

# Behavioural variation in natural populations. III: Antipredator displays in the garter snake *Thamnophis radix*

Albert Bennett


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## Behavioural variation in natural populations. VII. Maternal body temperature does not affect juvenile thermoregulation in a garter snake

STEVAN J. ARNOLD, CHARLES R. PETERSON & JEAN GLADSTONE  
*Department of Ecology and Evolution, University of Chicago*

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**Abstract.** This study was aimed to determine whether thermoregulation by juvenile garter snakes is affected by developmental temperature imposed by their mothers during pregnancy. Maternal temperature treatments were created by maintaining six groups of females at six different constant temperatures (24–32°C) during pregnancy. A seventh group of females was allowed to thermoregulate during pregnancy. The body temperatures of juveniles ( $N=324$ ) on thermal gradients were monitored in the morning and afternoon for 5 consecutive days, beginning 5 days after birth. The body temperatures of juveniles were consistent, apparently heritable and remarkably stereotyped. Juvenile body temperature was virtually unaffected by maternal temperature treatment

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Behaviour is the principal regulator of body temperature in reptiles, with physiological adjustments playing a secondary role (Bartholomew 1982). Many reptiles achieve relatively precise thermoregulation by behavioural choice of microhabitats and by postural adjustments (Cowles & Bogert 1944; Avery 1982; Firth & Turner 1982). Using surgically-implanted radiotransmitters that are temperature sensitive, field investigations indicate that individuals of our study species, the western terrestrial garter snake, *Thamnophis elegans*, usually maintain body temperatures in the range 28.0–32.5°C ( $\bar{X} \pm \text{SD} = 29.9 \pm 1.32^\circ\text{C}$ ) on warm days (Peterson 1987). Field studies of most snakes indicate modal activity temperatures within a similar range (Peterson et al. 1993). Because *Thamnophis* are viviparous, thermoregulation during pregnancy is particularly interesting. During a pregnancy that lasts approximately 100 days, a mother garter snake has behavioural control over the temperatures that her embryos experience.

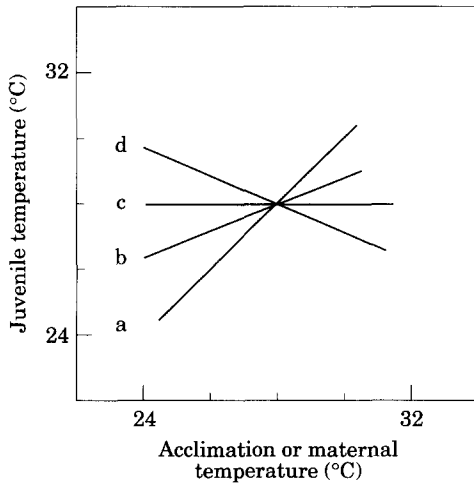
Developmental temperature can have major effects on some traits of reptilian embryos, but other traits are relatively immune to temperature influences. Developmental temperature affects sex

determination in crocodylians and many turtles (Yntema 1976; Ferguson & Joanen 1982; Janzen & Paukstis 1991a), as well as the postnatal size, growth and locomotor performance of some lizards and snakes (Burger 1989, 1990; Van Damme et al. 1992). Other traits appear to be buffered against the effects of developmental temperature. For example, scalation in water snakes is affected only slightly by developmental temperature (Osgood 1978), and sex determination in most lizards and snakes appears to be under strict genetic determination (Janzen & Paukstis 1991a; but see Dunlap & Lang 1990). Thus, recent discoveries of developmental temperature effects highlight the phenomenon of stability in the face of temperature perturbation.

Although there is a need to continue to inventory developmental temperature effects in reptiles, some investigators have stressed the adaptive significance of thermal lability versus stability (Charnov & Bull 1977; Bull 1983; Janzen & Paukstis 1991b; Janzen, in press). Although this new emphasis is likely to be important in the future, the conditions under which selection promotes lability versus stability are as yet poorly understood.

The question of whether the temperatures experienced during development affect subsequent thermoregulation has been neglected in the reptile literature. This issue has been explored in a crocodylian (Lang 1985), but apparently not in any viviparous reptile. In a viviparous reptile, we

Correspondence: S. J. Arnold, Department of Ecology and Evolution, University of Chicago, Chicago, IL 60637, U.S.A. (email: SJA1@midway.uchicago.edu). C. P. Peterson is now at the Department of Biological Sciences, Idaho State University, Pocatello, ID 83209-8007, U.S.A.



