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Incubation temperature and phenotypic traits of *Sceloporus undulatus*: implications for the northern limits of distribution

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Abstract Cold environmental temperature is detrimental to reproduction by oviparous squamate reptiles by prolonging incubation period, negatively affecting embryonic developmental processes, and by killing embryos in eggs directly. Because low soil temperature may prevent successful development of embryos in eggs in nests, the geographic distributions of oviparous species may be influenced by the thermal requirements of embryos. In the present study, we tested the hypothesis that low incubation temperature determines the northern distributional limit of the oviparous lizard *Sceloporus undulatus*. To compare the effects of incubation temperature on incubation length, egg and hatchling survival, and hatchling phenotypic traits, we incubated eggs of *S. undulatus* under temperature treatments that simulated the thermal environment that eggs would experience if located in nests within their geographic range at 37°N and north of the species' present geographic range at latitudes of 44 and 42°N. After hatching, snout–vent length (SVL), mass, tail length, body condition (SVL relative to mass), locomotor performance, and growth rate were measured for each hatchling. Hatchlings were released at a field site to evaluate growth and survival under natural conditions. Incubation at temperatures simulating those of nests at 44°N prolonged incubation and resulted in hatchlings with shorter SVL relative to mass, shorter tails, shorter hind limb span, slower growth, and lower survival than hatchlings from eggs incubated at temperatures simulating those of nests at 37 and 42°N. We also evaluated the association between environmental temperature and the northern distribution of *S. undulatus*. We predicted that the northernmost distributional limit of *S. undulatus* would be associated with locations that provide the minimum heat sum (~495 degree-days) required to complete embryonic development. Based on air and soil temperatures, the predicted northern latitudinal limit of *S. undulatus* would lie at ~40.5–41.5°N. Our predicted value closely corresponds to the observed latitudinal limit in the eastern United States of ~40°N. Our results suggest that soil temperatures at northern latitudes are not warm enough for a sufficient length of time to permit successful incubation of *S. undulatus* embryos. These results are consistent with the hypothesis that incubation temperature is an important factor limiting the geographic distributions of oviparous reptile species at high latitudes and elevations.

Keywords *Sceloporus undulatus* · Distributional limits · Cold climate hypothesis · Degree-days · Egg incubation

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Introduction

Cold environmental temperature is incompatible with oviparous reproduction by squamate reptiles judging

by distributional patterns of oviparous and viviparous species. The proportion of oviparous species in squamate faunas declines with increasing latitude and elevation, and in severely cold areas, all squamates are viviparous (Tinkle and Gibbons 1977; Shine and Berry 1978; Shine 1985). The presumed selective mechanisms that result in these geographic patterns are that cold temperature prolongs or prevents successful incubation of eggs in nests and reduces survival of hatchlings (Shine and Bull 1979; Shine 1985, 2002; Qualls and Andrews 1999). For temperate zone squamates, prolonged incubation as a result of cool incubation temperatures would thus delay fall emergence, leaving hatchlings with little time to feed and grow before cold temperatures preclude activity (Packard et al. 1977; Shine 1985). Moreover, cold temperatures during incubation may also adversely affect embryonic developmental processes, resulting in hatchlings with phenotypic traits associated with low fitness (Qualls and Andrews 1999; Shine 2002). For example, lizard eggs incubated at cool temperatures resulted in smaller hatchlings with reduced growth rates and slower sprint speeds compared to hatchlings incubated at warmer temperatures (Qualls and Andrews 1999).

The association between cold temperatures and the geographic limits of oviparous species on latitudinal and elevational gradients is well documented (Weeks 1933; Sergeev 1940; Greer 1968; Tinkle and Gibbons 1977; Shine and Berry 1978). In contrast, the direct association between the thermal requirements of egg incubation and the distributional limits of oviparous species has been made in only a few cases (Muth 1980; Shine 1987; Porter et al. 2002). For example, the geographic distribution of oviparous species of *Pseudechis* (Elapidae) is associated with regions where mean summer ambient temperatures are greater than 22°C (the minimum temperature required for successful development of *Pseudechis* embryos) (Shine 1987). An even more direct association is Shine's (2002) demonstration that eggs of the montane oviparous skink *Bassiana duperreyi* had reduced hatching success and hatchling viability when incubated at cool temperatures simulating those of nests at altitudes exceeding the elevational limit of the species' natural distribution (Shine 2002). If cold incubation temperature prevents oviparous species from colonizing habitats at high latitudes and elevations, then the distributional limits of oviparous species should be predictable from embryonic thermal tolerances and embryonic developmental rates as a function of temperature.

Oviparous squamate species with wide latitudinal distributions provide an opportunity to study the association between the thermal biology of embryos and geographic distribution. The oviparous lizard *Sceloporus undulatus*, for example, has a wide latitudinal distribution across the United States (US) and is one of the northernmost members of the genus *Sceloporus* (Fig. 1). In the central and eastern US the geographic range of *S. undulatus* extends maximally to about 40°N (Tinkle and Ballinger 1972; Stebbins 1985; Sites et al. 1992). *S. undulatus* occurs in a wide range of habitats, from arid deserts in the southwestern US to temperate deciduous forests in the eastern US. No obvious geographical or ecological barriers prevent a northern expansion of *S. undulatus*. It is unlikely that soil moisture plays an important role in determining the northern distributional limit of *S. undulatus* because precipitation in the US varies on a longitudinal, not latitudinal gradient. Moreover, *S. undulatus* is a generalist insectivore (Stebbins 1985; McGovern and Knisley 1986), individuals thus do not appear to have specialized dietary requirements. There is no evidence that competition, predation, or parasitism limit the northern distributional limit of *S. undulatus*. While we cannot rule out the possibility that factors other than incubation temperature may influence the geographic distribution of *S. undulatus*, the above considerations implicate temperature as an important determinant of its geographic distribution.

The objective of our study was to test the hypothesis that the thermal requirements for embryonic development determine the northernmost distribution of *S. undulatus*. Incubation temperature could affect

Fig. 1 Geographic distribution of *Sceloporus undulatus* in north-central and northeastern USA (*gray area*, see text for sources). Locations of *S. undulatus* nests where incubation temperatures were obtained are indicated: actual *Sceloporus undulatus* nests were at ~37°N in Blacksburg, Virginia (*VA*) (*open triangle*) and at 39°N near Pemberton, New Jersey (*NJ*) (*filled triangle*). Simulated nests were at 42°N (*filled circles*) and 44°N (*filled diamonds*). Soil temperatures for simulated nests were average daily soil temperatures during June–August (National Climate Data Center)

survival directly (e.g., by affecting developmental processes) or indirectly (by affecting time of hatching). We therefore, addressed this hypothesis in several ways. Our experimental protocol was to incubate eggs of *S. undulatus* under treatments that simulated temperatures of nests within its geographic range and those of nests outside of its northern distributional limit. We then evaluated the effect of incubation temperature on hatching success, incubation period, hatchling phenotypic traits (morphology and performance), and post-hatching survival. We predicted that hatchlings from eggs incubated at cool temperatures simulating nests at latitudes outside the northernmost distributional limit would have reduced hatching success, longer incubation period, reduced performance, and lower survival than hatchlings incubated at temperatures simulating nests within the geographic distribution.

