Phenotypic consequences of nest-site selection in black rat snakes (*Elaphe obsoleta*)

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Abstract: Nest-site selection is the only behaviour that can be considered parental care in most oviparous reptiles because eggs are abandoned after laying and because incubation conditions resulting from nest-site selection can have profound effects on offspring. During a 7-year study of black rat snakes, *Elaphe obsoleta* (Say in James, 1823), we investigated phenotypic effects of incubation temperature on hatchlings, monitored temperatures in nests, and determined the preferred nesting temperature. Temperatures of communal nests were higher than those of single-female nests. In the laboratory, females preferred to nest at temperatures most similar to those of communal nests. Hatchlings from eggs incubated at temperatures similar to those in the warmer communal nests hatched faster, were longer, swam faster, were less aggressive, and had fewer scale anomalies than hatchlings from eggs incubated at temperatures similar to those in single nests. A possible disadvantage of communal nests is that eggs in communal nests may be at greater risk to parasitism by *Nicrophorus pustulatus* (Herschel, 1807). The incubation experiment allowed a test of a key assumption of a model proposed to explain environmental sex determination. Contrary to that assumption, we found no evidence that incubation temperature affected males and females differently. Our results might explain why temperature-dependent sex determination appears not to occur in snakes.

Résumé : Chez la plupart des reptiles ovipares, le choix d'un site de nidification est le seul comportement qui puisse être considéré comme un soin parental, car les oeufs sont abandonnés après la ponte; en effet, les conditions d'incubation déterminées par le choix du site de nidification peuvent avoir d'importantes répercussions sur les rejetons. Au cours d'une étude de 7 années de la couleuvre obscure, *Elaphe obsoleta* (Say in James, 1823), nous avons analysé les effets de la température d'incubation sur les phénotypes des nouveau-nés, enregistré la température dans les nids et déterminé la température préférée de nidification. Les températures des nids communautaires sont plus élevées que celles des nids de femelles isolées. Au laboratoire, les femelles préfèrent établir leurs nids aux températures semblables à celles des nids communautaires. Les nouveau-nés provenant d'oeufs incubés aux températures des nids communautaires éclosent plus rapidement, sont plus longs, nagent plus vite, sont moins agressifs et leurs écailles présentent moins d'anomalies que les nouveaux-nés issus d'oeufs incubés aux températures des nids isolés. Un désavantage possible des nids communautaires est que les oeufs de ces nids risquent plus d'être parasités par *Nicrophorus pustulatus* (Herschel, 1807). Cette incubation expérimentale nous a permis de vérifier une des présuppositions principales d'un modèle proposé pour expliquer la détermination du sexe par les facteurs de l'environnement. Nous n'avons trouvé aucun indice que la température d'incubation affecte les mâles et les femelles de façon différente, ce qui infirme la présupposition. Nos résultats expliquent peut-être pourquoi il n'y a pas de détermination du sexe reliée à la température chez les couleuvres.

[Traduit par la Rédaction]

Introduction

Females of most oviparous species of reptiles abandon their eggs shortly after laying them. Thus, the only behaviour that can be considered to be parental care in these species is nest-site selection. It is now well established that incubation conditions can affect offspring phenotype in ways likely to affect their fitness (Qualls and Andrews 1999; Brana and Ji 2000; Janzen and Morjan 2002), including attributes as fundamental as offspring sex (Bull 1980; Rhen and Lang 1998; Valenzuela 2001). Given the consequences of incubation conditions, females should select nest sites that maximize the fitness of their offspring, and thus their own fitness. Despite the growing evidence of incubation-mediated phenotypic plasticity, however, few studies to date have put this phenomenon in an ecologically relevant context. Studies of incubation effects often lack information on female nest-site selection and on factors that affect the success of nest sites which are selected in the wild. Our general goal here is to investigate phenotypic effects of incubation temperature on hatchling black rat snakes, *Elaphe obsoleta* (Say in James, 1823), in an ecologically relevant context.

To achieve our goal of ecological relevance, we included two elements in this study in addition to conducting a conventional experiment to determine how hatchling phenotypes