**Figure 1.** Possible relationships between juvenile body temperature and maternal temperature, viewing maternal temperature as an acclimation temperature. Line a: juvenile temperatures are the same as acclimation temperatures (slope=1). Line b: the slope of the curve is less than 1 but greater than 0 (e.g. Pitt et al. 1956). Line c: juvenile temperature is unaffected by acclimation temperature (slope=0). Line d: the slope of the curve is negative (e.g. Garside & Tait 1958).

may view the temperatures imposed on developing embryos as possibly having maternal effects (Kirkpatrick & Lande 1989, 1992) on the traits of offspring. The focus of this paper is on whether maternal thermoregulation has the potential to affect the thermoregulation of neonates.

The literature on thermal acclimation provides some a priori expectations for our experimental results. Thermal acclimation is a phenomenon in which residence at a particular temperature (the acclimation temperature) for a period of days or weeks affects performance and/or physiology in subsequent tests (Rome et al. 1992). Thus, perturbed, constant maternal temperature could be viewed as acclimation temperature, and we can ask whether juvenile thermoregulation shows an acclimation response. Past work on temperature preferences of fish and lizards has revealed a variety of acclimation responses (Fry 1947; Precht 1958; Reynolds & Casterlin 1979) that can serve as alternative hypotheses for our experiment (Fig. 1). It is important to realize, however, that curves such as b or d in Fig. 1 are found when animals are tested within a few hours after removal from the acclimation temperature. After a day or more

such curves collapse or gravitate towards a flat line (curve c in Fig. 1). A flat line is perhaps the leading hypothesis in our experiments because tests began 5 days after animals were removed from the acclimation (maternal) temperature.

The present paper is part of a series of articles on inheritance and thermal biology in the garter snake *T. elegans*. Elsewhere we have described the thermal ecology of this species (Stevenson et al. 1985; Peterson 1987; Huey et al. 1989; Peterson et al. 1993) and outlined the conceptual connection between maternal thermoregulation and the inheritance of scalation (Arnold 1988). In later articles, we hope to report on the thermoregulation of free-ranging females during pregnancy and on how developmental temperature affects scalation.

This paper makes some methodological contributions to the study of behavioural variation. We used a sampling design that yielded multiple scores for the same behaviour (body temperature) for each individual. Many behavioural studies produce data sets with similar structure (e.g. samples of displays from cine or video records) and in recent years these are often analysed with repeated-measures analysis of variance. We employ such an analysis, somewhat more complicated than usual, and explore the effect of multiple records on estimates of stereotypy and repeatability.

## MATERIALS AND METHODS

### Animals and Temperature Treatments

Neonates were obtained from 67 gravid female *T. elegans* captured in the vicinity of Eagle Lake, Lassen County, California. The females were collected from six localities situated 1.3–21.3 km apart: Kephart's (1982) localities 1, 4, 10, 11, 20 and 22. We collected 57 of the females in a gravid state in April–August of 1987 and 1988, shipped them by air to the laboratory in Chicago and maintained them until their litters were born in June–September. Ten other females were bred in the laboratory to males from their native population and spent their entire gestation period in the laboratory. We assigned 51 females (49 wild-bred and two laboratory-bred) to one of six constant temperature treatments (24, 26, 28, 29, 30 and 32°C). Constant temperatures more extreme than this range will not support pregnancy. We attempted to distribute females from

each locality across all temperature treatments. These females were housed in groups of two to six on a crushed corn cob substrate in ventilated plastic boxes (33.5 × 24 × 10.5 cm high) situated in Percival incubators (model I-30BL), which maintained a natural (Chicago) photoperiod. Six boxes were stacked three deep in each incubator. The incubators maintained the set temperatures within ±0.5°C and showed no indication of vertical or horizontal temperature stratification. Records of incubator temperature (logged every 30 min throughout the period of maintenance) showed no instances of malfunction. Twice a week females were offered live minnows in a small plastic dish filled with water. Aside from these 60-min presentations, the females had no access to water, in order to reduce the opportunity for evaporative cooling. An additional set of 16 females (eight wild-bred and eight laboratory-bred, representing three of the six localities) were allowed to thermoregulate during pregnancy and will be referred to as the control group. These 16 females were maintained individually in glass cages (50 × 26 × 32 cm high), one end of which rested on a substrate heating element. All 16 females were kept on a crushed corn cob substrate, had constant access to water and were fed live minnows. Nine of the females (eight of which were laboratory-bred) experienced a daytime thermal gradient of 15–38°C and a night-time gradient of 15–20°C. Seven of the females were surgically implanted with radiotransmitters (as part of another study) and experienced a daytime thermal gradient ranging from a low of 20–26°C to a high of 38°C and a constant night-time temperature of 20–24°C. These thermal regimes bracket thermal options that are available in nature.

