

The effect of constant vs. fluctuating incubation  
temperatures on the phenotype and fitness of black rat  
snake (*Elaphe obsoleta*) hatchlings

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Thesis submitted to the Department of Biology in partial fulfillment of the  
requirements for the B.Sc. Honours degree, specialization Biology

BIO4900  
May 1<sup>st</sup>, 2007  
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## **Abstract**

According to the maternal manipulation hypothesis (Shine 1995), females can manipulate the phenotypes (and therefore the fitness) of their offspring by choosing appropriate incubation conditions. In oviparous black rat snakes (*Elaphe obsoleta*), females manipulate their offspring's phenotypes through nest-site selection. Females prefer to lay their eggs in communal nests, which have higher, more constant temperatures than single-female nests. The purpose of this study was to determine whether the thermal mean and variance of the incubation regime affects fitness-related traits in hatchlings. If high and constant incubation temperatures produce overall fitter hatchlings, then it is possible that female *E. obsoleta* use these criteria to assess the quality of nest sites. Hatchlings incubated at higher temperatures had more fitness-enhancing traits than those incubated at lower temperatures: they hatched earlier and more successfully, and were longer, faster, and less aggressive. Hatchlings incubated at constant temperatures hatched earlier and were longer. In terms of performance, there was a significant interaction between temperature mean and variance, meaning that the effect of variance depended on the mean temperature. Hatchlings incubated at 29°C were weaker, but swam and righted themselves faster at constant temperatures; whereas hatchlings incubated at 26°C swam faster, righted themselves more slowly and were equally strong at constant temperatures. Overall, constant incubation temperatures produced fitter hatchlings than fluctuating temperatures, but the effect of thermal variance on phenotype was not as pronounced as the effect of thermal mean. There is therefore some support for the hypothesis that black rat snakes prefer communal over single-female nests because these have higher, more

constant temperatures. This experiment also tested the differential fitness assumption of the Charnov-Bull hypothesis, but no support was found.

## **Introduction**

The phenotype of all organisms is the result of the interaction between genetic and environmental factors. The environmental conditions under which an embryo develops affect not only the rate of embryogenesis but also its trajectory (Shine and Harlow 1996). The early stages of development are particularly sensitive, and even small changes during embryogenesis can result in major phenotypic modifications (Brown and Shine 2004). In oviparous (egg-laying) reptiles, the temperature and humidity of the nest has been found to affect the incubation period, growth rate, morphology, behaviour and sexual differentiation of hatchlings (e.g. Hare *et al.* 2004, Brown and Shine 2005a, Xiang *et al.* 2006). Moreover, the phenotypic differences among neonates induced by incubation period may persist for many months after hatching (Shine and Harlow 1996) and affect the neonate's chances of survival (Jayne and Bennett 1990, Qualls and Andrews 1999, Hare *et al.* 2004).

According to the maternal manipulation hypothesis (Shine 1995), female ectotherms can manipulate the phenotypes of their offspring through the selection of an appropriate incubation environment. If a female chooses to expose her offspring to incubation conditions that produce phenotypic traits that maximize offspring survival, she enhances her offspring's fitness, and therefore her own. If adaptive modifications to offspring phenotype are achieved primarily through female choice of incubation conditions, natural

selection should act on female behaviour rather than on alleles “for” particular offspring traits. Examples of female behaviour that control the thermal conditions under which their young develop include behavioural thermoregulation during pregnancy in viviparous reptiles, egg-brooding, and nest-site selection (Shine 2003).

Since black rat snakes (*Elaphe obsoleta*) are an oviparous species, females control offspring phenotypes primarily through nest-site selection. Black rat snakes may choose to nest either in single-female or communal nests (Blouin-Demers *et al.* 2004). Indeed, females tend to prefer to lay eggs in communal nests: the use of communal nests is more traditional, with females returning to the same nest in subsequent years (Blouin-Demers *et al.* 2004). Blouin-Demers *et al.* (2004) observed that the temperature in communal nests tends to be higher and less variable than in single-female nests. It was also determined that females prefer to nest at higher mean temperatures, and that these temperatures produce hatchling traits that enhance fitness. However, the effect of temperature variance on female nest-site selection and hatchling phenotype was not explored. Since communal and single-female nests differ in thermal variance as well as thermal mean, it is possible that female *E. obsoleta* prefer communal nests not only because these offer higher mean temperatures, but also more constant temperatures. The hypothesis of this study is that female black rat snakes prefer to lay eggs in communal nests because these offer thermal conditions (in terms of variance as well as mean) that produce fitter phenotypic traits in hatchlings than do single-female nests. If this is the case, higher, more constant incubation temperatures should produce offspring phenotypes that enhance fitness.

A secondary goal of this study is to test the assumption of the Charnov-Bull differential-fitness hypothesis that incubation temperature differentially affects the fitness of male and female offspring (Charnov and Bull 1977). According to this hypothesis, temperature-dependent sex determination (TSD) enhances maternal fitness by enabling embryos to develop as the sex that is best suited to the particular conditions under which they are developing (reviewed in Shine 1999). Studies attempting to test this assumption in species with TSD are problematic because the effect of incubation temperature on offspring phenotype is confounded with the effect of sex on phenotype. One solution to this problem is to manipulate offspring sex using hormone treatments (Tousignant and Crews 1995), but these treatments can also affect offspring phenotype (Rhen and Lang 1995). Since black rat snakes have genetic sex determination, the effects of incubation temperature on offspring phenotype will not be confounded by other factors. If the assumption that incubation temperature affects males and females differently is correct, there should be an interaction between thermal mean and sex.

