

# Nest-Site Selection by *Psammodromus algirus* in a Laboratory Thermal Gradient

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**ABSTRACT.**—Oviposition site choice, which is one of the most important forms of parental care in oviparous reptiles, has major effects on incubation duration, reproductive success, and offspring phenotype. We determined the thermal characteristics of nest-sites selected by females of the lacertid lizard *Psammodromus algirus* in laboratory thermal gradients, and we linked results with information on the effects of temperature on incubation times and hatchling phenotypes. Temperatures at oviposition sites (mean 6 SD 5 26.0 6 1.9°C) were on average lower and had a lower variance than those at available sites (32.0 6 6.2°C). The duration of incubation decreased dramatically as temperature increased. In fact, the hatching dates predicted by the temperatures selected in the thermogradients were delayed with respect to the mean emergence dates observed in the field. Such disparity might occur because (1) natural incubation temperatures are variable rather than constant, (2) they are expected to rise later in the season, and (3) temperatures that are too high can severely compromise embryonic survival. In addition, females might select relatively low nest-site temperatures because hatching too early can force juveniles to face limited growth opportunities caused by food shortage in the Mediterranean environments in which they live.

In oviparous reptiles, two maternal decisions that have major effects on reproductive success are the allocation of the total investment into a few large versus many small offspring (Smith and Fretwell, 1974; Olsson and Shine, 1997a) and the selection of the nest-site (Kolbe and Janzen, 2002; Warner and Andrews, 2002; Brown and Shine, 2004). However, although the clutch size versus egg size trade-off has been the subject of numerous studies, much less is known about oviposition site choice, which is one of the most important forms of parental care in most oviparous species and certainly a critical component of both offspring fitness and maternal fitness. Physical characteristics of the nesting environment have important effects on embryogenesis and offspring traits. Temperature determines the rate of embryonic development (Van Damme et al., 1992; Shine, 2004) and, as a consequence, emergence dates. Hatching early may be especially advantageous in temperate regions if the time available for hatchling growth prior to winter is limited (Olsson and Shine, 1997b), but hatching too early can force juveniles to face serious difficulties caused by food shortage in Mediterranean climates where summer drought is severe (Iraeta et al., 2006). Because soft-shelled reptilian eggs increase their mass and volume during incubation by absorbing water from wet substrates (Packard, 1991; Ji

and Du, 2001), and this water exchange depends on the hydric potential of the substrate and its thermal conductivity, the selection of an appropriate nest environment is crucial for successful incubation. In addition, the thermal and hydric characteristics of the incubation environment influence several offspring phenotypic traits (Van Damme et al., 1992; Overall, 1994; Shine and Harlow, 1996; Brown and Shine, 2004), and some of these effects can persist for at least 20 weeks after hatching (Elphick and Shine, 1998).

Therefore, knowledge of the factors that determine nest-site selection is needed to fully understand the reproductive biology of any oviparous reptile. Within this context, our main objective was to determine the thermal characteristics of nest-sites selected by females of the Mediterranean lacertid *Psammodromus algirus* and to link that information with the effects of temperature on incubation times and hatchling phenotypes. Because oviposition sites selected in the wild are unknown (with the exception of sporadic observations of communal nestings; Pleguezuelos et al., 2004), we chose to design artificial thermogradients for oviposition to obtain baseline information for future research.

## MATERIALS AND METHODS

*Psammodromus algirus* is a medium-sized (adult snout-vent length 60–85 mm; mass 6–15 g), terrestrial, oviparous lacertid inhabiting

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shrub and woodland habitats in the western Mediterranean region. Hibernation takes place from October through February, courtship and egg-laying occur between April and June, and hatchlings are born in August and September (Veiga and Salvador 2001). Clutch size ranges between three and 12 eggs, and clutch frequency varies between one and two clutches per year.

During the first week of May 1995, we captured 25 lizards (12 males and 13 females) in a Mediterranean evergreen forest located near Madrid, Spain (El Pardo; 40°31'N, 03°47'W), and we transported them to the laboratory (Institute for Nature Conservation, Hasselt, Belgium) during the second week of May. We housed lizards in 100 cm long 34 cm wide 35 cm high terraria that provided standard accommodation, that is, refugia, water, and food (crickets and mealworms dusted with vitamin powder) ad libitum, and basking opportunities (120-W bulb suspended over one end of the terrarium, and subject to a 9.5:14.5 h light:dark cycle). All females shared their cage with a potential mate, and copulations were observed frequently. Whenever a female was found to bear oviductal eggs, she was moved to a thermogradient, where she was maintained in isolation until she laid her eggs. The female was then returned to her original cage and allowed to mate again so that she could produce a second clutch.