#### Juvenile Maintenance and Measurement of Body Temperatures

Gravid females were checked several times a day for births. Upon discovery of a recently-born litter, the female and her offspring were immediately removed from the gestation cage. The offspring were housed together overnight (17–24 h) in a ventilated plastic box with a water dish in a room maintained at 26–28°C with a natural (Chicago) photoperiod. The offspring were then weighed to the nearest 0.01 g, marked individually using a ventral scale clipping scheme similar to the

one described by Brown & Parker (1978), and placed in individual cage compartments. The 19 cages housing the juveniles from this point on were glass aquaria (50 × 26 × 32 cm high), partitioned with opaque plastic sheeting into three compartments (50 × 8 × 32 cm high) with a crushed corn cob substrate and individual water dishes. The cages were situated in a room maintained at 17°C with one end of the cage resting on a heating element so that the substrate of each compartment presented a thermal gradient ranging from 17°C in the front to 35°C in the back of the compartment. The lighting in the room was turned on each day from 0600 to 2000 hours and the cage heating elements were on from 0700 to 1900 hours.

The body temperature of each juvenile snake ( $N=324$ ) was recorded in the morning and afternoon for 5 consecutive days, beginning at post-natal age 5 days. Morning temperatures were recorded from 1030 to 1100 hours and afternoon temperatures from 1430 to 1500 hours. A thermocouple thermometer (Fluke model 52) was used to record body temperatures by inserting its thermocouple (diameter=1.5 mm) into the snake's cloaca. To minimize heat transfer during temperature recording, the snake was held by just the tail tip in the cotton-gloved fingers of the investigator. The procedure was performed in a few seconds, too short an interval for body temperature to change.

#### Statistical Methods

The standard error of the coefficient of variation was computed using an approximation given by Sokal & Rohlf (1981, page 139).

Repeatability of juvenile temperature records was analysed using methods described by Winer (1971). In particular, a statistical model was used in which the  $j$ th observation on the  $i$ th individual snake was

$$z_{ij} = \pi_i + \alpha_j + \eta_{ij}$$

where  $\pi_i$  is the true (temporally invariant) value of body temperature for the  $i$ th snake,  $\alpha_j$  is the contribution (main effect) of the  $j$ th observation period and  $\eta_{ij}$  is error of measurement. Repeatability (reliability) measures were computed in which differences between measurement periods (between  $\alpha_j$ ) do not contribute to error of measurement, using the 10 × 10 variance-covariance

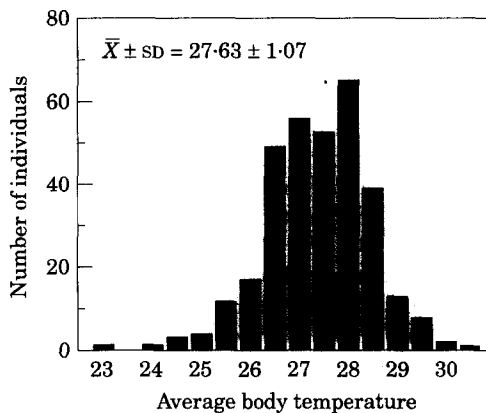
matrix for the records as described by Winer (1971, page 291). We used Winer's formulas for unbiased estimation of repeatability for the mean of two or more trials, but generally those formulas gave results that differed only at the third decimal place from results using the much simpler, standard formula (e.g. Falconer 1989).

Statistical analyses of effects on juvenile body temperature were done using the general linear model (GLM) procedure of the statistical analysis system (SAS 1988). Juvenile body temperature was analysed with a repeated-measures analysis of variance (ANOVA), computed using GLM in SAS. Maternal gestation temperature (Temp), and litter identity (Litter) were treated as classification variables and birth mass (Mass) was used as a covariate. A mixed model was fitted treating Litter as a random effect and Temp as a fixed effect, with Litter nested within Temp. The random statement in GLM was used to obtain expressions for the composition of expected mean squares. The repeated statement was used to specify two within subject variables: time of day (Time) and trial day (Day). The polynomial option was used to fit zero and first-order polynomials to time data points and zero- to fourth-order polynomials to the trial day data points. Pseudo *F*-tests were computed as described by Neter et al. (1990).