## **Methods**

### *Study site*

The study site for this experiment was the 10 km x 3 km area surrounding Queen's University Biological Station, approximately 40 km north of Kingston (Ontario, Canada). This area is 20 km from the northern extreme of the black rat snake distribution, and 200 km from the northern extreme of oviparous reptiles. In this area, black rat snakes nest in large piles of decomposing vegetation that are loose enough to allow them to burrow to

depths of up to 75 cm (Blouin-Demers *et al.* 2004). Nests include large leaf piles, compost piles, and hollow tree stumps.

### *Egg collection and incubation*

In July 2006, seven clutches of eggs were collected from known communal nests. I obtained an additional three clutches from gravid females caught opportunistically in the field. I placed these females on nesting substrate in plywood nesting chambers with wire mesh lids. The chambers were placed over heating pads and the substrate was checked every two days for eggs. Once collected, I equally and randomly divided each clutch among four treatments: two constant-temperature treatments (26 C and 29 C), and two treatments with means 26°C and 29°C, but fluctuating on a daily basis ( $\pm 3$  C) (*Figure 1*). These temperatures were chosen to roughly approximate those in communal and single-female nests (Blouin-Demers *et al.* 2004). I half-buried the eggs in 40 g of vermiculite mixed with 80 g of water, in Ziploc containers with perforated lids. The constant-temperature incubator was constructed from a plastic Rubbermaid closet (176 cm  $\times$  44.5 cm  $\times$  64.5 cm) insulated with Styrofoam and foam tape. The closet was divided into two thermally insulated compartments: the top one at 29°C and the bottom one at 26°C. Each compartment was equipped with a Nortron thermostat with a 100 W lightbulb that lit up when the temperature dropped, a fan (Nexxtech, 60 Hz) preventing any thermal gradients from building up in the incubator, and a miniature temperature datalogger (Dallas semiconductors, Maxim Integrated Products, Inc.) to monitor the temperature in the incubators. One incubator (Constant Temperature Control Limited Model LBC700) and one environmental chamber (Constant Temperature Control Limited

Model ER600) were used as fluctuating-temperature incubators. Due to logistical constraints, the environmental chamber was also used for performance trials. Eggs were temporarily placed in the constant-temperature 26°C incubator for a few hours every day for the duration of the performance trials. Every second day, I added water to each Ziploc container to return it to its initial mass. This compensated for any mass lost via evaporation. I also shuffled the containers when replacing them into the incubators, to compensate for any possible thermal gradients. Beginning in mid-August, I checked the eggs daily for signs of hatching, removed any neonates, and placed them in individually labeled containers with moist paper towel.

#### *Morphological and behavioural measurements*

Morphological and behavioural measurements were typically done within 24 hours of hatching. Firstly, I assessed hatchlings for aggression upon first handling. I scored aggression according to the following scale: 1- fleeing, 2- rattling tail, 3- gaping, 4- striking, 5- biting. Sex was assessed by eversion of the hemipenes. I also measured snout-vent length (SVL) and tail length (TL) to the nearest mm using a ruler taped to the table. The mean of two measurements was taken for SVL (Blouin-Demers 2003). I measured the mass to the nearest 0.1 g using an electronic scale, and counted the split ventral scales as an indicator of underlying skeletal deformities (Forsman *et al.* 1994).

#### *Performance measurements*

I assessed three measures of performance: swimming speed, righting time, and muscular strength. Each measure of performance was assessed at both 29 C and 26 C in an

environmental chamber to determine whether there was a temperature of optimal performance, and whether this depended on mean incubation temperature. I equilibrated the hatchlings at the appropriate temperature for at least 10 minutes prior to performance trials. In each case, I counted the fastest/strongest of two trials. Swimming speed was assessed rather than crawling speed because the latter typically provoked a defensive response. I ran the swimming trials in a 2 m-long trough filled with water, timing the swimming speed over the middle 1.5 m with a stopwatch. To encourage the hatchlings to swim at maximum speed, I gently prodded their tails. If the hatchling stopped swimming, the trial was stopped and started again. For righting time, hatchlings were smoothed out until straight, placed on their backs, and allowed to flip. Righting time was determined using the playback mode of a Cannon digital video camcorder (ER600). Muscular strength was measured with a 100 g pesola scale (Shine and Brown 2002). I used surgery tape to affix hatchlings' tails to the end of the pesola scale, which was taped to the table. Hatchlings were smoothed out and gently tapped below their cloacal area to encourage muscular contractions. I noted the strongest of the first five rapid contractions, allowed hatchlings to rest for ten seconds, and repeated the procedure a second time.

### *Statistical analyses*

All non-significant interactions that were not of interest were removed from the models. All terms of interest that were non-significant were removed from subsequent analyses (e.g. in the univariate F tests following a MANOVA). Since hatching success did not meet the normality or no-interaction assumptions of an unreplicated ANOVA, I used a non-parametric sign test. A multiway, unreplicated ANOVA was used for hatching date.



