

Evidence for Selection on Thermoregulation: Effects of Temperature on Embryo Mortality in the Garter Snake *Thamnophis elegans*

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Despite widespread belief that selection molds thermoregulatory behaviors, direct evidence for fitness effects is extremely rare. We studied the effect of developmental temperature on embryo mortality in a viviparous snake. Seventy-four female *Thamnophis elegans* were maintained at one of nine constant temperatures during pregnancy (21–33 C). The duration of pregnancy was recorded for each female as well as the sex, snout–vent length, mass, and survival of 504 newborns. Embryo survival was highest at an intermediate temperature (26.6 C). The developmental temperature of maximum survivorship in *T. elegans* corresponds to the temperature that induces minimum developmental abnormality and the optimum temperature for whole organism performance. These three thermal optima together correspond with the average temperatures imposed on embryos by free-ranging pregnant females (26.6 C). This correspondence implies that thermal optima are coadapted to thermoregulation in *T. elegans*.

THERMOREGULATION is often considered an adaptive activity, but few data directly support this conventional wisdom (Cowles and Bogert, 1944). Most studies that have examined the fitness effects of thermoregulation in vertebrates have used indirect measures of fitness, such as adult locomotor speed and offspring fluctuating asymmetry and body size (Beuchat, 1988; Shine and Harlow, 1993; Mathies and Andrews, 1997; Swain and Jones, 2000). While these indirect measures imply that selection likely acts on thermoregulation, this hypothesis has rarely been explicitly tested by looking for thermal effects on survival.

Indirect effects of thermoregulation on fitness have been well studied in the garter snake *Thamnophis elegans*. Two studies found evidence for the effects of thermoregulation on fitness using different indirect measures of fitness. First, a number of measures of whole organism performance have optima at or around 30 C, a temperature that coincides with the temperature chosen by thermoregulating *T. elegans* (Stevenson et al., 1985). The performance measures included swimming speed and crawling speed, for example, which probably facilitate escape from predators. Second, the incidence of scalation abnormalities in offspring is minimized at a developmental temperature of 27 C, which coincides with the average temperature of free-ranging, pregnant females (Arnold and Peterson, 2002).

While these studies have expanded our understanding of selection on thermoregulation, direct effects on fitness have not been reported. In fact, direct evidence for selection on thermoregulation is exceedingly rare. Here we report one of the first tests for direct effects of

thermoregulation on fitness. We tested for the effects of developmental temperature on embryo survival in *T. elegans*. We compare the optimum temperature for embryo survival to the average temperature chosen by free-ranging, pregnant females and to the known optima for minimizing developmental abnormalities and for maximizing whole organism performance. Based on studies conducted on the embryos of other oviparous vertebrates we expect temperature to affect the survival of these viviparous embryos, but such effects have not been previously reported, and studies of thermal effects on even oviparous snake embryos are rare (reviewed in Deeming and Ferguson, 1991; Packard and Packard, 1988; Bachmann, 1969; Wapstra, 2000).

The results reported here on offspring survival are from the same experiment previously described by Arnold et al. (1995) and Arnold and Peterson (2001). In those earlier reports, we described the effects of constant temperature treatments on juvenile thermoregulation (Arnold et al., 1995) and scalation (Arnold and Peterson, 2002). Sample sizes vary from report to report, however, because some litters were not tested for thermoregulation, litters from thermoregulating (control) females were included in the thermoregulation study, and scalation could not be scored in all litters.

MATERIALS AND METHODS

We obtained 504 neonates from 74 pregnant female *Thamnophis elegans* captured at eight localities in the vicinity of Eagle Lake, Lassen Co., CA, shipped by air to the laboratory and maintained in constant-temperature incubators until their litters were born. We immediately and

