

Why do female ball pythons (*Python regius*) coil so tightly around their eggs?

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ABSTRACT

Question: What benefits does brooding confer to offspring viability that outweigh its costs to the nest-attending female?

Organisms: Thirty captive *Python regius* females and their clutches.

Site: Vicinity of Lomé, Togo.

Background: It has previously been shown that brooding enhances ball python hatching success by reducing desiccation of eggs.

Methods: We captured wild, gravid females just before the time of egg-laying. Then we varied maternal attendance, allowing it to last 0, 15 or 60 days.

Conclusions: Brooding weakly influenced incubation temperature but markedly decreased egg mass loss owing to water loss and associated yolk coagulation. Brooded eggs produced larger, more active, faster swimming and more rapidly developing neonates than did non-brooded eggs.

Keywords: brooding, incubation, parental care, phenotypic plasticity, *Python regius*.

INTRODUCTION

Mathematical models suggest that whether or not a given life-history 'tactic' will evolve and continue to be expressed is dependent on the relative magnitude of two opposing forces: the costs and benefits that accrue from expression of that characteristic (e.g. Charnov and Krebs, 1974). One example of a complex behavioural trait with high costs is parental care (Alcock, 1993; Andersson, 1994). In many organisms, reproductive individuals experience substantial risks, and spend considerable energy in the process of raising their offspring (Clutton-Brock, 1988, 1991). Presumably, the compensating benefit in this case accrues to the offspring: higher parental investment may increase the offspring's probability of survival or its subsequent reproductive success (Townsend, 1986; Clutton-Brock, 1988; Clutton-Brock and Godfray, 1991). In cases where

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the parent provides direct protection against predators or nutritional input, the benefits to offspring fitness are well known (Woodruff, 1977; Forester, 1979; Woodside *et al.*, 1981; Gross and Sargent, 1985; Wolf *et al.*, 1988). However, the benefits of energetically expensive forms of parental care that do not involve protection or nutrient transfer to the neonates are less obvious.

Although parental care of eggs is relatively rare among squamate reptiles, a distinctive form of this behaviour appears to be ubiquitous in one lineage of snakes. Female pythons remain tightly coiled around their eggs throughout incubation (Noble, 1935; Cogger and Holmes, 1960; Hutchinson *et al.*, 1966; Shine, 1985; Somma, 1990). In cool climates (where almost all studies have been conducted), brooding females maintain high and constant temperatures within the clutch by shivering thermogenesis (Vinegar *et al.*, 1970), a behaviour that entails high energetic costs (Vinegar *et al.*, 1970; Harlow and Grigg, 1984; Slip and Shine, 1988). In addition, brooding females do not feed during incubation (Ellis and Chappell, 1987). Given these high costs, what are the benefits?

The most likely benefits involve the effects of maternally controlled incubation regimes on embryogenesis. Developmental trajectories in reptile embryos are highly sensitive to the physical conditions encountered during incubation (Andrews, 2004; Deeming, 2004; Shine, 2004). Eggs that experience conditions that are too dry or too wet, or too hot or too cold, either may die before hatching or may hatch but produce inferior hatchlings with a lowered probability of subsequent survival and growth (Fox, 1948; Taning, 1952; Licht and Moberly, 1965; Osgood, 1978; Muth, 1980; Burger *et al.*, 1987; Webb, 1987; Packard and Packard, 1988). This sensitivity has acted as a strong selective force on maternal behaviour in reptiles from a diverse array of phylogenetic lineages, and has favoured the evolution of careful nest-site selection in egg-laying species, and careful thermoregulation by pregnant females of viviparous taxa (Beuchat, 1988; Shine, 2004). Plausibly, the same kinds of selective forces have acted on maternal nest-attending behaviour (Shine *et al.*, 1997). That is, the benefit of maternal attendance might involve control over the physical conditions experienced by incubating eggs, in ways that enhance egg survival and/or hatchling phenotypes. The only obvious alternative hypothesis is that maternal attendance functions to reduce predation on the eggs, but this could be achieved by the mother simply remaining near the eggs rather than coiling around them and twitching. Indeed, this simpler form of parental care is seen in many other squamate species (Shine, 1985; York and Burghardt, 1988; Somma, 1990). Thus, attention focuses on two additional pathways by which maternal egg-brooding might enhance offspring viability: through changes to the thermal and/or hydric regimes experienced by the developing eggs.

The most detailed analysis of this question has come from research on water pythons (*Liasis fuscus*) from tropical Australia (Shine *et al.*, 1997; Madsen and Shine, 1999). Reproducing females display facultative nest attendance, depending upon thermal regimes inside the burrows where they lay their eggs (Madsen and Shine, 1999). Experimental incubation of eggs at a variety of thermal regimes (but without maternal attendance) suggested that maternal thermogenesis might substantially enhance offspring fitness, in some but not all natural nest sites (Shine *et al.*, 1997). This thermal effect was manifested both via increased hatching success and via modifications to phenotypic traits of hatchlings (Shine *et al.*, 1997). However, this study was conducted on artificially incubated clutches only and was restricted to temperature variations. Thus, Shine and colleagues' (1997) study provided no information on the determinants or consequences of hydric conditions during incubation, nor did it include maternally brooded clutches.