Independent factors were temperature mean (TM) and temperature variance (TV). For aggression, the normality assumption of a multiway ANOVA was not met. Therefore, I used a non-parametric Kruskal-Wallis test with Schreider-Ray-Hare extension instead. Independent factors were TM, TV, clutch, sex, the TM:TV interaction, and the TM:sex interaction. Morphological measurements were assessed using a MANOVA, and then (to see where the differences lay) with separate ANOVAs for SVL, TL and mass. Independent factors were TM, TV, clutch, sex, TM:TV and TM:sex. Since only five hatchlings had split ventral scales, this trait was excluded from analyses of morphology. I used a MANOVA to assess performance measurements, with TM, TV, clutch, sex, the temperature at which the hatchling was tested (TT), TM:TV, TM:sex and TM:TT as independent factors. Since there was a significant TM:TV interaction for performance, I conducted separate MANOVAs at each mean temperature and each temperature variance. I followed up the multivariate analyses with separate ANOVAs for swimming speed, righting time and muscular strength. Assumptions of multivariate normality and homoscedasticity were assessed following the methods suggested by Stevens (1996). The TM:sex interaction was used as an independent factor in these models to test the assumption of the Charnov-Bull hypothesis. The TM:TT interaction was used as an independent factor to determine whether hatchlings incubated at different mean temperatures performed better at different temperatures. Clutch was included as a control variable in all analyses because maternal or genetic effects may significantly influence phenotypic traits (Blouin-Demers *et al.* 2003). All statistical analyses were conducted with JMP version 5 (SAS Institute Inc, 2002) and S-PLUS (SPSS) statistical software.

## Results

The mean temperatures in the constant-temperature incubators were 25.7 +/- 0.02 °C and 29.5 +/- 0.02°C, respectively (*Figure 2*). There were strong clutch effects ( $P < 0.001$ ) in all analyses except for aggression ( $P = 0.36$ ) and hatching success ( $P = 0.06$ ). All marginally significant ( $0.05 \leq P < 0.1$ ) results were mentioned because the power in behavioural ecology studies tends to be low and so marginally significant results are probably significant (Nakagawa 2004).

### *Hatching*

The hatching success was 116/136 (85%), although, if we exclude the 11 eggs from clutch #10 (which all rotted and were assumed to be unfertilized) the hatching success was 93%. Eggs incubated at a mean temperature of 29°C had a significantly higher ( $P = 0.004$ ) hatching success than at 26°C, but there was no effect of temperature variance ( $P = 0.69$ ). For hatching date, there was a significant effect of temperature mean ( $R^2 = 0.55$ ,  $F_{[1,25]} = 560.99$ ,  $P < 0.001$ ) and temperature variance ( $R^2 = 0.023$ ,  $F_{[1,25]} = 23.53$ ,  $P < 0.001$ ). On average, eggs incubated at 29°C hatched 14 days earlier than those incubated at 26°C, and eggs incubated at constant temperatures hatched 3 days earlier than those incubated at fluctuating temperatures (*Figure 2*).

### *Behaviour*

For aggression, there was a significant effect of temperature mean ( $H_{[1,102]} = 7.93$ ,  $P = 0.005$ ) but not variance ( $H_{[1,102]} = 0.12$ ,  $P = 0.731$ ), or sex ( $H_{[1,102]} = 2.51$ ,  $p = 0.11$ ).

There was also no interaction between temperature mean and variance ( $H_{[1,102]} = 0.15$ ,  $P = 0.69$ ), or temperature mean and sex ( $H_{[1,102]} = 0.15$ ,  $P = 0.69$ ). Hatchlings incubated at a mean temperature of 26 °C were more aggressive than those incubated at 29°C.

### *Morphology*

Since only five hatchlings had scale anomalies, the sample size was deemed too small to analyze. However, since four out of the five were from the same clutch (and all four treatments were represented among the five), clutch effects were assumed to be the primary cause for the scale anomalies in this case. The whole model for the morphology MANOVA was significant (Wilk's  $\lambda = 0.04$ ,  $F_{[39,296.87]} = 15.36$ ,  $P < 0.001$ ), as was temperature mean (Wilk's  $\lambda = 0.62$ ,  $F_{[3,296.87]} = 20.66$ ,  $P < 0.001$ ), variance (Wilk's  $\lambda = 0.15$ ,  $F_{[3,296.87]} = 4.90$ ,  $P = 0.003$ ), and sex (Wilk's  $\lambda = 1.22$ ,  $F_{[3,296.87]} = 40.81$ ,  $P < 0.001$ ). The interactions between temperature mean and variance (Wilk's  $\lambda = 0.04$ ,  $F_{[3,296.87]} = 1.42$ ,  $P = 0.24$ ) and temperature mean and sex (Wilk's  $\lambda = 0.02$ ,  $F_{[3,296.87]} = 0.81$ ,  $P = 0.49$ ) were not significant. Thus, the mean and variance of the incubation temperature affected the morphology of hatchling black rat snakes. To determine the relative importance of SVL, mass and tail length to the multivariate main effects of incubation regime on morphology, I conducted univariate ANOVAs. Differences in mean temperature were due to SVL ( $R^2 = 0.18$ ,  $F_{[1,104]} = 37.13$ ,  $P < 0.001$ ) and to mass ( $R^2 = 0.01$ ,  $F_{[1,104]} = 4.84$ ,  $P = 0.03$ ), but not to tail length ( $R^2 = 0.004$ ,  $F_{[1,104]} = 1.52$ ,  $P = 0.22$ ). Hatchlings incubated at 29°C were longer than those incubated at 26°C, whereas those incubated at 26°C were heavier than those incubated at 29°C (*Figure 4*). Differences in temperature variance were also due to SVL ( $R^2 = 0.05$ ,  $F_{[1,104]} = 10.45$ ,  $P = 0.002$ ) and mass ( $R^2 = 0.02$ ,  $F_{[1,104]}$

