Body size, locomotor speed and antipredator behaviour in a tropical snake (*Tropidonophis mairii*, Colubridae): the influence of incubation environments and genetic factors

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Summary

1. The physical conditions experienced by reptile embryos inside natural nests can influence the size, shape and behaviour of the resultant hatchlings. Although most reptiles are tropical, the effects of incubation temperatures on offspring phenotypes have received little attention in tropical species.

2. The consequences of differences in thermal variance during incubation on offspring were studied in a tropical natricine snake (the Keelback *Tropidonophis mairii*), which lays eggs in soil cracks of varying depths. Some 253 eggs from 19 clutches were incubated under two thermal regimes with identical mean temperatures (25·6 °C), but temperatures in the 'variable' treatment fluctuated more (21·8–29·6 °C) than those in the 'constant' temperature treatment (25·2–26·5 °C). These thermal regimes were similar to those of shallow (20 cm deep) and deep (40 cm deep) soil cracks, respectively, and represent thermal conditions inside natural nests and potential nest sites.

3. Incubation temperatures affected body size, shape and antipredator behaviour of hatchling snakes. Snakes from constant temperature incubation were longer and thinner than snakes from high variance incubation. Clutch effects influenced all offspring traits, with significant interactions between clutch of origin and incubation treatment for body size, but not swimming speed or behaviour.

4. There was a significant interaction between incubation treatment and offspring sex on neonate swimming speed. Incubation under cycling thermal regimes significantly increased swimming speeds of females, but had little effect on males. Such sex differences in phenotypic responses of hatchling snakes support a major assumption of the Charnov–Bull hypothesis for the evolution of temperature-dependent sex determination.

Key-words: Offspring phenotypes, reptile, sex differences, thermal variance

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Introduction

In most oviparous reptiles, females select nest sites for oviposition (Wilson 1998). The physical conditions (temperature, moisture) inside natural nests can vary considerably depending on the soil type, depth and degree of vegetation cover (Burger & Zappalorti 1986; Burger 1993; Wilson 1998). Laboratory experiments show that the physical conditions experienced by embryos can profoundly influence the size (Gutzke & Packard 1987), behaviour (Burger 1989, 1990), locomotor performance (Miller, Packard & Packard 1987; Van Damme *et al.* 1992; Shine & Harlow 1996; Elphick

& Shine 1998), growth (Rhen & Lang 1995, 1999) and survivorship (Bobyn & Brooks 1994; Burger 1998) of the resultant hatchlings. In some taxa of crocodilians, turtles and lizards, incubation temperature also determines the sex of the offspring (Bull 1980; Janzen & Paukstis 1991; Lang & Andrews 1994). Thus, a female's choice of nest site can have profound effects on the phenotypes and survivorship of her offspring.

Despite the large number of experimental studies on phenotypic responses of hatchling reptiles, most research has focused on taxa with temperature-dependent sex determination (especially turtles and crocodilians, Bull 1980; Lang & Andrews 1994; Janzen 1995; Rhen & Lang 1998). Among species with genotypic sex determination, most studies have focused on lizards and turtles from temperate zone habitats (Bull

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1980; Georges 1989; Janzen & Paukstis 1991; Van Damme *et al.* 1992; Shine & Harlow 1996). Although much has been learnt from these studies, they do not provide a sufficient basis for interpreting the phenomenon of incubation-induced phenotypic plasticity among reptiles in general. For this, we need additional data on snakes (a diverse but less intensively studied group), and from tropical taxa (most reptiles are found in the tropics, but most attention has focused on temperate species). Furthermore, most laboratory studies have incubated eggs at constant temperatures, which may be biologically unrealistic. Temperatures inside natural nests show strong diel fluctuations, even in the tropics (Shine *et al.* 1997; Madsen & Shine 1999).

Here we investigate patterns of incubation-induced phenotypic plasticity in a tropical natricine snake, the Keelback *Tropidonophis mairii*. We combine field data on natural Keelback nests and measurements of temperatures in natural soil cracks to simulate thermal regimes in deep (40 cm deep) *vs* shallow (20 cm deep) nest sites in the laboratory. Our study focuses on three questions:

1. Does thermal variance about the same mean temperature influence offspring phenotypes?
2. What is the relative importance of incubation-induced modifications compared to clutch effects, and to interactions between clutch and incubation-induced effects?
3. Do the sexes display similar norms of reaction in response to incubation conditions? Two previous studies on squamate reptiles have reported sex differences in optimal incubation temperatures, as reflected in embryo mortality (Burger & Zappalorti 1988) and locomotor performance (Shine, Elphick & Harlow 1995; Elphick & Shine 1999).