We constructed thermogradients designed for oviposition in five terraria (100 34 35 cm) by placing a 7-m long, 5-mm diameter, 50-W heating cable (Rena™) on the glass floor of each terrarium to achieve a uniform depth of the heating source. The cable was coiled so that coil density was maximal at the end opposite to the heating bulb. Coil density decreased gradually until the last loop reached about one-half of the cage length. We subsequently covered the cable with sand until we achieved a uniform depth of 10 cm of sand over the glass floor. A 1-cm thick panel of Styrofoam was located over the sand under the heating bulb to impede focal overheating of the soil beneath, and a piece of bark was added on top of the Styrofoam to provide a basking substrate. Finally, we spread a thin layer of leaf litter (the type of substrate usually preferred by *P. algirus*) over the entire surface of the cage. The heating cable was connected to a thermostat with its sensor placed at a depth of 5 cm on the warm end of the thermogradient, and the thermostat temperature was set at 38°C. Preliminary measurements indicated that this set-up produced a regular, smooth gradient in soil temperatures ranging from 22–55.5°C. These values are within the wide range of temperatures available in the field, according to

hourly measurements taken in June 2005 at the study area using TidBit StowAway temperature Data Loggers (Onset Computer Corporation, Bourne, MA): mean temperatures (reported 6.1 SD with range in parentheses,  $N = 720$  in all cases) were 29.1 6.15.4°C (8.4–66.7) in full sun above the soil, 22.4 6.4.9°C (12.5–33.8) in the shade within a *Cistus* shrub, and 21.6 6.1.9°C (17.3–25.6) buried at a depth of 15 cm below the soil surface. Although females might avoid the warm end of the gradient (where the tape was densely coiled) to oviposit at the bottom of the terraria while keeping away from the heating tape, the middle zone of the gradients, where there was a low density of cable loops, still offered a wide choice of biologically relevant soil temperatures. In fact, several females selected this middle zone for digging their nests and laying their eggs. We watered the entire surface of the thermogradients daily, attempting to maintain constant and uniform soil humidity within the terraria. However, although we did not monitor soil moisture, it is likely that the temperature gradient induced a moisture gradient that mimicked the inverse relationship between temperature and moisture in Mediterranean soils.

Before placing any female, we measured soil temperature of the thermogradients with a thermocouple connected to an electronic digital thermometer (60.1°C precision) at depths of 2, 4, 6, and 8 cm at each of 40 points (overall sample 5160 points) determined with reference to a grid (cell size 5 10 3 10 cm) spanning the entire surface of the terrarium. Temperatures were measured with the 120-W light on, using a perforated Styrofoam plate to avoid overestimation of the temperatures directly underneath the bulb. This provided an accurate “map” of available ground temperatures that was subsequently used to characterize the thermal environment of the egg-laying sites. A general linear model with temperature at 8-cm depth (measured on two different terraria on three different days) as the dependent variable and the position on the grid as the categorical predictor explained 93.9% of the variance in soil temperatures ( $F_{39,80} = 531.7, P < 0.001$ ; standard error of estimate 5.2.2°C).

Once a female was placed in the thermogradient, she was monitored daily to detect changes in body shape or weight that might indicate egg-laying. Excavating activity was also noted, and the location of tunnels was registered with respect to the reference grid. After oviposition, we removed the female from the cage, and we recorded her snout-vent length (SVL) and body mass. We searched carefully for the clutch, and we recorded the following data: date, clutch size, location with

TABLE 1. Mean characteristics (6 1 SD) of the nest-sites selected by females for laying their first and second clutches and results of repeated measures ANOVAs with clutch order as the within-subjects effect.

	First clutches (N 5 13)	Second clutches (N 5 9)	$F_{1,8}$	$P$
Mean depth (cm)	8.7 6 1.5	9.8 6 0.7	3.94	0.082
Selected temperature (°C)	25.8 6 2.0	26.3 6 1.7	0.007	0.937

respect to the reference grid, and depth. We estimated the temperature of the substrate at the nest site by referring the location and depth of the nest site to the temperature map. The terrarium was then restored to its original condition and another gravid female was housed in it.

We placed each egg in an individually labelled 150 ml plastic container filled with moistened vermiculite (8 g of water per 10 g of vermiculite, equivalent to 2200 kPa) that covered completely all the egg surface. We distributed the eggs from each clutch, following a split-clutch design, in three incubators set at constant temperatures of 25.5, 27.0, and 30.5°C (6 0.5°C). We sealed the containers hermetically with a lid to minimize evaporation, and we inspected eggs weekly to verify their viability. At the end of incubation, we searched daily for newly hatched lizards and we recorded hatching date, SVL, and mass. After all laboratory work had finished, all lizards (adults and juveniles) were released at the study area.