Thermal requirements for development appear to influence geographic distributions for a variety of ectothermic organisms including plants (Beerling 1993), insects (Ungerer et al. 1999), and reptiles (Muth 1980; Shine 1999; Porter et al. 2002; Kearney and Porter 2004). We therefore also determined the association between environmental temperature and the northern distribution of *S. undulatus*. Because successful incubation of reptilian embryos is temperature dependent, we predicted that the northernmost distributional limit of *S. undulatus* would be associated with locations that provide the minimum heat sum (degree-days) required to complete embryonic development. We tested this prediction by assessing the geographic variation in environmental temperature at sites within and outside the northern distributional limits of *S. undulatus*.

Materials and methods

Collection and maintenance of gravid females

Gravid females of *S. undulatus* (n = 19) were collected from 17 June to July 6 2004, from sites within 35 km of Blacksburg, Montgomery County, Virginia. Females were placed singly in cloth bags and transported to Virginia Polytechnic Institute and State University (Virginia Tech, Blacksburg, Va.). Eggs from a single clutch were also collected on the day of oviposition in the field. Females were housed in plastic containers [73 length (L) × 48 width (W) × 22 height (H), two to three females per container] in the laboratory until oviposition occurred. Containers had a layer of moist sand to facilitate nesting and to prevent desiccation of eggs. Daily photoperiod was provided by laboratory windows. Fluorescent lighting (0800–1800 hours) and 100-W flood lamps suspended at one end of each container (0900–1700 hours) provided general lighting and a heat source, respectively. Containers were also provided with boards and rocks for basking and hiding places. Females were fed until satiation (crickets and mealworms dusted with mineral–vitamin supplement) every other day. Water was provided daily by misting the rocks, boards, and sides of the containers and from shallow ceramic dishes. Containers were checked several times daily for nesting females and eggs. After oviposition, females were released at the location of their capture.

Experimental design

Eggs were weighed to the nearest 0.01 g within a few hours of oviposition, and numbered consecutively within each clutch using a non-toxic waterproof marker. A single egg from each clutch was dissected and the embryo staged according to Dufaure and Hubert (1961) staging system. The remaining eggs of each clutch were incubated individually in 70-ml specimen jars containing vermiculite moistened with a sufficient quantity of distilled water (0.7:1.0 g H_2O:vermiculite) to produce a water potential of –200 kPa (determined by thermocouple psychrometry). The jars were covered with plastic food wrap and sealed with a rubber band.

Eggs from each clutch were allocated among three experimental temperature treatments. One of the treatments was based on temperatures observed in actual *S. undulatus* nests and the other two treatments were based on soil temperatures for localities north of the present distributional limit. The 22–32°C (mean = 27°C) treatment represented actual temperatures observed in *S. undulatus* nests at 37°N in Virginia (Andrews et al. 2000) and at 39°N in New Jersey (depth of 6 cm) (Angilletta et al. 2000). Soil temperature data were obtained from the National Climate Data Center (2005) for three localities at ~44°N (Waseca, Minn.; North West Michigan Research Farm, Mich.; and Canton, N.Y.) and four localities located at ~42°N (Chicago Botanical Garden, Ill.; Geneva Research Farm, N.Y.;; Fremont, Ohio; and Wanatah, Ind.). Soil temperatures at 44 and 42°N were average maximum and minimum soil temperatures during June–August, measured over a period of 5–30 years, at a soil depth of 10 cm. The 19–25 (mean = 22°C) and 21–27°C (mean = 24°C) treatments represented temperatures of simulated nests outside the northernmost distributional limit of *S. undulatus* at 44 and 42°N, respectively, if eggs were buried at depths similar to those of typical *S. undulatus* nests at low latitude.

The coldest temperature treatment (simulating nests at 44°N) was selected because 22°C is similar to the

lowest temperature regime where hatching could be successful. Eggs of *S. undulatus* fail to hatch when incubated at a constant temperature of 20°C (Sexton and Marion 1974). Eggs of *Sceloporus virgatus* (a member of the *undulatus* species group), however, hatched when temperatures fluctuated around a mean of 20°C (Andrews et al. 1997). Hatching can thus occur at a low average temperature as long as the diel cycle includes temperatures above the threshold temperature for development.

Eggs were placed, according to treatment, into programmable temperature chambers and incubated under a fluctuating temperature regime. Temperatures inside the chambers cycled linearly for 4 h between daily maximum (8 h) and minimum (8 h) temperatures. Incubation temperatures were measured inside a 70-ml jar containing moistened vermiculite. The temperature probe was placed in the center of the jar and buried ~1 cm below the surface of the vermiculite (the same depth as that of experimental eggs). The mean daily minimum, maximum, and overall mean temperatures observed for the 22°C treatment were 19.3, 25.1, and 22.3°C, respectively, for the 24°C treatment were 21.3, 27.1, and 24.2°C, respectively, and for the 27°C treatment were 21.7, 31.8, and 26.8°C, respectively. Racks of jars with eggs were rotated within the chambers every 3 days to minimize position effects on embryonic development. To ensure that eggs did not experience a negative water balance, the vermiculite was changed ~30 days after the start of incubation.

Morphology and husbandry of laboratory hatchlings

Hatchling sex, mass, snout–vent length (SVL), tail length (TL), and hind limb span (HLS) (maximum linear distance between the tip of the fourth digit on each hind limb), were recorded within 12 h of hatching. Each hatchling was numbered using a non-toxic marker and given a unique toe-clip for identification. Hatchlings were housed in plastic containers (73 L × 48 W × 22 H cm^3), with ten to 15 hatchlings per container. Hatchlings were fed (pinhead crickets and mealworms dusted with vitamin–mineral supplement) and watered by misting the interior and sides of the containers twice daily. Otherwise, husbandry was the same as for gravid females. Hatchlings were not fed on the day that locomotor performance trials were performed (see section below). Hatchlings were maintained in the laboratory for 10–13 days before release in the field. Mass, SVL, TL, and HLS were recorded at 10 days after hatching.