Materials and methods

STUDY SPECIES AND SITE

The Keelback *Tropidonophis mairii* (Gray 1841) is a small (to 80 cm snout–vent length, SVL, 280 g) nonvenomous natricine snake widely distributed in riparian habitats of eastern and northern Australia (Greer 1997). Our work was based at Fogg Dam 60 km east of Darwin, Northern Territory, Australia where temperatures are high year-round (monthly mean maxima 31–34 °C, minima 15–24 °C) but precipitation is highly seasonal (>75% of the annual rain falls within the 4-month wet season from December to March). At Fogg Dam Keelbacks lay eggs during the dry season (May–August) and the 'build-up' season (October–November: G. P. Brown and R. Shine, unpublished data). Both thermal and hydric conditions inside natural nests are likely to be very different at these two times of year. Our study focused only on the dry season, when snakes lay eggs in soil cracks of different depths. We investigated whether temperatures varied

with soil depth in the field, and used these data to simulate thermal cycles of natural nests in laboratory incubators.

TEMPERATURES INSIDE NESTS AND POTENTIAL NEST SITES

On the nights of 19 and 20 May 1989, one of us (R.S.) observed two female Keelbacks laying their eggs in soil-cracks on Fogg Dam wall. In both cases the females were found emerging from the ground with the clutch partly laid. One nest was 12–16 cm beneath the soil surface, under the roots of a small tree (*Melaleuca* sp.). The other nest was deeper (16–20 cm), 1 m from the water's edge and >5 m away from the nearest tree. The second clutch was laid between sods of dirt overturned a few days previously by a bulldozer. These observations suggest that Keelbacks lay eggs in soil cracks >10 cm deep. Most of the floodplains adjacent to Fogg Dam are treeless, and annual dry season wildfires produce large areas devoid of any vegetation. Thus, much of the potential thermal variation among natural nest sites may be due to differences in soil depth rather than the degree of shading by vegetation. To measure the temperatures of potential nest sites, we placed miniature temperature data loggers (Onset Corporation, Tidbit waterproof loggers) in exposed soil cracks (10, 20 and 40 cm deep) on the Adelaide River floodplain. Loggers recorded temperature every 15 min from 9 to 27 August 1998.
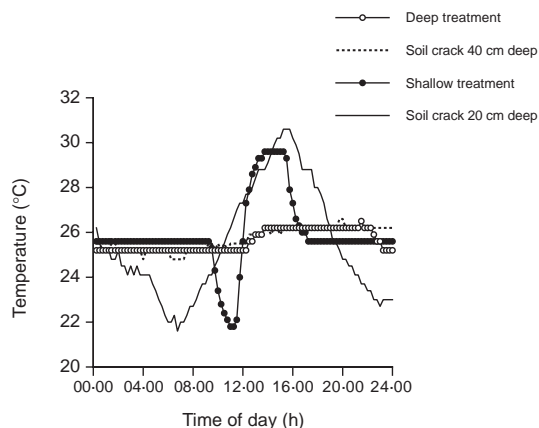
COLLECTION OF EGGS

One of us (G.B.) collected gravid female Keelbacks by hand at Fogg Dam during June and July 1999. The snakes were brought to a nearby field hut where they were measured (SVL, to nearest mm), weighed (to 0·1 g) and placed singly in shaded outdoor containers ($70 \times 50 \times 40$ cm³) provided with a water dish and a clump of damp grass clippings in which females could hide and oviposit. Cages were checked for eggs several times each day. All females laid eggs after 2–16 days (mean = 7 days). After egg-laying all females were weighed, marked and released at their point of capture. A total of 253 eggs from 19 clutches (mean clutch size = 13·3, range 10–17 eggs) were incubated in the experiment. A split-clutch design was used to assign eggs from each clutch to each temperature treatment. Because 'sibling' eggs were generally very similar in size, dividing clutches among treatments resulted in very similar mean sizes and masses of eggs in each treatment group. Depending on clutch size, between five and nine eggs were placed inside identical rectangular plastic containers ($15 \times 10 \times 8$ cm³) each half-filled with vermiculite (100% water by mass, yielding a water potential of −350 kPa, from calibration curve of Shine & Harlow 1996). The containers were sealed with plastic food wrap to retain moisture but allow oxygen exchange, and were placed in one of two incubators.

INCUBATION TEMPERATURES

The viability and hatching success of reptilian embryos are severely reduced at temperatures >38 °C (Burger 1976; Muth 1980; Wilson 1998). Temperatures in the 10-cm-deep crack exceeded 38 °C for 3·4 h each day (mean diel range 23·4–46·3 °C), so it is unlikely that embryo survivorship or hatching success would be high under these thermal conditions. Thus, temperatures in soil cracks 20 and 40 cm deep were used to represent thermal conditions in natural Keelback nests. Mean soil temperatures differed by only 3·0 °C (25·0 °C in deep crack *vs* 28·0 °C in shallow crack), but thermal variances were much smaller in the deep crack than in the shallow crack (mean diel range of 24·0–25·9 °C *vs* 23·6–35·1 °C for the shallow probe). Because our aim was to investigate the effects of variance in nest temperatures on offspring phenotypes (rather than the effects of mean and variance), two thermal treatments were used, both with a mean temperature of 25·6 °C. Temperatures in the constant temperature incubator ('deep nest') cycled between 25·2 and 26·5 °C while those of the variable incubation group ('shallow nest') cycled from 21·8 to 29·6 °C (Fig. 1). These thermal regimes were close to conditions in soil cracks at depths of 40 and 20 cm, respectively (Fig. 1).

