Thermoregulation during gravidity in the children's python (*Antaresia childreni*): a test of the preadaptation hypothesis for maternal thermophily in snakes

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Pregnant squamate reptiles (i.e. lizards and snakes) often maintain higher and more stable body temperatures than their nonpregnant conspecifics, and this maternal thermophily enhances developmental rate and can lead to increased offspring quality. However, it is unclear when this behaviour evolved relative to the evolution of viviparity. A preadaptation hypothesis suggests that maternal thermophily was a preadaptation to viviparity. Oviparous squamates are unique among oviparous reptiles for generally retaining their eggs until the embryos achieve one fourth of their development. As a result, maternal thermophily by gravid squamates may provide the same thermoregulatory benefits, at least during early development, that have been associated with viviparity. Thus, the evolution of viviparity in squamates may reflect an expanded duration of a pre-existing maternal thermoregulatory behaviour. Despite its evolutionary relevance, thermoregulation during gravidity in oviparous squamates has not yet been explored in depth. In the present study, we examined whether gravidity was associated with thermoregulatory changes in the oviparous children's python, *Antaresia childreni*. First, we discovered that, compared to most snakes, *A. childreni* is at an advanced stage of embryonic development at oviposition. Second, using surgically implanted temperature loggers, we detected a significant influence of reproductive status on thermoregulation. Reproductive females maintained higher and less variable body temperatures than nonreproductive females and this difference was most pronounced during the last 3 weeks of gravidity. Overall, these results highlight the continuum between oviparity and viviparity in squamate reptiles and emphasize the importance of thermal control of early embryonic development independent of reproductive mode.   © 2008 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2008, **93**, 499–508.

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INTRODUCTION

Temperature affects the dynamics of all biochemical processes and thereby influences many facets of an organism's life (Huey & Kingsolver, 1989; Hochachka & Somero, 2004). Such influences begin during embryogenesis, a crucial period of development (Farmer, 2000). Eggs of oviparous vertebrates are particularly vulnerable to environmental temperatures, and temperature can affect the developmental rate, hatching success, offspring phenotype, physiology, and behaviour (Burger & Zappalorti, 1988; Deeming & Ferguson, 1991; Shine, Elphick & Harlow, 1997; Laugen *et al*., 2003; Martell, Kieffer & Trippel, 2005; Watkins & Vraspir, 2006). Comparable effects have been demonstrated in viviparous reptiles and mammals despite the thermal buffering provided by viviparity (Lewis, 1993; Farmer, 2000; Faurie, Mitchell & Laburn, 2001). Taken together, these findings suggest that embryonic thermal sensitivity is a universal vertebrate feature. Conditions experienced early in life are evolutionary relevant not only

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because of immediate effects, but also for long-lasting consequences on individual growth, developmental stability, and fitness (Elphick & Shine, 1998; O'Steen, 1998; Lindström, 1999; Farmer, 2000; Lumma & Clutton-Brock, 2002; Shine, 2004a).

In this context, vertebrates have developed a wide diversity of parental strategies to reduce a potentially deleterious environmental impact on their progeny. Among endotherms, high basal metabolic rates and the associated thermogenesis permit maintenance of high body temperatures, and physiological changes during the period of embryonic development reduce deviation in fetal body temperature (Scribner & Wynneedwards, 1994; Farmer, 2000; Laburn *et al.*, 2002). Similarly, a variety of heterothermic mammals and birds display a marked shift in metabolic rate and body temperature (i.e. higher and less variable body temperature) tightly linked to embryo development (Farmer, 2000).

Although ectothermic vertebrates produce negligible metabolic heat, they also promote thermal regulation of their developing offspring, most notably through temperature-based selection of nest sites and behavioural thermoregulation (Shine & Harlow, 1996; Farmer, 2000). The reproductive mode also influences the degree to which females regulate the developmental environment of their offspring. Squamates reptiles (lizards and snakes) are characterized by a continuum between oviparity and viviparity (Shine, 1983). Viviparity within this group has evolved on more than 100 occasions (Shine, 1985), and the selective force responsible for these repeated transitions is probably linked to an improved control of developmental conditions (Shine, 1995, 2004b). Behavioural thermoregulation by the female buffers offspring developing in the oviduct from environmental fluctuation (Schwarzkopf & Shine, 1991; Tu & Hutchison, 1994; Charland, 1995; Shine, 1995; Rock, Andrews & Cree, 2000; Chiaraviglio, 2006). Ectotherms are also capable of physiological thermoregulation notably through changes in heat transfer (Seebacher, 2000; Labra, Soto-Gamboa & Bozinovic, 2001) and such mechanisms may contribute to the maintenance of an appropriate thermal environment (Labra *et al.*, 2001; Labra & Bozinovic, 2002).

Although the discussion of maternal thermoregulation and its resulting benefits is often restricted to viviparous species, oviparous squamates may provide similar care to the embryos for at least part of development. Oviparous squamates are particular among reptiles in that at least one fourth of embryo development is completed in the oviduct prior to oviposition (Shine, 1983; Blackburn, 1985; Andrews, 2004). Such intraoviductal development may allow oviparous squamates to behaviourally regulate the thermal conditions experienced by the embryos during early development. Shine (2006) recently proposed a pre-adaptation hypothesis, according to which modified maternal thermoregulation observed in viviparous species would be an extension of a pre-existing behaviour in oviparous species. An experimental study on a scincid lizard suggests that maternal thermophily before oviposition influences the developmental rate as well as the phenotypic traits of offspring (Shine, 2006).

