Evolutionary Ecology Research, 2004, **6**: 739–747

Developmental environment has long-lasting effects on behavioural performance in two turtles with environmental sex determination

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ABSTRACT

Charnov and Bull (1977) originally predicted that environmental sex determination (ESD) can evolve when the developmental environment differentially affects male and female fitness. While developmental temperature is known to affect hatchling performance in reptiles with ESD, this reaction norm can provide the conditions to favour ESD only if the effects are long-lasting. We incubated eggs of *Graptemys ouachitensis*, an emydid turtle with ESD, at temperatures previously found to affect hatchling righting response. We tested righting response in both hatchlings and turtles raised in the laboratory for 1 year, and also examined the effects of testing temperature and social interactions on righting response. Turtles incubated at 30°C righted more quickly than turtles incubated at 25°C both as hatchlings and yearlings, and this effect was robust to testing temperature or rearing conditions. We found a similar reaction norm of temperature on righting response in *Trachemys scripta elegans*, another emydid turtle with ESD. These results demonstrate that incubation temperature can have long-lasting effects on performance in reptiles with ESD, and may provide the conditions required to favour the evolution of ESD.

Keywords: differential fitness model, *Graptemys ouachitensis*, righting response, temperature-dependent sex determination, turtle.

INTRODUCTION

While the sex of many reptiles is determined by the temperature during incubation, the selective advantage of this unusual sex determining mechanism has remained unclear for decades. Charnov and Bull (1977) originally predicted that environmental sex determination (ESD) would be favoured by natural selection when male and female fitness is strongly and differentially influenced by environmental conditions. When the developmental environment is stochastic and affects selectively important phenotypic

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characteristics, selection should favour production of the sex whose fitness is enhanced by developmental environment in the optimal environment.

Recently, researchers studying the selective advantage of ESD have focused their attention on variation in post-hatching phenotype associated with incubation environment in reptiles (Shine, 1999). In short-lived organisms, seasonal variation in gender allocation can allow one sex more time to reach reproductive maturity. Environmental sex determination is adaptive when the sex that is produced earliest benefits more from attaining resources prior to reproduction, as shown for a short-lived amphipod (McCabe and Dunn, 1997). It is unlikely that turtles and crocodilians with ESD can benefit from seasonal variation in offspring gender distribution, since generation times are long and the seasonal difference in sex allocation should be washed out by variation in the factors that determine growth rate before reproduction. For differential effects of incubation environment on sex-specific fitness to explain the evolution and persistence of environmental sex determination in these reptiles, there must be long-lasting effects of incubation environment on phenotype.

Rhen and Lang (1995, 1999) found that male-producing incubation temperatures resulted in the fastest growing offspring in snapping turtles (*Chelydra serpentina*), while controlling for any effects of gender by reversing sex through hormone application. Male-producing incubation temperatures have also been found to optimize growth in snapping turtles elsewhere (Mcknight and Gutzke, 1993; Bobyn and Brooks, 1994; O'Steen, 1998). In this species, males apparently compete aggressively for mating access and thus male reproductive success is likely to be more dependent on body size and growth than is female reproductive success (Kiviat, 1980; Janzen and O'Steen, 1990). Studies on other turtles with ESD have shown that the temperature that produces the sex that is larger at maturity also produces the fastest growth rates (Roosenburg and Kelley, 1996; Freedberg *et al.*, 2001). These studies may offer empirical support for the Charnov-Bull model, since the observed effects of incubation temperature on growth and sex determination strongly suggest an adaptive reaction norm.

In addition to its effect on growth, incubation temperature has also been found to affect behavioural performance in reptiles, mostly from studies of species with genetic sex determination. Mid-range incubation temperatures result in maximized performance levels in three snake species (Burger, 1989), while incubation temperature affects sprint speed in scincid lizards (Shine *et al.*, 1997; Elphick and Shine, 1999), and warmer incubation temperatures enhance performance in hatchling softshell turtles (*Apalone mutica*) (Janzen, 1993).

