



The role of temperature and humidity in python nest site selection

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Parental care is a convergent trait shown by a broad range of taxa. Often, successful parents must balance multiple developmental variables (e.g. embryonic water balance and thermoregulation). Pythons have recently emerged as valuable parental care models because females show simple egg-brooding behaviours that significantly influence variables of widespread importance (i.e. embryonic predation, hydration, temperature and respiration). Nest site selection is an important parental behaviour that has been shown to enhance several developmental variables in numerous taxa. In pythons, where brooding can substantially mitigate environmental conditions to enhance the developmental environment, it is unclear to what extent females utilize environmental cues in selecting their nest site. Thus, we determined whether nest humidity and temperature influence python nest site selection because these variables influence python egg-brooding behaviour and are strongly associated with offspring fitness. We created a radial maze with three nest site options: O_{TH} : optimal temperature (31.5°C) and humidity ($23\text{ g/m}^3\text{ H}_2\text{O}$), as determined by previous studies; O_T : optimal temperature, suboptimal humidity ($13\text{ g/m}^3\text{ H}_2\text{O}$); O_H : suboptimal temperature (25°C) and optimal humidity. We monitored the locations of female Children's pythons, *Antaresia childreni*, during gravidity, at oviposition and when nonreproductive. Females significantly preferred O_{TH} over O_T and O_H during both reproductive stages; yet, female choice was not significantly different from random when females were nonreproductive. These results, when considered with previous results, demonstrate that female pythons sense environmental temperature and humidity and use this information at multiple time points (i.e. during gravidity, at oviposition and during egg brooding) to enhance the developmental environment of their offspring.

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Parental care represents an adaptation of widespread importance as it is a convergent trait exploited by a broad range of taxa (Clutton-Brock 1991). In addition to its direct impact on parent and offspring fitness, parental care may be inextricably involved in other evolutionary processes, such as the degree/direction of sexual selection and the evolution of endothermy (Trivers 1972; Clutton-Brock 1991; Farmer 2000). Broadly defined, parental care is any nongenetic contribution by a parent that appears likely to increase the fitness of its offspring, including parental care behaviours and physiological events (e.g. yolk deposition) (modified from Clutton-Brock 1991).

Despite the prevalence of care prior to parturition (i.e. pre-oviposition or preparturition), the majority of parental care research has focused on postparturition behaviours such as egg attendance, neonate feeding or offspring training (Clutton-Brock 1991). However, preparative decision making can have profound fitness implications on both parents and offspring. For example, relative to their cool-nesting counterparts, female water pythons, *Liasis fuscus*,

that choose to oviposit in warm nest sites attend their eggs for a shorter duration (mean: 7 days versus 58 days), and their offspring have higher rates of yearling survival (Madsen & Shine 1999). Because of this decision, warm-nesting females show higher survival rates and reproductive frequency than cool-nesting females (Madsen & Shine 1999). As a result of such effects, adaptive nest site or oviposition site selection is incredibly widespread among animal taxa (e.g. fruit flies: Dillon et al. 2009; butterflies: Rausher 1979; aquatic beetles: Brodin et al. 2006; treefrogs: Takahashi 2007; newts: Dvorak & Gvozdek 2009; turtles: Spencer 2002; passerine birds: Citta & Lindberg 2007).

Adaptive nest site selection generally results from the ability of females to incorporate cues from the biotic (e.g. evidence of predators or successful prior incubation) or abiotic (e.g. thermal or hydric characteristics) environment of potential nest sites. The latter is shown by taxa ranging from fruit flies (Dillon et al. 2009) to snakes (Brown & Shine 2004), because their embryos are significantly affected by the physical characteristics of their developmental environment (Deeming & Ferguson 1991; Deeming 2004). In the context of both pre- and postparturition parental care behaviour, pythons offer promising insight into the sensitivity of parental decision making to the thermal and hydric properties of the nest environment (Stahlschmidt & DeNardo 2009a, 2010). Python embryos are very

