Clutch size manipulation, hatching success and offspring phenotype in the ball python (*Python regius*)

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In a diverse array of avian and mammalian species, experimental manipulations of clutch size have tested the hypothesis that natural selection should adjust numbers of neonates produced so as to maximize the number of viable offspring at the end of the period of parental care. Reptiles have not been studied in this respect, probably because they rarely display parental care. However, females of all python species brood their eggs until hatching, but they do not care for their neonates. This feature provides a straightforward way to experimentally increase or reduce clutch size to see whether the mean clutch size observed in nature does indeed maximize hatching success and/or optimize offspring phenotypes. Eggs were removed or added to newly laid clutches of Ball Pythons (*Python regius*) in tropical Africa (nine control clutches, eight with 50% more eggs added, six with 42% of eggs removed). All clutches were brooded by females throughout the 2-month incubation period. Experimental manipulation of clutch-size did not significantly affect the phenotypes (morphology, locomotor ability) of hatchlings, but eggs in 'enlarged' clutches hatched later, and embryos were more likely to die before hatching. This mortality was due to desiccation of the eggs, with females being unable to cover 'enlarged' clutches sufficiently to retard water loss. Our results support the notion of an optimal clutch size, driven by limitations on parental ability to care for the offspring. However, the proximate mechanisms that generate this optimum value differ from those previously described in other kinds of animals. © 2003 The Linnean Society of London. *Biological Journal of the Linnean Society* 2003, **78**, 263–272.

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INTRODUCTION

One of the earliest and most influential hypotheses on life-history evolution was David Lack's (1947) suggestion that natural selection should adjust clutch sizes in birds (number of eggs produced) such as to maximize the number of offspring surviving to the end of the period of parental care. Lack's argument was that because the energy demands of parental care were greater than those related to the production of eggs, female birds should be physically capable of producing more eggs than they (and/or their partner) could raise through to fledging. Thus, selection should reduce the number of eggs laid until it approximately corresponded to the number of offspring that the parents were capable of caring for.

Lack's original hypothesis has long been known to be too simplistic: for example, it ignores potential effects of a larger clutch on parental survival or subsequent breeding. Many refinements have been added over time to reflect more accurately the complexity of natural situations (Williams, 1966; McGinley, Temme & Geber, 1987; Winkler & Wallin, 1987; Godfray, Partridge & Harvey, 1991; Morris, 1992; Sinervo, 1999). Nonetheless, Lack's idea of 'optimal clutch size' attracted considerable research because it was directly testable by experimental manipulation. Simply by adding or removing eggs early in the incubation period, students could measure the consequences of

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different clutch sizes for subsequent offspring fitness (Hardy, Griffiths & Godfray, 1992; Monaghan & Nager, 1997). Most of these experiments were carried out on birds, usually by manipulating the number of young in the nest after hatching (Pettifor, 1993) or, less often, by manipulating the number of eggs early in incubation (Monaghan & Nager, 1997). This brood size manipulation approach (mostly performed on birds and mammals) became a classic paradigm in evolutionary ecology, and generated many insights into the selective forces that act on fecundity. The general trend that emerged from empirical and experimental observations was that artificially enlarged clutch or brood size led to a lower survival of the offspring, decreased the phenotypic quality of the offspring and often induced greater costs for the parents. This result held true both in birds (Perrins, 1965; Clark & Wilson, 1981; Arcese & Smith, 1985; Baltz & Thompson, 1988; Moreno & Carlson, 1989; Smith, 1989; Moreno *et al*., 1991; Székely, Karsai & Williams, 1994; Siikamäki, 1995; Monaghan & Nager, 1997; Reid, Monaghan & Ruxton, 2000) and in mammals (Wurtman & Miller, 1976; Epstein, 1978; Eisen & Saxton, 1984; Koopman *et al*., 1990; Sikes & Ylönen, 1998).

Although the effects of clutch size manipulation have been studied in a wide range of birds and mammals, other vertebrates have attracted less scientific attention. Techniques have been developed to manipulate clutch sizes in reptiles, and have clarified issues such as costs of reproduction and trade-offs between egg size vs. number (Sinervo & Licht, 1991; Sinervo *et al*., 1992; Sinervo, 1999). However, this work has been conducted on reptile species without parental care, so that Lack's original hypothesis cannot be examined. In fact, because the phases of parental care tested have differed among studies on different kinds of organisms (Fig. 1), there is no straightforward way to compare the results of experimental manipulations of clutch size in birds and mammals vs. other vertebrate lineages. Even brood size manipulations in birds and mammals are not always directly comparable, because in mammals there are no experiments equivalent to clutch-size manipulation.