MEASUREMENT OF NEONATE SNAKES

All newborn snakes were measured (SVL, tail length, to nearest mm), sexed (by eversion of hemipenes) and weighed (nearest 0·01 g) within 24 h of hatching. Neonates were placed inside individual plastic containers (Click-Clack, model 9510, dimensions 20·9 × 15·1 × 10·5 cm³, with ventilation holes in lids and sides; Click-Clack International, Palmerston North, New Zealand) with paper towelling for shelter and a water dish. Cages were maintained in a constant temperature (CT) room at 20 °C and were placed on wooden racks with a heating cable at one end so that each cage contained a thermal gradient from 20·1 to 38·0 °C, to provide an opportunity for behavioural thermoregulation. Thermal gradients were maintained continuously but lighting mimicked the natural diurnal cycle.

MEASUREMENT OF SWIMMING SPEED

Keelbacks live close to water, and often swim to evade humans (and, presumably, other predators). Swimming speed may be a biologically relevant measure of locomotor performance. In August and September 1999 one of us (J.K.W.) measured swimming speed of 7-day-old Keelbacks in a plastic water trough (104 cm long, 8 cm wide, 18 cm high) placed inside a round plastic tub (108 cm in diameter, 42 cm high) partly filled with water (8 cm deep). Small (4 mm) holes in the sides of the swimming trough allowed water flow between the tub and the trough and facilitated temperature control. Water temperature was maintained at 27·0 °C (±0·1 °C) with a water bath heater (Ratek thermoregulator, model TH5; Ratek Instruments, Boronia, Vic., Australia). Measurements with a digital thermometer (Fluke Manufacturing Co. model 52K-J, Everett, WA, USA) confirmed that water temperature did not vary by more than 0·2 °C. All neonates were kept at 27 °C (±0·1 °C) in an incubator (Sanyo, Model MIR; Sanyo Sales & Supply Co., Itasca, IL, USA) for 24 h prior to testing. This temperature is similar to surface water temperatures at Fogg Dam around dusk when Keelbacks begin foraging (G. P. Brown, unpublished data). All measurements were carried out in a CT room at 26–28 °C at the Northern Territory University.

To begin a test, neonates were placed onto a rock at one end of the trough and were gently tapped with a paintbrush to stimulate swimming. Snakes were forced to swim four laps, with 10 s between each lap (to allow data to be recorded). For each lap we recorded: (1) number of taps to initiate swimming; (2) the number of stops; (3) swimming behaviour: 'surface swimming' with head above the water, or 'underwater swimming' with the body submerged; (4) the time to swim 1 m (with a stopwatch); and (5) 'hide under rock' if snakes hid beneath the rocks placed at each end of the trough. Whether snakes turned around during a trial was recorded, but as this behaviour was rarely observed (10 observations, 10 snakes) snakes were not made to swim an additional lap in such instances. All neonates (179 snakes from 16 clutches) were tested on two consecutive days (= 2 trials per snake). After the completion of testing, all neonates were marked and released at Fogg Dam.

STATISTICAL ANALYSES

A three-factor mixed-model (Type III) ANOVA with incubation treatment, clutch number (random factor) and offspring sex as the factors was used. This design enables us to evaluate not only whether hatchling traits were influenced by incubation treatment and/or by clutch effects (clutch of origin), but also whether offspring from different females responded differently to

Fig. 1. Thermal regimes in soil-cracks 20 cm ('shallow') and 40 cm below the surface ('deep'), compared with thermal regimes used in the egg-incubation experiment.

the two incubation treatments. The three-factor design also enabled us to investigate sex differences in morphological traits, antipredator behaviour and locomotor performance, and the ways in which the direction and magnitude of such sex divergences vary among clutches and are influenced by incubation temperatures, and potentially, the interactive effects of all three of these factors. Because unbalanced data sets can lead to difficulties for interpretation of analysis of variance (valid *F*-tests are hard to derive and the probability of Type I error increases, see Underwood 1997), data were omitted from three clutches with small sample sizes (due to failure of eggs to hatch). The remaining data set (159 neonates from 13 clutches) was better balanced (mean = 12·2 snakes per clutch, range 10–15). A single measure from each hatchling was used as the dependent variable (e.g. mean and maximum swimming speed) to avoid pseudoreplication. Prior to all analyses, a check was made to ensure that data were normally distributed (Shapiro–Wilk tests) and that variances were homogeneous (Bartlett's tests). Behavioural data (swim under water, hide under rock) were expressed as the proportion of trials that snakes displayed the behaviour and were arc-sin transformed prior to analysis of variance (Underwood 1997).

