

Plasticity in Preferred Body Temperature of Young Snakes in Response to Temperature during Development

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We investigated whether the thermal history of Black Rat Snakes (*Elaphe obsoleta*) and Northern Water Snakes (*Nerodia sipedon*) during development affected their preferred body temperatures (PBT) after hatching or birth. Eggs of black rat snakes were incubated at high or low temperatures. Gravid female water snakes were maintained in rooms under low, medium, or high ambient temperatures. PBT of neonates were measured in a controlled thermal gradient. We only found plasticity in PBT with developmental temperature for northern water snakes, where offspring that developed under warmer temperatures demonstrated a significant preference for warmer temperatures. Our results suggest that PBT are plastic in only some species of snakes and may explain some of the individual variation in PBT observed in adult snakes.

THE concept that reptiles have preferred body temperatures (PBT) is widely used in studies of thermal ecology (e.g., Hertz et al., 1993; Christian and Bedford, 1995; Christian and Weavers, 1996). PBT is the temperature (or narrow range of temperatures) selected by an individual in a controlled thermal gradient and is thought to represent the temperature at which numerous processes (e.g., digestion, locomotion) function at an optimal level (Stevenson et al., 1985). Investigations involving PBT have generally used a single PBT for a given population of animals (e.g., Shine and Madsen, 1996; Dorcas and Peterson, 1997). However, temporal, spatial, and individual variation in PBT have been reported (Gatten, 1974; Ellner and Karasov, 1993; Christian and Bedford, 1996). Thus, variation in PBT among individuals within populations appears to be common. For reptiles, short-term changes in PBT have been shown for individuals that are reproductive (Charland and Gregory, 1990; Cobb and Peterson, 1991) have recently fed (Lysenko and Gillis, 1980; Slip and Shine, 1988; Gibson et al., 1989) or are in the process of shedding (Gibson et al., 1989; reviewed in Peterson et al., 1993). However, even within such groups, there may still be variation in PBT among individuals (Peterson and Arnold, 1986), and the basis for that variation remains unknown. In this study, we investigate whether developmental temperature had an effect on PBT in Black Rat Snakes (*Elaphe obsoleta*) and Northern Water Snakes (*Nerodia sipedon*) and, thus, whether developmental temperature may contribute to individual differences in PBT.

Recent studies have shown that the thermal history of reptiles during development can have long-lasting effects on their morphology and be-

havior (Shine and Harlow, 1993; Alberts et al., 1997; Shine et al., 1997). Burger (1989, 1991) documented plasticity in morphology and behavior of hatchling Pine Snakes (*Pituophis melanoleucus*) in response to different incubation temperatures. Similarly, Shine and Harlow (1993) found that gravid female lizards that were allowed to bask for different periods of time had offspring that differed in morphology and behavior. In both studies, differences in behavior and morphology were still apparent after at least two months, suggesting long-term effects of incubation or gestation temperature. Thus, it seems plausible that the thermal history of individuals during development may affect their subsequent PBT. We investigated the effect of thermal history on PBT after hatching or birth in black rat snakes (*E. obsoleta*) and northern water snakes (*N. sipedon*). Studies of both species have documented substantial individual variation in PBT among older snakes. In a study involving 26 adult black rat snakes, all of which were postabsorptive, postshedding, and nonreproductive, PBT ranged from 24.2 C to 30.8 C with a mean of 27.7 ± 2.0 C (G. Blouin-Demers, unpubl. data). In a similar study of 13 northern water snakes, Brown and Weatherhead (in press) reported that individual PBT ranged from 25.1 C to 29.9 C, with a mean of 27.1 ± 2.4 C. Thus, the variability in PBT of older snakes gives ecological relevance to this study.

MATERIALS AND METHODS

As part of ongoing studies on the spatial ecology of black rat snakes, we collected 198 eggs (approximately 16 clutches) in July 1998 from three communal nesting sites and nine gravid females captured in the field that subsequently

laid in the laboratory. Each clutch was randomly divided in half and incubated in moist vermiculite under a constant temperature of 26 C (low temperature treatment) or 30 C (high temperature treatment). These incubation temperatures were chosen based on a mean temperature of 28 C measured in a communal black rat snake nesting site between late July and early September 1997. Individuals from eight clutches were used in the present study.