We have shown previously that some developmental temperatures that produce males yielded hatchling *Graptemys ouachitensis* that had lower survival, slower growth and poorer performance (Freedberg *et al.*, 2001). Furthermore, within the range of temperatures that produced only male offspring, a trend of cooler temperatures adversely affecting performance persisted, as warmer incubated males righted as well as females, demonstrating that the performance effects were attributable to incubation temperature and not gender. While the reaction norm of performance was compelling, the sex determining pattern cannot be considered adaptive unless the observed effects are enduring, so that they may affect sex-specific fitness. The key question in the current study is whether the performance differences persist. If they do, then ESD will be selectively favoured if it allows the most adversely affected sex to be produced at the temperatures that yield more favourable phenotypes.

Steyermark and Spotila (2001) cited evidence suggesting that righting response is a trait that is ecologically relevant to survivorship in turtles. In our earlier study, we found that hatchling turtles from warmer incubation temperatures righted in significantly less time and had significantly higher survivorship to the age of 9 months than did hatchlings from cooler incubation treatments (Freedberg *et al.*, 2001). Burger (1976) observed that hatchling turtles were frequently turned on their backs in the wild, and concluded that 'it is adaptive for hatchlings to have a well developed ability to right themselves', to avoid exposure to predation and thermal stress. In addition, righting response has been found to be a performance measure that turtles are highly motivated to perform (Burger *et al.*, 1998; Freedberg *et al.*, 2001), and thus variation in righting response time is probably indicative of the ability to perform other physical tasks in nature.

In the current study, we incubated *Graptemys ouachitensis* eggs at temperatures previously found to differentially affect hatchling righting response and tested this trait in both hatchlings and turtles that were maintained in the laboratory for 1 year after hatching. While we utilize two incubation temperatures that exclusively yield individuals of each sex, our earlier work demonstrated that the effect of incubation temperature on hatchling righting time in *Graptemys* was attributable to incubation temperature and not to gender. We examined the effects of testing environment on righting response by utilizing two different ambient testing temperatures. We also controlled for any possible effect of social interactions between turtles from the two incubation temperature treatments by housing turtles either mixed with turtles from the other temperature treatment or separate from them. In addition, we examined righting response in red-ear sliders (*Trachemys scripta elegans*) to determine if the same reaction norm is found in another emydid turtle.

METHODS

We collected eggs of *G. ouachitensis* in May 2000 along the West Fork of the White River in Daviess and Knox Counties, Indiana. Eggs were obtained both through collection from newly laid nests and from gravid females. Gravid females captured while nesting were induced to oviposit in the laboratory by injections of 0.7–0.8 ml oxytocin (Ewert and Legler, 1978) and then released. Eggs were incubated in plastic boxes with moistened vermiculite (vermiculite : water, 1 : 1 by mass; *c.* 170 kPa).

We randomly distributed a portion of eggs from 29 *G. ouachitensis* clutches among the two incubation temperatures. Eggs that were clearly infertile were not used. The distribution from each clutch was roughly proportional to the number of eggs allocated to each treatment in the entire study. In total, we placed 93 eggs at 30°C (a temperature which produces all females) and 93 eggs at 25°C (a temperature which produces all males). These temperatures had previously been found to differentially affect hatchling righting behaviour and to result in relatively high post-hatching viability (Freedberg *et al.*, 2001). Furthermore, these temperatures fall within the range of naturally occurring nest temperatures for *G. ouachitensis* (Vogt and Bull, 1984). After pipping, but before emerging from the shell, the turtles were placed in moist cups and maintained at room temperature. After the turtles had completely emerged from the eggshell, the shell was removed and enough water was added to completely cover the hatchling.

We ran performance trials on hatchlings at 2 weeks of age. On the following day, each turtle was weighed and marked for identification by shell notching. The turtles were then placed in one of six 30-gallon plastic tubs, measuring 86 × 58 × 20 cm, equipped with a filter and basking site. One tub housed only *G. ouachitensis* incubated at 30°C and one tub housed only *G. ouachitensis* incubated at 25°C. Two tubs housed a mixture of *G. ouachitensis* from each treatment. Room temperature was maintained at 30°C and water temperature at 25°C. Twice during the course of the study, tub positions within the room were rotated. Twice a week, the turtles were fed enough Trout Chow® pellets to ensure that there was excess remaining in each container, in order to limit any possible effects of competition for food.

We replicated the above methodology with eggs from 11 clutches of *Trachemys scripta elegans*. The 54 turtles that hatched were housed in two tubs with a mixture of turtles from each treatment.

