures that embryos experience in nests. This assumption must be roughly correct given the broad range of latitude (19–46) and elevation (24–2800 m) considered in this study, general climatic variation on such a scale (Crowe, 1971), and the general parallel between soil and air temperature (Russell, 1973; Parton, 1984). Information on nest temperatures, albeit limited, supports this assumption. For example, mean nest temperature of *S. aeneus* at 2700 m is 17 C and the mean nest temperature of *S. virgatus* at 1800 m is 25 C (Andrews and Rose, 1994, RMA, unpubl. data). For *S. aeneus* and *S. virgatus,* mean nest temperatures are 1 and 4 C higher, respectively, than mean air temperature during incubation (RMA, unpubl. data).

Ancestral values for incubation period were determined from the mean residual values for each species using the rooted minimized sum of squared changes (Huey and Bennett, 1987) option in the continuous character tracing section of the MacClade 3.02 phylogenetic program (W. P. Maddison and D. R. Maddison, Sinouer Assoc., Sunderland, MA, 1992, unpubl.).

Results

The dataset included observations made at different temperatures, both fluctuating and constant, and on species that differed in body size. Therefore, before testing the physiological adaptation hypothesis, potential biases resulting from these sources of variation in incubation period were evaluated and corrected when appropriate. We first determined the relationship between the incubation period (IP) and mean incubation temperature (T, C) and the mean live mass of hatchlings (HM, mg) by use of a stepwise multiple regression analysis that incorporated all 51 observations (Table 1). To correct for the exponential relationship between the length of incubation and temperature, values for incubation period were natural log-transformed prior to analysis. Incubation temperature entered the model on the first step and explained 81% of the variation in the length of incubation. Hatchling mass explained a further 12% of the variation in the length of incubation. The resultant regression equation was $\mathrm{LnIP} = 6.01 + 0.00076\mathrm{HM} - 0.092\mathrm{T}$; ($F_{2,48} = 330$, $P < 0.001$, $R^2 = 0.93$). The standardized residuals from this equation are temperature and hatchling size independent measures of the length of incubation, and these residuals were used for all further analyses.

We first determined whether the type of temperature regime affected the incubation period. The residuals of observations made at fluctuating temperatures did not differ from those made at constant temperatures ($F_{1,49} = 0.11$, $P = 0.74$, one-way ANOVA). Therefore, we did not include this variable in further analyses. We also checked for a potential bias due to differential responses to variation in incubation temperature within species by regressing residual values on incubation temperature. For the species with sufficient observations (*S. aeneus, S. hyacinthinus, S. scalaris,* and *S. virgatus*), these regressions were not significant ($P > 0.4$ in all cases).

The physiological adaptation hypothesis was first tested across the entire phylogeny. If short incubation periods compensate for low nest temperatures, then evolutionary decreases in the length of incubation (increases in developmental rate) should be correlated with decreases in nest temperature. The nine independent contrasts for incubation period, however, were not related to the independent contrasts for mean air (nest) temperature during incubation (Pearson correlation coefficient = 0.19, $P = 0.62$).

To determine whether incubation period exhibited systematic variation within or among lineages, we mapped mean residual values of incubation period for the 10 species in the dataset and the calculated values of their ancestors on a cladogram (Fig. 2; see Materials and Methods for details). Residual incubation periods for the most basal taxa, *U. ornatus, S. merriami,* and the *scalaris* group were similar to their ancestral values. Residual incubation periods for *S. clarki* and the *undulatus* species group decreased and increased, respectively, relative to their ancestral values. The overall pattern suggested that a short incubation period is an ancestral character for the *scalaris* group, and a long incubation period is a derived character for the *undulatus* species group. Overall, the mean residual incubation period of the *scalaris* group (0.41) was higher than that of the undulatus group (−0.68; $F_{1,42} = 17.5$, $P < 0.001$, one-way ANOVA).

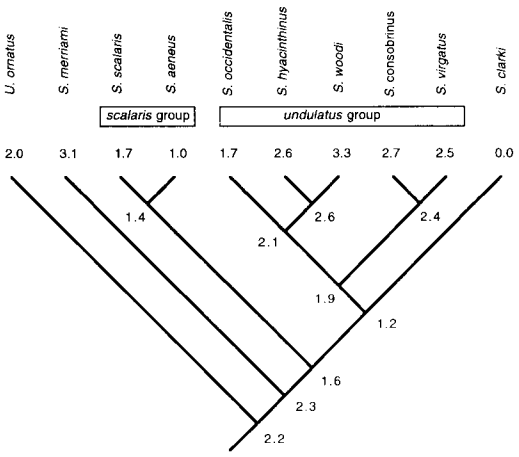

Fig. 2. Standardized values of residual incubation period of the 10 species in this study and their ancestors mapped on their phylogeny. See text for details. The cladogram is based on the more extensive phylogenies of Reeder and Weins (1996) and Weins and Reeder (1997).