Measurement of locomotor performance and growth of laboratory hatchlings

Locomotor performance was measured at 3–4 days after hatching using a 1-m-long electronically timed racetrack (Qualls and Andrews 1999; Warner and Andrews 2002). Five infrared photocells were connected to an electronic stopwatch; the five photocells were spaced at 0.25-m intervals along the length of the racetrack. The racetrack was placed in an environmental chamber (150 L × 80 W × 80 H cm^3) set at a constant 31°C. Two electric fans were situated at opposite ends of the chamber to promote air circulation and to ensure a uniform temperature distribution within the chamber. Observations were made between 1100 and 1530 hours. Hatchlings were acclimated for 30 min in the environmental chamber prior to locomotor performance trials. Locomotor performance was measured 3 times for each hatchling with at least a 2 min rest between trials. Hatchlings were placed at the beginning of the racetrack and prodded gently with a small paintbrush if they failed to run. Locomotor performance was assessed using three criteria: (1) the fastest running speed of the twelve 0.25-m intervals, (2) the fastest running speed of the three 1-m trials, and (3) the number of times that a hatchling stopped during the fastest of the three 1-m trials. The time required for hatchlings to cover the prescribed distance was expressed as meters per second. The number of times that a hatchling stopped along the 1-m distance was recorded using a manual counter.

Growth in the laboratory was measured over 10 days and was assessed as the difference between an individual's natural log-transformed SVL or mass at 10 days and at hatching divided by 10, the number of days between measurements.

Measurement of locomotor performance and growth of field hatchlings

In order to determine if laboratory-produced phenotypes were comparable to phenotypes produced in the field under comparable thermal conditions, we measured the phenotypes of hatchlings collected in the field ("field hatchlings"). These individuals ($n = 41$) were captured from 24 August to 19 September at one of the field sites. They were measured for mass, SVL, toe-clipped, and numbered with a non-toxic marker at the time of capture and at the time of release. They were returned to the laboratory for measurement of locomotor performance traits and growth rate. Field hatchlings were housed in separate containers from laboratory hatchlings but otherwise maintained under identical conditions (see Morphology and husbandry of

hatchlings above). Locomotor performance traits were measured 2–3 days after capture (as described for laboratory hatchlings). Field hatchlings were also maintained in the laboratory for 10–13 days before they were released at their location of capture.

Growth and survival of hatchlings in the field

Hatchlings (n = 47, 42, and 41 for the 22, 24, and 27°C treatments, respectively) were released on warm, sunny days between 1000 and 1600 hours from 27 August to 6 November at the site where field hatchlings were collected. The release site was ~600 m^2 in area and located on private land in Montgomery County, Va.. Piles of woody debris, and small shrubs scattered throughout the clearing provided suitable habitat for hatchlings. The clearing was surrounded on all sides by forest except for a narrow gravel access road that passed through the site. Hatchlings typically do not disperse through dense forest, and therefore tend to remain in the open areas where they hatched for at least the first month or two of life (Warner and Andrews 2002).

The clearing and periphery of the surrounding forest were searched thoroughly for hatchlings twice weekly from 27 August to 14 November. After capture, hatchlings were identified by their number and toe clip, weighed, and measured for SVL. After measurements were recorded, hatchlings were released at their site of capture.

Survival estimates in the field were based upon the assumption that disappearance of hatchlings was largely due to mortality rather than dispersal. This assumption is supported by previous studies on *Uta stansburiana* and *S. undulatus* that indicate that emigration is a relatively rare event and does not bias survival estimates (Wilson 1991; Andrews et al. 2000; Warner and Andrews 2002).

Calculation of degree-days

The heat sum accumulated during each treatment was expressed as:

$$D = \sum_{\text{day}=1}^{\text{day}=j} (t_{\text{m}} - t_{\text{o}}),$$

where D = sum of degree-days, j = incubation length in days, t_{o} = the threshold temperature for development, and t_{m} = the mean daily temperature (Zalom et al. 1983). Because development of *S. undulatus* embryos is arrested at 17°C (Andrews et al. 1997), we used 17°C as the minimum threshold temperature for degree-day calculations. Because embryos presumably would not complete development at a mean temperature less than 22°C (see above), our 22°C treatment represents the minimum number of degree-days required for successful incubation of *S. undulatus* embryos.

The number of degree-days accumulated above a threshold of 17°C was also estimated for 50 recording stations at latitudes ranging from 37 to 50°N. Mean monthly air temperatures (based on 30-year averages) during June–September were obtained from the Natural Resources Conservation Service's National Water and Climate Center (2005). Because long-term air temperature data are available for a large number of sites throughout the US, we used air temperature data instead of soil temperatures for extensive degree-day calculations. The use of mean air temperature to approximate nest temperature is reasonable because mean soil temperature is similar to mean standard air temperatures at shallow depths (2–10 cm) (Shine 1983). To verify the association between air and soil temperature for our study, we estimated the number of degree-days accumulated above a threshold of 17°C using soil temperature data from nine recording stations ranging in latitude from 37 to 47°N. Our observations were limited to nine stations where complete soil temperature data sets were available. Mean monthly soil temperatures recorded at a depth of 5.1 cm during June–September 2005 were obtained from the same source as air temperature. Degree-days accumulated from June–September were estimated by summing the number of degree-days above 17°C over the 122-day period for each locality. Because embryonic development does not occur below a threshold of 17°C, we assumed that degree-days were not accumulated during months where mean air or soil temperatures were less than or equal to 17°C.

Data manipulation and statistical analyses

Analyses of incubation length, morphology, growth, and locomotor performance in the laboratory

Statistical analyses were conducted using SAS statistical package version 9.1.2 (SAS Institute 2003). The effects of temperature treatment on incubation length, morphology, growth, and locomotor performance were analyzed using analysis of covariance (ANCOVA) and ANOVA. Preliminary analyses indicated that sex did not affect hatchling morphology, performance, or survival. Sex was therefore not considered in further analyses.

The effect of temperature treatment on incubation length was analyzed using stage at oviposition as a covariate. The effect of temperature treatment on SVL,

HLS, TL, and body mass at hatching was evaluated using egg mass as a covariate. The effect of treatment on hatchling mass at 10 days was evaluated using initial hatchling mass as a covariate. SVL at hatching was used as a covariate in analyses of the effect of temperature treatment on morphology at 10 days (SVL, TL, HLS) and running speed. Body condition (mass relative to SVL) was assessed as mass$^{0.3}$/SVL (Andrews 1982). When the covariate was not significant ($P > 0.05$), single-factor ANOVA was used to evaluate treatment effects. Analyses of treatment effects were based upon clutch means. Prior to ANCOVA analyses, the assumption of homogeneity of slopes was satisfied by testing for significance of the interaction of the covariate with treatment variables. For all ANCOVAs post hoc pair-wise comparisons were made using a least significant difference test on least squared means. For all ANOVAs, post hoc pair-wise comparisons were made using a Tukey's honestly significant difference test. The effect of temperature treatment on the frequency of kinked or bent tails was analyzed using a χ^2-test (Freq Procedure). Data are reported as mean ± SEM unless otherwise reported, and probability values less than 0.05 were considered significant.