randomly distributed the females among nine constant temperature treatments (21, 24, 26, 27, 28, 29, 30, 32, and 33 C) when they arrived at the laboratory. The average date at the onset of the temperature treatment was 26 May, with dates ranging from 8 April to 13 July. In laboratory-bred *T. elegans* that are permitted to thermoregulate during pregnancy, pregnancy lasts an average of about 100 days. For field-caught pregnant female *T. elegans* from Eagle Lake populations, the modal birth date is about 15 August. These values suggest that the average female was in the 20th day of pregnancy at the onset of our temperature treatments, with a range from preovulatory to the 48th day of pregnancy. The incubators maintained the set temperatures within ± 0.5 C and showed no indication of vertical or horizontal temperature stratification. Other details of maintenance procedures are described by Arnold et al. (1995). The number of females per temperature treatment ranged from 2 to 14 (mean = 8.2). We scored survivorship of young at the time of birth (stillborn versus live). In garter snakes stillbirths include embryos that die at any stage during pregnancy: dead embryos are not reabsorbed. All offspring (live and stillborn) were sexed by eversion of hemipenes or dissection of the base of the tail.

Because individuals within a litter do not represent independent observations, we treated whole litters (rather than individual offspring) as data points in all statistical analyses. We log-odds transformed all survival ratios and sex ratios to satisfy assumptions of uniform variance. We increased the numerator and denominator of all odds by one to avoid incalculable ratios (i.e., log of zero or zero in the denominator).

We first tested for an effect of temperature on the survival of the offspring, including both linear and quadratic terms in a linear regression on transformed data. To find the optimum temperature for offspring survival, we calculated the inflection point of the curvilinear (quadratic) regression equation by setting the first derivative equal to zero. The standard error for the inflection point was estimated using the delta technique (Bulmer, 1979). Because the condition of the mother might also affect survival of offspring, we added an index of the mother's condition as an additional potential covariate. We calculated the mother's condition index as the residual in a regression of the logarithm of mother's mass on mother's snout-vent length, using values taken the day after birth (Fig. 1).

Because thermal effects could be manifested as reduction in clutch size (either in terms of mass of individual offspring or as a reduction in the number of surviving offspring), we also tested for

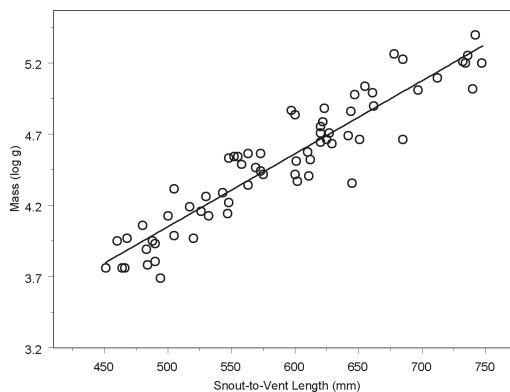


Fig. 1. Mother's mass as a function of snout-to-vent length. Mother's condition index is calculated as the residual from this regression. The regression is described by the equation: $\text{Log}(\text{mother's mass}) = (1.49 \pm 0.14\text{SE}) + (0.0051 \pm 0.0002\text{SE})x$, where x is the mother's snout-to-vent length in mm ($P < 0.001$, $R^2 = 0.87$).

effects of temperature on offspring mass. We included the same potential explanatory variables in this model as in the model for embryo survival.

Finally, we tested for linear and quadratic effects of temperature on sex ratio. Such effects could result from differential susceptibility of the two sexes to temperature. To test this hypothesis, we also tested for effects of temperature on survival of each of the sexes separately, to test whether effect on sex ratio might be a result of susceptibility to temperature in one sex but not the other.

RESULTS

Temperature had a significant curvilinear effect on the survival of offspring. Both a positive linear term and a negative quadratic term were statistically significant in a curvilinear regression of odds of survival on developmental temperature (Fig. 2, $R^2 = 0.20$, $P < 0.001$ for each term). Survival was maximal at $26.6 \pm 10.5\text{SE}$ C. A single litter at 21 C with low survivorship was suspected of having disproportionate influence on the analysis. Removing this litter from the analysis, however, did not change the conclusions. Mother's condition index did not significantly affect offspring survival after temperature effects were accounted for ($P = 0.65$).

