



RESEARCH PAPER

Social and Thermal Cues Influence Nest-site Selection in a Nocturnal Gecko, *Oedura lesueurii*

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Received: March 24, 2011
Initial acceptance: June 6, 2011
Final acceptance: June 11, 2011
(J. Wright)

doi: 10.1111/j.1439-0310.2011.01931.x

Abstract

In oviparous species lacking parental care, successful reproduction depends on females selecting nest sites that facilitate embryonic development. Such sites may be limited in the environment, which can lead to multiple females using the same nest site simultaneously. However, there are several alternative explanations for communal nesting, including natal homing, predator satiation, and adaptive benefits to offspring. We used laboratory experiments to evaluate three hypotheses about nest-site selection in velvet geckos (*Oedura lesueurii*), which often nest communally. We investigated whether the trend to nest communally is influenced by the following: (1) evidence of previous nesting (hatched eggshells); (2) body size; and/or (3) thermal regimes. When given the choice, females laid their eggs in shelters containing hatched eggshells rather than in empty shelters, and this was not influenced by body size. Females selected nest sites that were cooler than their own mean selected body temperatures, suggesting that thermal requirements of their developing embryos could outweigh their own thermoregulatory preferences. Field observations of natal homing and high predation rates on gravid females suggest that imprinting on nest sites and/or predator swamping also play roles in communal nesting. Collectively, our results suggest that female velvet geckos use multiple cues to select appropriate nest sites, and hence that multiple mechanisms result in communal nesting behavior in this species.

Introduction

In egg-laying species, females can influence egg survival, offspring phenotype, and fitness through nest-site selection (Resetarits 1996; Shine & Harlow 1996; Blouin-Demers et al. 2004; Brown & Shine 2004; Rudolf & Rödel 2005). Females should therefore select the best oviposition sites available (Jaenike 1978; Martin 1998). However, in many taxa, multiple females often lay their eggs communally, suggesting that maternal oviposition behavior is not solely dependent on nest-site quality but can be influenced by social facilitation (Jamieson 1995; Brown & Shine 2005; Radder & Shine 2007; Refsnider et al. 2010),

risk of predation upon the reproducing adult (Martin 1998; Spencer 2002), or a limited availability of suitable egg-laying sites (Rand & Dugan 1983; Blouin-Demers et al. 2004).

Communal egg-laying is very common in reptiles (known in >480 species: Doody et al. 2009), and this behavior often reflects active maternal preference rather than being an accidental by-product of limited nest-site availability (Brown & Shine 2005; Radder & Shine 2007). For example, in some scincid lizards (Radder & Shine 2007), colubrid snakes (Plummer 1981; Brown & Shine 2005), and frogs (e.g., Howard 1980), mothers are attracted to nest sites used by other females, even when suitable alternative nest

sites are available. However, the nest-site scarcity hypothesis is the most parsimonious explanation for communal egg laying (Doody et al. 2009) and predicts that suitable nest sites (e.g., those providing suitable thermal and/or hydric conditions) are a limited resource. This situation would favor communal nesting because there are not enough suitable alternative nest sites available (Howard 1980; Rand & Dugan 1983; Radder & Shine 2007). Rejecting the nest-site scarcity hypothesis is the first step in any attempt to discern the adaptive benefits of communal nesting (Doody et al. 2009).

Over half of the lizard species reported to nest communally are geckos (Doody et al. 2009), yet little is known about the mechanisms influencing nest-site selection in this diverse and widely distributed group. We studied velvet geckos (*Oedura lesueurii*), which inhabit rock outcrop habitats in southeastern Australia, and lay their eggs inside rock crevices. These crevices are scarce in the environment and maintain relatively moderate, stable thermal and hydric regimes, which prevent eggs from desiccating (Pike et al. 2010). Although velvet gecko nests can consist of a single clutch of eggs or multiple clutches from different females, 85% of nests laid inside crevices are communal (Webb et al. 2008; Pike et al. 2010). Together, these field observations suggest that social and/or abiotic cues (such as temperature or humidity) influence nesting behavior. Here, we use laboratory experiments to evaluate three hypotheses about nest-site selection in this species, by investigating whether (1) females use cues of previous nesting (hatched eggshells) to select nest sites; (2) female body size influences the propensity to nest communally; and (3) temperature is used as a cue for selecting nest sites. We offered females identical potential nest sites, half of which contained evidence of previous nesting by females and successful hatching (hatched eggshells). Importantly, body size may play a role in whether females nest communally; for example, smaller (presumably younger and thus inexperienced) females may be using the nest-site choices of other females to help locate suitable nest sites (Doody et al. 2009). For velvet geckos, the most readily available potential nest sites (loose surface rocks) are much too hot for successful embryonic development, and eggs laid there do not produce hatchlings (Pike et al. 2010). We thus predicted that females would use temperature as a cue when selecting nest sites and that nesting females would lay their eggs in sites that contain temperatures suitable for embryonic development (as opposed to, for example, their own preferred body temperatures).