Analyses of growth and survival in the field

The effect of temperature treatment on growth rate of laboratory hatchlings in the field was analyzed as described for growth rate in the laboratory. Because some hatchlings were not recaptured after release, no data on growth were obtained for some clutch/treatment combinations. Consequently, the number of clutches in the analysis was reduced to eight in the 22°C treatment, 15 in the 24°C treatment, and 13 in the 27°C treatment, respectively. Growth at 10–25 days after release in the field was measured as the difference between an individual's natural log-transformed SVL or mass at last capture and at the time of release in the field divided by the number of days between measurements. The effect of temperature treatment on hatchling survival at 10, 20, and 30 days after release in the field was analyzed using χ^2-tests. The overall association between phenotype and survival independent of treatment was assessed for each phenotypic trait using ANOVAs and ANCOVAs. Separate analyses were used to contrast survivors versus non-survivors for each of the three time periods.

Contrasts of field and laboratory hatchlings

Growth and locomotor performance of field hatchlings were compared to laboratory hatchlings. Because incubation conditions and clutch of origin of field hatchlings were unknown, individual values rather than clutch means were used in statistical analyses. Nine of the laboratory hatchlings ($n = 5$ in the 24°C treatment and $n = 4$ in the 27°C treatment) were inadvertently released before first obtaining data on locomotor performance. The number of hatchlings used in performance analyses was therefore reduced to 37 in both the 24 and 27°C treatments. The effect of temperature treatment on hatchling running speed was analyzed using ANCOVA with initial SVL as a covariate. We did not compare morphological traits (SVL, body mass, TL, HLS) between field and laboratory hatchlings because the morphology of field individuals at the time of hatching was unknown. Otherwise, statistical contrasts comparing growth and survival of field and laboratory hatchlings are the same as those described above for laboratory hatchlings (above).

Results

Effect of temperature treatment on egg and hatchling survival and incubation length

Survival of eggs and hatchlings was not affected by temperature treatment. Overall, 131 of 140 eggs hatched (94% survival), and survival exceeded 90% in all treatments. Survival of hatchlings in the laboratory was not related to temperature treatment. Only one (from the 22°C treatment) of 131 hatchlings died prior to release (99.2% survival). Survival of field hatchlings in the laboratory was 100%.

Incubation length differed among temperature treatments and was negatively associated with increasing temperature (Table 1). Gravid females laid eggs over a narrow range of embryo stages (mean stage = 28.8 ± 0.27; range 26–30, $n = 20$). Hatching occurred from 17 August to 6 September in the 27°C treatment, 1 September to 28 September in the 24°C treatment, and 29 September to 27 October in the 22°C treatment, with mean incubation periods of 56, 74, and 99 days, respectively.

Effect of temperature treatment on hatchling phenotypic traits

Hatchling SVL and body mass at hatching and at 10 days did not differ among temperature treatments (Table 1). Body condition at hatching was not affected by temperature treatment. At 10 days, however, hatchlings from the 22°C treatment were heavier for their length than hatchlings from the 27°C treatment.

Table 1 Mean values, SE (number of clutches), and results of statistical tests [ANOVAs and analyses of covariance (ANCOVAs)] for incubation period, phenotypic traits, locomotor performance, and growth of laboratory *Sceloporus undulatus* hatchlings from eggs incubated at 22, 24, and 27°C. Statistically significant results are in *bold*. Within each row, values with *different letters* are different from one another ($P < 0.05$). *SVL* Snout–vent length

Trait	Incubation at 22°C	Incubation at 24°C	Incubation at 27°C	Results
Incubation period (days)	**98.9 ± 0.7 (20)** a	**74.4 ± 0.9 (19)** b	**56.4 ± 0.3 (19)** c	$\mathbf{F_{2,54} = 996.9, P < 0.001}$
SVL (mm)				
At hatching	25.3 ± 0.2 (20)	25.4 ± 0.2 (19)	25.4 ± 0.2 (19)	$F_{2,54} = 0.1$, $P = 0.904$
At release	27.8 ± 0.3 (20)	27.8 ± 0.3(19)	28.4 ± 0.4 (19)	$F_{2,54} = 1.3$, $P = 0.292$
Body mass (g)				
At hatching	0.587 ± 0.013 (20)	0.589 ± 0.014 (19)	0.580 ± 0.015 (19)	$F_{2,54} = 0.2$, $P = 0.855$
At release	0.761 ± 0.034 (20)	0.725 ± 0.024 (19)	0.714 ± 0.027(19)	$F_{2,54} = 0.8$, $P = 0.433$
Body shape ($mass^{0.3}$/SVL)				
At hatching	0.034 ± 0.0001 (20)	0.033 ± 0.0001 (19)	0.033 ± 0.0002 (19)	$F_{2,55} = 0.8$, $P = 0.465$
At release	**0.033 ± 0.0002 (20)** a	**0.033 ± 0.0002 (19)** a, b	**0.032 ± 0.0004 (19)** b	$\mathbf{F_{2,55} = 3.8, P = 0.027}$
Tail length (mm)				
At hatching	**25.6 ± 0.6 (20)** a	**28.2 ± 0.5 (19)** b	**29.9 ± 0.47 (19)** c	$\mathbf{F_{2,54} = 18.9, P < 0.001}$
At release	**28.4 ± 0.7 (20)** a	**30.6 ± 0.6 (19)** b	**32.9 ± 0.56 (19)** c	$\mathbf{F_{2,54} = 18.7, P < 0.001}$
Hind leg span (mm)				
At hatching	**38.6 ± 0.9 (20)** a	**41.5 ± 0.3 (19)** b	**41.6 ± 0.4 (19)** b	$\mathbf{F_{2,54} = 8.4, P < 0.001}$
At release	**41.6 ± 0.4 (20)** a	**42.8 ± 0.4 (19)** b	**43.8 ± 0.4 (19)** b	$\mathbf{F_{2,54} = 9.6, P < 0.001}$
Locomotion (m/s)				
Speed over 0.25 m	0.69 ± 0.032 (19)	0.75 ± 0.056 (18)	0.78 ± 0.087 (18)	$F_{2,51} = 0.5$, $P = 0.629$
Speed over 1 m	0.42 ± 0.020 (19)	0.38 ± 0.020 (18)	0.42 ± 0.034 (18)	$F_{2,51} = 0.6$, $P = 0.569$
Number of stops over 1 m	3.0 ± 0.2 (19)	3.8 ± 0.2 (18)	3.7 ± 0.3 (18)	$F_{2,52} = 2.8$, $P = 0.068$
Growth in the laboratory				
SVL (mm/day)	0.25 ± 0.02 (20)	0.23 ± 0.02 (19)	0.29 ± 0.03 (19)	$F_{2,55} = 1.3$, $P = 0.269$
Mass (g/day)	0.017 ± 0.002 (20)	0.014 ± 0.002 (19)	0.013 ± 0.002 (19)	$F_{2,55} = 0.4$, $P = 0.682$
Growth in field				
SVL (mm/day)	0.10 ± 0.01 (8)	0.16 ± 0.02 (15)	0.19 ± 0.016 (13)	$F_{2,33} = 3.1$, $P = 0.06$
Mass (g/day)	**0.008 ± 0.017 (8)** a	**0.013 ± 0.017 (15)** a, b	**0.019 ± 0.001 (13)** b	$\mathbf{F_{2,33} = 4.9, P = 0.014}$