Temperature also had a significant effect on the mass of the live-born young ($R^2 = 0.14$, $P = 0.001$ for linear and quadratic terms), with the heaviest offspring occurring at intermediate temperatures. Adding the mother's condition

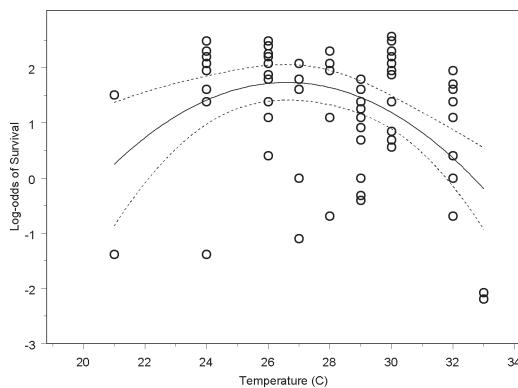


Fig. 2. Log-odds of embryo survival as a function of developmental temperature in *T. elegans*. The numerator and denominator of all odds ratios were increased by one to avoid incalculable ratios. The curve is a quadratic regression fitted to the data; Log-odds of survival = $(-31.6 \pm 9.8SE) + (2.51 \pm 0.71SE)x + (-0.0471 \pm 0.0129SE)x^2$, where x is developmental temperature in degrees C. The dashed lines represent the 95% confidence interval for the best-fit line.

index did not significantly improve the model ($P = 0.79$).

Temperature had no effect on the sex ratio of living offspring ($P = 0.08$ for both linear and quadratic terms; $R^2 = 0.04$). Mother's condition did not contribute significantly to the model ($P = 0.75$). In contrast to the results for live offspring, temperature had a significant effect on the sex ratio of stillborn offspring. The sex ratio of stillborn offspring increased with increasing temperature, with more males at higher temperatures (linear term, $P = 0.026$, $R^2 = 0.067$). A quadratic term did not add significantly to the fit of this model ($P = 0.95$). Male mortality was minimized at higher and intermediate temperatures ($R^2 = 0.19$; linear term, $P = 0.002$; quadratic term, $P = 0.001$). Mother's condition index also was not related to male mortality ($P = 0.49$). Female survival was not significantly affected by temperature ($R^2 = 0.07$, linear term, $P = 0.07$; quadratic term, $P = 0.06$). Mother's condition was also not related to female survival ($P = 0.47$).

DISCUSSION

In one of the few demonstrations of a direct effect of female thermoregulation on fitness, Beuchat (1988) found that embryo survivorship was highest at temperatures (28–34 C) surrounding the preferred temperature (32 C) of thermoregulating females in the viviparous lizard *Sceloporus jarrovi*. Our result that embryo survival in

Thamnophis elegans has an intermediate optimum temperature parallels this finding for *S. jarrovi*. We showed that the optimal temperature for embryo survival matched the average temperature that Arnold and Peterson (2002) found that embryos experienced in the field. It should be noted that our curvilinear regression of survival on developmental temperature has a broad peak, and thus the standard error for the estimate of the optimal developmental temperature is large. Nevertheless, the optimum temperature for offspring survival coincides with other thermal optima for *T. elegans*. These results for *S. jarrovi* and *T. elegans* indicate that selection acts on maternal thermoregulation.

Other studies of ectotherms provide less direct evidence for effects of thermoregulation on fitness. Various studies have shown that thermoregulating pregnant females choose temperatures that are optimal for production of healthy young. Pregnant lizards that were allowed to thermoregulate had larger offspring than females held at constant temperatures (Beuchat, 1988) or females with restricted access to heat (Qualls and Andrews, 1999; Swain and Jones, 2000). Likewise, pregnant females held at the preferred temperature of pregnant female lizards had larger offspring than females held at the temperature characteristic of postpartum females (Mathies and Andrews, 1997). Greater opportunity for thermoregulation by pregnant lizards also results in faster running speed in offspring (Shine and Harlow, 1993).

Our estimation of the optimal temperature for the survival of offspring coincides with known effects of temperature on aspects of adult performance in *T. elegans*. Various aspects of adult performance such as crawling speed, tongue flick frequency, and digestion have optima at temperatures that are very similar to the optima for embryo survival (around 30 C, Stevenson et al., 1985) and temperatures that minimize developmental abnormality in offspring (27 C, Arnold and Peterson, 2002). Taken as a whole, the reptile data provide strong evidence for stabilizing selection on thermal set points.