Despite its relevance, maternal thermoregulation in gravid oviparous squamates, and notably snakes, has attracted limited scientific evaluation (Shine, 2004a). Pythons are oviparous snakes that show distinct thermoregulatory behaviour before oviposition, where gravid females bask more frequently (Shine & Madsen, 1996) and periodically adopt an 'inverted' basking position (Ross & Marzec, 1990; Shine, 2004a). Hence, pythons provide a valuable opportunity to examine the thermal impact of gravidity in oviparous snakes. In the present study, we examined the extent of preoviposition embryonic development and the influence of gravidity on thermoregulation in the children's python (*Antaresia childreni*, Gray, 1842). We hypothesized that *A. childreni* has significant embryonic development prior to oviposition and that females would maintain higher and more stable body temperatures when gravid compared to the nonreproductive state.

MATERIAL AND METHODS

MODEL SPECIES AND MAINTENANCE

Antaresia childreni, is a medium-sized (up to 1200 mm snout–vent length, 600 g body mass), non-venomous, constricting snake that inhabits rocky areas in northern Australia from Kimberley, Western Australia, to the eastern Gulf of Carpentaria, Queensland (Wilson & Swan, 2003). Oviposition occurs towards the end of the dry season (July to August, Austral winter) when ambient conditions are relatively cool and dry. When gravid, *A. childreni* display a specialized inverted thermoregulatory behaviour prior to oviposition both in natural situations (Ross & Marzec, 1990) and captivity (D. DeNardo & O. Lourdais, pers. observ.). This posture is generally restricted to the posterior half of the body where the developing eggs are located. Clutch size is 3–12 with a mean of 6.5 ($N = 4$; Shine & Slip, 1990). After oviposition, females brood their eggs but are not facultatively endothermic.

The snakes in our study (49 females, plus 11 males used for breeding) are part of a long-term captive snake colony. Snakes were housed individually in cages ($91 \times 71 \times 46$ cm). The room was maintained at 25 °C under an LD 12 : 12 h photoperiod. Permanent

access to supplemental heat was provided using a subsurface heating element (Flexwatt, Flexwatt Corp.) below one end of each cage. The resulting thermal gradient was 26–42 °C. Water was available *ad libitum* in bowls, and snakes were fed mice (mean mass = 20 g) once every 2–4 weeks.

The adult snakes were cooled for 2 months (December to January) in a temperature-controlled room applying a 6 : 18 h daily temperature cycle of 25 : 15 °C. In early February, 2004, each female was surgically implanted with a miniature temperature data logger (Thermochron iButtons, Maxim). Loggers were programmed to sample temperature hourly and then coated with Plasti-Dip (PDI Inc.). Each snake was anaesthetized using isoflurane and the skin was locally disinfected using betadine. An approximately 1.5 cm ventro–lateral incision was made through the skin and body wall, and the logger was sutured in place caudal to the gall bladder (approximating the future location of the most cranial egg). The incision was closed with everting mattress sutures (3–0 Vicryl, Ethicon), and recovery from anaesthesia was relatively rapid (< 30 min).

Mating occurred from mid-February to mid-March and ultrasonography (Concept MCV, Dynamic Imaging) was used periodically to assess reproductive status. Twenty-one females commenced vitellogenesis (follicle size > 15 mm) and subsequently laid eggs between early April and mid-June 2004. In six other females, nonvitellogenic follicles were initially detected (< 10 mm) but vitellogenesis never occurred. For the remaining 22 females, no follicles were ever detected. Reproductive females typically refuse to feed after the cooling period. Thus, for consistency, we did not provide food to any of the snakes during the period between wintering and oviposition. The six females that stopped vitellogenesis and five others that produced only undeveloped ova were excluded from the analysis.

As reproductive stage (e.g. vitellogenic versus gravid) may affect maternal thermoregulation for different proximate reasons (e.g. energy allocation to follicular growth versus embryonic development), we aimed to assess thermoregulation during reproductive activity in general and gravidity (i.e. the presence of intraoviductal eggs) specifically. Unfortunately, the date of ovulation is difficult to determine consistently in snakes. However, in many snakes, an ecdysis occurs prior to oviposition. This 'prelaying shed', as it is commonly called, occurs approximately 1 week before oviposition in colubrid snake (Markel, 1990), but somewhat sooner in pythons (3–4 weeks preoviposition in *A. childreni*, Fyfe & Green, 2004). Ultrasonography indicated that postshedding reproductive pythons were always gravid (D. DeNardo & O. Lourdais, pers. observ.). Thus, although we could not iden-

tify the exact date of ovulation, date of ecdysis was used as a simple indicator of gravidity and temperature data obtained after shedding provided thermal data specific to gravidity.

VARIABLES EXAMINED

Embryo characteristics at oviposition
The range of embryonic development at oviposition is considerable among oviparous squamates (Andrews, 2004). Therefore, to ascertain the relevance of any thermoregulatory change during gravidity to embryonic development, we determined the embryonic stage at oviposition. We collected three eggs (one each from three different females) shortly after oviposition (< 6 h), fixed the embryos in 90% ethanol, and determined their embryonic stage using the classification of Hubert & Dufaure (1968).

Thermoregulatory behaviour
Although the significance of the inverted basking behaviour remains unstudied, this behaviour likely provides a thermoregulatory benefit. We estimated the frequency of inverted basking by recording the position of each snake in its cage for 40 days starting at the end of the mating season. Behavioural observations were conducted only once daily and concomitantly to cage cleaning to minimize disturbance. Observations were equally distributed between mornings and afternoons (10.00 h and 16.00 h, respectively). Snakes were classified as 'inverted basking' if they were found above the heat source in an inverted position.

Body temperature (T_b)
We compared T_b profiles of the 16 reproductive females with a group of 13 randomly selected nonreproductive females housed under the same conditions. For reproductive females, oviposition date was established as day 0, and we examined the recorded temperature for the 60 days preceding day 0. However, four reproductive females were used in another experiment and, for those individuals, the first third of the T_b records were not available. For nonreproductive females, we used a period of 60 consecutive days that coincided with the dates used for the reproductive females.