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sensitive to developmental temperature (Indian python, *Python molurus*: Vinegar 1973; African rock python, *Python sebae*: Branch & Patterson 1975; diamond python, *Morelia spilota spilota*: Harlow & Grigg 1984; *L. m. fuscus*: Shine et al. 1997) and humidity (Children's python, *Antaresia childreni*: Lourdais et al. 2007). Potentially, as a result, nest temperature and humidity have an interactive effect on egg-brooding behaviour in *A. childreni*. Female *A. childreni* spend less time tightly coiled around their clutches when nest temperatures are in the increasing phase of the daily temperature cycle (Stahlschmidt & DeNardo 2010). This decision increases the rate of clutch warming by reducing the resistance that the female's body provides between the relatively warmer environment and the clutch (Stahlschmidt et al. 2008; Stahlschmidt & DeNardo 2010). However, in order to maintain water balance, females do not show reduced tight coiling during increasing temperatures if nest conditions are dry (Stahlschmidt & DeNardo 2010).

While temperature and humidity have been shown to influence brooding behaviour, the roles of these variables in python nest site selection are unclear and have not been experimentally tested. We used a simple behavioural paradigm to test several competing hypotheses regarding maternal decision making in *A. childreni*. Because females typically affect the temperature and humidity of their eggs' incubation environment through egg brooding, adaptive nest site selection in pythons may not be as critical as it is in non-brooding species. Thus, it may be under minimal, if any, selective pressure (Hypothesis 1). This hypothesis predicts that python nest site selection will not be affected by nest temperature or humidity. Alternatively, python nest site selection may be influenced by a single abiotic characteristic of potential nest sites (Hypothesis 2). Hypothesis 2 predicts females will choose to oviposit in refuges that optimize one variable independent of the incubation quality of another variable. For example, if python nest site selection is solely affected by temperature, females would indiscriminately oviposit in refuges with optimal temperature (i.e. 31 °C, which approximates the preferred body temperature of *A. childreni* during gravidity; Lourdais et al. 2008) independent of nest humidity. Because multiple aspects of the developmental environment affect embryo fitness, python nest site selection may be influenced by multiple key abiotic factors of the nest (Hypothesis 3). For example, this third hypothesis would predict that females will predominantly choose to oviposit in refuges exhibiting optimal developmental temperature and humidity (23 g/m³ H₂O, which approximates the humidity at which brooded *A. childreni* eggs show high developmental and hatching success; Lourdais et al. 2007).

When considered with previous research (reviewed in Stahlschmidt & DeNardo 2011), our study shows the extent to which female pythons meet the thermal and hydric needs of their offspring. For example, females may meet these offspring requirements during retention of embryos (gravidity), at partition (oviposition) and postpartition (egg brooding). Our knowledge of such comprehensive regulation of developmental temperature and hydration is currently limited to highly derived taxa with more complex parental care such as mammals and mound-building megapode birds (Clutton-Brock 1991; Jones & Birks 1992). Our study also provides insight to the adaptive significance of a widespread parental care behaviour in a simpler parental care model.

METHODS

Study Species and Reproductive Assessments

To test our hypotheses, we used 11 reproductive female *A. childreni* from a long-term captive colony at Arizona State University. *Antaresia childreni* are medium-sized (up to 1.2 m snout–vent length, 700 g body mass), constricting snakes that

Table 1

Summary of *A. childreni* maternal and clutch characteristics ($N = 11$)

| | Mean ± SE |
|--|-----------|
| Maternal | |
| Gravid mass (g) | 522 ± 19 |
| Postoviposition mass (g) | 374 ± 10 |
| Relative clutch mass (clutch mass divided by maternal mass, %) | 32 ± 3 |
| Clutch | |
| Size (number of eggs) | 10 ± 1 |
| Mass (g) | 120 ± 13 |

inhabit rocky areas in northern Australia (Wilson & Swan 2008). Husbandry and breeding of the animals followed that described previously (Lourdais et al. 2007). During the reproductive season (January–May 2010), we determined vitellogenic or gravid status of female pythons through weekly ultrasonographic scans using a portable ultrasound system (Concept/MCV, Dynamic Imaging, Livingston, U.K.). Prior to oviposition (mean ± SE = 11 ± 3 days), we weighed (±1 g) each snake before moving it into a radial maze to assess refuge preference (Table 1). All procedures used in this study were approved by the Arizona State University Institutional Animal Care and Use Committee (protocol number 08-967R).