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vary in response to incubation temperature. First, we examined female preference for nest temperatures, an element that has been largely overlooked in this field to date. We predicted that females should prefer temperatures which are beneficial to offspring. Temperatures that promote early hatching should be preferred, particularly because our study population is at the northern extreme of the distribution of black rat snakes. For reptiles at higher latitudes, eggs slow to hatch are at risk of freezing (Bobyn and Brooks 1994). In addition, long-term phenotypic effects of incubation temperature can operate indirectly through variation in the date of hatching (Qualls and Shine 2000). Other attributes likely to enhance fitness in hatchling snakes include large size (Jayne and Bennett 1990; Bronikowski 2000) and superior locomotor performance (Jayne and Bennett 1990). Second, we also monitored temperature profiles of nests in the wild. Female rat snakes often nest communally, and use of communal nests appears to be more traditional (i.e., used in multiple years) than that of single nests (G. Blouin-Demers and P.J. Weatherhead, personal observation). Given these patterns of use, we predicted that, relative to single nests, communal nests should provide thermal conditions closer to those preferred by females and that are more beneficial to hatchlings. A secondary goal of this study was to use sex-specific effects of incubation temperatures to gain insight into temperature-dependent sex determination (Charnov and Bull 1977), despite the fact that this phenomenon is not known to occur in snakes. Most of the current models to explain temperature-dependent sex determination rely on the assumption that incubation temperature affects males and females differently (Shine 1999). One problem in testing this assumption has been that, in species with temperature-dependent sex determination, the effect of temperature on phenotype is confounded by the effect of sex on phenotype (some temperatures produce mostly males and others mostly females). To get around this problem, some researchers have used hormones to override the sex-determining effect of temperature (Tousignant and Crews 1995). However, hormone treatment also can affect phenotypes (Rhen and Lang 1995). One novel approach that has been used to test this critical assumption of the Charnov and Bull (1977) model is to use species with genetic sex determination to look for sex by incubation temperature interaction effects on offspring phenotypes (Elphick and Shine 1999). Although this method makes it more difficult to extrapolate the results to species with temperature-dependent sex determination, it avoids the pitfalls associated with hormonal manipulations and the confusion of sex effects with temperature effects. If the central assumption of the Charnov and Bull (1977) model is met, we predicted that male and female hatchling black rat snakes would be affected differently by incubation temperature.

Materials and methods

Study area

We conducted this study on black rat snakes from 1996 to 2002 at the Queen's University Biological Station, approximately 40 km north of Kingston (Ontario, Canada). The study location is less than 20 km from the northern extreme of the distribution of black rat snakes and less than 200 km from the northern extreme of the distribution of oviparous reptiles. Habitat in the 10 km × 3 km study area is primarily deciduous forest, with marshes, beaver ponds, rocky outcrops, and old hay fields providing openings in the forest canopy (Blouin-Demers and Weatherhead 2001*a*). Black rat snakes nest in decaying organic matter that is sufficiently loose to allow females to burrow up to 75 cm into the material. In our study area, nest sites used by rat snakes included the interior of hollow trees (both standing and fallen), leaf piles, and compost piles.