From these data sets, we calculated the mean daily T_b for the 60 consecutive days for each individual. Because mean values alone do not provide ample information to assess the efficiency with which ectotherms regulate their T_b, we also considered two derived variables to assess within female and among female variation in T_b. First, we determined the extent of within-female daily T_b variation by examining T_b standard deviation (SD) calculated for each day

and each female. Second, to measure the influence of reproductive status on interfemale variation in T_b, we calculated the absolute deviation between the individual mean daily T_b and the grand mean of daily T_b of the group of females sharing the same reproductive status. As the 60 days of data includes a period during which the females were vitellogenic and a period when they were gravid, we also examined mean daily T_b, daily T_b variation, and interfemale T_b variation restricting the data to the period between ecdysis and oviposition (i.e. including only days when the females were gravid) in reproductive females.

STATISTICAL ANALYSIS

All statistical analysis were performed using JMP (version 5.1, SAS Institute). Body temperatures were recorded hourly and synchronously for all individuals. We calculated mean T_b values and related variables for each day and each individual. First, we examined the influence of reproductive status on temperature profiles using univariate repeated measure analysis of variance (ANOVA) procedures treating reproductive status as the fixed factor, day as the repeated factor, and mean daily T_b, T_b SD, or interfemale T_b variation as the dependent variables. For these analyses, we included only individuals with the complete 60-day period of successive temperature records (12 reproductive and 13 nonreproductive females). Univariate repeated measures ANOVA assumes that the changes across levels (intervals among measurements in this study) are uncorrelated across subjects. The sphericity assumption was always tested, but no violations were detected in our repeated measures tests (Mauchley's sphericity tests, $P > 0.5$).

The influence of gravidity was later analysed using a mixed model analysis and treating individual identity as a random factor to control for pseudo-replication. We used a restricted maximum likelihood (REML) procedure (Patterson & Thompson, 1971). This method is appropriate because it balances the information on each individual level (multiple contributions) with the information on the variances across levels (fixed factors). Considering temperature records obtained after ecdysis in reproductive females, we compared the 16 gravid (i.e. postecdysis) with the 13 nonreproductive females treating reproductive status as the fixed factor and mean daily T_b (or alternate test variable) as the dependant variable. Finally, restricting the analysis to the 12 reproductive females with the complete 60-day period, we tested the influence of ecdysis status on T_b treating ecdysis status as a fixed factor. Unless otherwise stated, values are reported as mean ± SD.
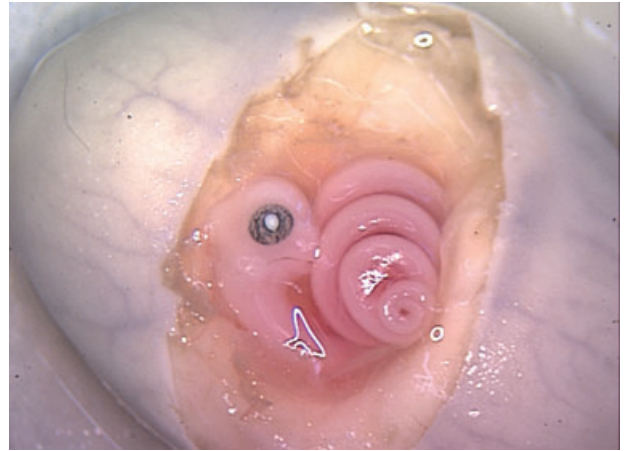

Figure 1. Photograph of an *Antaresia childreni* egg opened shortly after oviposition (< 3 h). The embryo was lying on its left side in a polar position. The eyes are pigmented, the lower jaws are well-defined, and the tail shows five distinct spires. The dark branches below the intact shell correspond to the circulatory network of the chorio-allantoic membranes.

RESULTS

EMBRYO CHARACTERISTICS AT OVIPOSITION

In each of the three eggs dissected at oviposition, we found embryos with characteristics that corresponded to stage 35 of Hubert & Dufaure (1968). Notable characteristics included pigmented eyes, well-defined lower jaws, and five distinct spires on the tail (Fig. 1).

BASKING BEHAVIOUR

A total of 103 'inverted basking' events were recorded during the 40 days of examination. A greater proportion of reproductive snakes (21 of 21 females) showed this behaviour compared to nonreproductive snakes (seven of 28 females, Yates corrected chi-square = 18.38, $P = 0.001$). Reproductive females had 95 total inverted basking events while nonreproductive females had only eight. Among reproductive females, inverted basking records were more frequent after than before ecdysis (65 versus 30 observations).

INFLUENCE OF REPRODUCTIVE STATUS ON T_B

Reproductive and nonreproductive females differed in their mean T_b over the 60 days with a progressive increase in mean T_b observed for reproductive females but not for nonreproductive females (Table 1, Fig. 2). Consistently, we found a strong relationship between mean daily T_b and date among reproductive females ($R^2 = 0.87$, $F_{1,59} = 4098.01$, $P < 0.001$) but not among nonreproductive females ($R^2 = 0.02$, $F_{1,59} = 1.21$, $P = 0.27$). Overall, reproductive females maintained

Table 1. Determinants of T_b in reproductive ($N = 12$) and nonreproductive ($N = 13$) *Antaresia childreni*

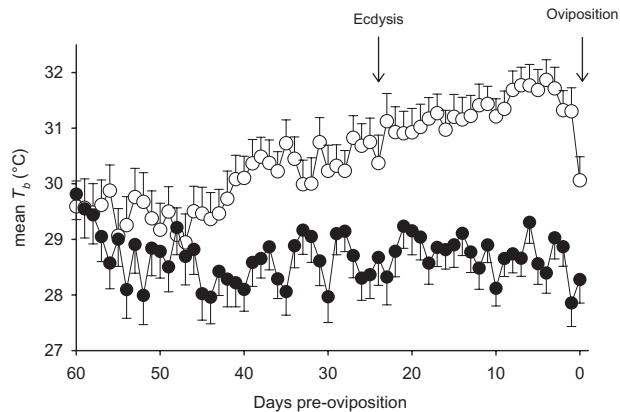| Source	Effect	d.f.	SS	F-ratio	P-value
Body temperature					
Reproductive status	Fixed	1	1146.00	47.69	< 0.0001
Days pre-ovipostion	Fixed	59	317.00	2.92	< 0.0001
Status × days	Fixed	59	329.00	3.03	< 0.0001
Standard deviation					
Reproductive status	Fixed	1	55.45	13.66	0.001
Days pre-ovipostion	Fixed	59	50.00	2.54	0.001
Status × days	Fixed	59	27.79	1.41	0.023

d.f., degrees of freedom; SS, sum of squares.