## RESULTS

Juvenile snakes were in a posture characteristic of thermoregulating snakes on nearly all (>95%) of the 3240 occasions on which they were approached to record body temperature. Generally, snakes were coiled motionless on the cage floor near the site of abrupt transition in the thermal gradient. The impression that the snakes were actively thermoregulating within the range of available temperatures (17–35°C) was reinforced by the observation of a relatively narrow distribution of average body temperatures.

The average ( $\pm$ SD) juvenile snake ( $N=324$ ) weighed  $3.19 \pm 0.71$  g at birth and showed an average body temperature of  $27.63^\circ\text{C}$  over the series of 10 trials. The distribution of average juvenile body temperatures (Fig. 2) approximates closely a normal distribution (Shapiro–Wilk *W* statistic = 0.984,  $P=0.51$ ). Nevertheless, the distribution is slightly skewed to the left ( $g = -0.434$ ,



**Figure 2.** Frequency distribution of average body temperature in a sample of 324 juveniles, disregarding maternal temperature treatment. Each juvenile's average temperature is based on 10 measurements.

$df=1$ ,  $P<0.01$ ) and leptotic ( $g=0.997$ ,  $df=2$ ,  $P<0.001$ ). The entire range of average scores was only  $7.5^\circ\text{C}$ , and 81% of the observations fell in a  $2.5^\circ\text{C}$  range ( $26.5\text{--}29.0^\circ\text{C}$ ). The standard deviation for observations pooled across all seven maternal temperature treatments was  $1.069$ , which is only slightly larger than the average standard deviation within maternal temperature treatments ( $0.990$ ). Similarly, the coefficient of variation for the pooled distribution (Fig. 2),  $3.9\%$  ( $\pm 0.2$  SE), is only slightly larger than the average coefficient within temperature treatments ( $3.7\%$ ). Three of the temperature treatments ( $24$ ,  $28$  and  $32^\circ\text{C}$ ) showed significant negative skewness ( $P<0.05$ ), and two treatments ( $24$  and  $32^\circ\text{C}$ ) showed significant leptosis ( $P<0.01$ ).

About half the variation in the means of 10 temperature observations results from intrinsic differences between juvenile snakes and about half results from variation within juveniles. The repeatability for the mean of 10 measurements of juvenile body temperature was  $0.47$  ( $F_{323,2907}=1.90$ ,  $P<0.001$ ). In other words, the expected correlation between replicate sets of 10 temperature measurements is about  $0.47$ . In contrast, the repeatability of a single temperature measurement is only  $0.09$ .

Preliminary analyses revealed no statistically significant ( $P>0.05$ ) year, cage, compartment or sex effects on body temperature (averaged over the 10 trials). In subsequent analyses these effects were not included in statistical models.

**Table I.** Repeated-measures analysis of variance for body temperatures of juvenile garter snakes

Source of variation	df	Mean square†	F	P
<b>Between subjects</b>				
Mass	1	49.60	5.41	*
Temp	6	48.72	3.29‡	**
Litter(Temp)	60	16.00	1.75	**
Error	256	9.16		
<b>Within subjects</b>				
Time	1	0.56	0.08	NS
Time*Mass	1	8.33	1.17	NS
Time*Temp	6	18.03	1.68§	NS
Time*Litter(Temp)	60	11.48	1.61	**
Error(Time)	256	7.13		
Day	4	18.44	1.66	NS
Day*Mass	4	14.21	1.28	NS
Day*Temp	24	21.25	1.57††	*
Day*Litter(Temp)	240	14.04	1.26	**
Error(Day)	1024	11.14		
Time*Day	4	5.91	1.10	NS
Time*Day*Mass	4	6.26	1.16	NS
Time*Day*Temp	24	4.83	0.90	NS
Time*Day*Litter(Temp)	240	5.96	1.11	NS
Error(Time*Day)	1024	5.38		
Total	3239			

†Based on type III sums of squares.

‡Pseudo *F*-test: *df*=6,75.