To facilitate comparisons with the intensively studied birds, we need a model organism that exhibits a prolonged period of parental care after laying (i.e. a long brooding period), and in which we can manipulate the number of eggs per clutch, and then monitor the effects of that manipulation on the fitness of the offspring at the end of the period of parental care. Although most squamate reptile species (snakes and lizards) do not care for their offspring after oviposition, maternal attendance of the developing eggs has been reported in a wide variety of species (Somma, 1985, 1990; Shine, 1998). Parental care in these taxa generally takes the form of the mother remaining near
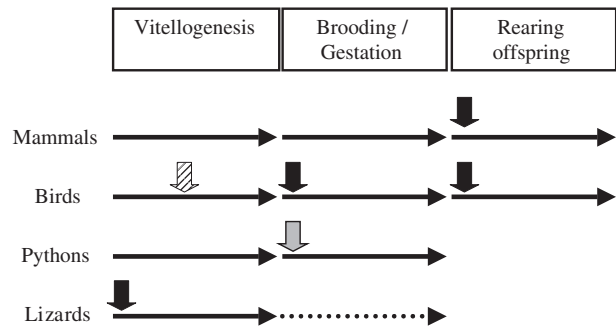
Figure 1. In vertebrates, maternal investment can be broadly separated into three main phases (boxes and horizontal black arrows): vitellogenesis, brooding (or gestation in viviparous species) and parental care devoted to the offspring. Vitellogenesis is the only obligatory phase: for example, many lizards abandon their eggs after laying (discontinuous arrow: incubation in a nest). Manipulations of clutch (litter) size have been conducted in different vertebrate lineages including mammals, birds and lizards. However, manipulation of offspring number generally has occurred at different stages (vertical black arrows) in these different groups, impeding direct comparisons among the taxa (see text for references). It should be noted that indirect manipulation of the clutch was also performed in birds (i.e. through hormonal manipulation: hatched arrow, i.e. Monaghan, Bolton & Houston, 1995; Visser & Lessells, 2001; Williams, 2001). The present study was carried out on a non-avian species that broods the clutch: the ball python. We manipulated clutch size at a stage (grey arrow) directly comparable to procedures adopted for studies of several bird species.

the eggs, presumably to guard them against predators but perhaps also to modify their thermal and hydric incubation environment (York & Burghardt, 1988; Somma & Fawcett, 1989; Somma, 1990; Shine, 1998). The most complex and energetically expensive form of parental care among squamate reptiles occurs in the Pythonidae family. Females of these large, muscular snakes coil around the eggs after oviposition, and remain with the eggs throughout the incubation period (Cogger & Holmes, 1960; Hutchinson, Dowling & Vinegar, 1966; Harlow & Grigg, 1984; Shine, 1998). Typically, females do not feed during this period (Madsen & Shine, 1999, 2000). The mother's presence may modify desiccation rates of the eggs (because her body covers the clutch) and rhythmic contractions of her musculature may generate heat to maintain high and stable incubation temperatures (Vinegar, Hutchinson & Dowling, 1970; Harlow & Grigg, 1984; Charles, Field & Shine, 1985; Slip & Shine, 1988; Shine *et al*., 1997a).

Pythons thus provide an ideal model to test Lack's 'optimal clutch size' hypothesis in a non-endothermic

vertebrate. Plausibly, the optimal clutch size for a python might be determined by her ability to brood her eggs successfully, in terms of either hydration or thermoregulation. We thus tested this idea by conducting an experimental study on the effects of clutch size manipulation on offspring fitness in a python species from tropical Africa. Besides the test of optimal clutch size, this paper presents the first quantitative ecological data for African pythons.