Figure 2. Influence of reproductive status on mean daily T_b. Reproductive snakes (open circles) and nonreproductive snakes (solid circles) were maintained in separate cages with permanent access to supplemental heat. The first arrow ('ecdysis') indicates the mean number of days prior to oviposition that ecdysis occurs in reproductive females (23.1 days). The second arrow ('oviposition') indicates oviposition date (day 0) in reproductive females. For statistical analysis, see text. Errors bars represent standard error, which reflects interfemale variation.
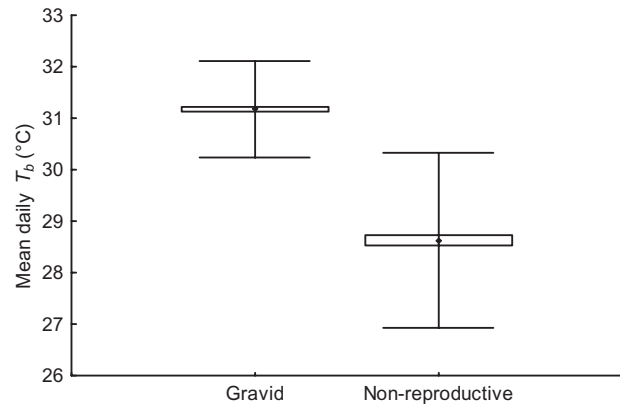

Figure 3. Mean daily T_b (°C) measured in gravid and nonreproductive female *Antaresia childreni*. Solid diamonds indicate the grand mean, whereas open squares indicate the standard error and error bars represent standard deviation to demonstrate the among-individual variation in mean daily body temperature for each group.

significantly higher mean daily T_b than nonreproductive females (30.44 ± 1.39 versus 28.69 ± 1.72 °C, $F_{1,23} = 45.28$, $P < 0.0001$, repeated measure ANOVA; Table 1). Reproductive females also showed less daily T_b variation than did nonreproductive females (mean values 1.46 ± 0.53 versus 1.91 ± 0.78 °C, coefficients of variation: 4.7 versus 6.6%, $F_{1,23} = 14.86$, $P = 0.001$, repeated measure ANOVA; Table 1). We also found a significant interaction between day and reproductive status (Table 1), reflecting a progressive decrease in a female's daily T_b variation over time among reproductive females ($R^2 = -0.87$, $F_{1,59} = 409.00$, $P < 0.001$) but not among nonreproductive females ($R^2 = 0.006$, $F_{1,59} = 0.35$, $P = 0.55$). We found a strong negative relationship between mean daily T_b and daily T_b variation in reproductive females ($R^2 = 0.57$,

$F_{1,59} = 81.99$, $P = 0.0001$), illustrating that the increase in mean T_b over time was linked to the decrease in daily T_b variation. Finally, the mean interfemale T_b variation was lower among reproductive females (mean values 0.85 ± 0.72 versus 1.28 ± 1.07 °C, $F_{1,23} = 16,67$, $P = 0.0004$; same procedure as above).

INFLUENCE OF GRAVIDITY ON T_B

The difference between reproductive and nonreproductive snakes in mean daily T_b and daily T_b variation was more pronounced when restricting the analysis to the postecdysis period for reproductive females. Gravid females maintained higher mean daily T_b ($F_{1,27} = 95,75$, $P < 0.0001$, REML procedure, status as a fixed factor and individual identity as a random factor; Fig. 3) and regulated their T_b with a smaller daily variation (coefficients of variation: 4.1
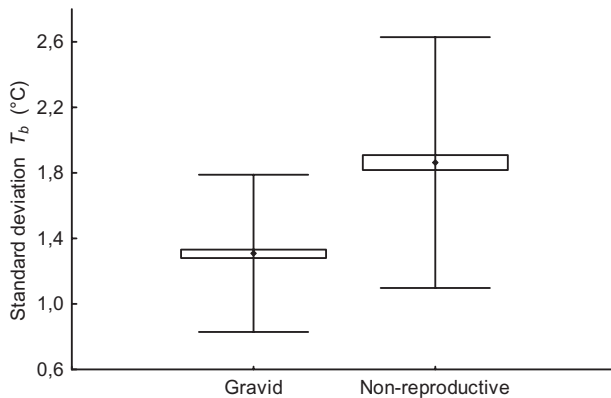
Figure 4. The precision with which individual females maintain body temperature as represented by the mean daily T_b standard deviation (°C) of gravid and nonreproductive female *Antaresia childreni*. Solid diamonds indicate the grand mean, open squares indicate the standard error, and error bars represent standard deviation.

Figure 5. Relationship between the time interval between ecdysis and oviposition date (days) and mean T_b maintained during that period (°C). Each open circle represents an individual snake.

versus 6.6%, $F_{1,27} = 25.54$, $P < 0.0001$, same procedure as above; Fig. 4) than nonreproductive females.