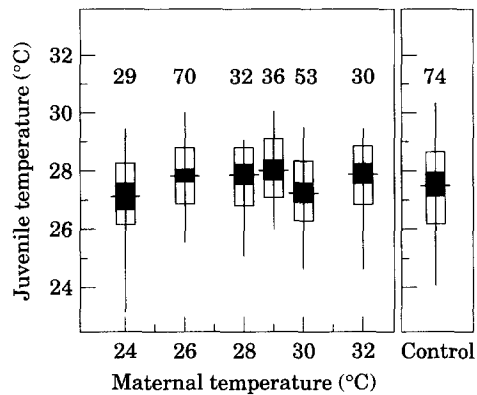
§Pseudo *F*-test: *df*=6,76.

††Pseudo *F*-test: *df*=24,325.

\**P*<0.05; \*\**P*<0.01.

The repeated-measures analysis summarized in Table I consists of two parts: a between-subjects part that tests for effects on the average body temperature of individual snakes, and a within-subjects part that tests for trends involving the repeated measurements of body temperature within individual snakes. In the within-subjects part of the analysis, each individual snake is considered as acting as its own control (Winer 1971).

The significant effect of birth mass (Mass) in the between-subjects part of the analysis (Table I) indicates that the regression slope relating birth mass to average body temperature is non-zero (*P*<0.05). Separate calculations show that this regression slope is positive (larger snakes tend to show higher body temperatures), but the effect is weak ( $\bar{X} \pm SE$  slope =  $0.37 \pm 0.16^\circ\text{C/g}$ , *P*=0.02; *r*=0.172, *P*=0.002) and does not vary between temperature treatments (*P*>0.05). The slight but

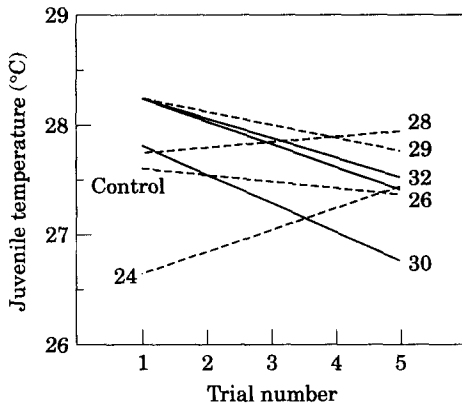


**Figure 3.** Average body temperatures of juveniles as a function of maternal temperature. Six groups of mothers were maintained at constant temperatures (24–32°C). The control group was allowed to thermoregulate during pregnancy. Means are indicated with horizontal lines, twice the SE with solid boxes, SD with open boxes and ranges with vertical lines. Sample sizes are shown.

significant effect of birth mass on juvenile body temperature is taken into account in all subsequent tests by the use of type III sums of squares.

The significant effect of maternal temperature (Temp) in the between-subjects part indicates that the average body temperatures of juveniles vary significantly (*P*<0.01) between maternal temperature treatment groups. However, these treatment differences are slight (Fig. 3) and show no obvious trends. Separate analyses showed that the linear and curvilinear regressions of average juvenile body temperature on maternal temperature (using the six constant maternal temperature groups) were statistically insignificant (*P*>0.05). Similarly, Duncan multiple-range tests revealed no trends between treatment groups. The significant Day\*Temp effect (*P*<0.05) indicates that maternal temperature treatment groups differ in how juvenile body temperature changes over the 5-day trial period (Fig. 4). Three of the maternal temperature treatment groups (26, 30 and 32°C) show statistically significant slopes (*P*<0.05 or <0.01) for the regressions relating average juvenile temperature to day number, and in all three cases the slopes are negative. Thus, there was no overall tendency for neonates from warmer temperatures to gradually lower their preferred temperatures, or for neonates from cooler temperatures to gradually raise their preferred temperatures.

The significant effect of Litter(Temp) in the between-subjects part indicates that litters vary in



**Figure 4.** Average juvenile body temperature as a function of trial day number for each of the seven maternal temperature treatment groups. Regression lines are shown for each treatment group. Groups shown with solid lines (26, 30 and 32°C) have regression slopes that differ significantly from zero ( $P < 0.05$ ). Groups shown with dashed lines (24, 28, 29°C and control) have regression slopes that do not differ from zero ( $P > 0.05$ ).

average juvenile body temperature within maternal temperature treatments ( $P < 0.01$ ). The within-subjects part of the analysis shows that litters also vary in morning versus afternoon differences in juvenile body temperature (Time\*Litter(Temp),  $P < 0.01$ ), as well as in how juvenile body temperature changes over the 5-day measurement period (Day\*Litter(Temp),  $P < 0.01$ ). Litters showed no significant variation in how the morning versus afternoon difference in juvenile body temperature varies over the 5-day measurement period (Time\*Day\*Litter(Temp),  $P > 0.05$ ).