= 9.99,  $P = 0.002$ ), but only marginally to tail length ( $R^2 = 0.009$ ,  $F_{[1,104]} = 3.28$ ,  $P = 0.07$ ). Hatchlings incubated at constant temperatures were longer and heavier than those incubated at fluctuating temperatures (*Figure 4*). The difference in sex was due to tail length ( $R^2 = 0.32$ ,  $F_{[1,104]} = 110.06$ ,  $P < 0.001$ ), with males having longer tails than females. Since there were no significant TM:sex interactions, the fitness of hatchlings at either mean temperature did not depend on sex.

### *Performance*

The whole model for the performance MANOVA was significant (Wilk's  $\lambda = 0.30$ ,  $F_{[45,618.7]} = 6.95$ ,  $P < 0.001$ ), as was temperature mean (Wilk's  $\lambda = 0.49$ ,  $F_{[3,618.7]} = 34.15$ ,  $P < 0.001$ ), temperature tested (Wilk's  $\lambda = 0.06$ ,  $F_{[3,618.7]} = 3.90$ ,  $P = 0.01$ ), and the interaction between temperature mean and variance (Wilk's  $\lambda = 0.11$ ,  $F_{[3,618.7]} = 7.86$ ,  $P < 0.001$ ). Temperature variance was marginally significant (Wilk's  $\lambda = 0.03$ ,  $F_{[3,618.7]} = 2.37$ ,  $P = 0.07$ ), whereas sex (Wilk's  $\lambda = 0.02$ ,  $F_{[3,618.7]} = 1.48$ ,  $P = 0.22$ ), the interaction between temperature mean and sex (Wilk's  $\lambda = 0.03$ ,  $F_{[3,618.7]} = 1.84$ ,  $P = 0.14$ ), and between temperature mean and temperature tested (Wilk's  $\lambda = 0.003$ ,  $F_{[3,618.7]} = 0.18$ ,  $P = 0.91$ ) were not significant. Because there was a significant interaction between temperature mean and variance, however, the main effects for each could not be interpreted. The data were separated by mean temperature, and then by temperature variance, and analyzed separately.

For the performance MANOVA at a mean temperature of 26°C, the whole model was significant (Wilk's  $\lambda = 0.03$ ,  $F_{[30,276.58]} = 4.80$ ,  $P < 0.001$ ), as was temperature variance

(Wilk's  $\lambda = 0.15$ ,  $F_{[3,276.58]} = 4.54$ ,  $P = 0.005$ ), but not temperature tested (Wilk's  $\lambda = 0.05$ ,  $F_{[3,276.58]} = 1.60$ ,  $P = 0.19$ ). Therefore, temperature variance had a significant effect on performance at 26°C. To determine the relative importance of righting time, swimming speed and muscular strength with respect to the multivariate effects, separate univariate ANOVAs were conducted. The significant effect of variance was due to righting time, marginally to swimming speed, and not at all to muscular strength (*Table 1*). For the performance MANOVA at a mean temperature of 29°C, the whole model was significant (Wilk's  $\lambda = 0.29$ ,  $F_{[30,311.81]} = 5.51$ ,  $P < 0.001$ ), as was temperature variance (Wilk's  $\lambda = 0.11$ ,  $F_{[3,311.81]} = 3.96$ ,  $P = 0.01$ ). Temperature tested was only marginally significant (Wilk's  $\lambda = 0.06$ ,  $F_{[3,311.81]} = 2.39$ ,  $P = 0.07$ ). Therefore, temperature variance significantly affected performance in hatchlings incubated at a mean temperature of 29°C. The significant effect of variance was due to muscular strength, righting time, and marginally to swimming speed (*Table 1*). Overall, at 26°C, hatchlings righted themselves faster at fluctuating temperatures, but swam faster at constant temperatures. At 29°C, hatchlings righted themselves faster and were stronger at constant temperatures (*Figure 5*).

For the performance MANOVA at constant temperatures, the whole model was significant (Wilk's  $\lambda = 0.28$ ,  $F_{[30,300.07]} = 5.40$ ,  $P < 0.001$ ), as was temperature mean (Wilk's  $\lambda = 0.89$ ,  $F_{[3,300.07]} = 30.15$ ,  $P < 0.001$ ), and temperature tested (Wilk's  $\lambda = 0.12$ ,  $F_{[3,300.07]} = 4.07$ ,  $P = 0.009$ ). Therefore, mean incubation temperature affected performance at constant temperatures. All three measures of performance contributed to the significance in temperature mean (*Table 1*). Significance in temperature tested was due to muscular strength ( $R^2 = 0.045$ ,  $F_{[1,104]} = 8.87$ ,  $P = 0.004$ ). Hatchlings were stronger when tested at

an ambient temperature of 26°C than at 29°C, regardless of the mean temperature at which they were incubated. For the performance MANOVA at fluctuating temperatures, the whole model was significant (Wilk's  $\lambda = 0.34$ ,  $F_{[30,288.33]} = 4.25$ ,  $P < 0.001$ ), as was temperature mean (Wilk's  $\lambda = 0.26$ ,  $F_{[3,288.33]} = 8.59$ ,  $P < 0.001$ ), but not temperature tested (Wilk's  $\lambda = 0.02$ ,  $F_{[3,288.33]} = 0.70$ ,  $P = 0.55$ ). Thus, mean incubation temperature has a significant effect in fluctuating temperature regimes. Significant differences in mean temperature were due to swimming speed, marginally to muscular strength, and not at all to righting time (*Table 1*). Overall, hatchlings incubated in fluctuating regimes swam faster when incubated at a mean temperature of 29°C, but were stronger at 26°C, and righted themselves at the same speed at both temperatures (*Figure 5*).