Righting response

At 14 ± 1 days post-pip, each turtle was placed upside down into one of nine open-top cubic containers, aligned in a 3 × 3 formation on a plastic tray. Trials were videotaped on a Hi8 recorder while the experimenter was out of the room. Runs were checked from a video monitor, so that the experimenter's presence would not distract the turtles. Videotaping was stopped either once all turtles had successfully righted or, failing that, after any turtles failed to attempt to right themselves after 1 h. Performance trials were run at ambient temperatures of both 25°C and 30°C for each turtle in both the summer of 2000 (as hatchlings) and the summer of 2001 (as yearlings). Turtles were randomly assigned to be tested at one temperature first, and were tested at the other ambient temperature the following day. Turtles were maintained at the testing temperature for 24 h before the trial.

'Righting time' is the amount of time each turtle spent actively trying to right itself. This was scored as the time elapsed from when the turtle began pushing off the surface of the container until the turtle successfully righted itself, but minus the time the turtle was motionless once the righting attempt had begun. Nine times, turtles actively tried to right themselves for more than 5 min without success; these turtles were assigned a righting time of 5 min. Five times, turtles failed to attempt to right themselves after 1 h and were excluded from the analysis.

Statistical analyses

Statistical analyses were done with SPSS® for Windows version 11.5. We determined the effect of incubation temperature on righting time with one-way analysis of variance. The righting data did not meet homogeneity of variance requirements and were transformed using a log-linear transformation. When transformed data still failed to meet homogeneity of variance requirements, they were analysed using a non-parametric Mann-Whitney *U*-test. The effect of temperature treatment on embryonic survival was analysed with a *G*-test.

RESULTS

In total, 154 *Graptemys* eggs produced hatchlings that survived to be tested and measured. Of these, 103 turtles survived to be measured and tested after 1 year; survival was higher in turtles from the warmer incubation treatment (61 of 82 at 30°C *vs* 42 of 72 at 25°C; d.f. = 1, $G = 4.47$, $P < 0.05$). Fifty-four *Trachemys* turtles were tested as hatchlings; 34 survived to be tested as yearlings. Again, more turtles survived from the 30°C incubation treatment than the 25°C treatment (19 of 21 at 30°C *vs* 15 of 33 at 25°C; d.f. = 1, $G = 12.5$, $P < 0.001$).

In a small fraction of righting trials (<10%), righting time could not be conclusively established from the videotape. These trials were excluded from the analysis. A total of 469 *Graptemys* and 141 *Trachemys* righting trials were scored and analysed. In the majority of analyses of incubation temperature on righting response, the data still failed to exhibit homogeneity of variance after log-linear transformation. The same results were statistically significant for each analysis for both the parametric and non-parametric tests. Thus, for consistency, all analyses are presented with the results of Mann-Whitney *U*-tests.

Graptemys righting response

For hatchlings, incubation temperature had a highly significant effect on righting time at both testing temperatures, with turtles incubated at 30°C flipping in less time than turtles incubated at 25°C. This was true for hatchlings (25°C: $Z = -5.500$, $P < 0.001$; 30°C: $Z = -5.407$, $P < 0.001$), for all yearlings combined (25°C: $Z = -5.399$, $P < 0.001$; 30°C: $Z = -5.937$, $P < 0.001$) (Fig. 1), and for yearlings with the treatments reared separately (25°C: $Z = -3.838$, $P < 0.001$; 30°C: $Z = -3.985$, $P < 0.001$) and together (25°C: $Z = -3.849$, $P < 0.001$; 30°C: $Z = -4.298$, $P < 0.001$).

Trachemys righting response

Hatchlings incubated at 30°C righted themselves in significantly less time than turtles incubated at 25°C when tested at 30°C ($Z = -3.219$, $P < 0.002$) but not at 25°C ($Z = -0.611$, $P = 0.541$). One-year-old turtles incubated at 30°C continued to right themselves in less time than turtles incubated at 25°C when tested at 30°C ($Z = -2.606$, $P < 0.01$) but not at 25°C ($Z = -0.908$, $P = 0.364$) (Fig. 2).