TL and HLS at hatching and at 10 days differed among temperature treatments (Table 1). At hatching, the TLs of hatchlings from the 22°C treatment were on average about 4 mm shorter than those of hatchlings from the 27°C treatment and differences in TL persisted over the 10 days that hatchlings were in the laboratory. HLS at hatching and at 10 days was shorter by about 3 mm in the 22°C treatment compared to the 24 and 27°C treatments. HLS did not differ between the 24 and 27°C treatments. Hatchlings from the 22°C treatment also exhibited a higher frequency of kinked tails than hatchlings from the 24 and 27°C treatments ($\chi^2 = 7.1$, $df = 2$, $n = 130$, $P = 0.03$). Approximately 21% of hatchlings from the 22°C had kinked tails compared to 2% of hatchlings in the 24°C treatment and 12% of hatchlings in the 27°C treatment, respectively. Hatchling growth in the laboratory over 10 days and locomotor performance did not differ among treatments (Table 1).

Growth rate and survival of laboratory hatchlings in the field

Growth in mass during the 10–25 days after release in the field was related to temperature treatment but growth in SVL was not (Table 1). Hatchlings from the 22°C treatment grew more slowly in mass and tended to grow more slowly in SVL than those from the 24 and 27°C treatments. Growth in mass of hatchlings incubated at 22°C was about 50% slower than that of hatchlings incubated at 24 and 27°C.

Survival of hatchlings in the field at 10, 20, and 30 days after release differed among temperature treatments (Table 2; Fig. 2) with lower hatchling survival in the 22°C treatment than in the 24 and 27°C treatments.

Correlates of survival of laboratory hatchlings in the field

Overall, individuals that survived 10 days after release had longer tails ($F_{1,126} = 5.9$, $P = 0.02$, ANCOVA, SVL used as the covariate) and higher average running speeds over 0.25 m ($F_{1,118} = 5.7$, $P = 0.02$, ANCOVA, SVL used as the covariate) and 1 m ($F_{1,118} = 7.9$, $P = 0.006$, ANCOVA, SVL used as the covariate) than individuals that did not survive (Fig. 3). While this pattern continued to 20 and 30 days after release, differences between survivors and non-survivors were not significant.

Table 2 Statistical tests comparing survival of laboratory- (contrasts of treatments) and field-incubated (contrasts of field versus laboratory) hatchlings of *S. undulatus* at 10, 20, and 30 days after release in the field. Overall contrasts of survival were performed using χ^2-tests. Significant differences are in *bold*

	Statistical test
Laboratory hatchlings	
Survival at 10 days	**χ^2 = 7.1, *df* = 2, *P* = 0.028**
Survival at 20 days	**χ^2 = 14.8, *df* = 2, *P* < 0.001**
Survival at 30 days	**χ^2 = 13.4, *df* = 2, *P* = 0.001**
Field versus laboratory hatchlings	
Survival at 10 days	χ^2 = 7.1, *df* = 3, *P* = 0.067
Survival at 20 days	**χ^2 = 15.5, *df* = 3, *P* = 0.001**
Survival at 30 days	**χ^2 = 13.5, *df* = 3, *P* = 0.004**

Estimation of field hatching dates and incubation times

Field-incubated hatchlings had a mean SVL of 27 mm at the mean date of first capture (4 September). Given the overall mean SVL of 25 mm at hatching and the overall mean growth rate of about 0.3 mm/day of laboratory-hatched individuals, the mean date of hatching in the field would have been 28 August. Assuming that oviposition of field eggs took place on 3 July (the median date of oviposition in the laboratory), the mean incubation period in the field would have been 56 days.

Locomotor performance and growth

Running speed of field hatchlings did not differ from that of laboratory hatchlings (Table 3). Field hatchlings, however, stopped more frequently over 1 m compared to laboratory hatchlings from the 22°C treatment ($F_{3,157}$ = 3.1, P = 0.03, ANCOVA, SVL used as the covariate).

Under laboratory conditions, field hatchlings grew in SVL about 40% more slowly than did hatchlings from the 27°C treatment ($F_{3,167}$ = 5.1, P < 0.01, ANOVA), while growth in mass did not differ between field and laboratory hatchlings (Table 3). In the field, growth of field hatchlings did not differ from that of laboratory hatchlings 10–25 days after release (Table 3). The average growth of field hatchlings was almost identical in both SVL and mass to that of laboratory hatchlings incubated at 24 and 27°C.

Survival in the field of field hatchlings was similar to that of hatchlings from the 24 and 27°C treatments at 10, 20, and 30 days after release (Table 2; Fig. 2). Survival of field hatchlings was, however, consistently higher than that of laboratory hatchlings from the 22°C treatment, but differences were only significant at 20 and 30 days after release in the field. At 20 and 30 days after release, survival of field hatchlings was 48 and 36%, respectively, compared to 19 and 12% survival for hatchlings from the 22°C treatment, respectively.

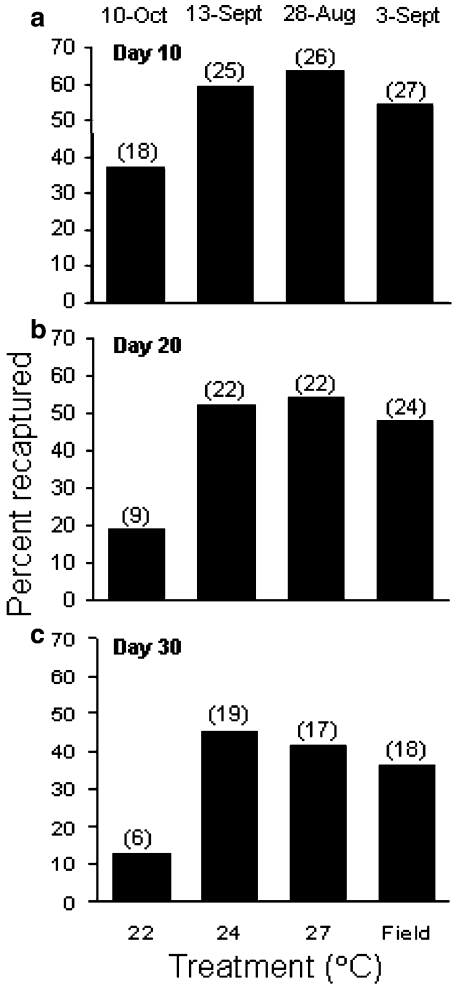

Fig. 2 Percent survival (recapture) of laboratory- and field-incubated hatchlings at **a** 10, **b** 20, and **c** 30 days after release in the field. **a** *Dates above bars* indicate mean date of hatching for laboratory individuals and the mean date of capture for field individuals. **a–c** *Numbers in parentheses* indicate the number of hatchlings recaptured at each time period from an initial release of 47, 42, and 41 hatchlings from the 22, 24, and 27°C treatments, respectively, and an initial capture of 50 field hatchlings. *Oct* October, *Sept* September, *Aug* August