We have addressed some of these missing elements with a study on ball pythons (*Python regius*) at a field site in equatorial Africa (Aubret *et al.*, 2003, 2005). This study is the third part of an experiment that investigated: (a) the effects of brooding on the female's energy

expenditure (Aubret *et al.*, 2005), (b) the influence of clutch size manipulation on hatching success and hatchling traits (Aubret *et al.*, 2003), and (c) the effects of brooding duration on the phenotype of neonates (this study). The questions addressed in each of the three constituents of the study diverge clearly: they focus respectively on the 'relationships between parental care and costs of reproduction' versus 'clutch size and reproductive success' versus 'brooding duration and offspring phenotype'. Although complementary, these issues were consequently considered separately. One part of the study showed that brooding females spent very little energy over the 2 month incubation period, and that such expenditure was independent of fecundity, challenging the notion that intensive parental care necessarily entails major energy costs (Aubret *et al.*, 2005). Experimental manipulation of clutch size in fully attended clutches (i.e. ignoring brooding duration) strongly influenced hatching success because females were unable to physically cover enlarged clutches, and thus some eggs desiccated and died (Aubret *et al.*, 2003). The current study focuses specifically on the effects of the duration of maternal brooding on phenotypic traits of hatchling snakes. Our current data thus bear directly on the effects of parental care duration on the phenotypic traits of offspring.

METHODS

Ball pythons (*Python regius*; Pythonidae) are small (up to 170 cm snout–vent length and 4 kg in weight) nocturnally active, non-venomous constricting snakes. The species extends over a vast area of Africa in terms of longitude, latitude and habitat types (Luiselli and Angelici, 1998; Chippaux, 2001). High-density populations occur from South Ghana to South Benin, especially in disturbed areas where rodents [the main prey of these snakes (Luiselli and Angelici, 1998)] are abundant. Our study was conducted in the extreme south of Togo (Lomé; 6°7'N, 1°13'E), an equatorial area characterized by high and relatively stable temperatures all year round (from 25 to 35°C).

In the study area, females lay 3–14 eggs in tortoise or rodent burrows or abandoned termite mounds, usually in early February (Aubret *et al.*, 2003). Females coil tightly around their clutches and adopt a defensive posture when disturbed (Fig. 1). Based on observations by professional snake hunters (and personal observations), almost all clutches are attended by females. However, some clutches are found without a female in attendance, suggesting that brooding may be interrupted (perhaps for short periods) or may be facultative in this species as it is in the water python (Shine *et al.*, 1997).

Experimental procedure

As this study is the last part of a larger experiment, material (e.g. animals) and methods overlap partly. However, all the current statistics are original. There is also a weak overlap between previous mean values [e.g. maternal and offspring characteristics (size and mass) of the control group, initial clutch sizes and initial maternal characteristics of the manipulated groups remain unchanged] and those presented here. Such overlap was inevitable, and necessary to provide links between the three studies. However, the current study focuses on offspring phenotype, behaviour and growth rate as a result of incubation regime; maternal and initial clutch characteristics are only provided to illustrate the homogeneity of the procedure during the allocation of the mothers in the three treatment groups.

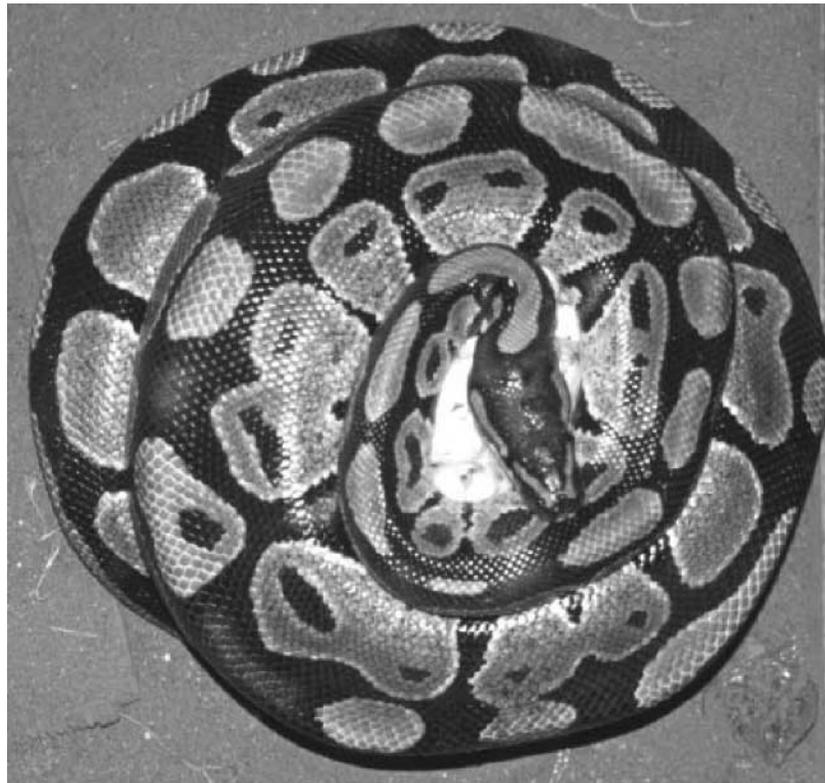


Fig. 1. Female ball python coiled around her clutch. The defensive posture adopted by the female allows observation of the eggs that are normally completely hidden.