Considering the 12 reproductive females with the complete 60-day period of records, we found that mean T_b was significantly higher after shedding (31.28 ± 0.93 versus 29.89 ± 1.36 °C, $F_{1,11} = 275.65$, $P < 0.001$, REML procedure using shedding status as a fixed factor and individual identity as a random factor). In addition, the mean daily T_b variation was significantly lower after shedding (mean values 1.29 ± 0.44 versus 1.57 ± 0.56 °C, coefficients of variation: 4.1 versus 5.2%, $F_{1,11} = 51.16$, $P < 0.001$, same procedure as above), as was the interfemale T_b variation (mean values 0.71 ± 0.51 versus 0.95 ± 0.81 °C, $F_{1,15} = 20.31$, $P < 0.001$ same procedure as above).

RELATION BETWEEN ECDYSIS, OVIPOSITION, AND T_B

Ecdysis occurred on average 23.1 ± 2.6 days before oviposition with values in the range 19–29 days. Significant interindividual variation was detected in mean T_b maintained between ecdysis and oviposition (ANOVA, $F_{1,15} = 21.64$, $P < 0.001$), and mean T_b during this period was inversely related to the ecdysis–oviposition interval ($R^2 = 0.60$, $F = 21.39$, $N = 16$, $P < 0.001$; Fig. 5).

DISCUSSION

The aim of the present study was to test the prediction that, in an oviparous squamate, embryonic development prior to oviposition should influence maternal thermoregulation (Shine, 2006). Despite its evolutionary relevance, thermoregulation during gravidity in oviparous squamates has received only limited scien-
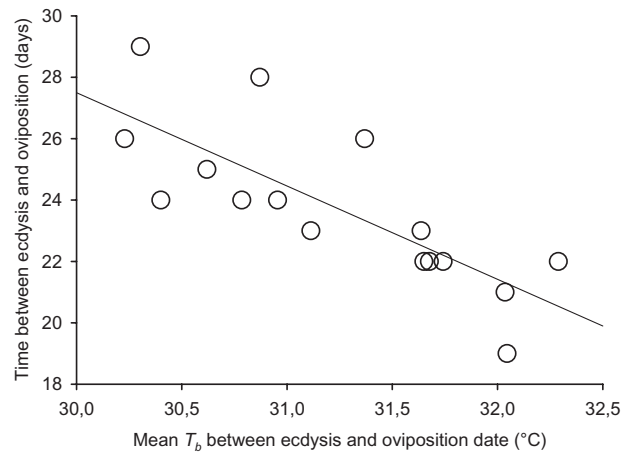
tific attention (Labra & Bozinovic, 2002; Shine, 2004a). Studies on the Montpellier snake (*Malpolon monspessulanus*; Blazquez, 1995) and the black rat snake (*Elaphe obsoleta*; Blouin-Demers & Weatherhead, 2001a) reported higher temperatures in gravid females than nongravid females. By contrast, a recent study on grass snakes (*Natrix natrix*) reported more variable temperatures in gravid compared to nongravid snakes (Isaac & Gregory, 2004). One major complication with previous studies is the lack of information on reproductive stage at the time of temperature measurements. This element is critical because thermal requirements of folliculogenesis (egg formation) and embryonic development likely differ.

To further assess the impact of gravidity on thermoregulation in oviparous squamates, we combined the examination of embryos at oviposition, behavioural observations, and T_b pattern analyses. This approach first indicates that a significant fraction of *A. childreni* development occurs in the oviduct. Second, we found that gravidity strongly influences thermoregulation with reproductive females maintaining higher and less variable T_b. Overall, our results are comparable to those obtained during pregnancy in many viviparous squamates, which show an increase in basking activities (Schwarzkopf & Shine, 1991) combined with elevated T_b (Dauf & Andrews, 1993; Charland, 1995; Rock *et al*., 2000) and more precise thermoregulation in gravid females (Gier, Wallace & Ingermann, 1989; Charland & Gregory, 1990; Graves & Duvall, 1993; Tu & Hutchison, 1994; Brown & Weatherhead, 2000; Rock, Cree & Andrews, 2002; Ka, Thompson & Seebacher, 2006; Webb, Shine & Christian, 2006). Meanwhile, several studies have demonstrated lower selected T_b during pregnancy

in lizards (Mathies & Andrews, 1997; Andrews, DelaCruz & SantaCruz, 1997; Le Galliard, Le Bris & Clobert, 2003). These results suggest different thermal requirements for adult activity and embryo development and possibly reflect higher preferred body temperatures in lizards (Lillywhite, 1987) that would be less compatible with incubation requirements. Despite this variation, evidence for more precise maternal thermoregulation also exits for species with lower thermal preference during pregnancy (Mathies & Andrews, 1997). Overall, the present study suggests that regulating thermal conditions of development is important from the onset of embryonic life regardless of reproductive mode, supporting Shine's (2006) preadaptive hypothesis for maternal thermophily. Below, we consider in more detail the reproductive characteristics that we have documented in *A. childreni*.