Experimental Design

We used a radial maze behavioural paradigm to determine environmental preference during gravidity, at oviposition and after reproduction. Because field data do not exist for *A. childreni* nests, we used temperatures that closely represented those found in the nests of *L. m. fuscus* (25 °C and 31 °C), which is sympatric with *A. childreni*, and nest humidities (13 or 23 g/m³ H₂O) that were ecologically relevant (Madsen & Shine 1999; Z. R. Stahlschmidt, D. F. DeNardo & R. Shine, unpublished data). Also, we have shown that female *A. childreni* alter the relative use of various brooding postures in response to shifts in these specific temperatures and humidity levels (Stahlschmidt & DeNardo 2010). Because of these ecological and biological aspects, we only used suboptimal levels of temperature and humidity as opposed to superoptimal levels (e.g. >31 °C and/or >23 g/m³ H₂O).

We created a radial maze with three nest site options: O_{TH}: optimal temperature (31.5 °C) and humidity (23 g/m³ H₂O); O_T: optimal temperature, suboptimal humidity (13 g/m³ H₂O); O_H: suboptimal temperature (25 °C) and optimal humidity (Fig. 1a). Each radial maze had three 46 cm long (5.1 cm internal diameter) plastic tunnels that terminated in a 1.9 litre refuge chamber

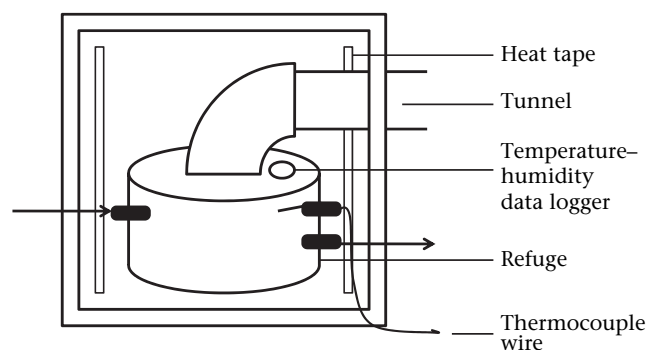


Figure 1. Schematic of the temperature-controlled refuge box placed at the terminus of each radial arm. Arrows denote humidity-controlled airflow (approximately 500 ml/min) through the refuge. Thermocouples and temperature–humidity data loggers were positioned inside each refuge with the thermocouple feeding back to a data logger that controlled the heat tape to precisely control nest site temperature.

(internal radius and height: 8.5 cm). We stacked four radial mazes on top of one another and randomly assigned the arrangement of refuges (i.e. O_{TH} , O_T , O_H) for each radial maze. We housed all radial mazes in a walk-in environmental chamber maintained at $25 \pm 1^\circ\text{C}$. Each refuge chamber was within a temperature-controlled insulated box ($42 \times 26 \times 35$ cm; Fig. 1). We heated O_{TH} and O_T refuges using heat tape (Flexwatt, Flexwatt Corp., West Wareham, MA, U.S.A.) positioned along the internal walls of the insulated boxes. We used a data logger (21X, Campbell Scientific Instruments, Logan, UT, U.S.A.) to control the power to the heat tape based on 21X input from a Type T thermocouple positioned 2 cm into each refuge (Fig. 1).