## **Discussion**

The results of this study are consistent with the hypothesis that both the mean and variance of the incubation temperatures experienced by *E. obsoleta* hatchlings affect their fitness-related phenotypic traits. In terms of mean temperature, this study is consistent with the results of Blouin-Demers *et al.* (2004): black rat snake hatchlings incubated at high mean temperatures hatched earlier and more successfully, were longer, swam and righted themselves faster, and were less reliant on aggressive defense. In terms of temperature variance, hatchlings incubated at constant temperatures hatched earlier, were longer, heavier, and (at high temperatures) swam and righted themselves faster and were stronger. At lower mean temperatures, hatchlings swam faster, but righted themselves more slowly. Mass was found to be greater at lower mean temperatures. Tail length was

unaffected by temperature mean, and neither tail length nor aggression was affected by the temperature variance.

Early hatching date is beneficial in *E. obsoleta* because this species hatches in late August to September, a few weeks prior to the first frost. Hatching earlier should minimize the risk of the egg freezing, maximize the chances of finding a suitable hibernaculum before the onset of winter, and allow neonates to begin their growth towards sexual maturity at 1050 mm SVL (Webb *et al.* 2001, Blouin-Demers *et al.* 2004). Large body size (Fox 1975, Ferguson and Fox 1984) and SVL in particular (Jayne and Bennett 1990, Brown and Shine 2005b) has been found to enhance the probability of survival in reptiles. Larger hatchlings may have enhanced locomotory performance, more successful handling of larger prey items (Booth *et al.* 2004), and have a better chance of surviving hibernation (Blouin-Demers *et al.* 2004). Hatchlings incubated at constant temperatures were also heavier than those incubated at fluctuating temperatures. A greater mass relative to length may indicate a larger amount of residual yolk and therefore greater energy reserves for the hatchling (Booth 2006). However, mass and body condition are poorer predictors of survival in neonate snakes than SVL (Jayne and Bennett 1990, Brown and Shine 2005b), and may indeed depend on the environment. Large body size with little residual yolk would be more advantageous in environments where food is easily obtained, but a smaller body size with a larger amount of residual yolk could provide hatchlings with energy for longer periods of time if food is scarce (Booth 2006). Regarding tail length, longer tails enhance locomotion in snakes (Shine and Shetty 2001), but even severe tail loss may not compromise survival (Aubret *et al.* 2005). Moreover, the difference in tail length between

males and females was much greater than the difference among incubation treatments, suggesting that the effects of tail length on survival may be negligible. With respect to anti-predator behaviour, aggression is presumed to be detrimental to neonates' survival; because it is a tactic opted by many species chiefly when unable to flee from danger (e.g. Hertz *et al.* 1982). In terms of behaviour, thermal variance did not affect hatchling phenotypes or fitness. In terms of incubation period and morphology, however, neonates incubated at high, constant temperatures were generally fitter than those incubated at fluctuating temperatures.

The results for performance were not as clear. Locomotor performance has been found to be a good predictor of early survival in garter snakes (Jayne and Bennett 1990). Although muscular strength has been assumed to affect the fitness of snakes (Shine and Brown 2002, Brown and Shine 2004), however, it has not been explicitly tested. A stronger hatchling would presumably be more likely to pull free from a potential predator than a weaker one. In this experiment, constant temperatures tended to produce "fitter" hatchlings than fluctuating temperatures when the mean temperature was high. At 29°C, performance at constant temperatures was better in terms of swimming speed and righting time, and poorer in terms of muscular strength. At 26°C, performance at constant temperatures was better in terms of swimming speed, poorer in terms of righting time, and not significantly different from fluctuating temperatures in terms of muscular strength. Interestingly, for strength and righting time, fluctuating temperatures seemed to equalize the performance of hatchling at different mean temperatures: the difference between hatchlings incubated at 26°C and 29°C was much greater at constant mean temperatures



than at fluctuating temperatures. In terms of performance, therefore, thermal variance appears to promote phenotypic uniformity within a population. Hatchlings in the fluctuating temperature regimes in this experiment experienced considerable overlap in incubation temperatures (*Figure 1*). This similarity in incubation temperatures may be responsible for the similarity in their performance. Temperature fluctuations have also been found to increase phenotypic uniformity (in terms of morphology) in smooth softshell turtles (Ashmore and Janzen 2003). In addition, the results for performance could potentially be explained in terms of the variance in the properties of snake muscles. Swimming is an aerobic activity dependent on slow oxidative muscle fibers, whereas righting and muscular strength rely primarily on the anaerobic fast glycolytic fibers (Hill *et al.* 2004). If thermal regime affects slow oxidative and fast glycolytic fibers differently, this could account for differences between swimming speed and the other two performance measurements. The other interesting result was that hatchlings incubated at lower temperatures were stronger than those incubated at higher temperatures. There may be a tradeoff between strength and speed, which would explain why the speedier group tended to be weaker. Since cells have a limited volume, increasing the amount of sarcoplasmic reticulum or glycogen (which increase the contraction rate) decreases the amount of myofibrils (required for strength) in a cell (Hill *et al.* 2004; Rome 2006). Faster locomotion may therefore result in lower strength. Alternatively, the results could simply be random. It has not been explicitly determined whether righting time and muscular strength are good predictors of hatchling survival in snakes. There may therefore be no selective pressure for these traits to be optimized under certain thermal regimes. Swimming speed, the most reliable predictor of hatchling survival, was optimized at