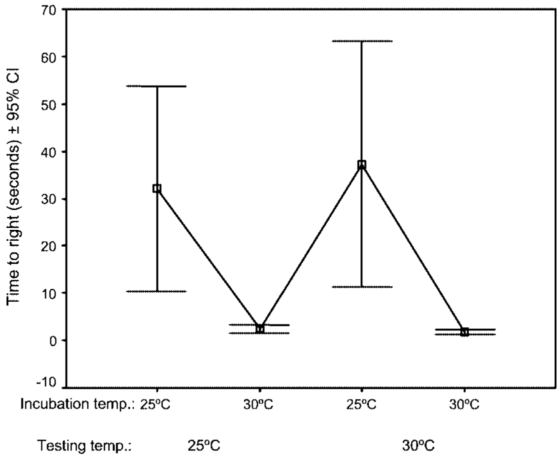

Fig. 1. Interaction plot showing righting time of yearling *Graptemys ouachitensis* incubated at 25 and 30°C when tested at ambient temperatures of 25 and 30°C.

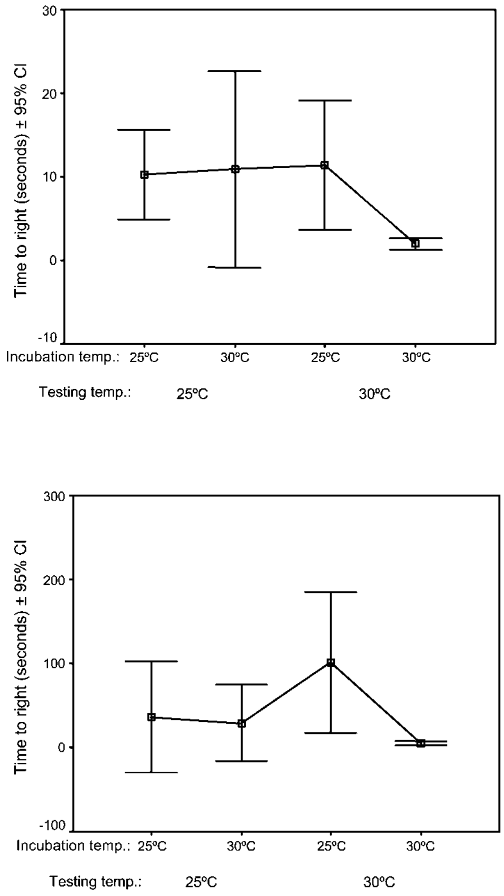

Fig. 2. Interaction plot showing righting time of *Trachemys scripta elegans* incubated at 25 and 30°C when tested as hatchlings (top) and yearlings (bottom) at ambient temperatures of 25 and 30°C.

DISCUSSION

We found that the temperature an individual *G. ouachitensis* experiences during incubation has a strong and lasting effect on phenotype. Specifically, we found that one-year-old turtles incubated at 30°C righted themselves more rapidly than yearlings incubated at 25°C. This is the first observation that incubation temperature has an enduring effect on performance in a reptile with environmental sex determination. Our findings suggest that incubation environment can differentially affect sex-specific fitness through long-lasting effects of incubation temperature on performance, and may help to explain the evolution and persistence of ESD.

We were able to examine the effects of testing temperature and housing arrangement on righting response. Map turtles incubated at 30°C righted in less time than turtles incubated at 25°C at both testing temperatures, whether raised separately from them or together with them. Thus the slower righting response of yearling turtles incubated at 25°C cannot be attributed to the presence of 30°C turtles inhibiting the growth or normal physical development of the 25°C turtles.

Incubation temperature similarly affected the righting response of another emydid turtle, *Trachemys scripta elegans*. Turtles incubated at 30°C righted themselves significantly faster than did those incubated at 25°C both as hatchlings and yearlings, but only when tested at 30°C and not when tested at 25°C (Fig. 2). The observation that incubation temperature had a similar lasting effect on righting response under warmer conditions in *Trachemys* as well as in *Graptemys* indicates that developmental temperature may have long-lasting effects on performance in a variety of turtles with ESD.

O'Steen (1998) found that juvenile snapping turtles incubated at a cooler temperature sought out warmer water and vice versa, suggesting that incubation temperature affects post-hatching thermal optima in turtles. Our results indicate that differences in thermal optima between incubation temperatures cannot explain differences in righting time in *G. ouachitensis*, as turtles incubated at 30°C righted more quickly than turtles incubated at 25°C at both testing temperatures. In contrast, we found that the effect of incubation temperature on righting response in *T. scripta* was dependent on testing temperature, as the strong effect observed at 30°C was not seen when turtles were tested at 25°C. These results imply that incubation temperature may affect thermal optima in *Trachemys*, with turtles incubated at 30°C performing better in a warmer ambient environment.