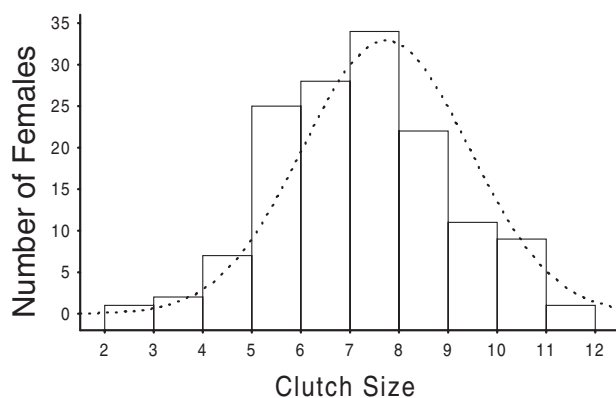## MATERIAL AND METHODS

STUDY SITE AND ANIMALS

The study was conducted from January to June 2000, in a tropical area of Africa (Lomé, south Togo). This area is characterized by high and relatively stable temperatures year-round (from 25 to 35 °C). The wet season usually begins in May and extends until August. The ball python (*Python regius*; Pythonidae) is a medium-sized nocturnal snake (body length averages 1–2 m, both in males and in females) that is abundant in anthropogenically disturbed habitats such as small fields in secondary forest. Females lay a clutch of 3–14 eggs, generally in February, in tortoise or rodent burrows. Following oviposition, the female coils around her clutch. Based on observations by professional snake hunters, most clutches are attended by females.

EXPERIMENTAL PROCEDURE

Snake hunters employed by Toganim (SARL) captured 28 gravid female pythons from the wild in the vicinity of Lomé, shortly before oviposition. The females were maintained in small wooden cages ($50 \times 50 \times 30$ cm) in a quiet, dark room. Water was provided to the snakes once per week. Each female was initially measured for total length (TL ± 0.5 cm), snout-vent length (SVL ± 0.5 cm) and body mass with an electronic scale (BM = body mass, resolution 1 g). The females produced their clutches 15–45 days after capture. As laying always occurred during the night, the whole clutch was weighed the following morning. The eggs were measured with callipers (maximum length and width). They were also weighed (individually where possible, but python eggs are often strongly adherent) at the beginning of the experimental period, and then every 15 days until hatching.

As soon as the females began to lay their eggs, we separated the clutches into three treatment groups (Fig. 2):
(1) ten unmanipulated clutches (6.88 ± 1.17 eggs);
(2) nine artificially enlarged clutches (initial clutch 6.50 ± 2.07 eggs increased to 9.75 ± 2.12 eggs: 50% increase of the initial clutch size);

Figure 2. The frequency distribution of natural clutch sizes (1–12 eggs) in ball pythons is non-normal; that is, most clutches are of intermediate size (i.e. larger than 5 eggs) rather than either very large or very small. The broken line shows the theoretical normal distribution.

(3) nine artificially reduced clutches (initial clutch 6.33 ± 2.07 eggs reduced to 3.67 ± 1.50 eggs: 42% decrease of the initial clutch size).

The clutches were inspected regularly to check for the presence of potential predators such as mice, beetles or ants, and to remove decomposing eggs that might otherwise have contaminated the whole clutch (although we have no field data on the importance of such factors on hatching success). Eggs that died during development were dissected, and we recorded the body mass and body length of the embryo (if any), and the residual egg mass.

Clutch size manipulation in pythons was not a straightforward task. Because eggs often adhere to each other, we could not redistribute eggs randomly among females. Also, eggs that are removed from an adherent group might be damaged in the process (e.g. eggshell damage might increase water loss). Lastly, females with artificially enlarged clutches may recognize and reject surrogate (foreign) eggs. For these reasons, eggs that were translocated to a new clutch ($N = 42$) may have a lower hatchling success or modified hatchling phenotype simply because they were manipulated, regardless of the clutch size treatment to which they were transferred. Because our experimental unit was the clutch and not the egg, we adopted the conservative approach of focusing on non-surrogate eggs ($N = 165$). Each egg was given an individual identification number.

Hatchlings generally took many hours between slitting the eggshell and fully emerging (see below), enabling us to link a hatchling with its egg of origin in most cases ($N = 142$ among 165; 86% for non-surrogate eggs; $N = 42$, 100% for surrogate eggs). For the clutches of five females (one from the unmanipu-

lated clutch group, one from the artificially enlarged clutch group and three from the artificially reduced clutch group), hatching was not observed. These five females were deleted from the analysis on hatchling phenotypes (so that the resulting sample size was 23 clutches: nine unmanipulated, eight enlarged and six reduced). Finally, 14 non-surrogate eggs belonging to different females died rapidly during incubation, and dissection revealed that they were infertile and relatively small (20% smaller than the other eggs on average). These 14 eggs were removed from the analyses on hatchling success and offspring phenotype.