higher, constant temperatures. Moreover, righting time was fastest in the constant 29°C treatment. For two out of three performance measurements, therefore, the combination of high thermal mean and constant thermal variance produces traits that presumably promote survival in neonate *E. obsoleta*.

The literature agrees with some of my results. Temperature variance has been found to have no effect on hatching success (Ashmore and Janzen 2003, Mullins and Janzen 2006) and SVL decreases with thermal variance (Webb *et al.* 2001, Webb *et al.* 2006, Du and Ji 2006) and mass increases (Webb *et al.* 2001). For all other traits, however, there is a general lack of agreement in the literature with respect to the effects of temperature variance on hatchling phenotype. Incubation period at fluctuating incubation temperatures has been found to be longer (Webb *et al.* 2006), and equal to (Webb *et al.* 2001) constant temperatures, or to be minimized at average variance (Ashmore and Janzen 2003). Temperature fluctuations have also been found both to increase (Ashmore and Janzen 2003, Du and Ji 2006) and to have no effect (Mullins and Janzen 2006, Demuth 2001) on locomotory performance. Clearly, therefore, the effects of incubation temperature variance on hatchlings are dependent on the study species and/or experimental design.

For black rat snakes, the effects of temperature variance on hatchling phenotype and fitness are mixed. Although constant temperatures provided no clear benefit for hatching success, aggression, righting time and muscular strength, they did enhance fitness for the three most reliable predictors of survival (incubation period, SVL and swimming speed). There is therefore support for the argument that female black rat snakes prefer to lay eggs

in communal nests because these have higher, more constant temperatures than single-female nests. However, thermal mean was found to be a much better predictor of hatchling fitness in *E. obsoleta* than thermal variance. To further assess the role of thermal variance in the nest-site selection in this species, I suggest that two studies be conducted. Firstly, it should be determined whether females do prefer to lay their eggs in substrates at constant temperatures to those at fluctuating temperatures. Secondly, a long-term study on hatchling snakes should be conducted to determine the reliability of the aforementioned phenotypic traits in predicting survivorship.

Finally, the results of this experiment did not support the assumption of the Charnov-Bull hypothesis of differential fitness, since there was no interaction between temperature mean and sex for any trait. However, this does not necessarily refute the hypothesis, because temperature-dependent sex determination is not known to occur in snakes. Indeed, TSD may not have evolved in snakes *because* the sexes are not differentially affected by mean temperature of incubation. It is possible that the assumption of differential fitness is supported in species with TSD. A better species on which to test this assumption would be a lizard or turtle with genetic sex determination that is closely related to species with TSD.

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## Appendix: Tables and Figures

Table 1. Effect of temperature variance (at mean temperatures 26°C and 29°C) and temperature mean (at constant and fluctuating temperatures) on the swimming speed, muscular strength and righting time in neonate *E. obsoleta*. All values are from ANOVAs following the appropriate MANOVA. Significant results ( $P < 0.05$ ) are bolded, and marginally significant results ( $0.05 \leq P < 0.1$ ) are italicized.

MANOVA	Swimming Speed	Muscular strength	Righting time
26°C	$F_{[1,99]} = 3.60, P = 0.06$	$F_{[1,98]} = 0.98, P = 0.33$	$F_{[1,100]} = 8.05, \mathbf{P = 0.006}$
29°C	$F_{[1,112]} = 2.98, P = 0.09$	$F_{[1,109]} = 4.40, \mathbf{P = 0.04}$	$F_{[1,112]} = 4.44, \mathbf{P = 0.04}$
Constant	$F_{[1,105]} = 26.61, \mathbf{P < 0.001}$	$F_{[1,104]} = 23.99, \mathbf{P < 0.001}$	$F_{[1,105]} = 22.96, \mathbf{P < 0.001}$
Fluctuating	$F_{[1,105]} = 20.23, \mathbf{P < 0.001}$	$F_{[1,102]} = 3.57, P = 0.06$	$F_{[1,106]} = 0.01, P = 0.91$