We analyzed our data using the General Linear Models (GLM) and Variance Components and Mixed-Model ANOVA/ANCOVA modules of the statistical package Statistica 6.1. The mixed-model, used for testing the significance of clutch (random factor) and temperature (fixed factor) on hatchling phenotypes, requires leaving the random effects independent of the fixed effects (Searle et al. 1992) by constructing appropriate error terms. Statistica 6.1 uses Satterthwaite's method of denominator synthesis to find the linear combinations of sources of random variation that serve as error terms for each effect. Thus, the degrees of freedom for the denominator mean square can be fractional rather than integer values, meaning fractions of sources of variation were used in synthesizing error terms for significance testing. Means are reported 6 1 SD.

## RESULTS

Of 13 females, nine laid two clutches, and four laid only one clutch. Nearly all females oviposited at the bottom of the terrarium (i.e., at the maximum possible depth) or close to it. Neither the temperature nor the depth of the selected sites differed significantly between first and second clutches (Table 1) or were related to

maternal SVL (temperature:  $r = 0.2089$ ,  $P = 0.351$ ; depth:  $r = 0.20102$ ,  $P = 0.964$ )

Temperatures at oviposition sites were on average lower and had a lower variance than those available in the egg-laying thermogradients (Fig. 1; mean nest temperature = 26.0 6 1.9°C, range = 22.9–29.1°C,  $N = 22$ ; mean of all available temperatures at all depths = 32.0 6 6.2°C, range = 22.9–55.5°C,  $N = 160$ ;  $t_{180} = 24.80$ ,  $P < 0.001$ ; Levene's test:  $F_{1,180} = 12.2$ ,  $P < 0.001$ ). Because females might choose deep sites for reasons not related to their thermal properties, we repeated the analysis using only the temperatures available at the deepest sites measured, and the same qualitative patterns were obtained (mean available temperature at 8 cm depth: 34.7 6 9.0°C, range = 22.9–55.5°C,  $N = 40$ ;  $t_{60} = 24.62$ ,  $P < 0.001$ ; Levene's test:  $F_{1,60} = 30.56$ ,  $P < 0.001$ ).

Four females excavated tunnels at the hot side of the egg-laying thermogradient and subsequently did not use them. Instead, they shifted to cooler sites to lay their eggs the following days (mean temperature of the tunnels excavated and not used: 48.3 6 1.4°C; mean temperature of the sites eventually used: 25.6 6 2.1°C; repeated measures ANOVA:  $F_{1,3} = 231.23$ ,  $P < 0.001$ ). This provides additional evidence of an active selection of relatively cool nest sites.

Temperature had a pronounced effect on incubation times ( $F_{2,78} = 2047.7$ ,  $P < 0.001$ ), explaining 98.1% of their variance. As expected,

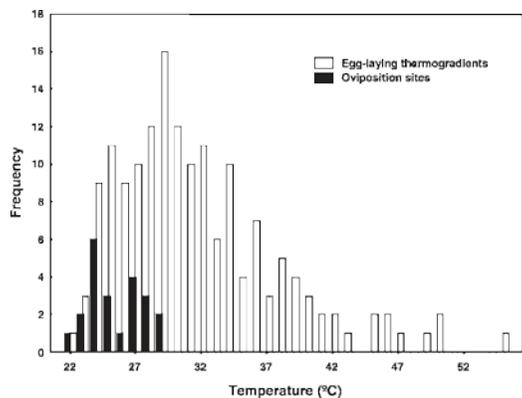


FIG. 1. Frequency distributions of temperatures available in the egg-laying thermogradients and temperatures selected at oviposition sites.

the duration of incubation decreased as temperature increased (mean duration: 94.9 ± 6.28 days at 25.5°C [ $N = 28$ ], 71.7 ± 6.23 days at 27°C [ $N = 28$ ], and 55.7 ± 6.12 days at 30.5°C [ $N = 25$ ]). Hatching success did not differ between incubation treatments ( $\chi^2 = 0.42$ ,  $P = 0.809$ ), but it was slightly lower at 30.5°C (0.80 at 25.5°C, 0.78 at 27°C, and 0.73 at 30.5°C).