Although our experimental design makes some improvements on past designs with reptiles (e.g., the use of nine test temperatures and a relatively large number of litters), our design could be improved in a couple of ways. First, snake embryos experience temperatures that cycle on a daily basis in the field, rather than constant temperatures (Arnold and Peterson, 2002). Although constant temperatures are a useful starting point in experimental work, it would be interesting to know if embryo survival is also

maximal at intermediate cycling temperatures that mirror natural cycles. Secondly, in our study experimental temperatures were imposed after embryos had experienced the early phases of development under a natural temperature regime. A study with captive-bred females could impose temperature treatments immediately after insemination and consequently might achieve more dramatic results.

Some reports indicate that incubation temperature may also play a role in determining the sex ratio of the offspring, even in species with strictly genetic sex determination, due to differential mortality of the offspring (Burger and Zappalorti, 1988; Weatherhead et al., 1998). Temperature had no effect on sex ratio of living offspring in *T. elegans*. However, temperature had a significant effect on the sex ratio of stillborn offspring; at higher temperatures, significantly more of the stillborn offspring were male. This effect appears to be mediated primarily by the effect of temperature on male mortality, rather than on female mortality. An earlier study revealed that males were more prone to show scalation abnormalities at high and low temperatures than were females (Arnold and Peterson, 2002). Other animal species show sexual differences in fluctuating asymmetry and early mortality (Clutton-Brock et al., 1985; Clarke, 1997; Monard et al., 1997). Such differences may be related to the general tendency for sexual selection to elaborate sexual characters in males (Darwin, 1871; Andersson, 1994). In a model for the evolution of sexual dimorphism, Lande (1980) showed that sexual selection that acts only on males can lead to maladaptation in males such that males, but not females, have suboptimal average phenotypes at evolutionary equilibrium. The sex ratio bias that we observed in stillborn embryos of *T. elegans*, as well as the greater sensitivity of their scalation to temperature effects, might be a consequence of maladaptation induced by sexual selection.

Many traits in reptiles seem to be buffered against developmental effects of temperature. Such absence of temperature effects may be important in understanding selection on thermoregulation because they illuminate the long-term consequences of selection on thermoregulation and thermal tolerance in embryos. Shine and Harlow (1993) imposed two temperature treatments (two or eight hours of basking per day) on pregnant lizards but were unable to detect effects on litter size or incidence of deformities in offspring or offspring mass. Developmental temperature does not affect offspring scalation averages (Osgood, 1978; Arnold and Peterson, 2002; but see Fox, 1948) or

thermoregulation (Arnold et al., 1995) in natrixine snakes. In the present study, we found no effect of developmental temperature on sex ratio or offspring mass. Why some traits are more buffered against temperature effects during development than other traits remains an open question.

Thermal optima for a variety of traits, including offspring survival, are similar in *T. elegans*. This coincidence of optima is evidence that these thermal optima and thermoregulatory behavior are coadapted traits and is among the first evidence of such coadaptation. We hypothesize that future tests, particularly of viviparous ectotherms with diverse thermoregulatory set-points, will show that these thermal optima and thermoregulatory behavior are indeed coadapted within many species.

ACKNOWLEDGMENTS

This work was supported by a Gaige Award from the American Society of Ichthyologists and Herpetologists to R. P. O'Donnell, a U.S. Public Health Service grant R01-GM35492 to S. J. Arnold, and a National Science Foundation grant DEB-9903934 to S. J. Arnold and M. Pfrender. We are grateful to J. Gladstone and C. Peterson for help with data collection and to R. Huey for discussions. The adult female garter snakes used in this study were collected under the auspices of California State Scientific Collecting Permits 1033, 906, and 909 to S. J. Arnold in 1986–8.