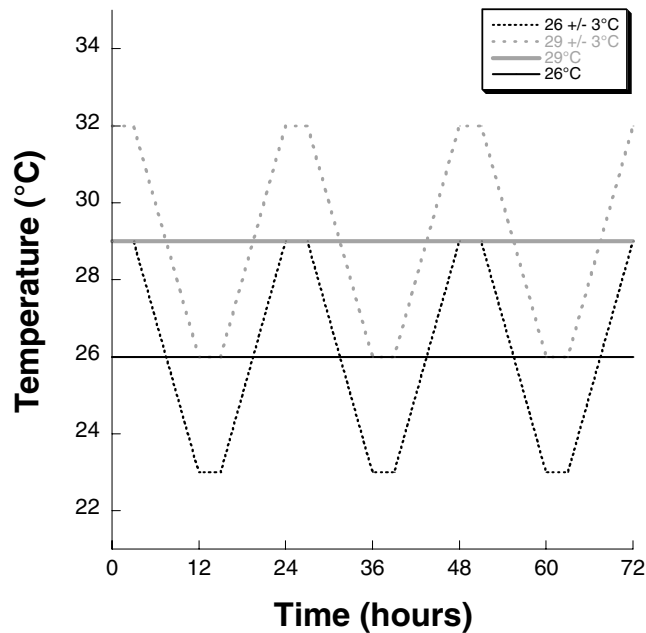
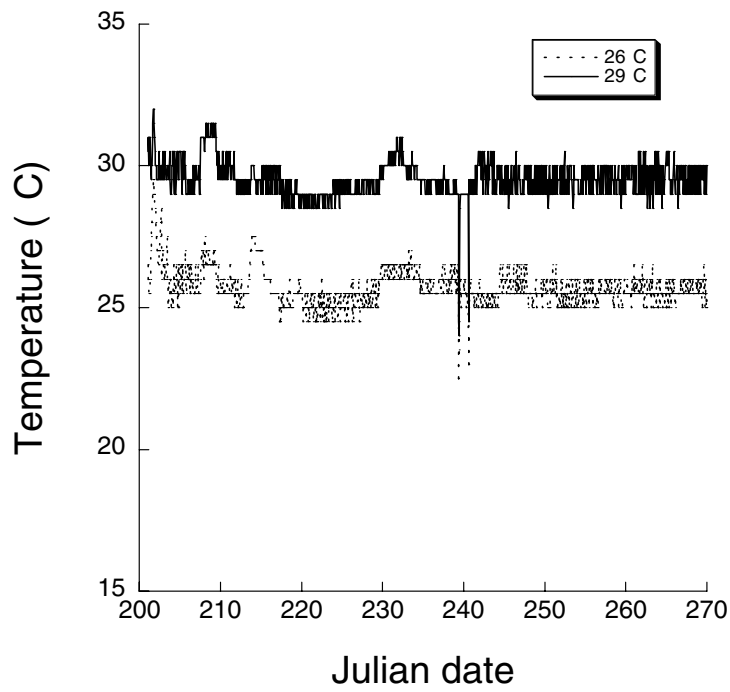
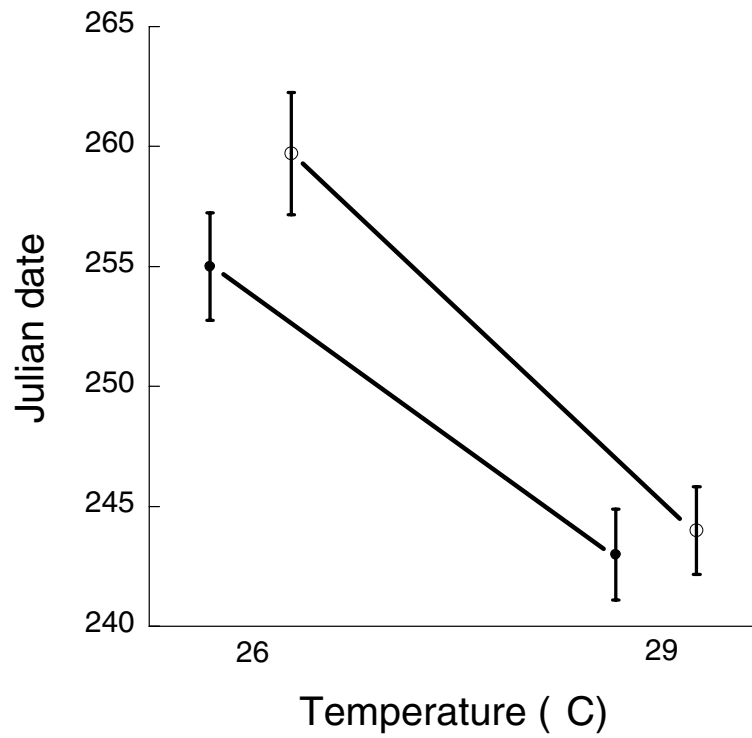


Figure 1. The four temperature treatments used in the incubation *E. obsoleta* eggs: 29°C, 29 +/- 3 °C, 26°C, and 26 +/- 3°C.



*Figure 2.* Temperature in the constant-temperature incubators throughout the incubation period, recorded with miniature temperature data loggers.





*Figure 3.* Effect of thermal mean (26 or 29°C) and variance (constant (●) and +/- 3°C (○)) of the incubation treatment on the hatching date of neonate black rat snakes. Values are means +/- SE. N = 116.