All other effects were statistically insignificant ( $P > 0.05$ ) in the within-subjects part of the analysis. Measurements of juvenile body temperature in the morning showed no overall difference from measurements taken in the afternoon (Time). The regression of morning temperature on birth mass did not differ in slope from the regression of afternoon temperature on birth mass (Time\*Mass). Maternal temperature had no discernible effect on the morning versus afternoon differences in juvenile body temperature (Time\*Temp). Juvenile body temperatures showed no overall difference between the five daily measurement periods (Day). The regressions of juvenile temperature on birth mass did not vary in slope between the 5 days (Day\*Mass). Finally, the difference between morning and afternoon tem-

peratures did not vary over the 5-day trial period (Time\*Day), and that contrast was unaffected by birth mass (Time\*Day\*Mass) and maternal temperature (Time\*Day\*Temp).

## DISCUSSION

### Juvenile Thermoregulation is Buffered from Maternal Effects

The present results suggest that juvenile thermoregulation is strongly buffered against maternal temperature effects. Compared to the several maternal-offspring temperature relationships suggested by the acclimation literature (Fig. 1), the results most resemble the flat line relationship that characterizes lack of acclimation. However, our assays of juvenile body temperature did not begin until 5 days after removal from maternal (acclimation) temperature treatments. Even when thermoregulation shows an acclimation response, the response usually collapses within 24 h (Reynolds & Casterlin 1979). Thus, if acclimation had occurred in our garter snakes, other studies indicate that it would have disappeared by the time we began our assays. Thermal acclimation of thermoregulation, due to differences in developmental temperature, is either absent or short-lived in *T. elegans*.

The absence of maternal effects on thermoregulation early in neonatal life (5–10 days postpartum) suggests that maternal effects on adult thermoregulation are unlikely. Generally, maternal effects are strongest early in development and wane with increasing time (Riska 1991). Under such a model one would not expect maternal temperature effects on adult thermoregulation in garter snakes. In particular, thermoregulation during pregnancy by a mother would not affect thermoregulation by her daughter during pregnancy. Thus, maternal effects probably do not contribute to any covariance that might exist between the thermoregulation of mothers and daughters.

The absence of a maternal temperature effect on thermoregulation is particularly striking given the wide range of maternal temperatures imposed in our study. That experimental range (24–32°C) represents virtually the entire range of constant temperatures that will support pregnancy. In a later paper, we plan to report the results of field studies of thermoregulation by gravid females in

our reference population of garter snakes. Those studies indicate that gravid females exhibit a modal temperature of 30°C with a standard deviation of less than 1°C during the midday period when the widest range of opportunities for thermoregulation are available (Peterson et al. 1993). Thus, in the field, garter snake embryos experience only a narrow distribution of temperatures during diurnal thermoregulation by their mothers. At night, however, maternal temperatures below 20°C are relatively common, and embryos may experience temperatures as low as 12°C. Although constant temperatures are a good starting point for experimental studies of maternal temperature effects, there is still a need to test for the effects of cycling maternal temperatures on juvenile thermoregulation. For example, if the intertidal fish *Girella nigricans* is exposed to naturally fluctuating conditions, it selects higher temperatures than if it is acclimated to constant temperatures in the laboratory (Norris 1963).

#### Relative Variation and Repeatability of Thermoregulation

Coefficients of variation are often used to compare the variation in two samples if sample standard deviations are known or thought to covary with the mean (Sokal & Rohlf 1981). For more detailed discussions of variation comparisons see Lewontin (1966), Wright (1968), Lande (1977) and Van Valen (1978).