INCUBATION AND HATCHLING CHARACTERISTICS

Incubation time was defined as the duration from the day of laying to the time when neonates began to slit the shell. We also measured the delay between the first shell slitting and the full emergence of the hatchling. On average, hatchlings required more than 24 h to emerge fully from the egg (see Results).

Once fully emerged, the hatchlings were measured for TL, SVL and BM (± 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 shape of the head was measured with callipers as follows: (1) jaw length (JL: from the tip of the snout to the quadrato-articular projection); (2) skull length (SL: from the tip of the snout to the base of the skull); (3) head width (HW: maximal width above the eyes, from the external margins of the supraoculars). We also weighed the remaining egg mass (shell plus remaining yolk). Incubation temperatures were recorded during the first 15 days of incubation using three automatic recorders attached to the clutches (one Tinytag Ultra [$-$ 40 to 85 °C] per batch; total $N = 415$ data points; delay between successive records 16 min 30 s; only 15 data points per logger were used to calculate mean values, in order to reduce autocorrelation between successive records).

LOCOMOTOR PERFORMANCE AND BEHAVIOUR OF HATCHLINGS

We performed several tests to quantify locomotor and physiological performances of hatchlings when they were 1 week old. Similar tests have been previously validated on other neonate reptiles, including snakes (Shine *et al*., 1997a; Shine, Elphick & Harlow, 1997b; Elphick & Shine, 1998; Flatt *et al*., 2001; Shine & Elphick, 2001). We measured their swimming ability (both neonate and adult ball pythons are good swimmers, pers. observ.) by placing them in a circular pool (1 m in external diameter, 0.9 m in internal diameter) at 28°C. When the hatchlings were placed in the water they usually started to swim after a few seconds to

search for a refuge. During a 3-min trial, we recorded the total number of laps swam and the total time spent swimming (disregarding the time during which the hatchling was immobile, or was trying to escape). We calculated the hatchling's swimming speed (distance covered in centimetres per minute), and the percentage of time spent swimming per trial. We also tested the crawling aptitude of the hatchlings. The young snakes were placed in an open area on sand, the most common natural substrate for ball pythons in South Togo. The experimenter was seated 3 m from the snake. Over a 2-min period we measured the distance travelled from the departure point to the final position. We also scored the total number of tongue flicks (using a manual counter) and defensive behaviour of the hatchlings. We assessed their propensity to strike defensively by continuously irritating them with a small object moving in front of them (a pen moved 10 cm from the snout). 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.

Finally, we evaluated growth rates of the hatchling snakes over their first 10 days of life. They were not fed during that period, so any changes in body mass or length must reflect utilization of energy stores originally present in the egg. Residual yolk can provide enough materials for a substantial increase in growth (Congdon, Dunham & Tinkle, 1982; Goulden, Henry & Berrigan, 1987; Ji *et al*., 1997; Ji & Sun, 2000). We also recorded the age when the snakes first shed their skin, another indicator of growth.

REPRODUCTIVE BIOLOGY OF SNAKES IN THE WILD

To determine whether or not the observed mean clutch size is optimal, we need an estimate of clutch sizes under natural conditions. Because no such published data are available for *Python regius*, we collected data on 138 gravid female pythons from the same area (in addition to the animals used in our experiment).

STATISTICS

As eggs within most clutches were strongly adherent to each other, we could not split clutches to allocate all the eggs randomly among each of our experimental treatment groups. Hence all the analyses on hatchling success ($N = 128$ eggs) and offspring phenotypes ($N = 103$, this reduced sample size was due to embryonic mortality during incubation) were based on non-surrogate eggs (except water loss during incubation; see Results). The analysis of hatchling success was performed using logistic regression with 'hatch vs. not

hatch' as the dependent variable and treatment as the independent variable. Brooding females were nested within treatment, thereby causing 'clutch' to become the experimental unit. Data for hatchling phenotypes were analysed in mixed-model ANOVAs or ANCOVAs. Hatchling traits were the dependent variables, treatment was a fixed factor and the incubating female was considered as a random factor. Null scores (i.e. no strike during the defensive behaviour test) were deleted from the analysis to avoid comparing behavioural measurements that may emerge from completely different decisions taken by the snakes (facing vs. escaping the danger). In order to compare temperature records, and avoid statistical problems due to non-independence of the data, we calculated maxima, minima and means for each day and used the daily numbers as quasi-independent data. All statistical tests were performed with Statistica 6.0 and SAS.