LITERATURE CITED

- ANDERSSON, M. 1994. *Sexual Selection*. Princeton Univ. Press, Princeton, New Jersey.
- ARNOLD, S. J., AND C. R. PETERSON. 2002. A model for optimal reaction norms: the case of the pregnant garter snake and her temperature-sensitive embryos. *Am. Nat.* 160:306–316.
- , ———, AND J. GLADSTONE. 1995. Behavioural variation in natural populations: VII. Maternal body temperature does not affect juvenile thermoregulation in a garter snake. *Anim. Behav.* 50:623–633.
- BACHMANN, K. 1969. Temperature adaptations of amphibian embryos. *Am. Nat.* 103:115–130.
- BEUCHAT, C. A. 1988. Temperature effects during gestation in a viviparous lizard. *J. Therm. Biol.* 13:135–142.
- BULMER, M. G. 1979. *Principles of Statistics*. Dover Publications, New York.
- BURGER, J., AND R. T. ZAPPALORTI. 1988. Effects of incubation temperature on sex ratios in pine snakes: differential vulnerability of males and females. *Am. Nat.* 132:492–505.
- CLARKE, G. M. 1997. The genetic basis of developmental stability. III. Haplo-diploidy: Are males more unstable than females? *Evolution* 51:2021–2028.

- CLUTTON-BROCK, T. H., S. D. ALBON, AND F. E. GUINNESS. 1985. Parental investment and sex differences in juvenile mortality in birds and mammals. *Nature* 313:131–133.
- COWLES, R. B., AND C. M. BOGERT. 1944. A preliminary study of the thermal requirements of desert reptiles. *Bull. Am. Mus. Nat. Hist.* 83:265–296.
- DARWIN, C. 1871. *The Descent of Man, and Selection in Relation to Sex*. Murray, London.
- DEEMING, D. C., AND M. W. J. FERGUSON. 1991. Physiological effects of incubation temperature on embryonic development in reptiles and birds, p. 147–171. *In: Egg Incubation: Its Effects on Embryonic Development in Birds and Reptiles*. D. C. Deeming and M. W. J. Ferguson (eds.). Cambridge University Press, Cambridge.
- FOX, W. W. 1948. Effect of temperature on development of scutellation in the garter snake, *Thamnophis elegans atratus*. *Copeia* 1948:252–262.
- LANDE, R. 1980. Sexual dimorphism, sexual selection, and adaptation in polygenic characters. *Evolution* 34:292–305.
- MATHIES, T., AND R. M. ANDREWS. 1997. Influence of pregnancy on the thermal biology of the lizard, *Sceloporus jarrovi*: Why do pregnant females exhibit low body temperatures? *Func. Ecol.* 11:498–507.
- MONARD, A.-M., P. DUNCAN, H. FRITZ, AND C. FEH. 1997. Variation in the birth sex ratio and neonatal mortality in a natural herd of horses. *Behav. Ecol. Sociobiol.* 41:243–249.
- OSGOOD, D. W. 1978. Effects of temperature on the development of meristic characters in *Natrix fasciata*. *Copeia* 1978:33–47.
- PACKARD, G. C., AND M. J. PACKARD. 1988. The physiological ecology of reptilian eggs and embryos, p. 523–605. *In: Biology of the Reptilia*. Vol. 16. C. Gans and R. B. Huey (eds.). Liss, New York.
- QUALLS, C. P., AND R. M. ANDREWS. 1999. Cold climates and the evolution of viviparity in reptiles: cold incubation temperatures produce poor-quality offspring in the lizard, *Sceloporus virgatus*. *Biol. J. Linn. Soc.* 67:353–376.
- SHINE, R., AND P. HARLOW. 1993. Maternal thermoregulation influences offspring viability in a viviparous lizard. *Oecologia* 96:122–127.
- STEVENSON, R. D., C. R. PETERSON, AND J. S. TSUJI. 1985. The thermal dependence of locomotion, tongue-flicking, digestion, and oxygen consumption in the wandering garter snake. *Physiol. Zool.* 58:46–57.
- SWAIN, R., AND S. M. JONES. 2000. Maternal effects associated with gestation conditions in a viviparous lizard, *Niveoscincus metallicus*. *Herpetol. Monogr.* 2000:432–440.
- WAPSTRA, E. 2000. Maternal basking opportunity affects juvenile phenotype in a viviparous lizard. *Func. Ecol.* 14:345–352.
- WEATHERHEAD, P. J., G. P. BROWN, M. R. PROSSER, AND K. J. KISSNER. 1998. Variation in offspring sex ratios in the northern water snake (*Nerodia sipedon*). *Can. J. Zool.* 76:2200–2206.
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