Examination of freshly oviposited eggs revealed an extended period of oviductal embryo development. Although most squamates lay eggs at approximately stage 30 and rarely beyond stage 32–33 (Shine, 1983; Blackburn, 1985; Radder, Shanbag & Saidapur, 1998; Andrews & Mathies, 2000), the results of the present study suggest further intraoviductal development in *A. childreni* until stage 35. This stage corresponds with major changes in embryonic physiology with an exponential increase in body mass and oxygen requirements (Andrews & Mathies, 2000; Andrews, 2002). Extended oviductal development may be true for other members of pythons (Shine, 1983), but further study is needed to determine the generality of this phenomenon and the selective pressure involved (Shine & Thompson, 2006). Extended egg retention can also occur in response to unfavourable laying sites and/or stress induced by captive conditions (Radder *et al*., 1998; Shanbhag, Saidapur & Radder, 2003). However, we doubt that egg retention was pathological in the present study for at least two reasons: (1) the snakes used in the present study were part of a long-term captive colony in which breeding conditions have been well-established and (2) maternal brooding behaviour in pythons effectively buffers the developmental environment from ambient conditions (Lourdais, Hoffman & DeNardo, 2007). We also found that oviposition in *A. childreni* occurs much later after ecdysis (19–29 days; average = 23.1 days) than it does in colubrid snakes (approximately 1 week; Markel, 1990). This extended ecdysis-to-oviposition interval may reflect the time required for the further embryonic development seen in *A. childreni*. However, such an explanation would rely on the preoviposition ecdysis occurring at some common and relatively fixed stage of the reproductive cycle. Unfortunately, data on the timing (or purpose) of the preoviposition ecdysis relative to female reproductive physiology are lacking. Interestingly, however, we found a significant inverse relationship between the ecdysis-to-oviposition duration and the mean T_b during that period (Fig. 5). Clearly, more work is needed to examine this interesting relationship and its functional significance, if any. Nevertheless, these preliminary results suggest a proximate influence of maternal T_b on the rate of embryo development in the oviduct as observed in viviparous species (Naulleau, 1986; Shine & Harlow, 1993; Lourdais *et al*., 2002, 2004). The fact that all females were gravid at the time of ecdysis provides a minimal duration of intraoviductal gestation. Considering that the postoviposition incubation time at 31 °C is 48 days on average, (O. Lourdais, unpubl. data), we know that a significant portion (i.e. at least 30%) of developmental time occurs in the oviduct.

Overall, the thermoregulatory changes that we report in gravid *A. childreni* are comparable in many ways to the results obtained in a variety of viviparous squamates (Tu & Hutchison, 1994; Charland, 1995; Shine, 2004a). In addition, our study clearly supports the existence of precise thermoregulation during early development because gravid females regulated their T_b with lower daily and interindividual variation compared to nonreproductive females. Although the present study was conducted in captivity, our behavioural results are ecologically relevant. Modified basking activity during embryo development is consistent with field observations in this species and others, including the water python (*Liasis fuscus*; Shine & Madsen, 1996) and black rat snake (*E. obsoleta*; Blouin-Demers & Weatherhead, 2001b). The evolution of reptilian viviparity and altered thermoregulatory behaviour during pregnancy is presumably linked to the fitness benefit derived from a better control of the thermal conditions experienced by the embryo (Shine, 1991, 1995). By maintaining high and, perhaps more importantly, stable T_b, the pregnant female can optimize developmental conditions and influence offspring phenotypes and fitness (Shine & Harlow, 1993, 1996; Shine, 1995; Waspra, 2000; O'Donnell & Arnold, 2005; Web *et al*., 2006). The results of the present study thereby support Shine's (2006) recent preadaptation hypothesis, which suggests that maternal thermoregulation may be a pre-existing trait in oviparous forms and that the transition from oviparity to viviparity in squamates may have favoured the extension of this pre-existing trait instead of imposing a major modification in maternal behaviour. Further studies are now required to examine experimentally the functional significance of thermoregulation early in embryonic development and specialized behaviours, such as inverted basking.

Interestingly, pythons are unique in showing complex parental care of their eggs, with females coiling tightly around the clutch after oviposition (Shine, 2004a; Somma, 2003). The costs of egg attendance encompass or even bypass those associated with viviparity (Shine, 1984) and thus it might be predicted that a transition to viviparity would have been facilitated, especially considering the vast number of times that such a transition occurred. Yet no pythonine snake is viviparous, possibly because of physiological constraints precluding oviduct egg retention as suggested by Shine (1984). However, we found that extended egg retention and associated embryonic development is achievable in this group. Overall, if egg retention is a general trait in pythonine snakes, it would present a distinct life history combination (extended oviductal embryo development followed by egg attendance) rather than a simple intermediate situation on the oviparity–viviparity continuum.

ACKNOWLEDGEMENTS

REFERENCES

Andrews RM. 2002. Low oxygen: a constraint on the evolution of viviparity in reptiles. *Physiological Biochemistry and Zoology* **75:** 145–154.

Andrews RM. 2004. Embryonic development. In: Deeming DC, ed. *Reptilian incubation: environment, evolution, and behaviour*. Nottingham: Nottingham University Press, 75–102.

Andrews RM, Mathies T. 2000. Natural history of reptilian development: physiological constraints on the evolution of viviparity. *Bioscience* **50:** 227–238.

Andrews RM, DelaCruz FRM, SantaCruz MV. 1997. Body temperatures of female *Sceloporus grammicus*: thermal stress or impaired mobility? *Copeia* **1997:** 108–115.

Blackburn DG. 1985. Evolutionary origins of viviparity in the Reptilia. II. Serpentes, Amphisbaenia, and Ichthyosauria. *Amphibia-Reptilia* **5:** 259–291.

Blazquez MC. 1995. Body temperature, activity patterns and movements by gravid and non-gravid females of *Malpolon monspessulanus*. *Journal of Herpetology* **29:** 264–266.

Blouin-Demers G, Weatherhead PJ. 2001a. Thermal ecology of black rat snakes (*Elaphe obsoleta*) in a thermally challenging environment. *Ecology* **82:** 3025–3043.

Blouin-Demers G, Weatherhead PJ. 2001b. Habitat use by black rat snakes (*Elaphe obsoleta obsoleta*) in fragmented forests. *Ecology* **82:** 2882–2896.

Brown GP, Weatherhead PJ. 2000. Thermal ecology and sexual size dimorphism in northern water snakes, *Nerodia sipedon*. *Ecological Monograph* **70:** 311–330.

Burger J, Zappalorti RT. 1988. Effects of incubation temperature on sex ratios in pine snakes: differential vulnerability of males and females. *American Naturalist* **132:** 492–505.

Charland MB. 1995. Thermal consequences of reptilian viviparity – thermoregulation in gravid and nongravid garter snakes (*Thamnophis*). *Journal of Herpetology* **29:** 383–390.

Charland MB, Gregory PT. 1990. The influence of female reproductive status on thermoregulation in a viviparous snakes, *Crotalus viridis*. *Copeia* **1990:** 1089–1098.