RESULTS

REPRODUCTIVE BIOLOGY OF SNAKES IN THE WILD

The 138 wild-caught female ball pythons averaged 116.4 ± 7.4 cm in SVL. The mean clutch size was 7.7 ± 1.7 eggs and mean clutch mass was 646.4 ± 173.5 g. Mean pre-laying and post-laying body masses for the females were 1944.1 ± 375.0 g and 1234.8 ± 241.3 g, respectively. Thus, relative clutch mass (clutch mass divided by post-laying maternal mass) averaged $51.7 \pm 9.3\%$. Clutch size was highly correlated with maternal SVL ($F_{1,136} = 41.4$; $P < 0.0001$; $r = 0.48$). Females laid 3–12 eggs, but 95% of the clutches contained at least five eggs. Clutch sizes were not normally distributed, with a strong over-representation of clutches containing five eggs (Shapiro-Wilk's $W = 0.96$; $P < 0.002$; Fig. 2).

DIFFERENCES AMONG TREATMENT GROUPS

There was no significant difference between the three experimental treatments in mean values for maternal body mass (ANOVA performed on the restricted sample [$N = 23$ clutches] with maternal body mass as the dependent variable and treatment as the factor, $F_{2,20} = 0.98$; $P = 0.39$), snout-vent length (same design ANOVA, $F_{2,20} = 0.92$; $P = 0.42$), body condition (ANCOVA with SVL as a covariate, $F_{2,19} = 0.48$; $P = 0.63$), mean clutch size ($F_{2,20} = 0.20$, $P = 0.82$) or date of oviposition ($F_{2,20} = 0.50$; $P = 0.61$). Our experimental manipulations, however, generated significantly different mean clutch sizes (control 6.9 ± 1.2; reduced 3.7 ± 1.5; enlarged 9.8 ± 2.1; $F_{2,20} = 23.68$; $P < 0.0001$) and clutch masses (control 606.2 ± 73.2 g; reduced 312.6 ± 111.5 g; enlarged 973.8 ± 210.6 g; $F_{2,20} = 37.00$, $P < 0.0001$) among the different treatment groups. These results remained unchanged when these analyses where performed on the complete sample ($N = 28$ clutches).

EGG TEMPERATURES

Difference in mean temperature was less than 1 °C between treatments: 30.8 ± 0.9 °C in the un-manipulated, 31.3 ± 0.7 °C in the reduced and 30.3 ± 0.6 °C in the enlarged clutches tested, respectively (Kruskal–Wallis ANOVA $H_{2,45} = 12.42$; $P = 0.002$).

SURROGATE VS. NON-SURROGATE EGGS

We compared hatching success and offspring phenotypes from the surrogate and non-surrogate eggs. None of the traits we measured was affected (mixed-model ANOVAs with egg traits as the dependent variable, surrogate or non-surrogate egg as the fixed factor and brooding female as a random factor; all P values >0.05), suggesting that the displacement of the eggs from their original clutch had little influence on hatching success and hatchling phenotype. Nonetheless, our subsequent analyses are generally based only on data for non-surrogate eggs in order to avoid more subtle problems: for example, possible rejection of foreign eggs by the brooding females.

HATCHING SUCCESS OF NON-SURROGATE EGGS

Overall, 80% of the eggs successfully hatched, producing 103 viable young pythons. However, hatching success differed among the three treatment groups (Logistic regression, $\chi^2 = 9.66$; d.f. = 2; $P = 0.008$), with 96.6% of eggs hatching in the un-manipulated clutches and 95.6% hatching in the artificially reduced clutches, but only 74.4% in the enlarged clutches. All the eggs that failed to hatch contained dead embryos embedded in coagulated yolk.

A few eggs (19 non-surrogate and 11 surrogate) did not adhere to other eggs, enabling us to record individual egg masses immediately after laying and shortly prior to hatchling. Eggs in all three treatments lost mass during incubation, owing to a net water loss (Packard & Packard, 1988; Rahn & Ar, 1974). On average, the eggs lost 30% of their initial mass over the 2 months of incubation. This mass loss comprised 22.9% of the initial egg mass in successfully hatched eggs (23.6% in non-surrogate eggs [$N = 14$] and 19.4% in surrogate eggs [$N = 3$]), vs. 39.4% in eggs that did not hatch successfully (47.0% in non-surrogate eggs [$N = 5$] and 34.7% in surrogate eggs [$N = 8$]). Because these eggs were scarce and randomly distributed across females, and because surrogate and non-surrogate eggs were indistinguishable for any trait we incorporated into analyses, we pooled the 30 'single eggs' to perform a repeated-measures ANOVA. As

expected, the magnitude of loss in egg mass differed significantly among the three treatment groups. Enlarged clutches exhibited the highest water loss rate (ANOVA with treatment group as the factor and repeated measures of egg mass over time as the dependent variable: Wilks $\lambda = 0.63$, $F_{4,52} = 3.41$, $P = 0.02$), reflecting the higher proportion of eggs that failed to hatch in the 'enlarged clutch' treatment.