To control refuge humidity, we created influent air of known humidity by flowing air through a heated water column and then sending the air through a condensation chamber held at the desired dew point. We used a 51-litre refrigerator maintained at 16°C as a condensation chamber to achieve $13\text{ g/m}^3\text{ H}_2\text{O}$, and we simply condensed water within the 25°C environmental chamber to achieve $23\text{ g/m}^3\text{ H}_2\text{O}$. For each refuge type (i.e. O_{TH} , O_T , O_H), we used a pump to create an airflow of approximately 2000 ml/min, and we split this airflow so that each refuge chamber of the given type received approximately 500 ml/min (Fig. 1). To determine real-time refuge characteristics, we programmed miniature temperature–humidity data loggers (DS1923, Maxim Integrated Products, Sunnyvale, CA, U.S.A.) to record temperature and humidity every 20 min and positioned them on the ceiling within each refuge chamber (Fig. 1). To verify the environmental conditions of each refuge type, we randomly sampled 10 000 data points from the temperature–humidity data loggers positioned in each refuge.

Daily, we checked and recorded each snake's position in its radial maze to determine each snake's refuge preference during gravidity. To avoid disturbance, we kept the snakes in darkness except during these daily checks. At oviposition, we briefly removed the female and her clutch to determine their masses (Table 1). Then, we allowed snakes to brood their eggs as described previously (Stahlschmidt & DeNardo 2008; Stahlschmidt et al. 2008). We washed each radial maze with warm water and mild detergent between trials. At least 1 month after reproduction, we determined postabsorptive refuge preference for each female by monitoring the female in a radial maze for 1 week as was done during gravidity. Because we took multiple point samples during gravidity and after reproduction, we also determined the preferred refuge fidelity for each female by measuring the percentage of sampling points in which a female was observed in her most commonly used refuge during each of these sampling periods.

Statistical Analyses

Because of sample size constraints ($N = 11$), we used log likelihood ratio tests to determine whether refuge choice was nonrandom among the three refuge types during gravidity, at oviposition and after reproduction. To determine whether preference for refuges differed significantly, we used log likelihood ratio tests and accounted for α inflation with sequential Bonferroni corrections. We determined significance at $\alpha < 0.05$ for all tests and present all results as means \pm SE.

RESULTS

The temperature and dew point of the three refuge types were as follows: $O_{TH} = 30.9 \pm 0.0^\circ\text{C}$ and $25.1 \pm 0.0^\circ\text{C}$ ($22.8 \pm 0.0\text{ g/m}^3\text{ H}_2\text{O}$); $O_T = 30.8 \pm 0.0^\circ\text{C}$ and $15.9 \pm 0.0^\circ\text{C}$ ($12.9 \pm 0.0\text{ g/m}^3\text{ H}_2\text{O}$); $O_H = 25.1 \pm 0.0^\circ\text{C}$ and $24.5 \pm 0.0^\circ\text{C}$ ($22.4 \pm 0.0\text{ g/m}^3\text{ H}_2\text{O}$).

During reproduction, females nonrandomly occupied refuges (gravidity and oviposition: $G_2 = 13.7$, $P < 0.001$; Fig. 2). During

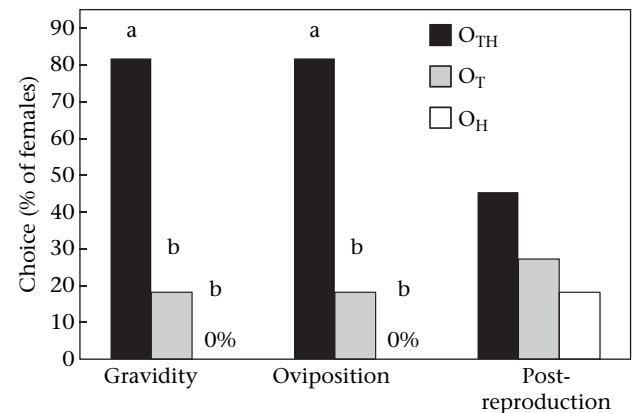


Figure 2. Percentage of females choosing each refuge type (O_{TH} : optimal temperature and humidity; O_T : optimal temperature and suboptimal humidity; O_H : suboptimal temperature and optimal humidity) during gravidity, at oviposition and after reproduction ($N = 11$). For each stage, different letters denote significant differences between refuge types.