Chiaraviglio M. 2006. The effects of reproductive condition on thermoregulation in the Argentina boa constrictor (*Boa constrictor occidentalis*) (Boidae). *Herpetological Monographs* **20:** 172–177.

Dauf EF, Andrews RM. 1993. The effect of pregnancy on thermoregulatory behavior of the viviparous lizard *Chalcides ocellatus*. *Journal of Herpetology* **27:** 6–13.

Deeming DC, Ferguson MWJ. 1991. Physiological effects of incubation temperature on embryonic development in reptiles and birds. In: Deeming DC, Ferguson MWJ, eds. *Egg incubation: its effects on embryonic development in birds and reptiles*. Cambridge: Cambridge University Press, 147–171.

Elphick MJ, Shine R. 1998. Longterm effects of incubation temperatures on the morphology and locomotor performance of hatchling lizards (*Bassiana duperreyi*, Scincidae). *Biological Journal of the Linnaean Society* **63:** 429–447.

Farmer CG. 2000. Parental care: key to understanding endothermy and other convergent features in birds and mammals. *American Naturalist* **155:** 326–334.

Faurie AS, Mitchell D, Laburn HP. 2001. Feto-maternal relationships in goats during heat and cold exposure. *Experimental Physiology* **86:** 199–204.

Fyfe G, Green D. 2004. *Keeping children pythons*. Bendigo: Australian Reptile Keeper Publications.

Gier PJ, Wallace RL, Ingermann RL. 1989. Influence of pregnancy on behavioral thermoregulation in the northern pacific rattlesnakes *Crotalus viridis oreganus*. *Journal of Experimental Biology* **145:** 465–469.

Graves BM, Duvall D. 1993. Reproduction, rookery use, and thermoregulation in free-ranging, pregnant *Crotalus v. viridis*. *Journal of Herpetology* **27:** 33–41.

Hochachka PW, Somero GN. 2004. *Biochemical adaptation: mechanism and process in physiological evolution*. Oxford: Oxford University Press.

Hubert J, Dufaure JP. 1968. Table de développement de la vipère aspic: *Vipera aspis* L. *Bulletin de la Société Zoologique de France* **93:** 135–148.

Huey RB, Kingsolver JG. 1989. Evolution of thermal sensitivity of ectotherm performance. *Trends in Ecology and Evolution* **4:** 131–135.

Isaac LA, Gregory PT. 2004. Thermoregulatory behaviour of gravid and non-gravid female grass snakes (*Natrix natrix*) in a thermally limiting high-latitude environment. *Journal of Zoology (London)* **264:** 403–409.

Labra A, Bozinovic F. 2002. Interplay between pregnancy and physiological thermoregulation in *Liolaemus* lizards. *Ecosciences* **9:** 421–426.

Labra A, Soto-Gamboa M, Bozinovic F. 2001. Behavioral and physiological thermoregulation of Atacama desert-dwelling *Liolaemus* lizards. *Ecosciences* **8:** 413–420.

Laburn HP, Faurie A, Goelst K, Mitchell D. 2002. Effects on fetal and maternal body temperatures of exposure of pregnant ewes to heat, cold, and exercise. *Journal of Applied Physiology* **92:** 802–808.

Laugen AT, Laurila A, Rasanen K, Merila J. 2003. Latitudinal counter gradient variation in the common frog (*Rana temporaria*) development rates – evidence for local adaptation. *Journal of Evolutionary Biology* **16:** 996–1005.

Le Galliard JF, Le Bris M, Clobert J. 2003. Timing of locomotor impairment and shift in thermal preferences during gravidity in a viviparous lizard. *Functional Ecology* **17:** 877–885.

Lewis SE. 1993. Effect of climatic variation on reproduction by pallid bats (*Antrozous pallidus*). *Canadian Journal of Zoology* **71:** 1429–1433.

Lillywhite HB. 1987. Temperature, energetics and physiological ecology. In: Seigel RA, Collins JT, Novak SS, eds. *Snakes: ecology and evolutionary biology*. New York, NY: Macmillan, 422–477.

Lindström J. 1999. Early development and fitness in birds and mammals. *Trends in Ecology and Evolution* **14:** 343–348.

Lourdais O, Bonnet X, Shine R, DeNardo D, Naulleau G, Guillon M. 2002. Capital-breeding and reproductive effort in a variable environment: a longitudinal study of a viviparous snake. *Journal of Animal Ecology* **71:** 470–479.

Lourdais O, Shine R, Bonnet X, Guillon G, Naulleau G. 2004. Climate influences embryonic development in a viviparous snake (*Vipera aspis*). *Oikos* **104:** 551–560.

Lourdais O, Hoffman T, DeNardo D. 2007. Maternal brooding in the Children's python (*Antaresia childreni*) promotes egg water balance. *Journal of Comparative Physiology B* **177:** 569–577.

Lumma V, Clutton-Brock T. 2002. Early development, survival and reproduction in humans. *Trends in Ecology and Evolution* **17:** 141–147.

Markel RG. 1990. *Kingsnakes and milk snakes*. Neptune City, NJ: TFH Publication.

Martell DJ, Kieffer JD, Trippel EA. 2005. Effects of temperature during early life history on embryonic and larval development and growth in haddock. *Journal of Fish Biology* **66:** 1558–1575.

Mathies T, Andrews RM. 1997. Influence of pregnancy on the thermal biology of the lizard, Sceloporus jarrovi: why do pregnant females exhibit low body temperatures? *Functional Ecology* **11:** 498–507.

Naulleau G. 1986. Effect of temperature on gestation in *Vipera aspis* and *V. berus* (Reptilia: Serpentes). In: Rocek Z, ed. *Studies in herpetology.* Prague: Charles University, 489–494.