Degree-days and effect of latitude on incubation length

The number of degree-days accumulated during each treatment ranged from 495 at 22°C, 521 at 24°C, to 564 at 27°C. The number of degree-days accumulated at recording stations based upon air temperature decreased with latitude (r^2 = 0.57, $F_{1,48}$ = 63.0, P < 0.001)

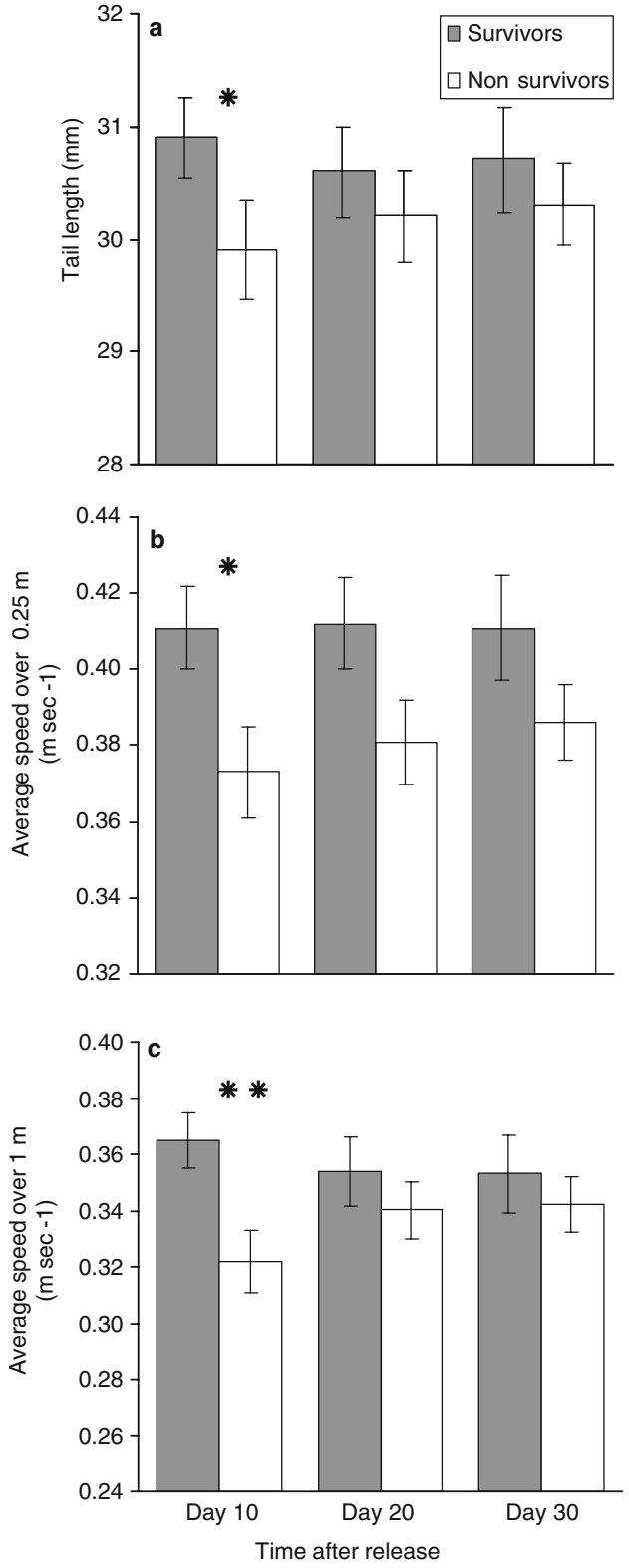

Fig. 3 Comparisons of **a** tail length, **b** average running speed over 0.25 m, and **c** average running speed over 1 m (overall mean, ±SE) independent of temperature treatment for laboratory-incubated hatchling *S. undulatus* that survived (*solid bars*) and did not survive (*open bars*) to 10, 20, and 30 days after release in the field. $*P < 0.05$, $**P < 0.01$

and ranged from >900 at 37°N to 103 at 50°N (Fig. 4a). Based upon a minimum requirement of ~495 degree-days above a threshold of 17°C to complete embryonic development, the predicted northern latitudinal limit of *S. undulatus* would lie at ~40.5°N. Similar results were obtained when soil temperature was used in degree-day calculations. The predicted latitudinal limit of *S. undulatus* based on soil temperature would lie at ~41.5°N (Fig. 4b). Eighty-four percent of the locations from 37 to 40°N (northernmost extent of the geographic distribution of *S. undulatus*) had ≥495 degree-days accumulated during June–September (based on air temperature). In contrast, only 11% of locations at latitudes from 41 to 50°N had ≥495 degree-days accumulated over the same period of time.

Discussion

A central question in ecological and evolutionary physiology is whether the variables measured in laboratory studies are ecologically relevant indicators of fitness under natural conditions (Irschick 2003). One difficulty is that the association between phenotypic traits and fitness is complex and poorly understood (Travis et al. 1999; Andrews et al. 2000). For example, the effects of incubation conditions on phenotypic traits of hatchling lizards may exist transiently during ontogeny and may therefore have relatively little effect on hatchling fitness (Irschick 2000; Qualls and Shine 2000). Another difficulty is that studies examining the association between incubation temperature and phenotypic traits such as locomotor performance and growth rate often yield conflicting results. For example, cool incubation temperatures may be associated with increases (Qualls and Andrews 1999) or decreases (Elphick and Shine 1998) in running speed. A further complication is that lizards in nature may rarely achieve running speeds recorded under laboratory conditions (Braña 2003; Irschick 2003). Running speed may depend on multiple interacting factors such as distance from refugia, energy expenditure, substrate temperature, benefits of prey capture, or the perceived threat of a predator. With this in mind, we determined whether the measures of fitness we selected were ecologically relevant by assessing the overall association between phenotype of laboratory hatchlings and subsequent survival in the field. Individuals that survived through the 30-day observation period had longer tails and faster average running speeds than individuals that did not survive this long (Fig. 3). TL and fast speed were thus relevant to the survival of hatchlings.