Snake hunters employed by the registered farm TOGANIM (SARL) captured 30 gravid female pythons from the wild in the vicinity (<50 km) of Lomé at the beginning of the laying season in January 2000. Each female was initially measured for total length (± 0.5 cm) and snout–vent length (± 0.5 cm) with a flexible ruler, and body mass with an electronic scale (resolution 1 g, precision $\pm 0.2\%$). The snakes were maintained in small wooden cages (50 \times 50 \times 30 cm) in a quiet, dark room. Water and food (pre-killed mice) were provided to the snakes once a week. Although the females drank regularly, they refused to eat [as in reproductive females of many snake species (Lourdais *et al.*, 2002a)]. The 30 females produced their clutches 15–45 days after capture. The clutch was weighed less than 6 h after oviposition. Abnormal eggs (i.e. undersized or with incomplete shell) were discarded to avoid possible mould contamination to the entire clutch. The mass, maximum length and width of the eggs were recorded at the beginning of the experimental period, and then every 15 days until hatching. Because python eggs are strongly adherent, it was not always possible to separate them without damaging the shell. In such cases, the mass of each egg was inferred from the mass of the clutch divided by egg number instead of weighing the eggs individually. An estimate of the volume of the eggs was obtained using the equation to calculate the volume of an ellipsoid: $4/3\pi ab^2$, where $a = 1/2$ the length of the egg and $b = 1/2$ the width of the egg (Mayhew, 1963). As soon as the females began to lay their eggs, they were randomly allocated to one of the three treatment groups:

1. Ten 'maternally brooded' clutches were left with their mothers until hatching (i.e. control group).
2. Ten 'partly brooded' clutches were left with the mother for the first 15 days after laying; then the female was removed and the eggs left without maternal attendance.
3. Ten 'artificially incubated' clutches were separated from the mother immediately after laying.

The clutches left without maternal attendance were placed in boxes ($50 \times 50 \times 20$ cm) filled with wood shavings. The eggs were placed in the middle of each box, close to the surface, and were covered by a thin layer of shavings. Similar artificial incubators are used at TOGANIM. However, the room we used was large and well ventilated, while local farmers incubate the eggs in small and closed rooms. Despite the fact that the boxes we used were watered once a week to keep the uppermost shavings damp, the humidity (not measured) may have fallen below 100% at times. The high ambient temperatures in Lomé were buffered in the incubators in a similar way as occurs in natural nests inside the burrows of tortoises (see below). The clutches were inspected several times a week and any eggs affected by mould were removed. Eggs that died during development were dissected, and we recorded the body mass and body length of the embryo and the residual egg mass.

At the end of the experiment, all the females were apparently healthy and in good body condition (Aubret *et al.*, 2005). The females were released along with 10% of the neonates, under regulations set down by local wildlife authorities. The rest of the neonates were legally exported to the USA, Japan or Europe. None of the animals involved in our study were mistreated, sick or injured. Our study was carried out under the ongoing legal activity of TOGANIM. The IUCN recently undertook a survey on ball python populations of Togo suggesting that this species adapts well to this legal trade (further information is provided at the following IUCN site: <http://www.iucn.org/themes/ssc/programs/togoreptiles.html>).

Temperature records

Incubation temperatures were recorded using two data loggers per treatment (Tinytag Ultra –40 to 85°C; 1929 data for each recorder; delay between each record of 16 min and 30 s). We attached the loggers ($n = 6$) to the clutch. We placed two other temperature recorders in potential natural nest sites: one in a termite mound and the other in a tortoise burrow. This allowed us to compare potential natural incubation temperatures without maternal attendance to those we monitored in our experiment. We also recorded the ambient temperature of the room that housed the three sets of clutches. For analysis, we focused on the first 2 weeks of incubation, because early development is the period of highest sensitivity of the embryos in squamates as well as many other vertebrates (Gerhart and Kirschner, 1997; Shine, 1999; Shine and Elphick, 2001; Andrews, 2004). All our clutch data loggers recorded temperatures within a narrow range [extremes were recorded in the 'natural nest site' (25.9°C) and ambient room temperature (33.0°C)], and with similar means (28.8–30.5°C). Because the conditions were further buffered in the cages and boxes, all the embryos experienced similar temperatures; there were subtle differences among treatment groups, however. On average, mean temperatures of the maternally brooded or partly brooded clutches were slightly higher (1°C) than those of the artificially incubated clutches (30.5°C in maternally or partly brooded clutches versus 29.5°C in artificial incubators; the extremes ranged from 26.3 to 32.2°C). As expected

with such a narrow range of temperatures, the variances were low (all standard deviations lower than 1.3).