gravidity and at oviposition, females significantly preferred O_{TH} over O_T ($G_1 = 4.8$, $P = 0.028$) and O_H ($G_1 = 12.5$, $P < 0.001$), while there was no difference in preference between O_T and O_H ($G_1 = 2.8$, $P = 0.096$; Fig. 2). Notably, all females oviposited in the same refuge they preferred during gravidity, suggesting that nest site preference is determined well before oviposition. In contrast to the selective nature of refuge choice during reproduction, females' preference for refuge type after reproduction did not differ significantly from random ($G_2 = 1.4$, $P = 0.24$; Fig. 2).

Although sample size constraints precluded the use of inferential statistics, there were trends in preferred refuge fidelity suggesting that females investigated several refuges prior to making a selection, and they did so more actively during gravidity than after reproduction. For example, females that preferred O_{TH} showed $64 \pm 9\%$ and $83 \pm 9\%$ fidelity for this refuge during gravidity and after reproduction, respectively. Also, females that preferred O_T showed $58 \pm 8\%$ and $94 \pm 6\%$ fidelity for this refuge during gravidity and after reproduction, respectively. Although no females preferred O_H during gravidity, females showed $75 \pm 25\%$ fidelity for O_H after reproduction.

DISCUSSION

Hypothesis 1 was not supported because female *A. childreni* preference for refuges at oviposition did not differ significantly from random; thus, environmental conditions influenced nest site selection (Fig. 2). The role of one or more abiotic factors in nest site selection by taxa lacking postpartitive parental care is widespread because nest site selection is the final parental decision for these animals (Clutton-Brock 1991). However, other taxa exhibiting postpartitive parental care may also demonstrate nest site selection that influences abiotic aspects of the developmental environment. For example, chestnut-collared longspurs, *Calcarius ornatus*, prefer to orient their nests in a manner that increases nest temperature (Lloyd & Martin 2004).

Nest site selection in *A. childreni* was influenced by more than one environmental variable, which supports Hypothesis 3, but not Hypothesis 2. Specifically, female *A. childreni* preferred nest sites of ideal temperature and humidity (Fig. 2). Similar to those of other taxa, python embryos are profoundly affected by developmental temperature. For example, *L. m. fuscus* embryos incubated at a stable, ideal temperature (32°C) showed shorter incubation periods, faster growth rates, better body condition as hatchlings

and a greater willingness to feed relative to those incubated under cooler, more variable thermal regimes of ecological relevance (Shine et al. 1997). Furthermore, nest humidity can also dramatically affect the fitness of python embryos in the absence of maternal attendance, which substantially increases the hydric conditions to which the eggs are exposed (Stahlschmidt et al. 2008). For example, *A. childreni* eggs that are incubated at preferred incubation temperature (30.5 °C) and 75–80% relative humidity have an 80% hatching success when brooded by the female, but 100% mortality in the absence of maternal attendance (Lourdais et al. 2007). Given embryos' sensitivities to the developmental environment, we show that pythons show adaptive nest site selection because their decisions at parturition meet the thermal and hydric needs of their developing offspring. Such adaptive decision making at parturition creates increased flexibility in postparturitive maternal decision making. For example, female *L. m. fuscus* choosing thermally favourable nest sites attended their eggs for a shorter duration than females ovipositing in less thermally ideal nest sites (Madsen & Shine 1999).