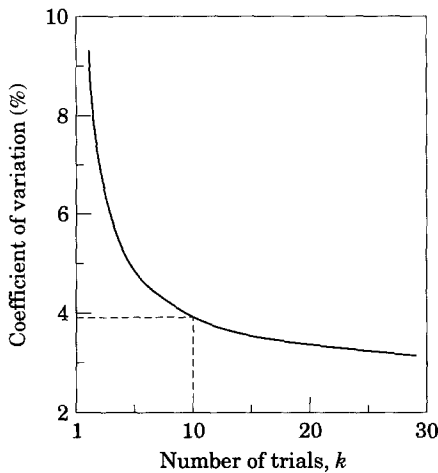
The observed coefficient of variation (3.9%) for average juvenile body temperature seems to indicate an extraordinary degree of behavioural stereotypy. DeWitt & Friedman (1979) surveyed studies of body temperature in 13 species of amphibians, reptiles, fishes and insects and found an average coefficient of variation of 15% (range=8–24%). Ferguson (1971) analysed two to three push-up displays for between eight and 25 individual male lizards (*Uta stansburiana*) from each of 12 localities. Average coefficients of variation ranged from about 12 to 27% within localities for temporal components of the display. Dane et al. (1959) analysed the durations of 13 kinds of social and epigamic displays for an unknown number of individual male goldeneye ducks, *Bucephala clangula*, and recorded an average coefficient of variation of 21% (range=5–72%) for an average total sample of about 29 measurements per display (range=11–66). Rothblum & Jenssen

(1978) analysed an average of 16 push-up displays by 11 individual male lizards (*Sceloporus undulatus*) and recorded coefficients of variation ranging from 9 to 15% for components of the display and 7% for total display duration. Stamps & Barlow (1973) analysed four to 11 push-up displays by each of five lizards (*Anolis aeneus*) and recorded coefficients of variation ranging from about 12 to 39% for durations of display components. Thus, the average juvenile body temperature in *T. elegans* is apparently more stereotyped than any displays so far subjected to cine or video analysis.

The number of observations per individual must be taken into account, however, in comparisons of coefficients of variation. This point seems to have not been appreciated in some general discussions of behavioural variation (e.g. Barlow 1968; Slater 1978). Thus, if  $k$  observations of behaviour are made on each individual in a sample, the expected value for the variance of the mean of  $k$  observations is  $V_k = V_b + (1/k) V_w$ , where  $V_b$  and  $V_w$  are, respectively, the within and between individual components of variance (Sokal & Rohlf 1981). The total variation in behaviour shrinks as the number of observations per individual increases because of a contraction in the contribution of variation within individuals. In contrast, the expected value of the mean does not change with  $k$  (unless there are systematic changes in the mean through time). Consequently, the coefficient of variation (the square root of total variance divided by the mean and expressed as a %) decreases as the number of observations is increased. The coefficient of variation for average juvenile body temperature as a function of  $k$  is shown in Fig. 5. We see that the expected coefficient of variation ranges from 9.3% when one observation is made on each individual, to 2.7% when an infinite number of observations are made on each individual. Juvenile body temperature is very stereotyped even when the number of observations per individual is taken into account.

DeWitt & Friedman (1979) noted that frequency distributions of ectotherm body temperatures often show negative skewness (a wider range of variation in temperature below the median than above it) and they argued that this negative skewness is a consequence of the regulation of some exponential temperature-dependent rate process with a normal curve of error. Our results with garter snakes suggest that the distribution of temperature set points may be negatively skewed.



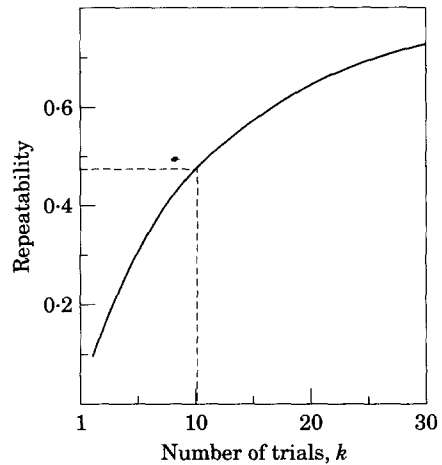


**Figure 5.** The expected coefficient of variation for the mean body temperature for  $k$  trials as a function of the number of trials with each individual.

According to the DeWitt–Friedman model, this skewness would mean that the intrinsic values for the hypothesized rate process (as well as errors for the process) are normally distributed.

The single trial repeatability of juvenile body temperature (9%) estimated in this study is low compared with the repeatabilities of behavioural displays and locomotor performance (running speed, endurance, etc.), the most comparable traits for which we could find published values. For example, repeatabilities of components (durations) of lizard displays range from 27 to 46% of *A. limifrons* (Jenssen & Hover 1976), 1 to 80% in *A. townsendi* (Jenssen & Rothblum 1977) and 57 to 96% in *S. undulatus* (Rothblum & Jenssen 1978). Austin & Shaffer's (1992) survey of locomotor performance studies in four species of amphibians and five species of reptiles revealed single trial (1 day between trials) repeatabilities ranging from 30 to 84%.