Incubation and hatchling characteristics

As soon as the neonatal snakes began to slit their eggshells, the eggs were removed from the females or from the incubator and placed in individual containers. The time elapsing between oviposition and egg-slitting was recorded (incubation time). The delay between the first shell-slitting and the full emergence of the hatchling was also recorded. Most hatchlings required more than 24 h to fully emerge from the egg (see Results), perhaps because the snakes slit the shell before absorbing their residual yolk completely. On a few occasions with unusually dry eggshells, the young snakes were unable to slit an opening large enough to escape from the egg. After 1 day of unsuccessful attempts, we opened a window (1 cm incision) with a scalpel. This reduced the total time necessary for emergence, but prevented unnecessary mortality. Data on 'delay of emergence' for these animals were not used in our analyses.

After full emergence, hatchlings were measured for body length, snout–vent length and body mass (± 0.1 g with an electronic scale). We counted the number of ventral scales, recorded scale abnormalities, and determined sex by eversion of hemipenes. The size and the shape of the head were measured with callipers as follows: (1) jaw length (from the tip of the snout to the quadrato-articular projection); (2) skull length (from the tip of the snout to the base of the skull); and (3) head width (maximal width above the eyes, from the external margins of the supraoculars). The remaining egg mass was weighed [shell plus remaining yolk (Deeming 1989)]. Water was provided to the neonates immediately after completion of the first measurements.

Locomotor performance and behaviour of hatchlings

Locomotor performances of 1-week-old neonates were assessed by several tests, similar to those previously used to quantify phenotypic quality in neonate reptiles (Van Damme *et al.*, 1992; Shine *et al.*, 1997; Aubret *et al.*, 2003). Swimming ability was recorded in a circular pool (1 m in external diameter; 0.9 m in internal diameter; water temperature 28°C). Dropped from 5 cm above the water, the hatchlings usually started swimming after a few seconds. During a 3 min trial, we recorded the total number of laps swum and the total time spent swimming (disregarding the time during which the hatchling was immobile, or was trying to escape). Hatchling swimming speed (distance covered in centimetres per minute) and the percentage of time spent swimming per trial were calculated.

The crawling aptitude of the hatchlings was assessed in an open area of sand, a common natural substrate for ball pythons in South Togo. The experimenter sat 3 m from the snake to minimize disturbance. Over 2 min, the distance travelled from the departure point to the final position was recorded, as well as the total number of tongue flicks (using a manual counter). Scores on this test may reflect a combination of variables such as the vigour with which the animal attempted to sample cues (tongue licking) and to escape (crawling speed) from a potentially dangerous open area.

The defensive behaviour of the hatchlings was also recorded. Their propensity to strike defensively at a small object (a pen moved at 10 cm from the snout) was assessed. The first strike started the test, and then we counted the total number of strikes during the next 30 s.

If the snake refused to strike after 3 min of harassment, or had adopted a passive defensive position such as curling itself into a compact ball by that time, we scored the trial as null.

We also measured growth rates of the hatchling snakes from birth to 10 days after emergence. Water was available, but the snakes were not fed during that period, so variations in body mass or body size must reflect utilization of energy stores originally present in the egg, including yolk conversion into new tissues and the associated water intake. Notably, residual yolk can provide enough materials to sustain growth in body size (Congdon *et al.*, 1982; Ji and Sun, 2000). Finally, we recorded the age when the snakes first shed their skins.

Statistical analyses

It was not possible to allocate the eggs randomly among treatments because they were often strongly adherent to each other. Consequently, to control for the variance due to a possible maternal effect, we used mixed-model analyses of variance (or analyses of covariance) with maternal identity as a random factor, experimental treatment as a fixed factor, and morphological traits of the eggs or of the hatchlings as the dependent variables. We also used mixed-model analyses of variance to assess physiological performances post hatching. Snake body condition (mass relative to length) was analysed using analyses of covariance with body mass as the dependent variable and snout–vent length as the covariate (Garcia-Berthou, 2001). Because the neonates used in the behavioural tests were chosen randomly within different clutches and equally distributed among groups, it was not necessary to control for maternal (i.e. clutch) identity. Indeed, the mean number of neonates per mother included in the behavioural analyses was 1.14 (range 1–2), removing a potential maternal (pseudo-replication) effect. Growth trajectories were analysed using multivariate analysis of variance with repeated measures of snout–vent length and body mass over time; maternal identity was included as a random factor [multivariate analysis of variance (O'Brien and Kaiser, 1985)]. For all behavioural tests, several variances were not homogeneous (even after log transformation), so we used non-parametric Kruskal-Wallis analyses of variance for these tests. Null scores (i.e. no strike during the defensive behaviour test) were taken into account in the analysis to avoid comparing behavioural measurements that may emerge from different 'decisions' taken by the snakes (facing versus escaping the danger). All statistical tests were performed with Statistica 6.1.