We demonstrate that refuge preference is not fixed in *A. childreni* because females' preference for warm, humid refuges disappeared after reproduction (Fig. 2). Furthermore, our results suggest that females may be choosier in selection of refuges during reproduction. Together, these results agree with and expand upon previous research showing that female *A. childreni* alter their thermoregulatory patterns during gravidity to create a higher and more stable temperature for developing embryos (Lourdais et al. 2008). Temperature regulation during gravidity clearly benefits offspring; yet, the relative benefits of occupying high-humidity refuges during this stage are less straightforward. Although they show some degree of nest site fidelity (Madsen & Shine 1999), female pythons may use the stage of gravidity to find an appropriate oviposition site. Thus, sensitivity to refuge temperature and humidity during gravidity is simply a prestep to adaptively choosing an oviposition site based on both its thermal and hydric qualities. Alternatively or additionally, humid refuges may benefit gravid pythons. *Antaresia childreni* probably transfer a significant proportion of water into their eggs over the final 2 weeks of gravidity (Stahlschmidt et al., in press). Thus, humid refuges may slow the rate of evaporative water loss by females during a period of high water demand.

Postparturition, brooding pythons regulate developmental temperature (Burmese python, *Python molurus*: Vinegar et al. 1970; diamond python, *Morelia spilota spilota*: Harlow & Grigg 1984; black-headed python, *Aspidites melanocephalus*: Johnson et al. 1975; southern African python, *Python natalensis*: Alexander 2007), water balance (ball python, *Python regius*: Aubret et al. 2005), or both (*A. childreni*: Lourdais et al. 2007; Stahlschmidt & DeNardo 2009a, 2010). Thus, the adaptive significance of egg brooding in pythons may be to maintain embryonic temperature and hydration. Combined with previous research, our results suggest that the adaptive significance of python parental care in general (i.e. both pre- and postparturition) may be to meet these two critical needs of developing embryos.

Combined with those of previous studies, our results demonstrate that female pythons use abiotic information to enhance multiple developmental variables throughout all aspects of the parental care period (i.e. before, during and after oviposition). To our knowledge, such thorough regulation of developmental temperature and hydration is rivaled only by parental care in mammals (i.e. preparative placental control and postparturitive nursing) and some mound-building megapode birds (i.e. preparative internal provisioning and postparturitive manipulation of nest substrate) (Clutton-Brock 1991; Jones & Birks 1992). Yet, like other examples of parental care in endotherms, these parental care

systems are highly derived and relatively complex. In contrast, python egg brooding typically entails two easily quantifiable behaviours, tight coiling and postural adjustment, that significantly affect embryonic predation, temperature, water balance and respiration (reviewed in Stahlschmidt & DeNardo 2011). Thus, establishing the existence of adaptive nest site selection in pythons further supports the value of pythons as models for studies of parental care (reviewed in Stahlschmidt & DeNardo 2011).

Although maternal care in pythons is emerging as a simple yet valuable parental care model, several critical aspects of this system remain unknown. Future research should focus on the roles of refuge temperature and humidity in preparative decision making in other python species because significant variation in habitat (desert versus tropical), ecology (terrestrial, arboreal, semiaquatic), geography (low versus high latitude), evolution (Afro-Asian versus Indo-Australian clades; Rawlings et al. 2008) and physiology (nonthermogenic versus facultatively thermogenic, Stahlschmidt & DeNardo 2011) exist within the Pythonidae. Factors other than temperature and humidity affect the fitness of python offspring, as well as those of other taxa; yet, their role in python nest site selection are currently unknown. For example, some pythons show intra- and interpopulation variation in brooding duration, with some females brooding their clutch for the entire length of incubation and others brooding for only the beginning of the incubation period. Nest temperature can account for a portion of this variation (Madsen & Shine 1999); however, certain biotic factors (e.g. predator scent, evidence of successful prior incubation, or clutch size) may account for remaining variation in this important maternal decision. Also, subterranean nests may create hypoxic developmental conditions, which could constrain embryonic respiration and negatively affect offspring phenotype (Stahlschmidt & DeNardo 2008, 2009b). Thus, nest O₂ and CO₂ may influence nest site selection. Finally, pythons present a unique model for the future study of how pre- and postparturitive behaviours interactively affect parental decisions and offspring fitness.

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