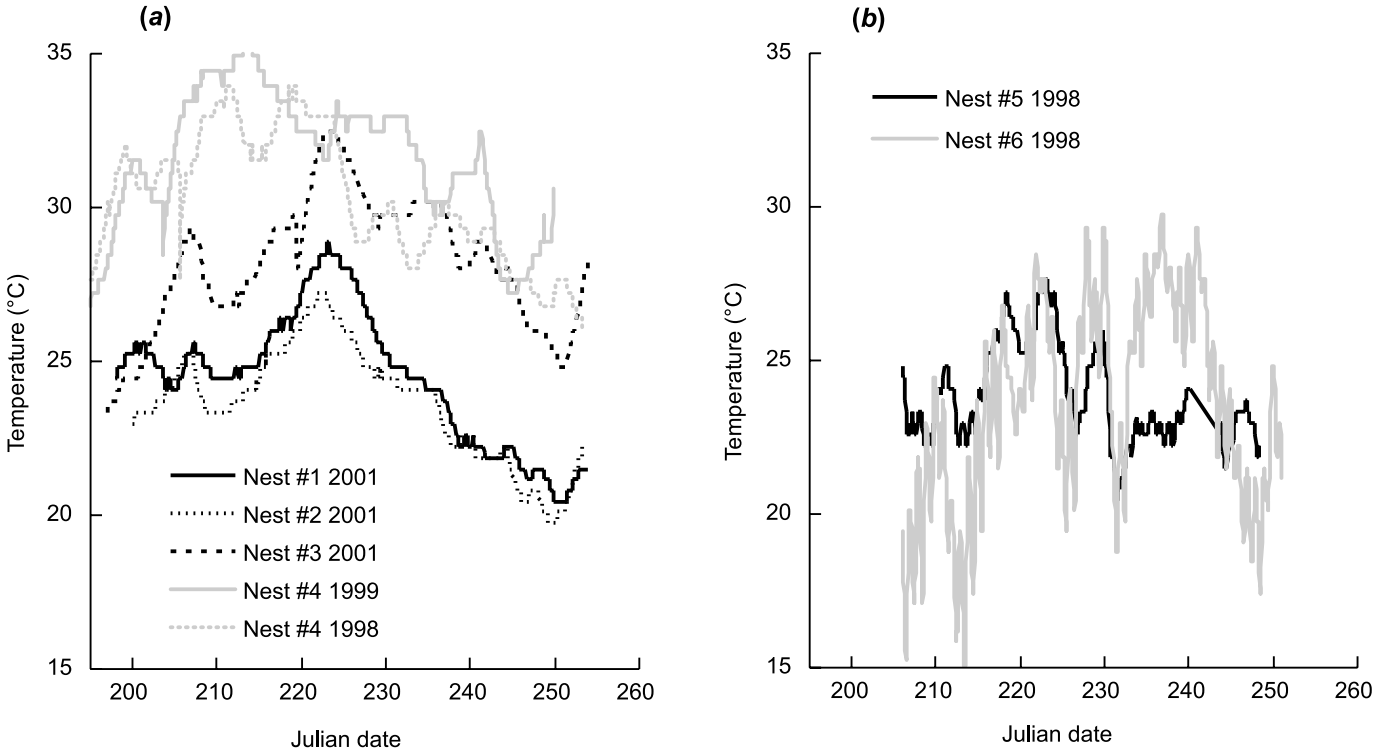
Location of nests

We located black rat snake nests by radio-tracking gravid females until they oviposited. Females were captured during spring emergence from hibernacula (Blouin-Demers et al. 2000*a*) or opportunistically during the active season (Prior et al. 2001) and had radiotransmitters (SI-2T, Holohil Systems, Carp, Ont.) surgically implanted in their body cavity using sterile techniques and under isoflurane anesthesia (Blouin-Demers et al. 2000*b*). From 1996 to 2002, we followed 64 females for periods ranging from 3 weeks to 4 years. Twenty-six of the 64 radio-tracked females were gravid at least once (4 females were gravid twice and 1 female was gravid three times). Females with radiotransmitters led us to 12 communal nests and 6 single nests. At communal nests, we regularly encountered gravid females without radiotransmitters. To confirm whether nests were communal or single, we also searched the nesting substrate for eggs on several occasions through the nesting season. At most nests, we used miniature temperature dataloggers (Hobo Temp and Hobo RH; Onset Computers, Pocasset, Mass.) to measure temperature and relative humidity in the nest where the eggs had been laid. Females continued to nest in sites in which we had placed dataloggers, indicating that our activity did not change the nesting sites dramatically.

Preferred nesting temperature

To determine the preferred nesting temperature of female black rat snakes independently of other factors, we adapted the approach commonly used to determine the preferred body temperature range in studies of thermal ecology (Hertz et al. 1993; Blouin-Demers and Weatherhead 2001*b*). Instead of placing snakes in a thermal gradient to determine their preferred body temperatures, we placed gravid females in a thermal gradient that included nesting substrate to determine their preferred temperature for laying their eggs. In the laboratory, we constructed a plywood nesting chamber with a thermal gradient. The chamber was 200 cm long, 240 cm wide, and 50 cm high. We subdivided the chamber into eight compartments measuring 200 cm × 30 cm using 50 cm high partitions. A removable mesh lid was placed over the top of the chamber to prevent snakes from escaping. The thermal gradient was created by placing one end of the chamber over heating pads and the other end over a coil of tubing circulating well water. The chamber was positioned so that each compartment had the same thermal gradient. We filled each compartment to a depth of 25 cm with a mixture of approximately 25% leaf litter and 75% moist peat moss. After several days of equilibration, the chamber offered a smooth and stable thermal gradient ranging from 10 to 40 °C. In July 2002, 10 gravid female black rat snakes were captured opportunistically from the wild and placed individually in com-

Fig. 1. Temperatures recorded in five communal (*a*) and two single-female (*b*) nests of black rat snakes, *Elaphe obsoleta*, at the Queen's University Biological Station in Ontario.



partments of the chamber. Every second day, all partitions were searched gently by hand to minimize disturbance of the nesting material, females, or eggs. When a clutch was found, we removed the female from the chamber and carefully placed a mercury thermometer in the centre of the clutch with minimal disturbance to the nesting material. After 10 min of equilibration, the nesting temperature was recorded and the eggs were removed. Several initial trials confirmed that after equilibration, the temperature in the "nests" remained constant.

Incubation of eggs

In 1998, we collected all of the eggs from natural nests used by radio-implanted females and brought them to the laboratory for artificial incubation. In addition, gravid females without radiotransmitters that we encountered opportunistically in the field were captured and were brought to the laboratory. Each of these females was housed alone and was provided with a nesting box filled with a mixture of moist peat and sphagnum moss in which it laid within a few days. We obtained 206 eggs from 17 clutches (clutch size range = 7–17), but 24 eggs were already parasitized by *Nicrophorus pustulatus* (Herschel, 1807) when collected in the wild, including all 12 eggs from 1 clutch. Thus, we incubated 182 eggs from 16 clutches. We randomly divided each clutch in half and assigned one half to a constant low-temperature incubator (91 eggs at 25 °C) and the other half to a constant high-temperature incubator (91 eggs at 30 °C). These two incubation treatments were selected to approximate the lower and upper limits of the preferred body temperature of black rat snakes (Blouin-Demers and Weatherhead 2001*b*). Fortuitously, these temperatures also approximated mean temperatures of single-female nests and of communal nests (Fig. 1). We chose constant temperatures for incubation because temperatures in natural nests are buffered from pronounced daily temperature cycles by decomposing vegetation.