RESULTS

Maternal and clutch characteristics

The mean characteristics of the females and of their clutches allocated to the three treatment groups are given in Table 1. We did not find any significant differences among the three batches in the mean body lengths of the mothers (ANOVA with treatment as the factor: $F_{2,27} = 0.47$, $P = 0.63$), their body masses ($F_{2,27} = 0.71$, $P = 0.50$), their body condition (same design ANCOVA with maternal size as a covariate: $F_{2,26} = 0.31$, $P = 0.74$), their clutch sizes (same design ANOVA: $F_{2,27} = 0.68$, $P = 0.51$), clutch masses (same design ANOVA: $F_{2,28} = 0.19$, $P = 0.82$), or laying dates (same design ANOVA: $F_{2,27} = 0.59$, $P = 0.56$; $P > 0.50$ in all *post-hoc* tests for these five analyses of variance). Similarly, egg mass at oviposition (mixed-model ANOVA with maternal identity as a random factor and treatment as the

Table 1. Maternal and clutch characteristics of the three brooding treatment groups of ball pythons ($n = 10$ females in each group)

Variable	Maternally brooded	Partially brooded	Not brooded
Snout–vent length (cm)	112.5 ± 2.2	114.5 ± 1.6	115.3 ± 2.6
Body mass (g)	1844.2 ± 98.5	1933.3 ± 91.2	2032.3 ± 139.2
Pre-laying body condition (g)	1910.2 ± 71.3	1917.7 ± 70.6	1981.9 ± 71.0
Clutch size	7.3 ± 1.2	7.4 ± 1.1	8.0 ± 1.9
Egg masses at laying (g)	86.9 ± 1.9	88.7 ± 1.1	87.9 ± 2.8
Egg volume at laying (cm ³)	80.0 ± 1.0	86.0 ± 1.2	84.8 ± 1.7

Note: Pre-laying maternal body condition represents maternal body mass adjusted by size. Sample sizes for egg mass ($n = 12, 4, 12$) and individual egg size at laying ($n = 62, 64, 65$) for the maternally brooded, partially brooded and the ‘artificially’ incubated group, respectively. Egg volume = volume of an ellipsoid ($4/3\pi ab^2$, where $a = 1/2$ the length of the egg and $b = 1/2$ the width of the egg). Mean values are given with standard errors.

main factor: $F_{2,9} = 0.02$, $P = 0.98$) and egg volume at laying (same design mixed-model ANOVA: $F_{2,26} = 1.36$, $P = 0.28$) did not differ among the three groups. Therefore, any difference in relevant traits among our treatment groups should reflect the influence of incubation regimes on incubation periods and on the phenotypes of hatchlings.

Incubation regime, incubation period and morphology of the hatchlings

Incubation periods averaged 2 months and did not differ significantly among the three treatment groups (Table 2). However, many traits we measured were affected by the incubation regime (Table 2). Maternally incubated neonates were larger, heavier and had longer jaws than neonates in the other two groups (all $P < 0.001$). The artificially incubated hatchlings were small and in poor body condition. However, our experimental treatments did not induce any significant difference in many other traits, such as length or width of the heads, or scale counts (total number of ventral scales, number of abnormal scales). Importantly, the mass of the material remaining after hatching (yolk + shell) was significantly lower in the maternally brooded group, intermediate in the partly brooded group and higher in the ‘artificially’ incubated group. This pattern suggests that maternal brooding allowed hatchlings to incorporate their available yolk material into the body cavity before leaving the egg. As expected, the mass of the remaining material was negatively correlated with the body mass of the hatchlings ($n = 79$, $r = -0.40$, $P = 0.0002$).

Due to a net loss of water (Wangensteen *et al.*, 1970; Rahn and Ar, 1974; Packard, 1991), the eggs lost mass from laying to hatching in all three groups. However, the amount of water lost differed significantly among the treatments (repeated measures of mass over time: Wilks’ $\lambda = 0.53$, $F_{2,19} = 6.34$, $P < 0.008$). Maternally brooded eggs lost, on average, 16% of their initial mass, partly brooded eggs lost 37% of their initial mass, while the artificially incubated eggs lost 51% of their initial mass. This result clearly suggests that the presence of the mother limited desiccation of the eggs.

Considering only maternally brooded hatchlings, greater hatchling mass was associated with longer incubation ($n = 21$, $r = 0.54$, $P < 0.01$) [Fig. 2; mean values per clutch plotted – in these analyses we also used supplementary data from a parallel experiment in which females were allowed to completely brood their clutches (Aubret *et al.*, 2003)]. Also, larger eggs gave rise to larger hatchlings ($n = 18$, $r = 0.47$, $P < 0.04$).