To examine relationships among correlated variables, residual scores were calculated from general linear regression between the variables of interest. For example, our measure of 'body shape' was the residual score from the linear regression of ln mass against total length, whereas relative tail length was the residual score from the linear regression of tail length against SVL.

Results

A total of 179 neonates from 16 clutches hatched from the original 253 eggs (from 19 clutches). Three clutches failed to hatch irrespective of their incubation treatment. Two of these clutches contained only unfertilized eggs while a third consisted of five full-term embryos and nine unfertilized eggs. Two partially successful clutches had a high proportion of unfertilized eggs (8 of 14 and 7 of 11 eggs, respectively). A third clutch included three dead full term embryos. The remaining 13 clutches contained on average, 1·2 unfertilized eggs (range 0–4).

INCUBATION PERIOD AND SEX RATIOS

Incubation temperatures did not influence incubation periods. On average, snakes from the constant temperature treatment hatched at the same time as snakes from the variable temperature treatment (mean incubation periods of 56·9 *vs* 56·7 days). The incubation treatment did not affect hatchling sex ratios (47 females and 38 males from the variable temperature treatment *vs* 43 females and 51 males from the constant temperature treatment, $\chi^2 = 1·63$, 1 df, $P = 0·20$). Nor did either sex ratio deviate significantly from 50/50 ($\chi^2 = 0·95$ and 0·68, respectively, 1 df, $P > 0·05$).

MORPHOLOGY

Incubation temperature and clutch identity affected the morphology of hatchling snakes (Table 1). Significant clutch effects were evident on all hatchling morphological traits whereas incubation temperature affected the body length and shape of the hatchlings, but not their mass (Table 1). Neonates from the variable incubation treatment were shorter (SVL), had shorter tails relative to SVL, but were heavier relative to their total length than were snakes from the constant temperature incubation treatment (Table 2). However, there were significant interactions between clutch identity and incubation temperature for the SVL and relative tail length of the hatchlings (Table 1). These interactions show that some clutches responded to incubation temperature differently from others. For example, constant temperature incubation produced longer offspring than did variable incubation for 11 clutches, whereas this pattern was reversed in three clutches. Male and female hatchlings did not differ significantly in the mean values of any of the morphological traits that were measured, but this apparent lack of dimorphism concealed a far more complex situation. Significant interaction terms (Table 1) show that incubation temperatures affected male and female hatchlings in different ways and, in some cases, temperature affected sexually dimorphic traits differently from one clutch to another.

SWIMMING SPEED

Maternal identity strongly influenced both our measures of swimming speed. Thus, offspring from some clutches were faster swimmers than were neonates from other clutches. The main effect of incubation temperature did not influence swimming speed (Table 2), and there was no significant interaction between clutch identity and incubation temperature for either measure of swimming speed (Table 1). Despite the lack of overall sexual dimorphism in morphological traits, male and female hatchlings differed significantly in locomotor performance (Table 1): female hatchlings swam faster than did males. Surprisingly, the sexes also differed in the way in which incubation treatment affected locomotor speeds. The three-factor ANOVA detected significant interactions between incubation treatment and offspring sex for both mean swimming speed and maximum burst speed. That is, male and female hatchlings responded differently to the incubation treatments. Swimming speed of males did not differ between the two incubation treatments, but females from the variable incubation treatment swam faster than males incubated under the same conditions (Fig. 2).

ANTIPREDATOR BEHAVIOUR

Significant effects of clutch identity were evident for all our measures of antipredator behaviour (last four

Table 1. Results of three-factor ANOVAS for effects of clutch, incubation temperature and offspring sex on phenotypes of neonate Keelback snakes *Tropidonophis mairii*. Relative tail length and body shape are residual scores from the general linear regressions of tail length against snout–vent length and mass against total length, respectively. See text for explanation of other variables. Table shows *F*-values and levels of significance

	Mass	SVL	Relative tail length	Body shape	Mean speed	Maximum speed	Number of taps	Number of stops	Swim underwater	Hide under rock
Incubation	0·13	10·12**	5·45**	12·33***	2·87	0·00	0·53	4·77*	1·73	6·58*
Clutch	32·92***	22·69***	12·56***	13·35***	5·84***	2·95**	1·91*	3·79***	7·12***	4·60***
Sex	0·00	3·24	0·12	1·27	19·12***	10·98***	0·35	19·82***	4·68*	0·77
Incubation × clutch	1·61	2·37**	1·91*	1·51	1·72	1·11	1·18	1·55	0·64	0·79
Incubation × sex	0·37	0·29	8·75***	4·79*	5·32*	5·45*	0·01	0·15	0·05	1·06
Clutch × sex	2·25*	2·25*	1·79	1·67	0·69	0·53	0·70	1·06	1·37	1·33
Incubation × clutch × sex	1·56	1·36	1·70	0·94	1·39	0·93	0·54	1·27*	1·53	0·12

*$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$.

columns in Table 1). Incubation temperature influenced only two of four behaviours: the number of stops made during a swim and the tendency to hide under the rock at the end of a swim. Interpretation of this result is, however, complicated by a significant higher-order interaction: the number of times that a hatchling snake stopped during a trial was affected by an interaction between sex, maternal identity and incubation temperature (Table 1). Male and female hatchlings also differed significantly in antipredator behaviour for two of the four traits that we examined (Table 1). Males (which were slower swimmers than their sisters: see above) stopped more often during swimming than did females, whereas females swam underwater more often than did males (see Fig. 2).