The incubators were built with fiberboard laminated with melamine and were 120 cm high, 90 cm wide, and 30 cm deep. Shelves supporting the egg containers were 20 cm wide, providing a 5-cm space front and back for air circulation. A small fan in continuous operation in each incubator prevented thermal gradients from developing. Temperature was maintained in each incubator with a 100-W light bulb activated by an electronic thermostat (CT3300; Honeywell, Morristown, N.J.). A miniature temperature datalogger (Hobo Temp, Onset Computers) was placed in the centre of each incubator to monitor incubation temperatures. Eggs were grouped by clutch in 15 cm × 15 cm × 6 cm plastic containers with perforated lids and were half-buried in 3 cm of moist vermiculite (2:1 ratio by mass of water and vermiculite). The containers with the eggs and incubation medium were weighed at the onset of incubation, and to compensate for evaporation, water was added weekly to maintain total mass. This weekly manipulation also allowed shuffling of the position of the containers within the incubators to prevent potential position effects. Toward the end of incubation, we checked incubators twice a day to record hatching date.

Phenotypic measurements

Within 1 day post hatching, we removed the babies from the incubators and scored their aggressiveness in response to first handling (1, escaping; 2, gaping the mouth; 3, striking; and 4, biting). The next day, we measured the hatchlings'

swimming speed at 25 °C in a 2 m long trough filled with water (Blouin-Demers et al. 2003). Hatchlings were encouraged to swim at maximum speed by gently tapping their tails during the trial. We made each hatchling swim the distance twice, measured the time required using a stopwatch, and recorded the faster of the two trials. We measured swimming speed instead of crawling speed because we wanted to ensure that hatchlings were performing at maximum speed, and prodding of crawling hatchlings produced defensive responses instead of increased speed. Several hours after the swimming trials, we measured righting time by placing each hatchling on its back and recording the time required for it to right itself. Finally, we took morphological measurements of the hatchlings and determined their sex by everting the hemipenes. Hatchlings were weighed to the nearest 0.1 g on an electronic scale. Snout–vent length (SVL) and tail length were measured to the nearest 1 mm by laying the hatchlings against a metal ruler taped to a table. For SVL, we used the mean of two measurements (Blouin-Demers 2003). We also counted the number of split ventral scales as an index of scale anomalies (Merila et al. 1992; Forsman et al. 1994). All phenotypic measurements were completed within 3 days post hatching.

All of our work was conducted with the approval of the animal care committees at Carleton University and at the University of Illinois and was in accordance with the principles and guidelines of the Canadian Council on Animal Care.