We then tested whether the length of the incubation period exhibited systematic variation within the *scalaris* and the *undulatus* groups and in each case explicitly tested the hypothesis that the incubation period of taxa from colder climates is shorter than taxa from warmer locations. We used elevation and latitude as indices of climate because of the fine scale of analyses.

The first set of comparisons involved the 15 observations on the *scalaris* species group. We predicted that the incubation periods of *S. aeneus* at 2800 m and of the *S. scalaris* population at 2600 m (both from colder climates) would be shorter than that of the lower elevation

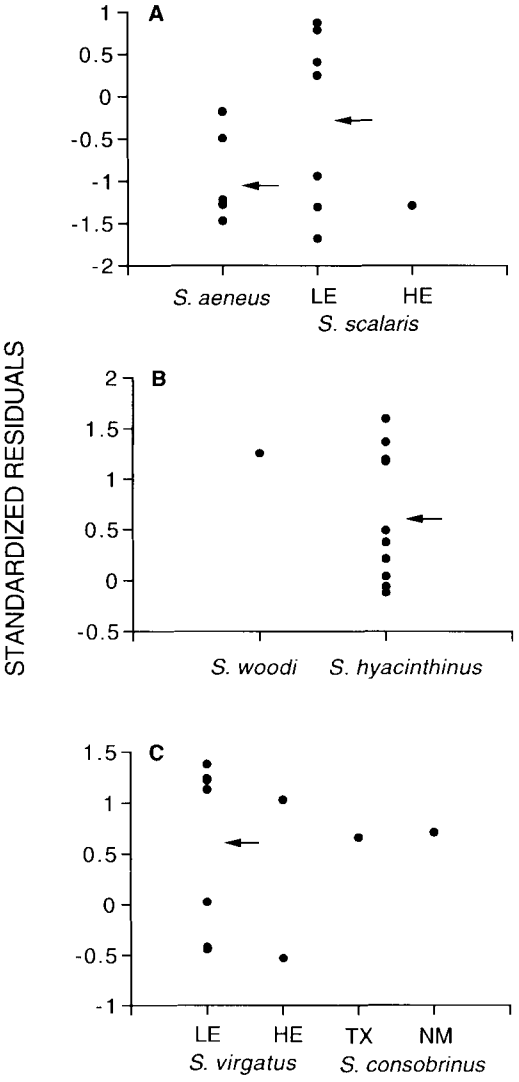

Fig. 3. Standardized residuals of incubation period for selected populations or species of *Sceloporus*. (A) *Scalaris* species group, (B) *Undulatus* species group (eastern lineage). (C) *Undulatus* species group (western lineage). Arrows indicate the mean residual for the reference taxon (−1.05, −0.23, 0.59, and 0.59 for *S. aeneus*, *S. scalaris* LE, *S. hyacinthinus*, and *S. virgatus* LE, respectively). LE and HE refer to low and high elevation populations, respectively.

(1460 m) and warmer climate population of *S. scalaris*. The mean incubation period of *S. aeneus* was shorter than the mean incubation period of low elevation *S. scalaris* (Fig. 3A), but the difference was not significant ($F_{1,12} = 3.5$, $P = 0.087$, two-way ANOVA). Similarly, although the incubation period of the high-elevation population of *S. scalaris* was relatively short, this observation was only 1.0 standard deviation below the mean incubation period for low-elevation *S. scalaris* ($P = 0.16$, one-tailed *t*-test, Rohlf and Sokal, 1981). We therefore concluded that high-elevation taxa in the *scalaris* species group do not have shorter incubation periods than the low-elevation population of *S. scalaris*.

Another comparison involved the eastern lineage of the *undulatus* species group from which our 12 observations included *S. woodi* from a warm climate (Florida) and Virginia and Missouri populations of *S. hyacinthinus* from colder climates (Fig. 3B). We predicted that the incubation period of *S. woodi* would be longer than that of their more northerly relatives. The incubation period of *S. woodi* was 1.1 standard deviations higher than the mean incubation period of *S. hyacinthinus*, but this difference was not significant ($P = 0.30$, one-tailed *t*-test). The incubation period of *S. woodi* was thus not longer than that of close relatives from cooler climates.