BODY SIZE *VS* SWIMMING SPEED

Neonate total length did not influence swimming speed (correlation between total length and mean speed: $r = 0.13$, $n = 159$, $P = 0.11$; total length and maximum speed: $r = 0.11$, $P = 0.17$). However, heavier hatchlings swam faster than did smaller neonates (correlations between mass and mean swimming speed: $r = 0.35$, $n = 159$, $P < 0.0001$; mass and maximum swimming speed: $r = 0.24$, $n = 159$, $P = 0.002$). Because swimming speed covaried with body size (mass), mass was included as a covariate in three-factor mixed (Type III) model ANCOVAS (with clutch identity, sex and incubation treatment as factors, and mean or maximum swimming speed as dependent variables). To select the final model all the non-significant interaction terms were successively dropped from the initial model. The final model for mean swimming speed revealed a significant effect of clutch identity ($F_{12,130} = 4.62$, $P = 0.0001$), incubation treatment ($F_{1,130} = 5.20$, $P = 0.02$), sex ($F_{1,130} = 20.72$, $P = 0.0001$) and the interaction between incubation treatment and sex ($F_{1,130} = 3.97$, $P = 0.049$), but all the other interaction terms were not significant. The final model for maximum swimming speed revealed a significant effect of clutch identity ($F_{12,130} = 2.49$, $P = 0.006$), sex ($F_{1,130} = 13.49$, $P = 0.0001$) and the interaction between incubation treatment and sex ($F_{1,130} = 5.93$, $P = 0.049$), but the main effect of incubation treatment was not significant, as were the other interaction terms. These analyses show that locomotor differences were not a secondary consequence of morphological effects: that is, the effects of clutch identity, sex and the interaction between incubation treatment and sex on swimming speed remained significant for both measures of swimming speed. These results are consistent with the results of our initial analysis using a three-factor ANOVA (Table 1).

Discussion

Increased thermal variance about the same mean temperature during incubation significantly influenced the

Table 2. Offspring phenotypes of hatchling Keelbacks (*Tropidonophis mairii*) incubated under thermal regimes to simulate conditions in deep (40 cm) *vs* shallow (20 cm) soil cracks. Mean temperatures were identical in both treatments, but thermal variance was higher in the variable incubation treatment. Table shows means (± SE) for each trait for each incubation treatment. *P*-values show level of significance for the main effect of incubation temperature from three-factor ANOVAs with clutch, incubation temperature and offspring sex as the main factors. Relative tail length and body shape are residual scores from the general linear regressions of tail length against snout–vent length and mass against total length, respectively. See text for explanation of other variables