MORPHOLOGY OF HATCHLINGS

Incubation periods differed slightly (but significantly) among treatments. The delay between eggshell-slitting and emergence from the egg, however, did not differ (Table 1). Our clutch-size manipulations had very little effect on the phenotypic traits of hatchling snakes. In fact, neonatal characteristics did not differ significantly among the three treatment groups for mean values of any trait that we measured (Table 1). Hatchlings from the three treatment groups were indistinguishable in regard to their physical performance at birth, and to their phenotypic traits at 10 days of age (Table 1). Despite the absence of food, all young pythons grew in snout-vent length and in body mass from hatching to the age of 10 days. Importantly, water was available *ad libitum* over this period.

DISCUSSION

Our experimental results are relatively straightforward. Artificial reduction of the size of the clutch did not translate into any benefit to the female or to the offspring, but an artificial increase in litter size increased embryonic mortality and thus increased the

Table 1. Effects of clutch size manipulation (nine un-manipulated clutches, eight enlarged clutches and six reduced clutches) on phenotypes of hatchling ball pythons. Mean values are expressed ± SD; the number of neonates involved is also given in parentheses. The analyses were based on non-surrogate offspring exclusively and were performed using mixed-model ANOVAs or ANCOVAs (see text). Hatchling traits were the dependent variables, treatment was a fixed factor and the incubating female was considered as a random factor. For behavioural traits and physiological performance, neonates were selected randomly for measurements and null scores were deleted from the analysis (see text), leading to reduction of the sample sizes

Trait	Control	Reduced	Enlarged	d.f.	F	P
	(N = 51)	(N = 21)	(N = 31)			
Incubation period (days)	60.72 ± 1.09	61.14 ± 1.42	62.76 ± 1.20	2, 20	6.99	0.005
Emergence period (days)	1.51 ± 0.58	1.71 ± 0.56	1.75 ± 0.57	2, 20	1.27	0.31
Morphology at hatchling	(N = 51)	(N = 21)	(N = 31)			
Body mass (g)	55.0 ± 6.5	52.9 ± 8.9	60.3 ± 14.6	2, 20	2.10	0.15
Snout–vent length (cm)	39.3 ± 2.0	38.9 ± 2.4	40.2 ± 3.9	2, 20	0.99	0.39
Size-adjusted body mass (g)	55.6 ± 6.3	54.6 ± 6.3	58.0 ± 6.4	2, 20	0.90	0.42
Skull length (mm)*	9.82 ± 0.36	9.81 ± 0.36	9.98 ± 0.37	2, 20	1.41	0.27
Jaws length (mm)**	27.27 ± 0.88	26.80 ± 0.88	26.93 ± 0.89	2, 20	1.83	0.19
Head width (mm)**	4.83 ± 0.28	4.81 ± 0.28	4.85 ± 0.28	2, 20	0.05	0.95
Number of ventral scales*	206.8 ± 3.4	206.1 ± 3.4	206.3 ± 3.4	2, 20	0.18	0.84
Abnormal ventral scales***	2.4 ± 2.0	2.4 ± 2.0	2.3 ± 2.0	2, 20	0.61	0.56
Physical performances						
Number of strikes elicited	5.6 ± 3.7 (N = 19)	6.4 ± 3.6 (N = 14)	6.2 ± 3.8 (N = 23)	2, 17	0.19	0.83
Distance swam (m)	6.8 ± 3.7 (N = 35)	7.0 ± 3.0 (N = 18)	5.7 ± 2.6 (N = 27)	2, 16	0.55	0.59
Swimming speed (m min^{-1})	3.16 ± 1.33 (N = 35)	3.35 ± 1.04 (N = 18)	3.10 ± 1.30 (N = 27)	2, 16	0.10	0.90
Percentage of activity	69.8 ± 19.1(N = 35)	69.0 ± 18.6 (N = 18)	60.9 ± 19.4 (N = 28)	2, 16	1.17	0.35
Distance on ground (m)	1.2 ± 0.9 (N = 33)	0.7 ± 0.8 (N = 15)	0.7 ± 0.6 (N = 30)	2, 17	3.54	0.07
Number of tongue flick	112.1 ± 46.9 (N = 36)	84.0 ± 51.0 (N = 18)	86.6 ± 51.7 (N = 31)	2, 17	3.80	0.07
Physiological performances						
Delay of first shed (days)	10.5 ± 0.92 (N = 32)	10.9 ± 1.1 (N = 16)	10.5 ± 1.0 (N = 22)	2, 13	1.19	0.35
BM after 10 days (g)	58.2 ± 6.6 (N = 34)	52.6 ± 10.1 (N = 18)	62.6 ± 14.3 (N = 24)	2, 13	1.79	0.21
SVL after 10 days (cm)	44.3 ± 1.7 (N = 34)	43.6 ± 2.4 (N = 18)	45.9 ± 3.6 (N = 24)	2, 13	1.53	0.26
Change in BM in 10 days (g)	1.49 ± 358 (N = 34)	0.58 ± 2.10 (N = 18)	2.18 ± 2.92 (N = 24)	2, 13	0.39	0.67
Change in SVL in 10 days (cm)	5.06 ± 1.66 (N = 34)	5.00 ± 1.04 (N = 18)	5.60 ± 1.71 (N = 24)	2, 13	0.38	0.69