A third set of comparisons involved the western lineage of the *undulatus* species group for which our 11 observations included HE and LE populations of *S. virgatus* and populations of *S. undulatus consobrinus* from New Mexico and Texas. We predicted that the incubation period of the HE population (colder climate) of *S. virgatus* would be shorter than that of the warmer climate LE population (Fig. 3C). In addition, assuming that the more southerly (TX) and even lower elevation (NM) populations of *S. consobrinus* normally experience warmer incubation temperatures than the LE population of *S. virgatus*, we predicted that the incubation periods of these populations would be longer than that of the LE population of *S. virgatus*. The mean incubation period of the HE population of *S. virgatus* was 0.41 standarad deviations below the mean incubation period of the LE population, a nonsignificant difference ($P = 0.34$, one-tailed *t*-test), and the incubation periods of the New Mexico and Texas populations of *S. consobrinus* were not longer than the LE population of *S. virgatus* (0.15 and 0.85 standard deviations greater than the mean for the LE population of *S. virgatus*, $P = 0.44$ and 0.20, respectively, one-tailed *t*-tests). Incubation period therefore does not seem to be related to climate within the western lineage of the *undulatus* species group.

The final comparison involved six observations on incubation periods of *S. occidentalis*. Because no one population had enough observations to serve as a statistical baseline, we used the standardized residuals in a stepwise multiple regression in which residual incubation period was the dependent variable and elevation and latitude were the independent variables. Eleva-

tion entered the model in the first step but did not explain a significant amount of variation in incubation period ($F_{1,4} = 0.08$, $P = 0.79$), and the model that included latitude did not explain additional variation ($F_{2,3} = 0.04$, $P = 0.96$). The incubation period of *S. occidentalis* was therefore not related to climate, based on elevation and latitude.

Discussion

We predicted that short incubation periods should be associated with cold climates. This hypothesis was not supported by a phylogeny-wide comparison of incubation period and an index of nest temperature. Although the *scalaris* species group had shorter incubation periods than the *undulatus* species group, the present geographic distribution of these two groups suggests that variation in incubation period is not related to climate. The *scalaris* species group is found at mid- to high elevations from central Mexico to the southwestern United States, and the *undulatus* species group is found at low- to high elevations from northern Mexico to the northern United States (Sites et al., 1992); the ranges of the two groups thus overlap broadly.

The hypothesis that embryos within lineages would exhibit physiological adaptation to local climatic conditions was also not supported. In none of seven intraspecific comparisons were incubation periods for taxa from cold climates shorter than those from warm climates. DeMarco's (1992) conclusion that short incubation periods are associated with elevation (as an index of temperature) was based, in part, on the observation that embryos of the high-elevation population of *S. scalaris* had short incubation periods in comparison with embryos of lower-elevation members of the *undulatus* species group. However, our observations suggest that his analysis was confounded by phylogenetic effects; incubation periods of the entire *scalaris* species group are shorter than those of the *undulatus* species group. In related studies, *Sceloporus* embryos from different climates did not exhibit associated variation in mortality following cold exposure or in their low-temperature thresholds for development (Andrews et al., 1997), further refuting the physiological adaptation hypothesis for *Sceloporus*.

Despite a lack of association between incubation period and climate, the analyses did reveal variation that was associated with phylogeny. Relatively short incubation periods appear to be an ancestral character in the genus (Fig. 2). Short incubation periods persist in the *scalaris* species group and in *S. clarki*. In contrast, relatively long incubation periods characterize the *undulatus* species group with the exception of a possible reversal of this trait by *S. occidentalis*. Although the adaptive nature (if any) of phylogenetic variation in incubation period and thus developmental rate is far from obvious at this point, it is clear that comparative studies of the rates of embryonic development must consider phylogeny as an important contributing variable.

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(RMA, TM) DEPARTMENT OF BIOLOGY, VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY, BLACKSBURG, VIRGINIA 24061; AND (CPQ, FJQ) P.O. BOX 8255, MOU-3, DEDEDO, GUAM 96912. E-mail: (RMA) randrews@vt.edu. Send reprint requests to RMA. Submitted: 9 July 1998. Accepted: 28 Dec. 1998. Section editor: R. G. Bowker.