Offspring phenotypes	Constant (deep nest) 25·2–26·5 °C (*n* = 84)	Variable (shallow nest) 21·8–29·6 °C (*n* = 75)	*P*
Mass at hatching	2·54 ± 0·03	2·55 ± 0·03	NS
SVL at hatching	162·1 ± 0·79	160·2 ± 0·81	<0·01
Relative tail length at hatching	0·35 ± 0·43	−0·39 ± 0·44	<0·01
Body shape at hatching	−0·027 ± 0·02	0·036 ± 0·03	<0·01
Mean swimming speed	0·298 ± 0·005	0·312 ± 0·005	NS
Maximum swimming speed	0·389 ± 0·007	0·395 ± 0·008	NS
Number of taps	1·20 ± 0·03	1·23 ± 0·03	NS
Number of stops	0·68 ± 0·05	0·51 ± 0·04	<0·05
Swim underwater	0·60 ± 0·03	0·63 ± 0·04	NS
Hide under rock	0·26 ± 0·02	0·31 ± 0·02	<0·05
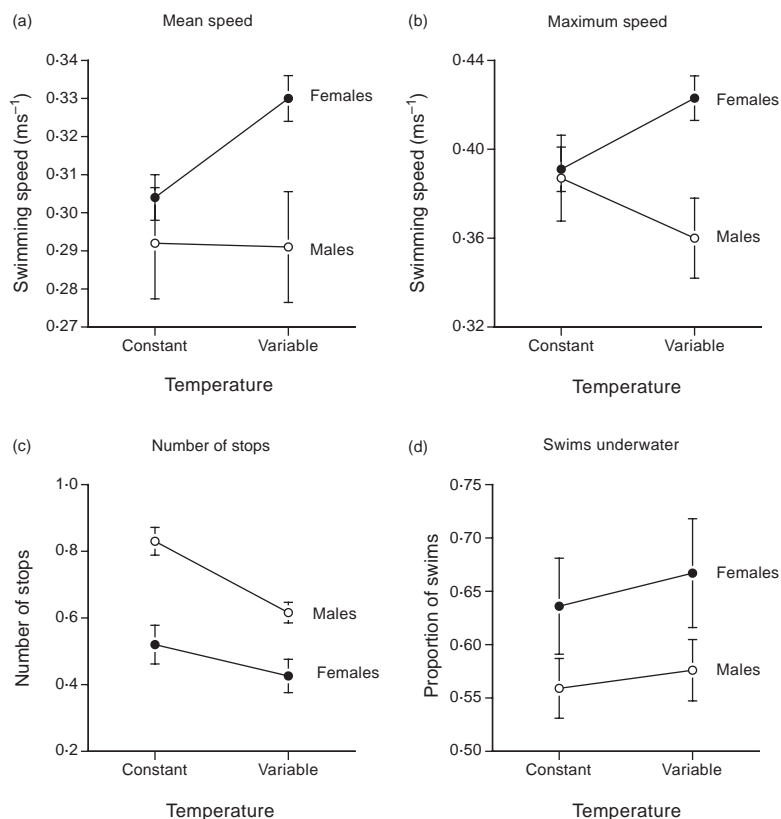
Fig. 2. Norms of reaction for locomotor performance of male and female hatchling Keelbacks (*Tropidonophis mairii*) in response to two different thermal regimes during incubation. Mean incubation temperatures were identical, but the variable temperature treatment had a much higher thermal variance than the constant temperature treatment. The graphs show sex-specific responses for the following traits: (a) mean swimming speed; (b) maximum swimming speed; (c) mean number of stops per trial; and (d) mean proportion of trials in which the hatchling swam underwater. The figure shows mean values with associated standard errors.

morphology (size and shape) and behaviour of hatchling *Tropidonophis mairii* from the wet–dry tropics. Previous studies on squamate reptiles have shown that differences in mean incubation temperatures can sig-

nificantly affect offspring phenotypes (Gutzke & Packard 1987; Burger 1989, 1990, 1998; Van Damme *et al.* 1992), but our study confirms that thermal variance is important in this respect also (Shine & Harlow 1996). Changes to thermal variance should not increase the mean incubation period provided that temperatures remain above the minimum temperature for embryogenesis (Georges 1989; Shine & Harlow 1996). Our results support this conclusion. Thermal variance in the variable incubation treatment was moderate (7·8 °C, see Shine & Harlow 1996) and temperatures in both treatments remained above the lower threshold temperature for embryogenesis for Keelbacks (Greer 1997). As a result, incubation periods were very similar in the constant and variable incubation treatments (56·9 *vs* 56·7 days, respectively).

Many species of temperate zone reptiles lay eggs in shallow nests with high mean temperatures and high thermal variances (e.g. Burger 1976; Packard *et al.* 1985; Shine & Harlow 1996). In cold climates, this strategy can accelerate embryogenesis and thus permit early hatching, which in turn may enhance survival probability and growth rates of the hatchling prior to hibernation (Shine & Harlow 1996; Burger 1998). However, it is unclear whether similar selective pressures operate in the tropics. For example, it may be more important for tropical reptiles to buffer their eggs from extreme thermal conditions rather than to exploit the hottest available nesting site (Georges 1992). Although we have insufficient data on natural Keelback nests to investigate this possibility, studies on water pythons (*Liasus fuscus*) at Fogg Dam have suggested that thermal variance is a significant criterion for nest-site selection in this species. Burrow-nesting water pythons select nest sites with the lowest thermal variance (Shine *et al.* 1997; Madsen & Shine 1999). Although our study focused on temperature, the prolonged (6 month) breeding season of Keelbacks during the dry season means that both thermal and

hydric regimes of Keelback nests may show strong seasonal changes (e.g. Muth 1980; Packard *et al.* 1985), which could significantly affect offspring phenotypes (Packard *et al.* 1983; Gutzke & Packard 1987) and survivorship in the field (Snell 1984).

Our variable thermal regime was similar to that experienced in natural nests (Fig. 1), so our experimental findings should be relevant to natural conditions. At Fogg Dam, several species of predatory water birds forage in areas inhabited by Keelbacks. Our locomotor trials may mimic the situation where neonates attempt to escape from a predatory bird's thrusting beak (or, in our case, a paintbrush) by swimming away or hiding under water. Presumably some offspring traits (e.g. propensity to swim under water) will influence long-term survivorship, but we are reluctant to interpret our results in terms of the advantages of maternal selection of nest-sites (Roosenburg 1996) for two reasons. Firstly, we do not know which offspring traits will enhance fitness. For example, a study on Garter Snakes found that offspring locomotor ability influenced survivorship in the field (Jayne & Bennett 1990), whereas a study on hatchling turtles found that immobility in the face of a 'predator' was a better predictor of subsequent offspring survival than was the ability for rapid escape (Janzen 1995). Secondly, differences between traits from the two incubation treatments were small (Table 2), and we do not know whether these differences persist during later life. Previous work has shown that some incubation-induced effects in squamates remain stable for several months (Burger 1989; Elphick & Shine 1998), whereas others do not (Qualls & Shine 1996).