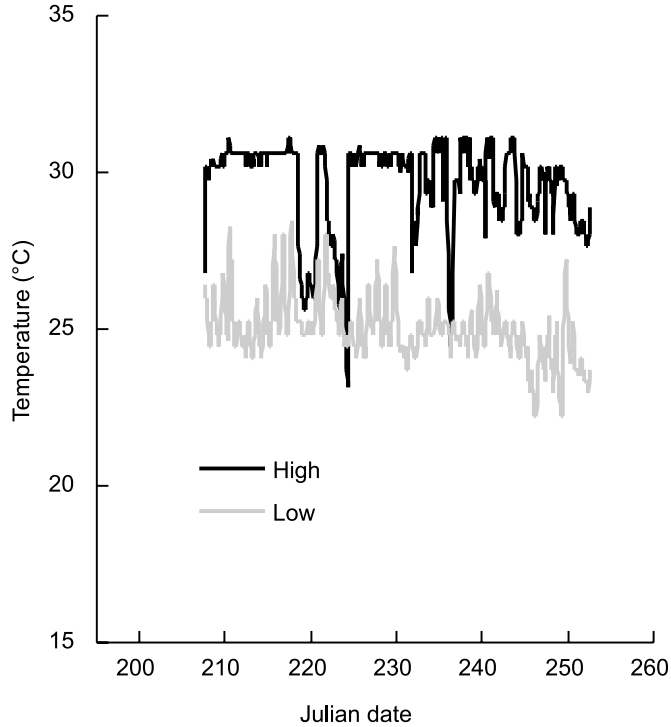
Analyses

We analyzed the effect of incubation temperature on the morphology of offspring with multivariate analysis of variance (MANOVA). Dependent variables were SVL, mass, and tail length and independent variables were incubation temperature, sex, and their interaction. We analyzed the effect of incubation temperature on behaviour and performance of hatchlings with MANOVA. Dependent variables were swimming speed, righting time (natural logarithmically transformed), and response to handling and independent variables were incubation temperature, sex, and their interaction. We confirmed that dependent variables followed a multivariate normal distribution in each group and that population covariance matrices for dependent variables were equal following the methods outlined by Stevens (1996). We used nominal logistic regression to analyze the effects of incubation temperature on scale anomalies. The dependent variable was presence or absence of split ventral scales and independent variables were incubation temperature, sex, and their interaction. In each of the preceding analyses, we included clutch as a control variable because maternal and (or) genetic factors can affect offspring morphology and performance (King 1997; Blouin-Demers et al. 2003). All analyses were conducted using JMP version 5 (SAS Institute Inc. 2002). We considered statistical results significant at α = 0.05 in two-tailed tests. All values are means ± 1 SE.

Results

Communal nests had higher and less variable temperatures than single-female nests (Fig. 1). During the period 15 July to 15 September between 1996 and 2002, the mean temperature of nine communal nests (27.93 ± 0.81 °C, range = 23.6–31.8 °C) was significantly higher ($t_{[10]}$ = 2.90, P = 0.016) than the mean temperature of three single nests (23.22 ± 1.41 °C, range = 22.2–23.8 °C). The relative humidity in all nests was 100%. The mean nesting temperature selected by the 10 females in the thermal gradient was 31.10 ± 0.92 °C (range 28–36 °C), which is significantly higher than the mean temperature of communal nests ($t_{[17]}$ = 2.46, P = 0.025). With other factors controlled, females preferred to nest at temperatures only available in some communal nests (Fig. 1).

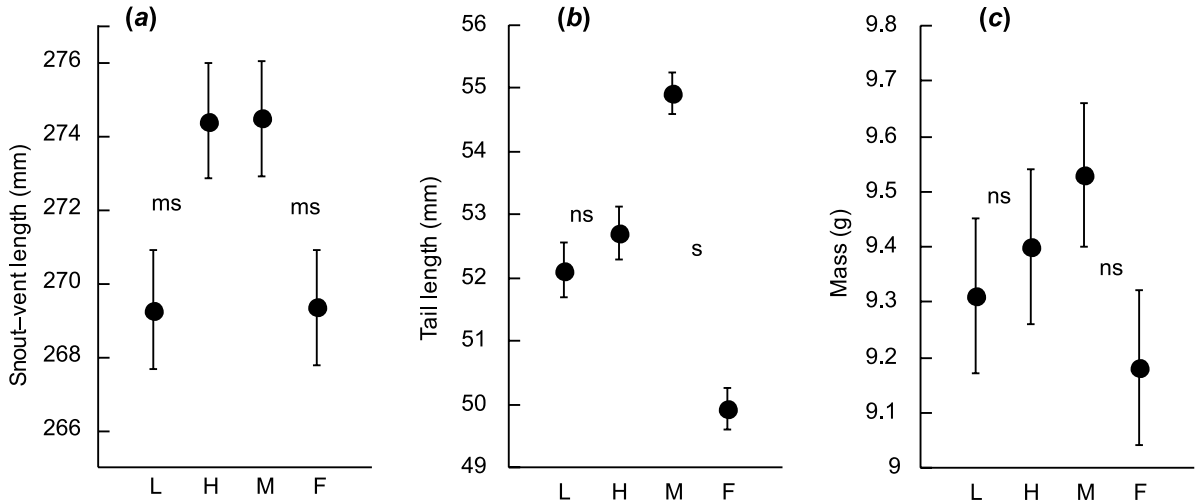
Fig. 2. Temperatures inside the low-temperature incubator and the high-temperature incubator experienced by black rat snake eggs from the Queen's University Biological Station in Ontario.



The low-temperature incubator had a mean temperature of 25.03 ± 0.03 °C and the high-temperature incubator had a mean temperature of 29.63 ± 0.06 °C (Fig. 2). Of the 182 eggs from 16 clutches placed in the incubators, 170 eggs hatched: 85 of the 91 eggs (93.4%) hatched in each treatment. The sex ratios were almost exactly 1:1 in each treatment (low temperature = 42 females and 43 males; high temperature = 43 females and 42 males). Taking the mean hatching date for each of the 16 clutches in each treatment, the 4.6 °C difference in incubation temperature resulted in a mean difference of 9.5 ± 1.1 days in hatching date between the low- and the high-temperature eggs. Taking the laying date for the 10 clutches for which it is known exactly (females that nested in the laboratory) and the mean hatching date for each of these clutches for each treatment, the low-temperature eggs had a mean incubation period of 61.9 ± 0.9 days, whereas the high-temperature eggs had a mean incubation period of 53.4 ± 2.2 days.