A mixed-model ANOVA with incubation temperature as the fixed factor and clutch as the random factor showed that neither temperature nor clutch had a significant effect on hatchling SVL (temperature:  $F_{2,25.5} = 0.7$ ,  $P = 0.485$ ; clutch:  $F_{13,24.7} = 1.5$ ,  $P = 0.187$ ). However, the interaction term was highly significant ( $F_{26,30} = 3.5$ ,  $P = 0.001$ ), meaning that in some clutches hatchling size increased with temperature, whereas in others the opposite was true. The body mass of hatchlings was unaffected by temperature treatments, but it differed significantly among clutches (temperature:  $F_{2,26.5} = 0.6$ ,  $P = 0.561$ ; clutch:  $F_{13,21.5} = 9.7$ ,  $P = 0.001$ ; interaction:  $F_{26,30} = 1.0$ ,  $P = 0.485$ ), even when controlling for the effects of egg mass (mixed model ANCOVA; egg mass:  $F_{1,13.1} = 54.84$ ,  $P = 0.001$ ; temperature:  $F_{2,19.9} = 1.01$ ,  $P = 0.380$ ; clutch:  $F_{13,23.1} = 2.67$ ,  $P = 0.019$ ; temperature × clutch interaction:  $F_{26,29} = 1.02$ ,  $P = 0.478$ ).

#### DISCUSSION

Our results indicate that females did not lay their eggs at random locations but were selective in their choice of oviposition sites (Shine and Harlow, 1996; Warner and Andrews, 2002; Brown and Shine, 2004). This selection was based on substrate temperature, because the temperatures of the locations they used were on average much lower and within a much narrower range than those available in the egg-laying thermogradients. It should be noted that our experimental set-up reflected the sort of temperature conditions nesting females will have to choose from in the field, where nest-sites with diurnal temperatures in the range 23–29°C should be readily available within the first 10 cm of soil depth according to our field data about soil temperatures.

The mean temperature of the nesting sites (26°C) was surprisingly low in light of our results on the thermal dependence of incubation times. Indeed, eggs incubated at a constant temperature of 25.5°C took about three months to hatch. This would delay hatching in the field until late September, which is later than the usual emergence of newborns (August and September; Civantos et al., 1999; Iraeta et al., 2006). We can offer some possible explanations for such disparity. First, our incubation experiments were performed at a constant tempera-

ture for each thermal regime, and it is known that seasonal and daily variation in incubation temperatures, independently of mean values, can substantially reduce incubation times (Overall, 1994; Shine and Harlow, 1996; Shine, 2004). Second, and perhaps more important, ground temperatures tend to increase throughout the summer. Surface ground temperatures at the lizards' capture site were on average 4.5°C higher in July than in June, a difference that could be much greater at certain locations (e.g. with low vegetation cover) or times of day (Díaz and Cabezas-Díaz, 2004). This seasonal variation, which could hasten embryonic development even if thermal variations are buffered at higher soil depths (Shine, 2004), would elevate the mean temperature of incubation sites well above the initial value of approximately 26°C. This would promote earlier hatching while reducing the potential negative effects of high temperature, that are less pronounced in the later stages of embryonic development (Flatt et al., 2001; Shine and Elphick, 2001). Third, an alternative explanation for the low temperatures selected, and associated prolongation of incubation times, is that hatching too early can force juveniles to face limited growth opportunities caused by food shortage in Mediterranean climates with a severe summer drought (Iraeta et al., 2006). Fourth, another possible reason may be that cool incubation temperatures produce better quality offspring (Van Damme et al., 1992). However, our data indicate that the effects of temperature on hatching success or hatchling size (which is a good predictor of juvenile survivorship; Díaz et al., 2005) are unimportant within the 25.5–30.5°C range tested (although the significant interaction between the effects of temperature and clutch on SVL might be indicative of different reaction norms). Finally, females may not have based their selection of oviposition sites on temperature, but on an associated characteristic such as soil humidity, or on a combination of temperature and moisture. As such, they may trade off the benefits of increased egg development at higher temperatures with the requirements of water absorption by the eggs during incubation.

We emphasize the importance of knowing the thermal characteristics of the oviposition sites selected by females for a proper evaluation of habitat quality. Thus, the lack of potential nest-sites can determine the limits of distribution ranges (Shine et al., 2003), and, as a consequence, it can have implications for the management and conservation of populations (Castilla and Swallow, 1995). In fact, only a direct approach such as the one used here can provide reliable data about the temperatures selected by females to lay their eggs. Estimating these temperatures

on the basis of the thermal preferences of females during the breeding season (circa 33°C in May; Díaz et al., 2006), or the thermal dependence of incubation times (results of this study), would clearly lead to an overestimation of nest-site temperatures.

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