Table 3 Pair-wise contrasts of performance and growth between laboratory hatchlings from eggs incubated at 22, 24, and 27°C, and field-incubated hatchlings of *S. undulatus*. Values are mean ± SEM (number of hatchlings). Because incubation conditions and clutch of origin for field hatchlings were unknown, individual values instead of clutch means were used in statistical contrasts of field and laboratory hatchlings. Statistical tests were ANOVAs and ANCOVAs. Significant results are in *bold*. Within each row, values with *different letters* differ at $P < 0.05$

Trait	Field hatchlings	Laboratory hatchlings		
		22	24	27
Locomotion (m/s)				
Speed over 0.25 m	0.84 ± 0.032 (41)	0.69 ± 0.026 (47)	0.76 ± 0.044 (37)	0.77 ± 0.059 (37)
Speed over 1 m	0.47 ± 0.018 (41)	0.41 ± 0.018 (47)	0.39 ± 0.020 (37)	0.42 ± 0.021 (37)
Number of stops over 1 m	**3.7 ± 0.16 (41)** a	**3.2 ± 0.19 (47)** b	**3.6 ± 0.19 (37)** a, b	**3.7 ± 0.24 (37)** a, b
Growth in laboratory				
SVL (mm/day)	**0.19 ± 0.019 (41)** a	**0.23 ± 0.020 (47)** a, b	**0.21 ± 0.022 (42)** a, b	**0.30 ± 0.03 (41)** b
Mass (g/day)	0.015 ± 0.002 (41)	0.016 ± 0.002 (47)	0.013 ± 0.002 (42)	0.014 ± 0.002 (41)
Growth in field				
SVL (mm/day)	0.19 ± 0.02 (22)	0.11 ± 0.01 (10)	0.18 ± 0.03 (20)	0.19 ± 0.01 (22)
Mass (g/day)	0.019 ± 0.003 (22)	0.008 ± 0.001 (10)	0.015 ± 0.002 (20)	0.018 ± 0.001 (22)

Our results also support a direct connection between the phenotypic traits produced in the laboratory and in the field. For example, nest temperatures at our field sites near Blacksburg average about 27°C (Andrews et al. 2000). We therefore predicted that the incubation period and phenotypes of field hatchlings would be most similar to hatchlings from our 27°C treatment. In general, our observations supported this prediction: field hatchlings and the hatchlings from the 27°C treatment had similar incubation periods and survival in the field, while both groups differed from hatchlings from the 22°C treatment. For most performance traits, field hatchlings did not differ from laboratory hatchlings from the 24 and 27°C treatments.

Egg incubation temperature as a determinant of geographic distributions

Incubation of *S. undulatus* eggs at temperatures simulating those of nests at 44°N (22°C treatment) substantially increased incubation length, affected hatchling phenotypic traits (Table 1) and reduced survival in the field (Table 2; Fig. 2). In contrast, incubation at temperatures simulating those of nests at 42°N (24°C treatment) resulted in a relatively modest increase in incubation length, and with the exception of TL, hatchlings did not differ in any aspect of phenotype, locomotor performance, or survival compared to hatchlings incubated at a mean temperature of 27°C.

Our prediction that the northern latitudinal limit of *S. undulatus* would be associated with the minimum number of degree-days required for successful development of *S. undulatus* embryos was upheld (Fig. 4a, b). Based upon a minimum requirement of ~495 degree-days above a threshold of 17°C, the predicted northern distributional limit of *S. undulatus* in the central and eastern US would lie at ~40.5–41.5°N. Our predicted value based on air and soil temperatures corresponds to the observed northern distributional limit of ~40°N in the eastern US. One inherent limitation to our correlative model is that it is impossible to discern whether soil temperature is responsible for determining the northern distributional limit or whether it is set by some other factor not directly related to

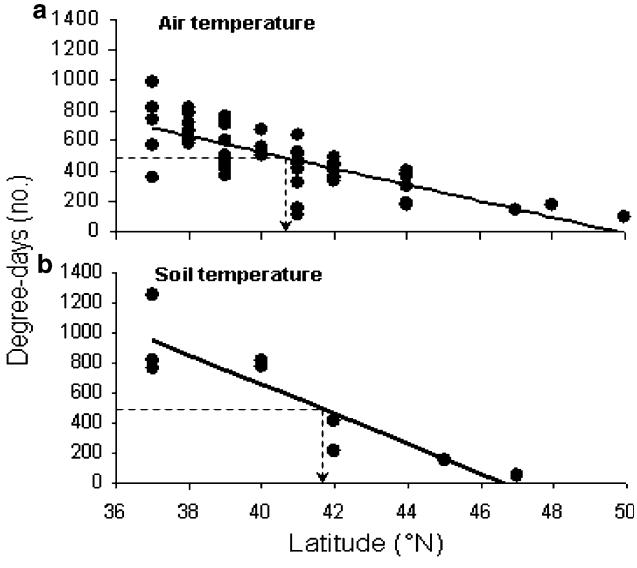

Fig. 4 Relationship between number of degree-days above 17°C accumulated over 122-day (June–September) season based upon **a** air temperature and **b** soil temperature as a function of latitude (37–50°N). *Dashed horizontal line* indicates the minimum estimated number of degree-days (495 days) required for successful incubation of *S. undulatus* embryos. *Arrow* indicates the predicted northern latitudinal limit for *S. undulatus* based upon 495 degree-days

incubation temperature The close association between our predicted and observed latitudinal limit, however, in combination with the results of our incubation experiments, suggests that soil temperature plays an important role in determining the northern distributional limit of *S. undulatus*.

How does incubation temperature limit populations of *S. undulatus* to ~40°N? Cool soil temperatures could influence geographic distribution by affecting embryo survival in nests. In contrast to this prediction, however, hatching success was high (>90%) in all treatments, suggesting that eggs could at least survive to hatching in nests at a mean temperature of 22°C at latitudes as far as 44°N. Because embryos appear to be most susceptible to the effects of cold temperatures late in development prior to hatching, however, eggs in nests at high latitudes could be exposed to lethally low temperatures during late summer and early autumn (Yntema 1978; St. Clair and Gregory 1991; Bobyn and Brooks 1994).

Temperature-induced effects on physiological processes during development could also influence the geographic distribution of *S. undulatus.* Hatchlings from the 22°C treatment had shorter SVL relative to mass, shorter tails, shorter HLS, and a higher frequency of tail deformities compared to hatchlings in the 24 and 27°C treatments (Table 1). Of these morphological traits, hind limb length in particular, has been shown to be a developmentally plastic trait, and is an important component of locomotor performance in lizards (Losos et al. 2000; Kolbe and Losos 2005). In *Anolis* lizards, for example, hatchlings with longer hind limbs had faster running speeds on broad surfaces whereas individuals with shorter limbs had greater maneuverability on narrow surfaces (Kolbe and Losos 2005). These observations suggest that incubation temperature could have important implications for both locomotor performance and microhabitat use for populations of *S. undulatus* in the field. Contrary to our prediction, however, locomotor performance did not differ among temperature treatments. While we were not able to determine whether lower survival in the 22°C treatment was directly related to hatchling morphology or performance, our results indicate that relatively long tails and relatively high speeds enhanced juvenile survival in populations of *S. undulatus* in the field (Fig. 3). In general, cool incubation temperature has a negative effect on hatchling fitness (e.g., Shine et al. 1997; Qualls and Shine 1998; Qualls and Andrews 1999; Andrews et al. 2000; Blouin-Demers et al. 2004). For example, hatchling *S. virgatus* incubated at a mean temperature of 20°C were smaller and ran more slowly than hatchlings from eggs incubated at a mean temperature of 25°C (Qualls and Andrews 1999). In other examples, hatchling black rat snakes (*Elaphe obsoleta*) incubated at 25°C had reduced locomotor performance compared to hatchlings from eggs incubated at 30°C and for pine snakes (*Pituophis melanolucus*), incubation at 23°C resulted in abnormal hatchling behaviors such as increased emergence time from nests compared to hatchlings from eggs incubated at 28°C (Burger 1991; Blouin-Demers et al. 2004).