O'Donnell RP, Arnold SJ. 2005. Evidence for selection on thermoregulation: effects of temperature on embryo mortality in the garter snake *Thamnophis elegans*. *Copeia* **4:** 930–934.

O'Steen S. 1998. Embryonic temperature influences juvenile temperature choice and growth rate in snapping turtles *Chelydra serpentina*. *Journal of Experimental Biology* **201:** 439–449.

Patterson HD, Thompson R. 1971. Recovery of inter block information when block sizes are unequal. *Biometrika* **58:** 545 & 1971.

Radder R, Shanbag BA, Saidapur SK. 1998. Prolonged oviductal egg retention arrests embryonic growth at stage 34 in captive *Calotes versicolor*. *Herpetological Review* **29:** 217.

Rock J, Andrews RM, Cree A. 2000. Effects of reproductive condition, season, and site on selected temperatures of a viviparous gecko. *Physiological and Biochemical Zoology* **73:** 344–355.

Rock J, Cree A, Andrews RM. 2002. The effect of reproductive condition on thermoregulation in a viviparous gecko from a cool climate. *Journal of Thermal Biology* **27:** 17–27.

Ross RA, Marzec G. 1990. *The reproductive husbandry of pythons and boas*. Stanford, CA: Institute for Herpetological Research.

Schwarzkopf L, Shine R. 1991. Thermal biology of reproduction in viviparous skinks, *Eulamprus tympanum*: why do gravid females bask more? *Oecologia* **88:** 562–569.

Scribner SJ, Wynne Edwards KE. 1994. Disruption of body-temperature and behavior rhythms during reproduction in Dwarf Hamster (*Phodopus*). *Physiology and Behavior* **55:** 361–369.

Seebacher F. 2000. Heat transfer in a microvascular network: the effect of heart rate on heating and cooling in reptiles (*Pogona barbata* and *Varanus varius*). *Journal of Theoretical Biology* **203:** 97–109.

Shanbhag BA, Saidapur SK, Radder RS. 2003. Lowering body temperature induces embryonic diapause during prolonged egg retention in the lizard. *Calotes Versicolor Naturwissenschaften* **90:** 33–35.

Shine R. 1983. Reptilian reproductive modes: the oviparity-viviparity continuum. *Herpetologica* **39:** 1–8.

Shine R. 1984. Physiological and ecological questions on the evolution of reptilian viviparity. In: Seymour R, ed. *Respiration and metabolism of embryonic vertebrates*. The Hague: Junk, 147–154.

Shine R. 1985. The evolution of viviparity in reptiles: an ecological analysis. In: Gans C, Billet F, eds. *Biology of the Reptilia*, Vol. 15. New York, NY: John Wiley and Sons, 605–694.

Shine R. 1991. Influences of incubation requirements on the evolution of viviparity. In: Deeming DC, Ferguson MWJ, eds. *Egg incubation: its effects on embryonic development in birds and reptiles*. Cambridge: Cambridge University Press, 361–270.

Shine R. 1995. A new hypothesis for the evolution of viviparity in reptiles. *American Naturalist* **145:** 809–823.

Shine R. 2004a. Adaptive consequences of developmental

plasticity. In: Deeming DC, ed. *Reptilian incubation: environment, evolution and behaviour*. Nottingham: Nottingham University Press, 187–210.

Shine R. 2004b. Does viviparity evolve in cold climate reptiles because pregnant females maintain stable (not high) body temperatures? *Evolution* **58:** 1809–1818.

Shine R. 2006. Is increased maternal basking an adaptation or a pre-adaptation to viviparity in lizards? *Journal of Experimental Zoology* **305A:** 524–535.

Shine R, Harlow P. 1993. Maternal thermoregulation influences offspring viability in a viviparous lizard. *Oecologia* **96:** 122–127.

Shine R, Harlow PS. 1996. Maternal manipulation of offspring phenotypes via nest-site selection in an oviparous lizard. *Ecology* **77:** 1808–1817.

Shine R, Madsen T. 1996. Is thermoregulation unimportant for most reptiles? An example using water pythons (*Liasis fuscus*) in tropical Australia. *Physiological Zoology* **69:** 252–269.

Shine R, Slip DJ. 1990. Biological aspects of the adaptive radiation of Australasian pythons (Serpentes: Boidae). *Herpetologica* **46:** 283–290.

Shine R, Thompson MB. 2006. Did embryonic responses to incubation conditions drive the evolution of reproductive modes in squamate reptiles? *Herpetological Monographs* **20:** 159–171.

Shine R, Elphick MJ, Harlow PS. 1997. The influence of natural incubation environments on the phenotypic traits of hatchling lizards. *Ecology* **78:** 2559–2568.

Somma LA. 2003. *Parental behavior in lepidosaurian and testudinian reptiles, a literature survey*. Malabar, FL: Krieger Publishing Co.

Ka R, Thompson MB, Seebacher F. 2006. Thermal biology of a viviparous lizard with temperature-dependant sex determination. *Journal of Thermal Biology* **31:** 292–301.

Tu MC, Hutchison VH. 1994. Influence of pregnancy on thermoregulation of water snakes (*Nerodia rhombifera*). *Journal of Thermal Biology* **19:** 255–259.

Waspra E. 2000. Maternal basking opportunity affects juvenile phenotype in a viviparous lizard. *Functionnal Ecology* **14:** 345–352.

Watkins TB, Vraspir J. 2006. Both incubation temperature and posthatching temperature affect swimming performance and morphology of wood frog tadpoles (*Rana sylvatica*). *Physiological and Biochemical Zoology* **79:** 140–149.

Webb JK, Shine R, Christian KA. 2006. The adaptive significance of reptilian viviparity in the tropics: testing the maternal manipulation hypothesis. *Evolution* **60:** 115–122.

Wilson S, Swan G. 2003. *A complete guide to reptiles of Australia*. Frenchs Forest, Australia: New Holland Publishers.