During the course of an ongoing investigation of maternal effects on offspring phenotypes in northern water snakes, 30 apparently gravid females were collected in the field in May and June 1998, and 10 were randomly assigned to each of three temperature treatments: low, medium, and high. The females assigned to the low temperature treatment were kept in a room with a mean temperature of 20.9 ± 2.1 C and were provided with an electric heating rock for 2 h per day. The females in the medium temperature treatment were kept at 23.5 ± 2.3 C and had access to a heating rock for 2 h per day. The females in the high temperature treatment were kept at 25.2 ± 2.2 C and had continuous access to a heating rock. The surface temperature of the heating rocks was approximately 38 C, and therefore the females from the medium and low temperature treatments were able to thermoregulate to some extent during the day. Therefore, the exact body temperatures of the females during pregnancy are not known, but our treatments did provide different opportunities for thermoregulation. In the present study, we used individuals from one litter from the low temperature treatment and three litters from each of the medium and high temperature treatments.

Female water snakes started giving birth in mid-August (one, three, and three females gave birth from the low, medium, and high temperature treatments, respectively). There was a 38-day difference in the mean parturition date between the low and medium temperature treatments and 18 days between the medium and high temperature treatments. Black rat snake eggs started hatching in late August, and the mean difference in hatching dates for the two temperature treatments was seven days. For each species, we tested PBT for 30 neonates from each temperature treatment that were born to at least three different mothers (except for the low temperature treatment for water snakes where we only obtained a single litter of 17 neonates). The testing took place from five to 10 days after hatching or birth. We tested the two species and individuals from different temperature treatments separately.

We measured PBT using a thermal gradient chamber in the laboratory. The chamber was constructed using a plastic tank (120 cm \times 60 cm \times 30 cm) that was oval in shape to eliminate edge effects. A fluorescent light over the chamber provided constant and homogeneous illumination. We established a thermal gradient by placing one end of the chamber over a coil of tubing through which cold water was constantly circulating, and the other end over a heating pad, producing a smooth gradient from 15 to 40 C. Ten thermal probes were positioned equidistant along the gradient to measure temperature at each position.

Twelve hours prior to testing, an average of 12 snakes (from eight to 16) were given unique paint codes for identification and were introduced into the chamber to acclimatize to the setting. Individuals were tested in small groups, but some individuals were also tested individually as a control (see Results). Once recording of temperatures began, we recorded the temperature for each individual based on its position in the gradient approximately once an hour (never less than 45 min). We recorded a total of 15 temperatures per individual snake. The small size of neonate rat snakes and water snakes (between 5 and 10 g) means their thermal inertia is very low, and thus body temperatures approximate the substrate temperature in the absence of a radiant heat source (Stevenson, 1985). When an individual was positioned over a thermal probe, the selected substrate temperature was the temperature indicated by the probe. When an individual was between two probes, we used the mean of the temperatures of the two probes as the selected substrate temperature. We used the mean of the 15 readings of selected substrate temperature as PBT for each individual snake in our analyses.

In an attempt to control for clutch effects in black rat snakes, we used a split-clutch design. Each clutch was randomly divided between the two temperature treatments for incubation, and we attempted to use members of the same clutch, but from different temperature treatments, while testing for PBT. Controlling statistically for clutch effects in black rat snakes by including clutch as a variable in a two-way ANOVA was unfortunately not practical in this case for two reasons. First, 16 of 60 black rat snakes tested could not be assigned with absolute certainty to a clutch because these eggs were collected from communal nests. Second, because some eggs failed to hatch, some clutches were only represented by one or two individuals in each treatment, and two of eight clutches used in PBT testing were only represented in one of

the treatments. Therefore, we analyzed differences in PBT using a one-way ANOVA for black rat snakes. For northern water snakes, we controlled for litter effects by including temperature treatment and litter as variables in a nested ANOVA. Statistical analyses were conducted on JMP version 3.0 for the Macintosh. We verified that the assumptions of normality and homogeneity of variance were upheld by inspecting Box plots (Stevens, 1996). We used $\alpha = 0.05$ as our significance level. Means are reported ± 1 SE.

RESULTS

For black rat snakes, the mean PBT of 35.3 ± 0.3 C for hatchlings that developed under the low incubation temperature was not significantly different from the mean of 34.7 ± 0.3 C for hatchlings that developed under the high incubation temperature (ANOVA: $F_{1,58} = 1.91$, $P = 0.17$). Thus, hatchling black rat snakes chose similar substrate temperatures regardless of their incubation temperature. For water snakes, mean PBT of offspring born to females in the three temperature treatments were significantly different (ANOVA: $F_{2,70} = 49.99$, $P < 0.0001$), and there were significant litter differences in PBT (ANOVA: $F_{4,70} = 101.05$, $P = 0.01$). Posthoc Tukey-Kramer HSD tests revealed that all means for the three temperature treatments were significantly different from one another (Fig. 1). A caveat must accompany this result because only a single litter was obtained for the low temperature treatment and our analyses demonstrated significant litter effects. Therefore, the difference we observed for this group could be a result of genotype and may not reflect the maternal thermal environment. However, the difference observed in PBT between the medium and high temperature treatments seems real.