Table 2. Effects of incubation regime on incubation period, remaining egg mass and hatchling traits (mean \pm standard error) in the ball python, *Python regius*

Variable	Maternally brooded	Partially brooded	Not brooded	d.f.	F/H	P
Incubation period (days) *	60.73 \pm 0.15	60.55 \pm 0.35	61.83 \pm 0.83	2,19	0.74	0.49
Emergence duration (days)	1.51 \pm 0.08	2.07 \pm 0.15	2.60 \pm 0.60	2,16	5.43	<0.01
Remaining egg mass (g)	5.50 \pm 0.43	13.14 \pm 2.00	17.18 \pm 3.58	2,19	11.31	<0.001
<i>Morphology at hatching</i>						
Body mass (g)	55.05 \pm 0.91	44.31 \pm 3.23	38.35 \pm 2.96	2,19	9.00	0.001
Snout–vent length (cm)	39.26 \pm 0.28	35.01 \pm 0.81	35.83 \pm 0.95	2,19	15.66	<0.001
Body condition index	48.38 \pm 1.22	47.65 \pm 1.54	40.68 \pm 2.80	2,19	1.51	0.24
Jaw length (mm) **	26.95 \pm 0.14	25.25 \pm 0.19	25.67 \pm 0.37	2,19	21.09	<0.001
Skull length (mm) ***	9.65 \pm 0.07	9.51 \pm 0.09	9.45 \pm 1.16	2,19	0.75	0.48
Head width (mm) **	4.76 \pm 0.04	4.61 \pm 0.06	4.80 \pm 0.11	2,19	2.23	0.13
Number of ventral scales	207.04 \pm 0.48	205.82 \pm 0.65	205.33 \pm 1.09	2,19	0.82	0.45
Abnormal ventral scales (n)	2.37 \pm 0.26	3.55 \pm 1.10	3.00 \pm 0.68	2,19	1.32	0.28
<i>Behavioural traits</i>						
Distance swum (m)	6.62 \pm 0.56	3.52 \pm 0.74	3.31 \pm 0.14	2,63	11.04	<0.005
Swimming speed (m \cdot min ⁻¹)	3.07 \pm 0.27	2.45 \pm 0.34	1.49 \pm 0.64	2,63	8.17	<0.02
Percentage of activity	67.95 \pm 4.34	47.62 \pm 5.69	56.18 \pm 10.64	2,63	7.86	<0.02
Distance covered on ground (m)	1.12 \pm 0.14	0.70 \pm 0.18	0.62 \pm 0.34	2,63	3.32	0.19
Number of strikes elicited	5.57 \pm 0.98	6.08 \pm 1.18	9.00 \pm 1.91	2,37	0.99	0.61
Number of strikes elicited †	2.94 \pm 0.73	3.76 \pm 0.96	7.50 \pm 1.79	2,63	3.18	<0.02
Number of tongue flicks	112.11 \pm 7.67	95.05 \pm 10.03	103.83 \pm 18.76	2,63	2.73	0.26
<i>Physiological performance</i>						
Delay to first slough (days)	10.47 \pm 0.16	12.25 \pm 0.62	12.50 \pm 0.96	2,10	9.21	<0.01
BM (g) (10 days old)	58.18 \pm 1.13	52.31 \pm 2.64	40.50 \pm 3.28	2,12	8.12	<0.01
SVL (g) (10 days old)	44.31 \pm 0.30	42.44 \pm 0.73	40.10 \pm 1.05	2,12	5.43	<0.02
Increase in BM (g) ****	1.90 \pm 0.72	3.62 \pm 0.78	0.59 \pm 1.51	2,12	0.96	0.41
Increase in SVL (cm) ***	5.83 \pm 0.29	6.22 \pm 0.34	4.08 \pm 0.62	2,12	2.05	0.17

Note: All comparisons among the three treatments were performed using mixed-model analyses of variance with maternal identity as a random factor, except for behavioural traits where Kruskal-Wallis analyses of variance were used (see text for details). BM = body mass; SVL = snout–vent length.

Maternally brooded = clutch left with their mother until hatching ($n = 51$ neonates); partially brooded = clutch left with their mother during the first 2 weeks, then placed into an artificial incubator ($n = 22$ hatchlings); artificially incubated = clutch placed into an artificial incubator throughout incubation ($n = 6$ hatchlings); * mean value per clutch; ** relative to skull length; *** relative to initial snout–vent length; **** relative to initial body mass; † number of strikes including null scores.

Locomotor performance and behaviour of hatchlings

Incubation regimes affected several aspects of hatchling performance. First, maternally brooded hatchlings emerged more rapidly from their eggs than did the partly brooded snakes, which in turn emerged more rapidly than did the artificially incubated hatchlings (Table 2). Larger neonates emerged from the egg more rapidly than smaller snakes, as indicated by a negative correlation between body size and the duration to escape from the egg (snout–vent length: $n = 66$, $r = -0.39$, $P < 0.001$; body mass: $n = 66$, $r = -0.47$, $P < 0.001$). Thus, maternally brooded snakes were not only larger, but they also emerged

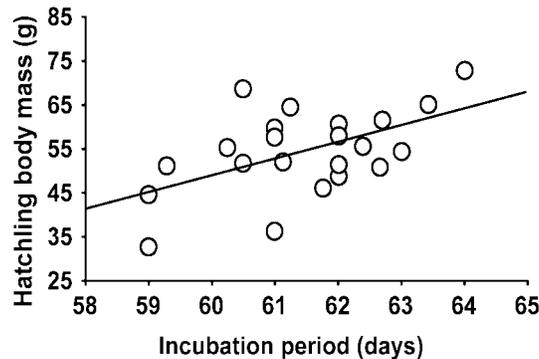


Fig. 2. Relationship between the duration of incubation and the body mass of hatchlings in the ball python. These two parameters are plotted as means per clutch.

from the egg more quickly. However, this latter effect may have been a consequence of the former: when the effect of neonate size was taken into account, statistical significance was lost using an analysis of covariance with clutch identity nested within incubation treatment as factors, hatchling mass as covariate and escape time as the dependent variable ($F_{2,47} = 2.96$, $P < 0.061$), but not with snout–vent length as covariate ($F_{2,47} = 4.95$, $P < 0.011$).