Means: * adjusted to SVL, ** adjusted to skull length, *** adjusted to the number of ventral scales.

'cost' paid per viable neonate by the mother. Once clutch size falls below the level that a female can effectively brood, we do not expect to see any additional advantages accruing to further reduction. In such a situation, a clutch of less than about five eggs is unlikely to maximize maternal fitness. The female still has to pay all the 'costs' of brooding, including the lack of feeding during two months (Bonnet *et al.*, 2002; Lourdais, Bonnet & Doughty, 2002a), and the energy expended in shivering thermogenesis (Harlow & Grigg, 1984; Slip & Shine, 1988; Madsen & Shine, 2000), but she gains less benefit in fitness terms than she would from a larger clutch. The 'optimal clutch size' hypothesis thus predicts that very small clutches should be rare in nature, and our data support this prediction: >95% of natural clutches contained at least five eggs (Fig. 2).

Artificially enlarged clutches produced a higher number of neonates (6.6 on average) than controls (6.0 on average). At first sight, this suggests that our results contradict Lack's hypothesis: females can brood larger clutches than they generally produce in nature. However, this apparent refutation does not take into account the mother's efficiency in energy use: that is, the energy content of viable neonates divided by the energy content of ovulated eggs. Hatching success was strongly reduced in the enlarged clutches (74.4 vs. 96.6%), so the energy invested per viable neonate increased substantially with increasing clutch sizes. In snakes, long-term body reserves strongly influence fecundity and maternal survival (Bonnet *et al.*, 1999, 2001). Thus, any saving of energy by the female during her current reproductive episode may well strongly enhance her future reproductive success.

Overall, our results falsify the simplest version of Lack's hypothesis (i.e. female pythons *can* physically brood more eggs than they usually produce in nature) but the data nonetheless support a more sophisticated version of the hypothesis that incorporates possible future 'costs' to parental fecundity rather than simply dealing with immediate success. Clutch sizes that deviate strongly from the average observed in natural conditions will be less effective in converting maternal resources (time, energy and materials) into viable offspring. The study organism (a python) used in the present investigation is very different from the ones on which Lack's idea was first developed (birds), and hence the proximate mechanisms that generate the effect differ also. Because parental care is limited to brooding in pythons, adding eggs to a clutch could affect offspring fitness in one of two ways: by reducing survival rates during incubation, or by altering hatchling phenotypic traits of the surviving offspring in ways that would later translate into differences in viability. We found strong evidence for the former effect, but little sign of the latter.