Methods

Gecko Collection and Husbandry

We collected gravid velvet geckos from Morton National Park in Oct 2008 and Royal National Park in Oct 2009. Both sites are located in southeastern New South Wales, Australia. Geckos were brought back to the laboratory, measured (snout-vent length [SVL] to 1 mm and body mass to 0.01 g), and housed individually in ventilated plastic cages (200 × 140 × 55 mm) inside a temperature-controlled room (constant 20.0°C; overhead lights on a 12:12 cycle). Cages were situated on timer-controlled heating racks to create a thermal gradient of 24–40°C during the day, which dropped to room temperature at night. Geckos were fed crickets dusted with vitamin supplements two to three times weekly, and water was available *ad libitum*. Each cage contained a substrate of moist vermiculite, a water dish, and two plastic shelters consisting of opaque plastic tubes cut in half lengthwise. Shelters were identical and measured 80 × 40 mm in size, but their configuration within the cage differed in our two nest-site selection experiments. Cages were checked twice daily for freshly laid eggs, the location of any eggs was recorded, and the vermiculite was remoistened so that any eggs laid would not desiccate.

Nest-site Selection Experiments

In the first experiment, we tested whether the presence of hatched eggshells influenced nest-site selection of gravid females (collected from Morton National Park) and whether female body size affected this selection. We offered females the choice of laying eggs within a shelter containing hatched eggshells ($n = 2$, the clutch size of a single female; Doughty 1997) or within an identical shelter that did not contain any eggshells. The two shelters were arranged parallel to one another and situated along the long axis of the cage, such that each experienced equivalent thermal regimes. The hatched eggshells were collected from a communal nest in the field and randomly placed into one of the shelters. We compared the proportion of females nesting in each shelter type using a chi-squared test. Our null hypothesis was that the presence of hatched eggshells would not influence nest-site selection, and hence, females would use both shelter types as nest sites. Female experience could also play a role in nest-site selection, and we predicted that smaller females (i.e., younger and potentially less experi-

enced nesters; Webb et al. 2008) would be more selective in their nest-site choice than larger (i.e., more experienced) females. Our mark/recapture studies have shown that as velvet gecko body size increases, growth rate decreases and that older reproductive females are larger than younger reproductive females (Webb et al. 2008). We tested whether body size (SVL and body mass) influenced nest-site selection using *t*-tests.

In the second experiment, we tested whether thermal cues influenced nest-site selection (using geckos collected from Royal National Park). Although nests laid in the field differ in both thermal and hydric regimes from available nest sites, these two variables are intercorrelated (Pike et al. 2010). Here, we control for hydric conditions and focus exclusively on temperature differences. For this experiment, we arranged the two shelters end to end, creating one contiguous shelter spanning most of the cage length. This provided females access to temperatures within the shelters ranging from 24.5 to 35°C during the day. Instead of creating a thermal gradient allowing lizards access to this entire range of temperatures, the small cage size (200 × 140 × 55 mm) resulted in two distinct regions of temperature as follows: a 'hot' end (one-third of the cage, situated on the heating strip) that ranged from 30 to 35°C and a 'cool' end (the remaining two-thirds of the cage, away from the heating strip) that ranged from 20 to 24.5°C. The temperature between these two distinct regions changed very abruptly (i.e., from 24.5 to 30°C over <0.5 cm). These temperatures are similar to those found beneath rocks (hot end) and inside nest crevices (the cool end) in the field (Pike et al. 2010). Rocks are by far the most commonly available retreat site in the field but are rarely used as nest sites (e.g., 13% of nests were laid beneath rocks, none of which hatched); by contrast, crevices are rare in the environment but are the most commonly used nest sites (Pike et al. 2010).