Spatial and yearly thermal variation of nesting habitats has been shown to affect mean nest temperatures of turtles in the wild. In map turtles, variation in mean nest temperatures in one year encompassed a range of 24.5–29.5°C (Bull, 1985), and yearly temperature variation at this site (NOAA weather records, 2003) suggests the total range of mean nest temperatures in this population will be several degrees greater. The fact that incubation environment predictably produces variation in physical ability provides the conditions that can favour the evolution of labile sex determination in response to the environment if the fecundity of one sex is more strongly affected by physical ability.

In an earlier study, we showed that hatchlings from part of the range of naturally occurring male-producing temperatures (24–25°C) took longer to right than hatchlings from female-producing temperatures (Freedberg *et al.*, 2001). Those results, in combination with our findings that these effects of temperature on righting response are persistent and irrespective of both testing temperature and among-treatment social interactions, suggest that some male-producing incubation temperatures may permanently hinder some aspects of physical ability relative to female-producing temperatures.

In many turtle species, including map turtles, there may be stronger selection for maximized performance in females than in males. Adult male map turtles weigh less than half of what females weigh and males apparently do not establish territories or actively combat for mates (in contrast to *C. serpentina*). Female map turtles, however, must perform several complex tasks associated with nesting. Female turtles must leave the water every year to nest, and nest construction is an extremely important component of fitness, since most nests fail to produce viable hatchlings. Cochran (1987) observed 22 cases of adult mortality in *Graptemys* and *Chrysemys* due to depredation, and noted that all identifiable remains were from females that had been preyed upon during nesting attempts.

Although it is not clear how frequently females must right themselves when avoiding predators, righting response time likely serves as an indicator of coordination and/or physical strength, as the turtles in our study appeared strongly motivated to right themselves once they began trying. If cool incubation temperatures adversely affect physical aptitude in adult map turtles, as our results suggest, female fitness should be more adversely affected by development at cool incubation temperatures than male fecundity. Thus, our findings that incubation temperature can permanently affect performance in a reptile with ESD are consistent with the conditions that may favour the evolution of ESD.

One plausible explanation for the more robust effect in *Graptemys* is that the disparity in the strength of selection between the sexes for adult condition may be greater in *Graptemys* than in *Trachemys. Trachemys scripta* males wander widely on land (Thomas and Parker, 2000), and did so more frequently than did *Graptemys* males at our collecting sites (S. Freedberg, personal observation). Furthermore, the sexual size dimorphism of *G. ouachitensis* is much greater than that of *T. scripta* (Ernst *et al.*, 1994). Because a sex difference in the strength of selection is important for the evolution of sexual size dimorphism (Shuster and Wade, 2003), this disparity in the degree of size dimorphism may indicate a greater sex difference in the strength of selection in *Graptemys*.

It is not immediately clear how, mechanistically, incubation temperature permanently affects righting response in reptiles. No obvious morphological differences were apparent between turtles from the different temperature treatments. Differences in muscular composition, coordination or other physiological attributes, if present, could account for the differences in righting response. While effects of developmental temperature on gonadal differentiation have been known for decades (Bull and Vogt, 1979; Ewert and Nelson, 1991), the effects of incubation environment on other aspects of reptilian physiology are only more recently becoming evident. In leopard geckos (*Eublepharis macularius*), incubation temperature affects the volume of ventromedial hypothalamus and preoptic area of the brain (Crews *et al.*, 1996) as well as reproductive behaviour (Flores *et al.*, 1994). Incubation temperature affected thyroid hormone concentration and metabolic resting rate in juvenile snapping turtles (O'Steen and Janzen, 1999). Our observation that cool incubation temperatures adversely affect performance and survival indicates that these environments may impair physiological development. We are currently studying the effects of incubation temperature on the physiology of hatchling turtles.

ACKNOWLEDGEMENTS

We are grateful to A.R. Cook and J. Gass for assistance with behavioural trials and animal husbandry. The work was partially funded by the Department of Biology, Indiana University.

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