Our study confirms a consistent result from previous work on reptilian reproduction: clutch-of-origin effects were significant for every offspring trait (Table 1). Offspring from different clutches differed in antipredator behaviours, locomotor speeds and body sizes, and responded differently to thermal regimes during the incubation period. These 'clutch-of-origin' effects could reflect differences in offspring genotype (direct genetic effects), differences in maternal phenotype (maternal environmental effects) or differences in the effect of maternal genotype (maternal genetic effects; Bernardo 1996). Because our experimental design did not replicate clutch effects (no female had more than one clutch in one treatment), and we confounded clutch and cage effects (all eggs of a clutch were placed in the same container) we could not determine the causes of these clutch effects. Future studies could avoid these problems by placing eggs singly in individual containers and by obtaining multiple clutches from females. Alternatively, future studies could investigate the magnitude of genetic *vs* maternal effects by manipulating maternal food intake and thermoregulation in gravid females in the laboratory (e.g. Shine & Harlow 1993; Sorci & Clobert 1997).

One of our most interesting findings was that male and female *T. mairii* responded differently to incuba-

tion conditions. Hatchlings of both sexes were very similar in size and shape but displayed strikingly different locomotor speeds and antipredator tactics (Fig. 2). Females swam faster than their brothers and were more likely to swim underwater and less likely to stop during a trial. Incubation temperatures modified locomotor ability of female hatchlings, but had little effect on swimming speeds of their brothers (Fig. 2). This result is similar to the sex differences in reaction norms found in the alpine lizard *Bassiana duperreyi* (Shine *et al.* 1995; Elphick & Shine 1998, 1999). These sex differences in phenotypic responses to incubation temperature have significant implications for theoretical models for the evolution of temperature-dependent sex determination (TSD). No snake species has been reported to show TSD (Bull 1980), and sex ratios in our incubation experiment and previous work (Shine 1991) show that Keelbacks possess genotypic sex determination (GSD) rather than TSD. Nonetheless, information on GSD species may provide valuable insights into the temperature dependence of developmental responses in TSD species as well. A widely supported model for the evolution of TSD suggests that the sexes differ in optimal incubation temperatures; thus, TSD may enable a reproducing female to match her offspring's sex to the nest temperatures that they encounter (Charnov & Bull 1977).

Acknowledgements

References

Bernardo, J. (1996) Maternal effects in animal ecology. *American Zoologist* **36**, 83–105.

Bobyn, M.L. & Brooks, R.J. (1994) Interclutch and interpopulation variation in the effects of incubation conditions on sex, survival and growth of hatchling snapping turtles (*Chelydra serpentina*). *Journal of Zoology (London)* **233**, 233–257.

Bull, J.J. (1980) Sex determination in reptiles. *Quarterly Review of Biology* **55**, 3–21.

Burger, J. (1976) Temperature relations in nests of the northern diamondback terrapin, *Malaclemys terrapin terrapin*. *Herpetologica* **32**, 412–418.

Burger, J. (1989) Incubation temperature has long term effects on behaviour of young pine snakes (*Pituophis melanoleucus*). *Behavioural Ecology and Sociobiology* **24**, 201–207.

Burger, J. (1990) Effects of incubation temperature on behaviour of young black racers (*Coluber constrictor*) and kingsnakes (*Lampropeltis getulus*). *Journal of Herpetology* **24**, 158–163.

Burger, J. (1993) Colony and nest site selection in lava lizards *Tropidurus spp.* in the Galapagos Islands. *Copeia* **1993**, 748–754.

Burger, J. (1998) Effects of incubation temperature on hatchling pine snakes: implications for survival. *Behavioural Ecology and Sociobiology* **43**, 11–18.

Burger, J. & Zappalorti, R.T. (1986) Nest site selection by pine snakes, *Pituophis melanoleucus*, in the New Jersey Pine Barrens. *Copeia* **1986**, 116–121.

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

Charnov, E.L. & Bull, J.J. (1977) When is sex environmentally determined? *Nature* **266**, 828–830.

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

Elphick, M.J. & Shine, R. (1999) Sex differences in optimal incubation temperatures in a scincid lizard species. *Oecologia* **118**, 431–437.

Georges, A. (1989) Female turtles from hot nests: is it duration of incubation or proportion of development at high temperatures that matters? *Oecologia* **81**, 323–328.