By offering gravid females two distinct temperature ranges, we could test whether thermal cues influence nest-site selection. As both temperature regimes encompassed different amounts of area within the cage, if gravid females are selecting nest sites randomly (i.e., without regard to thermal cues), we would expect one-third of the females to lay their eggs in the hot end and two-thirds to lay in the cool end. We used a contingency table analysis to compare the number of females laying in each thermal regime to these expected values.

Temperature Selection by Gravid Females

Because female geckos could lay their eggs in sites that match their own selected body temperatures (as opposed to selecting a nest site suitable for embryonic development), we quantified temperature selection by gravid females collected from Royal National Park and compared this with: (1) nest temperatures under field conditions and (2) temperatures of nest sites selected in the laboratory. To create a thermal gradient, we housed geckos individually in large plastic cages (290 × 200 × 100 mm) with one end of the cage sitting on a heating strip. Inside the cage were two sets of two terra cotta tiles (110 × 220 mm). The tiles contained grooves, which when stacked together formed crevices (approx. 2–3 cm wide) in which geckos could fit. Tiles were arranged along the long axis of the cage, such that geckos could move within the crevices and access temperatures ranging from 22.5 to 35.4°C during the day. We allowed geckos to acclimate for 48 h before measuring female body temperatures using a non-contact infrared thermometer (Raytek Ranger ST) following the methods of Hare et al. (2007). These values were compared to a smaller number of cloacal temperatures taken simultaneously, and in all cases were well within 1°C of each other (with no systematic bias). To measure female body temperatures, we lifted the tiles and immediately measured the gecko's temperature using the infrared thermometer.

We compared the mean selected body temperatures of gravid females with the daily maximum temperature of the nest sites selected by females in the field and in the laboratory. We used miniature dataloggers (Thermochron iButtons) to record temperatures inside gecko nest crevices in the field (see Pike et al. 2010 for full details). Temperatures were measured at 1.5 h intervals, and we used these data to calculate daily maximum temperatures within nest crevices over a 2-wk period at the beginning of the incubation period (Nov. 5–18, 2007). For each nest crevice, we calculated the average daily maximum temperature during this period and compared this with female selected body temperatures using a *t*-test. For the laboratory comparison, we used a one-sample *t*-test to compare female selected body temperatures with the daily maximum temperature at the selected end of the cage (see Results). All cages had similar thermal regimes and thus represented a single sample for analysis. These two analyses test the null hypothesis that gravid females select nest sites within their preferred temperature range,

as opposed to other factors such as the thermal tolerance of developing embryos.

Results

In both nest-site selection experiments, all gravid females laid their eggs inside the shelter sites. Velvet geckos preferentially nested in locations that provided evidence of prior successful nesting; 73.4% of new clutches were laid in the shelter containing hatched eggshells ($n = 34$; $\chi^2 = 7.53$, $df = 1$, $p = 0.006$). However, body size did not influence nest-site selection; there was no significant difference in the sizes of females that nested with hatched eggshells and those that nested away from hatched eggshells (SVL: $t_{33} = 0.86$, $p = 0.40$; mass: $t_{33} = -0.96$, $p = 0.35$).

Velvet geckos also nested non-randomly with respect to thermal regimes inside shelter sites ($n = 11$; $\chi^2 = 4.89$, $df = 1$, $p = 0.02$); all of the gravid females (100%) laid their eggs in the coolest part of the cage. One explanation for this pattern is that females preferred cooler temperatures (i.e., reaching a maximum of 24.5°C). However, in thermal gradients, gravid females ($n = 18$) selected higher daytime temperatures (24.4–34.1°C, mean = 30.8°C) than temperatures inside nests laid in the field ($N = 17$ nest crevices, mean daily maximum temperature = 23.9 ± 0.4 ; $t_{33} = 10.55$, $p < 0.0001$) and in the laboratory (daily maximum of 24.5°C; one-sample t -test: $t_{17} = 11.79$, $p < 0.0001$).