Why did our manipulations of clutch size affect hatching success? Brooding female pythons may enhance the survival of their offspring in three ways: by repelling potential egg predators; by maintaining high and constant incubation temperatures; and by retarding desiccation of the eggs (Fitch, 1954; Fitch & Fitch, 1967; Somma & Fawcett, 1989; Ackerman, 1991; Phillips & Packard, 1994; Alberts *et al.*, 1997; Packard, 1999). Thus, the proximate mechanisms by which additional eggs reduce offspring fitness plausibly involve disruption to one or more of these factors. Because we studied captive snakes, nest predation was unimportant in our experiment, although plausibly predation is a major factor in the field. Our data suggest that artificially enlarged clutches made it more difficult for brooding females to control hydric (and perhaps thermal) conditions as precisely as could females with control or reduced clutches.

(1) Hydric conditions. Typically, female pythons coil so tightly around the clutch that the eggs are not visible from the outside (pers. obs.). This behaviour may reduce exposure to the air, and thus reduce desiccation of the eggs (Bels & Van den Sande, 1986; Fitch, 1954; Somma, 1985, 1990; York & Burghardt, 1988; Somma & Fawcett, 1989). In our experiment, females with artificially enlarged clutches were unable to cover them completely (pers. obs.) and, hence, the higher rate of desiccation of these eggs is not surprising. Severe desiccation of the eggs (> 30% to 50% of loss mass) caused coagulation of the yolk and was fatal to neonates. Such desiccation caused substantial (approximately 23%) mortality prior to hatching.

Despite the massive morphological and ecological differences between birds and snakes, the mechanisms that we have identified above have close parallels to avian studies. For example, Moreno *et al.* (1991) found that adding eggs to the nests of collared flycatchers lowered hatching success because the female was unable to cover all her eggs efficiently, and hence could not maintain sufficiently high, stable incubation temperatures. Thus, in birds as in pythons, a female's body size imposes an upper limit to the number of eggs that she can fully cover. Although experimentally increasing a python's clutch-size reduced hatching success and slightly prolonged incubation, reducing her clutch size had no detectable effect on offspring traits (Table 1). Maximum clutch size relative to SVL in this species may be determined by the ability of the female to cover her clutch. The distribution of clutch sizes in the wild again fits with this model, with < 8% of clutches consisting of >10 eggs. The less-than-expected level of variation in natural clutch sizes (compared to a nor-

mal distribution) also fits with the idea that these pythons have evolved to display a relatively narrow range of clutch sizes. Presumably, factors such as local energy availability adjust clutch sizes within this range (Olsson & Shine, 1997; Lourdais *et al*., 2002b).

(2) Thermal conditions. In many squamate reptiles, phenotypic traits of hatchlings are more sensitive to temperatures than to hydric conditions during incubation (Van Damme *et al*., 1992; Shine *et al*., 1997a,b; Elphick & Shine, 1998; Ji & Braña, 1999; Flatt *et al*., 2001; Shine & Elphick, 2001). Female ball pythons given artificially enlarged clutches maintained the eggs at slightly lower temperatures than did females in the other treatment groups. Also, incubation periods (which are strongly temperature-sensitive in pythons, as in other reptiles: Shine *et al*., 1997a) were longer for the artificially enlarged clutches. We suggest that the female's inability to completely cover the enlarged clutch with her coils meant that the eggs were less effectively insulated from fluctuations in ambient temperature. Nonetheless, this effect was very subtle, with mean temperature differing among treatments by less than 1 °C. Presumably in consequence, we did not detect marked effects on the phenotypes of hatchlings (Table 1). Although the phenotypes of hatchling reptiles (including pythons) are sensitive to the thermal conditions experienced during incubation (Shine *et al*., 1997a; Shine & Elphick, 2001; references therein), the absence of such an effect in our study reflects the minor thermal differences induced by clutch-size manipulation, which in turn may reflect the relatively high and constant ambient temperatures in our study area. Clutch-size manipulations would probably induce much greater fluctuations in incubation regimes, and hence in offspring phenotypes, in a temperate-zone python species (Slip & Shine, 1988).

In summary, our data support the generality of Lack's 'optimal clutch size' hypothesis, and suggest that some of the proximate mechanisms acting on this trait in birds (as revealed by previous studies) may also operate in a reptile species with prolonged but relatively 'simple' parental care. Thus, although clutch sizes in most squamate reptiles may have evolved to match female abdominal volume, and have been influenced by various costs of reproduction (Shine, 1980; Sinervo & Licht, 1991; Shine & Schwarzkopf, 1992; Sinervo, 1999), parental care may have imposed an additional selective force on fecundity in pythons. That is, clutch sizes in pythons may have evolved to match one specific aspect of the reproducing female's body size: her ability to cover the clutch throughout the 2-month incubation period.

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