For the effect of incubation temperature on the morphology of hatchlings, the whole-model MANOVA was significant (Wilks' λ = 0.12, $F_{[57,442.11]}$ = 7.84, P < 0.001). The

Fig. 3. Effects of high (H) or low (L) incubation temperatures and of sex (M, male; F, female) on the snout–vent length (*a*), tail length (*b*), and mass (*c*) of hatchling black rat snakes at the Queen's University Biological Station in Ontario. Values are means ± 1 SE. Results of univariate *F* tests following a significant MANOVA are either nonsignificant (ns), marginally significant (ms), or significant (s).



main effects of incubation temperature ($F_{[3,148]}$ = 4.40, P = 0.005) and sex ($F_{[3,148]}$ = 49.37, P < 0.001) were significant. The main effect of the interaction between sex and incubation temperature, however, was not significant ($F_{[4,147]}$ = 0.77, P = 0.549). The main effect of clutch was significant (Wilks' λ = 0.24, $F_{[48,440.986]}$ = 5.62, P < 0.001). Thus, incubation temperature and sex affected the morphology of hatchling black rat snakes. To identify the relative importance of SVL, mass, and tail length to the multivariate main effects of incubation temperature and sex on morphology, we conducted univariate F tests, adjusting α to 0.017 because we conducted three tests (i.e., 0.05/3) in each case. For the effect of incubation temperature, SVL contributed marginally to the significance ($F_{[1,168]}$ = 5.23, P = 0.023), while mass ($F_{[1,168]}$ = 0.19, P = 0.660) and tail length ($F_{[1,168]}$ = 0.94, P = 0.334) did not contribute. Hatchlings from the high incubation temperature were longer than hatchlings from the low incubation temperature (Fig. 3). For the effect of sex, tail length contributed substantially to the significance ($F_{[1,168]}$ = 117.52, P < 0.001), SVL contributed marginally to the significance ($F_{[1,168]}$ = 5.27, P = 0.023), and mass did not contribute ($F_{[1,168]}$ = 3.37, P = 0.068). Male hatchlings had longer tails and were longer overall than female hatchlings (Fig. 3). In relative measurements, males had tails that averaged 20.0% of their SVL whereas females had tails that averaged 18.5% of their SVL.

For the effect of incubation temperature on behaviour and performance of hatchlings, the whole-model MANOVA was significant (Wilks' λ = 0.46, $F_{[57,439.13]}$ = 2.27, P < 0.001). The main effect of incubation temperature ($F_{[3,147]}$ = 20.04, P < 0.001) was significant. The main effects of sex ($F_{[3,147]}$ = 0.45, P = 0.721) and of the interaction between sex and incubation temperature ($F_{[3,147]}$ = 1.00, P = 0.394) were not significant. The main effect of clutch was again significant (Wilks' λ = 0.61, $F_{[48,438.01]}$ = 1.65, P = 0.005). Thus, incubation temperature affected the behaviour and performance of hatchling black rat snakes. To identify the relative importance of swimming speed, righting time, and response to handling to the multivariate difference in performance and behaviour, we conducted univariate F tests, adjusting α to 0.017 because we conducted three tests (i.e., 0.05/3). For the effect of incubation temperature, swimming speed ($F_{[1,167]}$ = 9.56, P = 0.002) and response to handling ($F_{[1,167]}$ = 34.71, P < 0.001) both contributed to the significance, whereas righting time did not ($F_{[1,168]}$ = 1.38, P = 0.242). Hatchlings from the high incubation temperature swam faster but were less aggressive than hatchlings from the low incubation temperature (Fig. 4).