Discussion

Our laboratory experiments revealed that velvet geckos use both biotic and abiotic cues to select nest sites. Gravid females preferred to lay eggs inside shelters that provided evidence of successful hatchling emergence (hatched eggshells) over identical, but empty, shelters. Female body size did not influence this behavior. Females also selected nest sites that maintained relatively cool, stable temperatures and avoided high temperatures (with dry conditions) that cause egg mortality under field conditions (Pike et al. 2010). However, this result was not caused by females preferring cooler temperatures; in fact, gravid females selected high body temperatures that were more similar to the temperatures at the warm end of the cage, which nesting females avoided. In the field, gravid females shelter beneath rocks that are too hot and dry for egg incubation, but they lay their eggs nest inside crevices with thermal and hydric conditions suitable for embryonic development

(Webb & Shine 1998; Webb et al. 2008; Pike et al. 2010). Collectively, these results suggest that velvet geckos use multiple independent cues to select suitable nest sites.

Nesting communally has many adaptive benefits and has been hypothesized to reduce predation (on gravid females and/or offspring via predator swamping), increase egg survival (by facilitating thermal or hydric exchange with nearby eggs), and save females the effort of having to find their own nest site (reviewed by Doody et al. 2009). Communal nesting also can evolve when nest sites are inherited (Graves & Duvall 1995); sea turtles provide a classic example of natal homing (Meylan et al. 1990), and this behavior has also been recorded in snakes (Brown & Shine 2007). During a mark/recapture study of velvet geckos, we recorded one hatchling returning to her natal nest as a gravid female (Webb et al. 2008), suggesting that nest sites may be inherited, which could help explain patterns of communal nesting in this species. We have recaptured 13-yr-old gravid female velvet geckos (J. K. Webb, unpublished data), and because females mature at age 3–4 yr (Webb et al. 2008), there should be substantial overlap in nesting by mothers and their offspring. These ideas could be tested using molecular techniques (e.g., Meylan et al. 1990). However, natal homing does not explain why females exposed to novel nest sites in the laboratory preferred to nest with hatched eggshells, suggesting other adaptive benefits. One plausible explanation is to reduce predation risk on gravid females; we have observed predatory broad-headed snakes (*Hoplocephalus bungaroides*) waiting inside gecko nest crevices, and 35% of the velvet geckos consumed by this species were gravid females (Webb et al. 2008). Females can plausibly reduce their risk of predation by aggregating inside nests and laying eggs synchronously; in such situations, the risk of predation to individual females may be low (Doody et al. 2009). Furthermore, a snake waiting to prey upon gravid females entering the nest crevice will likely consume geckos before they are able to lay eggs, and thus, the presence of eggshells may indicate that snakes are either unable to access the nest crevice or at least have not been present in the area in previous nesting seasons. Avoidance of predators while nesting could be especially important in this long-lived, late maturing species that produces a single clutch annually (Webb et al. 2008). In the field, predation on eggs is low, and hatching success was similar in single and communal nests (suggesting no difference in egg predation rates; Pike et al. 2010). Importantly, the natal

homing and predator satiation hypotheses are not independent; predator satiation may be a by-product of natal homing, which complicates any attempt to determine the causal mechanism behind communal nesting. Finally, velvet geckos lay soft-shelled eggs that are extremely vulnerable to desiccation (Packard 1991), and contact with other eggs may improve water transfer, thereby reducing desiccation risk and increasing hatchling body size (Radder & Shine 2007). Together, our field observations and laboratory experiments indicate that nest-site selection depends on both biotic and abiotic factors and that there may be multiple, concurrent reasons for communal nesting within a single species.

Acknowledgements

We thank Betsy Roznik for help with fieldwork, Amanda Lane for gecko husbandry, and Rick Shine for discussion and encouragement. Funding was provided by the Australian Reptile Park, Australian Research Council, Department of Environment, Climate Change, and Water New South Wales, Forests New South Wales, the Hawkesbury-Nepean Catchment Authority, Royal Zoological Society of New South Wales (Ethel Mary Read Grant to DAP), and Zoos Victoria. DAP was supported by an Endeavour International Postgraduate Research Scholarship and an International Postgraduate Award funded by the Australian Department of Education, Science and Training and the University of Sydney. RMA was supported by NSF award DEB-0844523 (PI Aaron Bauer) and a 2009 Visiting Fellowship for Women from the School of Biological Sciences, University of Sydney.

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