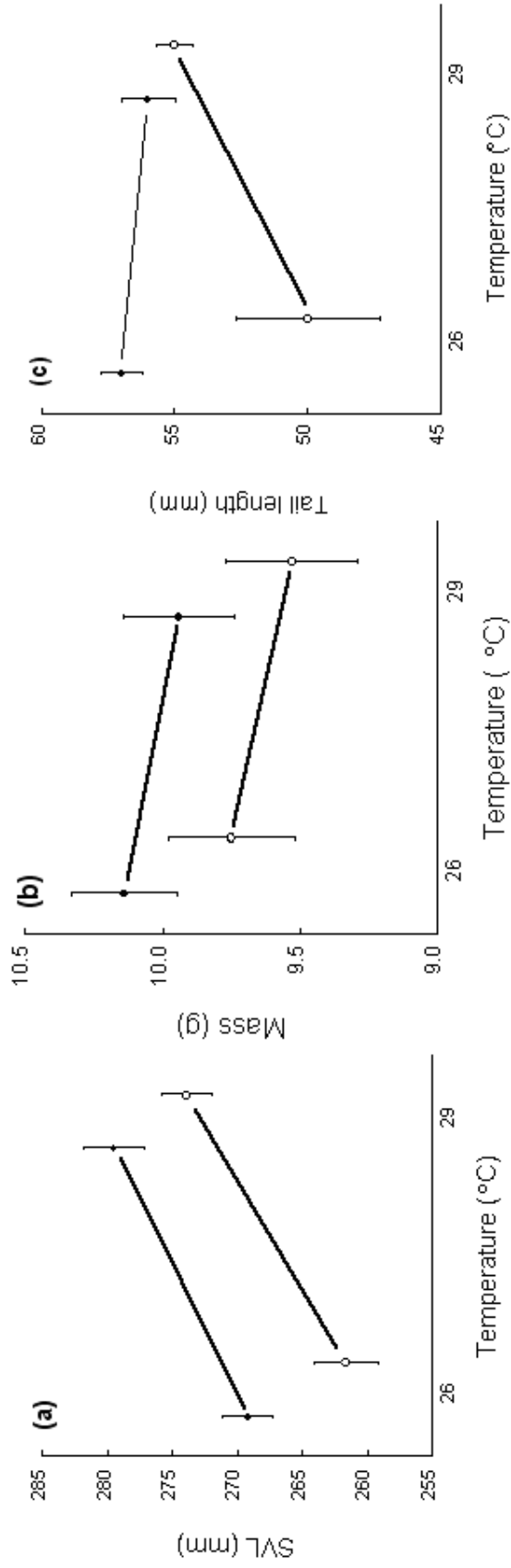


Figure 4. Effect of thermal mean (26 or 29°C) and variance (constant (●) and +/- 3°C (○)) of the incubation treatment on the (a) snout-vent length, (b) mass and (c) tail length of black rat snake hatchlings. Values are means +/- SE, N = 116.

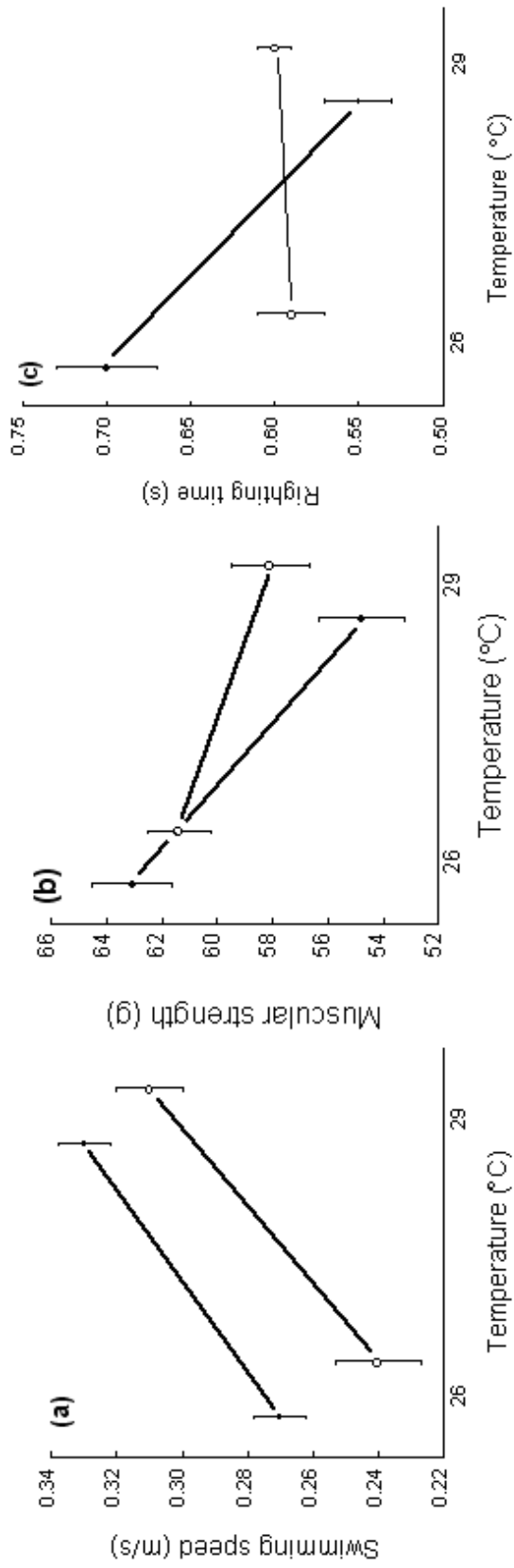


Figure 5. Effect of thermal mean (26 or 29°C) and variance (constant (●) and +/- 3°C (○)) of the incubation treatment on the (a) swimming speed (N = 116), (b) muscular strength (N = 114) and (c) righting time (N = 114) of black rat snake hatchlings. Values are means +/- SE.