Although 9% seems to be a low repeatability, it must be remembered that repeatability of an average (or sum) is an increasing function of the number of observations (Winer 1971; Falconer 1989). Furthermore, the behaviour and ecology of the organism can help us identify the relevant range for the number of observations that make up the sum or average (Houck et al. 1985; Arnold 1994). For example, Jayne & Bennett (1990) have shown that larger juvenile garter snakes show improved survivorship. Juvenile thermoregulation



**Figure 6.** The expected repeatability of the mean body temperature for  $k$  trials as a function of the number of trials with each individual. Extrapolations are based on the observed repeatability of the mean of 10 trials (dashed line) using formulas given by Winer (1971).

may very well affect growth rate (Arnold & Peterson 1989) and hence survivorship. The relationship between body temperature and growth rate, however, is played out over scores, hundreds or even thousands of days. Thus, 10–1000 is the ecologically relevant number of observations (or days) for assessing repeatability of juvenile body temperature. On that scale the repeatability of juvenile body temperature ranges from 47 to nearly 100% (Fig. 6).

Repeatability is a useful statistic because it places an upper bound on heritability, which in turn affects genetic responses to selection (Falconer 1989). Thus, repeatability tells us how much effort must be expended to detect heritable variation (e.g. how many families must be scored and how many measurements must be made on each individual). The present results (Fig. 6) indicate that a considerable gain in statistical power would be experienced by making up to 10 temperature measurements on each individual, but making more than 20 measurements would probably not repay the effort.

#### Variation between Litters in Thermoregulation

We detected variation between litters in multiple aspects of thermoregulation. In particular, litters of neonates differed in average body temperature, in morning versus afternoon

temperature profiles and in temperature trends over a 5-day period. On the average, garter snake litter-mates are nearly full-siblings, despite the presence of some multiple paternity of broods (Schwartz et al. 1989). Thus, the resemblance between litter-mates detected in our experiment could reflect additive genetic variance, dominance variance, the common prenatal environment of litter-mates and maternal effects (Willham 1963, 1972). We controlled statistically for litter-mate resemblance in body size and so eliminated one potential source of common environment effects. Furthermore, our experiment suggests that one important class of maternal effects is unlikely. Thus, additive and non-additive genetic effects emerge as the leading candidates for the resemblance between litter-mates in thermoregulation that we detected in our experiment. Additive genetic variance for multiple aspects of thermoregulation is especially important, because it would allow thermoregulation to evolve in response to selection. Whereas the results so far do not demonstrate conclusively that garter snake thermoregulation is heritable, they do point in the direction of heritable multivariate variation and should encourage more sophisticated tests and breeding designs (e.g. assays of father-offspring and paternal half-sibling resemblance).

#### Acclimation and Maternal Effects on Thermoregulation in Reptiles

Few studies have addressed the effects of acclimation and maternal environment on thermoregulation in reptiles. Wilhoft & Anderson (1960) acclimated adult lizards (*Sceloporus*) for 14 days at three constant temperatures (12, 25 and 35°C). Lizards from the high temperature treatment selected lower body temperatures than controls, but lizards from the low lower acclimation treatments showed no acclimation response. Although Scott & Pettus (1979) showed that the preferred body temperatures of adult *T. elegans* differed between snakes collected in late summer and snakes maintained over winter under laboratory conditions (total darkness and 4°C), we know of no snake studies that have examined the effects of thermal acclimation independent of changes in photoperiod. Lang (1985) is the only previous study of the effects of developmental temperature on thermoregulation by neonatal reptiles. Lang incubated the eggs of a crocodile (*Crocodylus*

*siamensis*) at two constant temperatures (28 and 32.5°C) and then monitored the body temperatures of hatchlings on thermal gradients. Hatchlings from the higher incubation temperature selected higher body temperatures. However, as Lang noted, incubation at 28°C produced females and incubation at 32.5°C produced males, so the difference in thermoregulation may reflect a sexual difference or an interaction between sex and incubation temperature. Furthermore, Lang's crocodiles were housed and tested in groups, so social effects on thermoregulation may have

complicated the results. Thus, the evidence for effects of developmental temperature on thermoregulation in hatchling crocodilians is equivocal. In our experiment, we found no sexual differences in thermoregulation, and we housed each snake in its own compartment to avoid social effects.

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