These results indicate that water snakes selected temperatures that reflected their relative gestation temperatures. One difference between the two species was that the PBT of neonatal water snakes overlapped extensively with that of adults, whereas hatchling black rat snakes preferred temperatures approximately 5 C warmer than adults.

By testing snakes in groups, it is possible that individual PBT values might not be independent if the snakes affected each other's behavior. There are several reasons that make this seem unlikely to be a problem in our study. First, our observations of snakes suggested that individuals were behaving independently. Some snakes did coil together, but individuals often moved to different locations between measure-

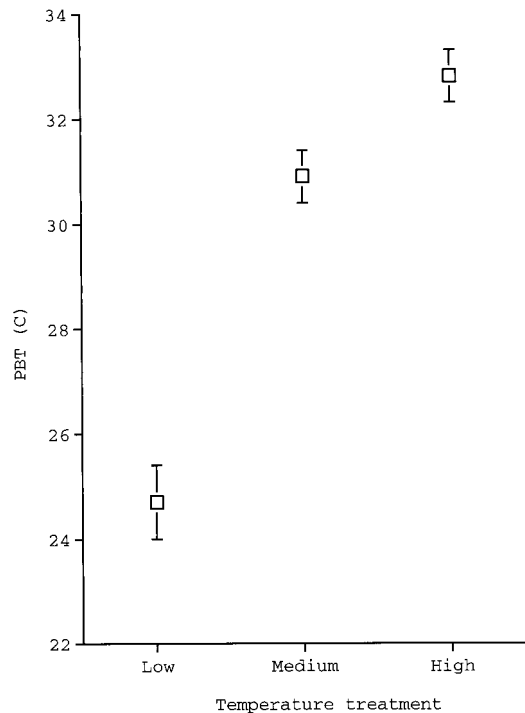


Fig. 1. Mean and standard deviation of the preferred body temperature of northern water snakes from the low, medium, and high temperature treatments (see text for details).

ments. Snakes moving between locations readily crawled over one another, suggesting that individuals did not impair the ability of other snakes to move freely. Second, we conducted four tests with single rat snakes (two from each of the temperature treatments). The PBT of these individuals was not significantly different when we tested them individually or in groups (paired t -test: $t_3 = 0.382$, $P = 0.73$), and their movements in the gradient were also qualitatively similar to the movements of individuals tested in groups. Third, we conducted separate analyses using group mean PBT instead of individual PBT (Stevens, 1996). Qualitatively, our results for the effect of temperature treatment remained unchanged for both species (black rat snakes $F_{1,2} = 0.13$, $P = 0.75$; water snakes $F_{2,3} = 9.01$, $P = 0.05$). Therefore, we have only presented analyses using individual PBT.

DISCUSSION

We found that developmental temperature affects PBT in neonatal water snakes but not in black rat snakes in our populations. Our results for water snakes contrast those of Arnold et al. (1995). In their study involving six temperature

treatments (24–32 C), these authors found that maternal thermoregulation had no effect on body temperatures selected by neonate garter snakes. A difference between our study and Arnold et al.'s (1995) is that our female water snakes were allowed to thermoregulate to some extent during the day, whereas their female garter snakes were kept at constant temperatures. Fluctuating body temperatures are probably more representative of conditions experienced by embryos in the wild.

One possible explanation for developmental temperature affecting PBT in water snakes but not black rat snakes may be that it is related to the different reproductive mode of the two species. Developmental temperature of black rat snakes is entirely dependent upon the temperature of the nest, whereas female northern water snakes can mediate the effects of ambient temperature on development through thermoregulation. Mean nest temperatures of seven black rat snake nests monitored 24 h/day throughout July and August 1998 varied from 22–30 C (GB-D, unpubl. data), whereas the mean body temperatures of female northern water snakes monitored 24 h/day by telemetry over the same period only varied from 26.8–27.7 C (Brown and Weatherhead, in press). Thus, selection may have favored greater tolerance to variation in incubation temperatures in black rat snakes. In contrast, water snake embryos normally experience much more stable developmental temperatures than those they experienced in this study. The change in PBT in response to gestation temperature that we observed in water snakes could reflect an adaptive response, where neonates behave in a fashion suited to the environment predicted by their gestation temperature. Alternatively, the response could be a nonadaptive artifact of having gestation occur at suboptimal temperatures.

Could developmental temperatures account for variation in PBT observed in older snakes? Our results for rat snakes suggest not, whereas the results for water snakes suggest it is possible. The next step will be to determine whether neonatal PBT reflects PBT later in life. More important, a central goal in the study of thermal ecology should be to understand the ecological and evolutionary consequences of individual variation in PBT.

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