Maternally brooded hatchlings swam faster and over a longer distance than did young snakes from the other treatments. We found a positive influence of snout–vent length on locomotor ability. However, when significant, this relationship was always very weak ($n = 63$; $0.02 < r^2 < 0.08$, $0.25 < P < 0.03$ in all correlations between locomotor performances and snout–vent length), and including snout–vent length as a covariate in the analyses did not affect the results. Consequently, the better swimming performance of maternally incubated hatchlings was mostly attributable to the effect of maternal attendance *per se* rather than a by-product of size. The maternally incubated hatchlings were also more active (see Table 2). Our results suggest that on average maternally brooded hatchlings tended to travel greater distances over the ground and explored their environment more intensively by tongue flicking. However, the results for tongue-flicking rates and distance travelled over the ground did not reach statistical significance, perhaps due to high variability for these traits. The maternally incubated hatchlings did not display more intense defensive behaviour when harassed during experimentation. In fact, the reverse trend was observed, and this effect was significant when null scores were included. The proportion of snakes that struck the pen or adopted a defensive passive posture differed, although not significantly so (due to the small sample size of several cells in the contingency table), between treatments: 53% of the maternally brooded snakes decided to strike, versus 62% for the partially brooded and 83% for the non-brooded neonates ($\chi^2 = 2.11$, d.f. = 2, $P = 0.34$).

Post-hatching growth rates

Despite the absence of food, all young pythons increased their snout–vent length and body mass from hatching to the age of 10 days (Table 2). Importantly, water was available *ad libitum* over this period. This early growth undoubtedly was sustained by abdominal yolk or other body reserves (Ji et al., 1997; Ji and Sun, 2000). Hatchlings from all groups showed similar increases in size and mass (Table 2), but maternally brooded snakes maintained a significant

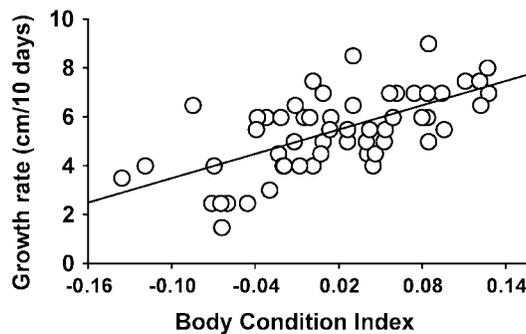


Fig. 3. Relationship between body condition index at hatching and growth in snout–vent length until the age of 10 days in neonate ball pythons.

advantage in terms of body size and body mass after 10 days (Table 2). Partially brooded snakes were also larger and heavier than the ‘artificially’ brooded offspring. Snakes with the highest body condition index at the time of hatching, and hence with greater body reserves, exhibited a higher post-natal growth rate (growth rate after hatching was positively correlated with initial body condition: $n = 54$, $r = 0.64$, $P < 0.0001$) (Fig. 3).

Maternally brooded hatchlings also sloughed their skins earlier than did other hatchlings. The delay from birth to the first slough was significantly correlated with the initial body mass of hatchlings ($n = 46$, $r = 0.52$, $P < 0.0002$). When this effect of body size was controlled through an analysis of covariance (with body mass as the covariate), the influence of treatment on sloughing delay was not statistically significant ($F_{2,34} = 1.25$, $P = 0.30$). Thus, incubation treatment influenced sloughing mainly via its effect on the size of neonates; small neonates tended to delay their first slough until they had attained a larger size. Overall, our data suggest that maternally brooded hatchlings were in ‘better’ condition (larger, faster, more active, faster-developing) than those that were partly brooded. The hatchlings that were ‘artificially’ incubated were in the poorest condition.

DISCUSSION

Our data show that for ball pythons, parental care over a prolonged period strongly affected not only hatching success of the eggs (Aubret *et al.*, 2005), but also the phenotypic traits of hatchlings that emerged from the viable eggs (this study). The variety of traits that we measured support the inference that maternally incubated eggs gave rise to ‘better’ hatchlings. That is, not only was hatching success much higher from maternally incubated clutches, but the hatchlings that emerged were larger, more active, swam faster and for longer, and developed more rapidly post hatching than did offspring from artificially incubated clutches.