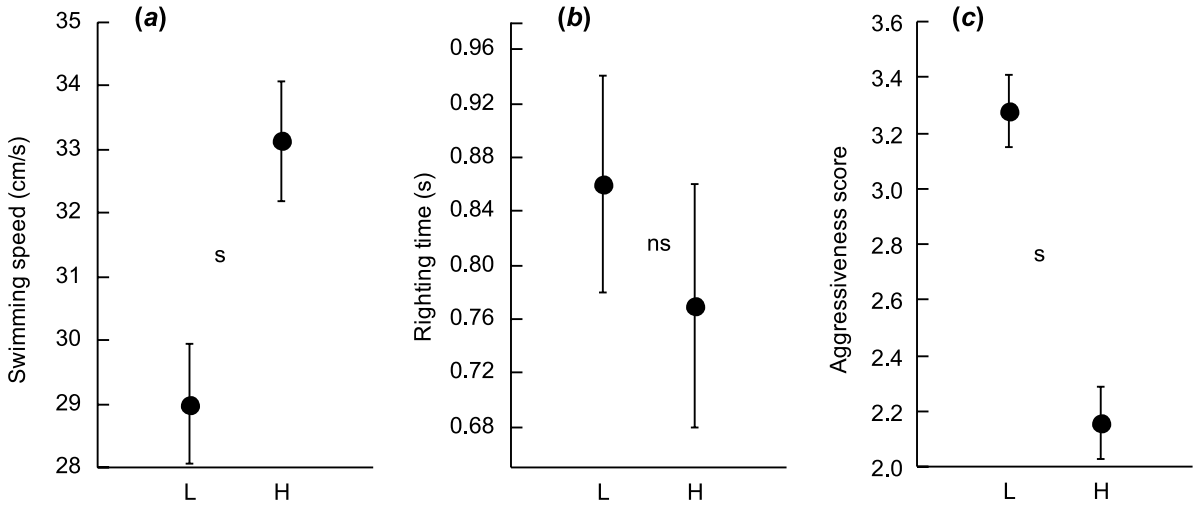
For the effect of incubation temperature on scale anomalies, nominal logistic regression revealed that the probability of having split ventral scales was affected by incubation temperature (Wald's $\chi^2_{[1]}$ = 5.74, P = 0.017) but not by clutch (Wald's $\chi^2_{[16]}$ = 14.03, P = 0.597), sex (Wald's $\chi^2_{[1]}$ = 1.37, P = 0.242), or the interaction between incubation temperature and sex (Wald's $\chi^2_{[1]}$ = 0.01, P = 0.923). In the low incubation temperature treatment, 27 of the 83 (32.5%) hatchlings had split ventral scales, whereas in the high incubation temperature treatment, only 17 of the 87 (19.5%) hatchlings had split ventral scales.

Discussion

Temperature and nesting ecology

The mean temperature in communal nests was ≈5 °C higher than the mean temperature in nests used by single females. In a thermal gradient, female black rat snakes laid their eggs in positions where temperatures were in the range found only within communal nests used by free-living females, although the mean selected temperature in the gradient was higher than the mean temperature of communal nests. Eggs incubated at temperatures similar to mean temperatures in communal nests produced hatchlings that differed in several ways from those incubated at temperatures more similar to those in single nests. Eggs incubated at the warmer temperature hatched 9.5 days earlier, approximately 16% less time than required at the cooler temperature. Controlling for clutch effects, hatchlings from the warmer incubator were larger, faster, and less reliant on aggressive defence and had fewer scale anomalies.

Fig. 4. Effects of high- (H) or low- (L) incubation temperatures on the swimming performance (*a*), righting (*b*), and aggressive (*c*) behaviours of hatchling black rat snakes at the Queen's University Biological Station in Ontario. Values are means ± 1 SE. Results of univariate *F* tests following a significant MANOVA are either nonsignificant (ns) or significant (s).



All of the differences attributable to incubation temperature are consistent with our prediction that temperatures preferred by females for incubation should be those most beneficial to hatchlings. Earlier hatching should be advantageous in this northern population because eggs hatch within a few weeks of the time that adult snakes start entering hibernation. In black rat snakes, adults hibernate communally at traditional underground hibernacula, but hatchlings rarely join communal hibernacula before they are 7 years old (Blouin-Demers et al. 2000*a*; Blouin-Demers and Weatherhead 2002). In the interim, hatchling and juvenile black rat snakes hibernate singly and thus cannot rely on odour cues from other snakes to locate hibernacula (G. Blouin-Demers and P.J. Weatherhead, personal observation). Therefore, early hatching would provide hatchlings from communal nests more time to find a suitable hibernaculum before the onset of winter. Hatching early may also provide hatchlings with a head start on growth towards sexual maturity, which occurs at approximately 1050 mm SVL or 9 years old (Blouin-Demers et al. 2002). In lizards, the benefits of early hatching are long term and their magnitude can even surpass that of other phenotypic effects of incubation temperature (Qualls and Shine 2000).

Phenotypic attributes of hatchlings associated with warmer incubation should also enhance fitness. Garter snakes (species of *Thamnophis* Fitzinger, 1843) that are larger at birth have higher survival early in life (Jayne and Bennett 1990; Bronikowski 2000), as do those with superior locomotor performance (Jayne and Bennett 1990). Also, larger size at birth may be advantageous for surviving hibernation, especially in our population where hatching occurs just a few weeks before the onset of hibernation. The fact that hatchlings incubated at warmer temperatures relied more on flight than fight when handled also suggests that they would have an advantage at avoiding predators. Reptiles that normally flee from threats often adopt alternative tactics (crypsis, active defence) when mobility is impaired by exhaustion or cool temperatures (Hertz et al. 1982; Arnold and Bennett 1984; Scribner and Weatherhead 1995). Thus, flight would appear to be the superior tactic. Finally, the greater incidence of scale anomalies among hatchlings from the lower incubation temperature suggests that normal development might have been impaired in these snakes. Scale anomalies in reptiles have been associated with skeletal anomalies including fused vertebrae and duplicated ribs and with reduced neonatal survival (Osgood 1978; Arnold and Bennett 1988; Merila et al. 1992; Forsman et al. 1994).