Georges, A. (1992) Thermal characteristics and sex determination in field nests of the pig-nosed turtle, *Carettochelys insculpta* (Chelonia: Carettochelydidae), from northern Australia. *Australian Journal of Zoology* **40**, 511–521.

Greer, A.E. (1997) *The Biology and Evolution of Australian Snakes*. Surrey Beatty & Sons, Sydney.

Gutzke, W.H.N. & Packard, G.C. (1987) Influence of the hydric and thermal environments on eggs and hatchlings of bull snakes *Pituophis melanoleucus*. *Physiological Zoology* **60**, 9–17.

Janzen, F.J. (1995) Experimental evidence for the evolutionary significance of temperature-dependent sex determination. *Evolution* **49**, 864–873.

Janzen, F.J. & Paukstis, G.L. (1991) Environmental sex determination in reptiles: ecology, evolution and experimental design. *Quarterly Review of Biology* **66**, 149–179.

Jayne, B.C. & Bennett, A.F. (1990) Selection on locomotor performance capacity in a natural population of garter snakes. *Evolution* **44**, 1204–1229.

Lang, J.W. & Andrews, H.V. (1994) Temperature-dependent sex determination in crocodilians. *Journal of Experimental Zoology* **270**, 28–44.

Madsen, T. & Shine, R. (1999) Life history consequences of nest-site variation in tropical pythons (*Liasis fuscus*). *Ecology* **80**, 989–997.

Miller, K., Packard, G.C. & Packard, J.J. (1987) Hydric conditions during incubation influence locomotor performance of hatchling snapping turtles. *Journal of Experimental Biology* **127**, 401–412.

Muth, A. (1980) Physiological ecology of desert iguana (*Dipsosaurus dorsalis*) eggs: temperature and water relations. *Ecology* **61**, 1335–1343.

Packard, G.C., Packard, M.C., Boardman, T.J., Morris, K.A. & Shuman, R.D. (1983) Influence of water exchanges by flexible-shelled eggs of painted turtles *Chrysemys picta* on metabolism and growth of embryos. *Physiological Zoology* **56**, 217–230.

Packard, G.C., Paukstis, G.L., Boardman, T.J. & Gutzke, W.H.N. (1985) Daily and seasonal variation in hydric conditions and temperature inside nests of common snapping turtles (*Chelydra serpentina*). *Canadian Journal of Zoology* **63**, 2422–2429.

Qualls, C.P. & Shine, R. (1996) Reconstructing ancestral reaction norms: an example using the evolution of reptilian viviparity. *Functional Ecology* **10**, 688–697.

Rhen, T. & Lang, J.W. (1995) Phenotypic plasticity for growth in the common snapping turtle: effects of incubation temperature, clutch, and their interaction. *American Naturalist* **146**, 726–747.

Rhen, T. & Lang, J.W. (1998) Among-family variation for environmental sex determination in reptiles. *Evolution* **52**, 1515–1520.

Rhen, T. & Lang, J.W. (1999) Temperature during embryonic and juvenile development influences growth in hatchling snapping turtles, *Chelydra serpentina*. *Journal of Thermal Biology* **24**, 33–41.

Roosenburg, W.M. (1996) Maternal condition and nest-site choice: an alternative for the maintenance of environmental sex determination? *American Zoologist* **36**, 157–168.

Shine, R. (1991) Strangers in a strange land: ecology of the Australian colubrid snakes. *Copeia* **1991**, 120–131.

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

Shine, R. & Harlow, P.S. (1996) Maternal manipulation of offspring phenotypes: nest-site selection in an oviparous reptile. *Ecology* **77**, 1808–1817.

Shine, R., Elphick, M.J. & Harlow, P.S. (1995) Sisters like it hot. *Nature* **378**, 451–452.

Shine, R., Madsen, T.R.L., Elphick, M.J. & Harlow, P.S. (1997) The influence of nest temperatures and maternal brooding on hatchling phenotypes in water pythons. *Ecology* **78**, 1713–1721.

Snell, H.L. (1984) *Evolutionary ecology of Galapagos land iguanas (Iguanidae:* Conolophus*)*. *PhD Thesis*, Colorado State University, Fort Collins, CO.

Sorci, G. & Clobert, J. (1997) Environmental maternal effects on locomotor performance in the common lizard (*Lacerta vivipara*). *Evolutionary Ecology* **11**, 531–541.

Underwood, A.J. (1997) *Experiments in Ecology: Their Logical Design and Interpretation Using Analysis of Variance*. Cambridge University Press, Cambridge.

Van Damme, R., Bauwens, D., Brana, F. & Verheyen, R.F. (1992) Incubation temperature differentially affects hatching time, egg survival, and hatchling performance in the lizard *Podarcis muralis*. *Herpetologica* **48**, 220–228.

Wilson, D.S. (1998) Nest-site selection: microhabitat variation and its effects on the survival of turtle embryos. *Ecology* **79**, 1884–1892.