Cool soil temperature could also affect hatchling survival indirectly by prolonging the incubation period and delaying the time of hatching. Eggs incubated at a mean temperature of 22°C (44°N) had an incubation period of ~100 days. Hatching would thus occur during the first part of September assuming eggs were laid at the beginning of June. Soil temperatures at that latitude, however, decrease toward the end of the summer (National Climate Data Center). For example, the average soil temperature at 44°N decreased from a mean of about 22°C in August to 19°C in September (National Climate Data Center 2005). At high latitudes, therefore, the incubation period would presumably be even longer than in our experiments due to decreasing temperatures during September and October. The putative benefit of early hatching is that hatchlings experience more favorable environmental conditions and thus are able to grow more and accumulate greater fat stores than hatchlings that emerge late in the season (Ferguson and Fox 1984; Shine 1997; but see Andrews et al. 2000). In our study, hatchlings from the 22°C treatment that were released late in the season had substantially slower growth in mass and lower survival than hatchings from the warmer treatments (Tables 1, 2; Fig. 2). For example, the average mass of hatchlings from the 22°C treatment was about 0.8 g at 10–25 days after release in the field compared to about 1 g for hatchlings from the 24 and 27°C treatments. Because growth rate in the laboratory did not differ among treatments, the reduced growth and survival of hatchlings from the 22°C treatment was presumably attributable to environmental factors such as reduced activity period, increased competition, and/or reduced food supply associated with hatching in October rather than August and September.

An unanswered question, however, is why does *S. undulatus* occur in localities such as Blacksburg which has a relatively cool climate for its latitude (37°N). According to our model, ~364 degree-days are accumulated during June–September while about 495 degree-days are required for successful development. One possible explanation is that gravid females could enhance embryonic development through prolonged egg retention. The selective basis for prolonged egg

retention in cold climates is that embryos would develop faster inside the thermoregulating female than in a nest (Packard et al. 1977; Shine 1983). The capacity of gravid females of *S. undulatus* to retain eggs, however, does not appear to vary among populations in cool versus warm climates. For example, the mean embryonic stage at oviposition for *S. undulatus* females from a population near the northern latitudinal limit at 39°N in New Jersey was very similar to that of females from a southern population at 33°N in South Carolina (New Jersey mean stage = 28.4 versus South Carolina mean stage = 28.8) (Parker et al. 2004). These values are similar to the mean embryonic stage at oviposition of 28.8 observed for *S. undulatus* females in Virginia. An alternative explanation is that *S. undulatus* embryos have evolved physiological adaptations to cold environmental temperature. *S. undulatus* embryos from populations in cool and warm climates, however, have similar thermal tolerances and similar developmental rates at a given temperature irrespective of population of origin (Andrews et al. 1997, 2000). Thus, there is no evidence of thermal adaptation by *S. undulatus* embryos to local climate conditions. The most likely explanation seems to be that gravid females compensate for low ambient temperature associated with high elevation (~625 m) through selection of warm sites on both landscape and micro-habitat scales (Andrews 2000; Shine 2004). In mountainous southwestern Virginia, *S. undulatus* is typically found on south-facing slopes. Moreover, females oviposit in open areas where nest sites are exposed to the sun for most of the day (A. Roberts and R. Andrews, personal communication). Selection of warm microhabitats for oviposition by gravid females has been observed for other species of reptiles inhabiting high latitudes and high elevations, including *Sceloporus* lizards (Andrews 2000), snakes (Blouin-Demers et al. 2004), skinks (Hecnar 1994; Shine 2004), and turtles (Litzgus and Brooks 1998). With increasing latitude and elevation, thermally favorable nesting sites would presumably become increasingly rare resulting in a patchy distribution of isolated populations. Availability of thermally appropriate nesting sites would thus appear to be critically important for populations of oviparous species living in cool climates near their latitudinal and elevational limits.

Our research suggests that the distributional limits of oviparous species in cold climates are determined both by absolute temperature and the length of time eggs are exposed to a favorable range of incubation temperatures. The effect of cold on egg incubation is complicated by the nature of seasonal temperature variation. For example, tropical latitudes are characterized by low seasonal temperature variation irrespective of elevation (Janzen 1967). Mean monthly temperatures at high elevations at tropical latitudes may vary by only a few degrees between the warmest and coldest months of the year. In contrast, temperate latitudes are characterized by high seasonal temperature variation. Temperatures at temperate latitudes, even at high elevations, are considerably warmer during summer than in winter. These latitudinal differences in thermal regimes could have important implications for the evolution of viviparity, or conversely the maintenance of oviparity. First, because mean monthly temperatures at high elevations at tropical latitudes are relatively cool all year round, embryonic development in these environments should be limited by low absolute temperature. Second, because high latitudes are relatively warm during summer but cold during winter, embryonic development in these environments should be limited both by ambient temperature and by the length of the season during which environmental temperatures are warm enough to support development of embryos. The above considerations suggest that the strength and/or nature of selective pressures on reproductive mode (oviparity versus viviparity) in these environments may not be the same. For example, relatively cool temperatures all year round at high elevations in tropical latitudes would likely constitute a substantial barrier to oviparous reproduction. Because winter temperatures in tropical latitudes are not severe, even at high elevations, selection for prolonged egg retention would not be constrained by seasonal temperature variation. Prolonged reproductive periods with gestation during winter and birth in spring are characteristic of several high-elevation tropical lizard species (Méndez-de la Cruz et al. 1998). Conversely, at high latitudes, both low absolute temperature and relatively short summers could prevent successful egg incubation and/or recruitment of hatchlings. Selection for prolonged egg retention at high latitudes would presumably enhance embryonic developmental rate and therefore result in earlier hatching compared to eggs in a nest. To our knowledge, no comprehensive studies of the thermal limits of oviparous reproduction (or recent origins of viviparity) at high elevations at tropical latitudes versus high latitudes have been conducted. Environmental constraints on reproductive mode related to embryo thermal biology remains a complex and productive area for future research.

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