Females that used communal nests appeared to provide their eggs with superior incubation conditions relative to conditions experienced by eggs in single nests. Given this result, why do some females use single nests? One possibility is that the thermal disadvantages of single nests are compensated for by lower risk of egg parasitism by *N. pustulatus*. Madsen and Shine (1999) found that some female water pythons (*Liasis fuscus* Peters, 1873) nest in burrows of varanid lizards, while others nest in the roots of paperbark trees. Superior thermal conditions allow females that nest in varanid burrows to desert their eggs soon after laying and resume feeding (resulting in a 1-year reproductive cycle). Although posthatching survival of "burrow" hatchlings was high, egg mortality in burrows may also have been high, with the lizards being likely predators. In contrast, females that nest in paperbark roots remain with their eggs and warm them through shivering thermogenesis. These females forgo feeding, resulting in higher mortality and a multiyear reproductive cycle. Madsen and Shine (1999) proposed that one explanation for the coexistence of these two nesting strategies could be that the various trade-offs resulted in similar fitness payoffs for females adopting either strategy. The same may be true for single and communal nesting by female rat snakes, although more data on rates of egg parasitism, hatching success, and posthatching survival are required to assess this possibility.

A plausible alternative explanation may be that females which nest singly have not yet located one of the superior communal nests. This would explain why single nests appear to be used only once and is also consistent with the fact that all females in the thermal preference chamber selected tem-

peratures only available within communal nests. However, females that nest singly are not smaller (i.e., younger) than females that nest communally ($t_{[38]}$ = 0.51, P = 0.613), which is not consistent with singly nesting females not yet having located communal nests, although the power of this test was low because we had few females that nested singly.

Temperature and sex

We found that male hatchlings had longer tails and were longer overall than female hatchlings. These differences reflect the sexual dimorphism that occurs in adults (Blouin-Demers et al. 2002). More important for its relevance to environmental sex determination, however, we did not find a sex by incubation temperature interaction for morphology or performance of hatchling black rat snakes. A major assumption of Charnov and Bull's (1977) hypothesis for the evolution of temperature-dependent sex determination in reptiles is that the effect of incubation temperatures on offspring phenotypes is sex dependent. Webb et al. (2001) reasoned that if incubation effects are sex dependent in reptiles generally, those effects should occur in reptile species lacking temperature-dependent sex determination. Accordingly, they incubated snake eggs under two regimes that differed only in variance around the same mean temperature. They found significant interactions between incubation treatment and sex on swimming speed, body shape, and relative tail length and interpreted this interaction as supporting the assumption of the Charnov–Bull hypothesis.

One reason that our results differ from those of Webb et al. (2001) may involve how they analyzed their data. Webb et al. (2001) used 10 three-factor ANOVAs to test for significance of the 10 potential effects without adjusting α to 0.05/10 = 0.005 for the multiple comparisons being made. The authors did not report exact P values, but based on the levels of significance reported, three of the four interactions were not significant at α = 0.005 (the fourth interaction may or may not have been significant depending on the exact P value). Burger and Zappalorti (1988) documented differential mortality of male and female embryos of pine snakes, *Pituophis melanoleucus* (Daudin, 1803), at different incubation temperatures in the laboratory. Many of the laboratory incubation temperatures, however, were outside the range of temperatures recorded in natural nests of pine snakes. Indeed, the sex ratios (expressed as the number of males divided by the number of females) only ranged from 0.95 to 1.14 between 28 and 32 °C, the range of temperatures recorded in natural nests (Burger and Zappalorti 1988). Therefore, it remains unknown whether the results of this experiment reflect biologically realistic phenotypic plasticity that could be under the influence of natural selection.

Differences in methods (manipulation of mean versus variance in incubation temperatures) and analysis make it difficult to assess the available evidence for sex-specific effects of incubation in snakes. If future studies find a general sex-specific effect of incubation temperature in snakes, that result would support the assumption of models for the evolution of environmental sex determination (Shine 1999) while simultaneously begging the question of why that phenomenon appears to be absent in snakes. Alternatively, consistent failure to document sex by incubation treatment interactions in snakes could explain the absence of temperature-dependent sex determination, despite this phenomenon having evolved at least once in all other reptile groups.

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