Why did maternal brooding enhance hatching success and generate ‘superior’ hatchling phenotypes in our experiment? Previous discussions of the benefits of maternal brooding for offspring fitness have generally focused on thermal regimes (Vinegar *et al.*, 1970; Vinegar, 1973; Harlow and Grigg, 1984; Shine *et al.*, 1997). Notably, in pythons, enhanced embryonic survival and development has been attributed to the maintenance of high stable temperatures via shivering thermogenesis. More generally, many studies on squamate reptiles have concluded

that hatchling phenotype temperatures are more sensitive to thermal than to hydric conditions during incubation (Van Damme *et al.*, 1992; Shine *et al.*, 1997; Flatt *et al.*, 2001; Shine and Elphick, 2001). However, several studies have reported strong hydric effects (e.g. Warner and Andrews, 2002; Brown and Shine, 2004). Our results support this latter conclusion. Although we acknowledge that our experimental design did not separate out thermal and hydric effects, the consequences of maternal brooding for hatching success and offspring phenotypes are more likely to reflect hydric than thermal factors. We base this conclusion on four observations:

1. Maternally brooded eggs lost less water than eggs in the other treatments, and the partly brooded clutches lost less water than the non-attended ones [see also Aubret *et al.* (2003, 2005) for the difficulties faced by the mother in covering an artificially enlarged clutch during incubation].
2. The low hatching success of ‘artificially’ incubated eggs was due to yolk desiccation: many of these hatchlings left substantial solidified yolk behind in the egg, rather than incorporating it into their bodies before hatching (note the negative correlation between hatchling mass and residual egg mass; and the heavier residual mass of non-brooded eggs). Yolk in ‘artificially’ incubated eggs began to solidify, especially on the desiccation-prone upper surface. The solid mass of yolk may have directly impaired the hatching process.
3. Mean hatching dates did not differ among treatments. In reptiles, incubation period and gestation length are strongly influenced by mean temperatures during development (Blanchard and Blanchard, 1941; Naulleau, 1986; Lourdais *et al.*, 2002b), so the similarity in incubation periods infers a similarity in mean temperature.
4. We did not find any difference in the number of ventral scales or in the occurrence of scale anomalies among the three groups. These traits are also sensitive to incubation temperatures (Fox, 1948; Osgood, 1978; Lourdais *et al.*, 2004). Overall, the differences in temperature experienced by the embryos generated by our experiment were probably too small ($\approx 1^\circ\text{C}$) to induce any major effects.

How can the mother’s presence modify the hydric balance of her clutch? Female ball pythons coil so tightly around the clutch that the eggs are completely hidden (Fig. 1; personal observation). This ‘bell’ surrounding the clutch could create a saturated microclimate around the eggs, substantially reducing evaporation (O. Lourdais and D. DeNardo, personal communication). Thus, our results differ from those of most previous research by advocating an important hydric rather than thermal benefit for parental care in pythons. If optimal incubation conditions generate optimal hatchling phenotypes, there will be strong selection for maternal behaviours that expose embryos to such conditions [for example, temperature-dependent sex determination processes (Shine, 1999); nest-site selection, shivering thermogenesis (Shine *et al.*, 1997)]. In such cases, natural selection can act at two levels: on genes that code for maternal influence on the clutch (e.g. thermal criteria for nest-site selection or the intensity of shivering thermogenesis), and on genes involved in norms of reaction during embryogenesis.

The mechanism that generated phenotypic variation among our hatchling pythons is one that has not attracted previous interest – yolk coagulation due to egg desiccation that reduces the amount of resources available to the embryos (Sinervo, 1990). We note that the relative importance of hydric control on fitness versus that of thermic control remains an open question in wild-brooding pythons. However, the eggs of many species that are not

maternally incubated (reptiles, insects) often suffer from desiccation; hydric control may allow pythons to incubate their eggs in areas where the clutch would potentially desiccate if left alone (Snell and Tracy, 1986; Tracy and Snell, 1986).

A plausible scenario for the evolution of parental care in pythons involves an initial step whereby the female's presence benefited offspring survival by discouraging egg predators (Shine, 1985). The subsequent change to the female's posture (coiling tightly around her eggs) may have been favoured because of the resultant substantial reduction in water loss from the clutch at a very low additional cost (Aubret *et al.*, 2005). Lastly, advantages associated with high and more stable incubation temperatures may have resulted in the evolution of shivering thermogenesis. Our results suggest that even a brief initial period (2 weeks) of maternal brooding enhances hatching success and improves phenotypes compared with artificially incubated eggs. If offspring fitness is enhanced even by brief parental attendance, it is easy to imagine the evolution of obligate brooding through a series of intermediate stages that involved a gradual increase in the duration of this behaviour (Mell, 1929; Shine, 1985; Farmer, 2000, 2003). Any such adaptationist scenario is speculative, and relies upon data from present-day taxa to extrapolate back to the ancestral condition. We may be misled by adaptive changes that have occurred subsequent to the evolution of brooding. For example, if maternal brooding maintains high constant humidity around the clutch, eggs may evolve a lowered resistance to desiccation (because they are never exposed to this problem): the sensitivity to desiccation might be secondary, notably to allow embryonic respiration (Wangenstein *et al.*, 1970). In keeping with this possibility, the eggs of ball pythons are more vulnerable to desiccation than are the eggs of most other squamate reptiles (Alberts *et al.*, 1997). Comparative data on desiccation rates of eggs from brooding species and their non-brooding relatives could clarify the validity of this hypothesis.

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