The fitness correlates of scalation asymmetry in garter snakes *Thamnophis sirtalis parietalis*

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Summary

1. In a field population in Manitoba, about 16% of Red-Sided Garter Snakes (*Thamnophis sirtalis parietalis*) have a different number of ribs on either side of the body, as indicated by anomalous ventral scales. A study on congenerics shows that this asymmetry reflects disruption of embryogenesis by environmental factors, including suboptimal maternal thermoregulation during pregnancy.

2. Correlates of this scalation asymmetry in adult snakes can clarify the degree to which (and pathways by which) the thermal environment that an individual experiences during embryogenesis affects its later fitness.

3. Data on >4000 free-ranging snakes reveal no significant association between asymmetry and fitness-related traits such as body size, body condition, locomotor speeds or female ability to resist harassment from males.

4. However, adult male snakes with scale asymmetry dispersed from the den-based mating aggregations sooner than did symmetric males. In outdoor arenas, asymmetric males were less able to obtain matings than were symmetric males; and if asymmetric males did mate, they consistently used the hemipenis on the side of the body opposite to their extra rib.

5. Our data suggest that careful thermoregulation by a female garter snake during pregnancy provides a fitness benefit to her sons after they mature, via sexual selection.

Introduction

In all viviparous (live-bearing) animals, the mother controls many aspects of the embryo's environment during early development. Minor variations in the conditions experienced by an embryo can translate into major phenotypic modifications (Henry & Ulijaszek 1996; Deeming 2004), suggesting that reproducing females should be under intense selection to optimize incubation regimes for their offspring (Vinegar 1974). One of the most important abiotic influences on both the rate and the trajectory of embryonic differentiation is temperature; even minor fluctuations in maternal body temperature can exert significant impacts on the phenotypic traits of neonates (Lourdais *et al*. 2004). Although trivial for most endotherms, diel and seasonal fluctuations in maternal body temperature are considerable in many ectotherms, especially terrestrial taxa (Hertz, Huey & Stevenson 1993; Peterson, Gibson & Dorcas 1993). Thus, we might expect that during pregnancy, viviparous ectotherms would attempt to maintain body-temperature regimes that would optimize embryogenesis (Beuchat 1986, 1988; Beuchat & Ellner 1987; Arnold & Peterson 2002).

In support of this prediction, pregnancy induces shifts in thermoregulatory tactics in many terrestrial ectotherms. For example, gravid females of many viviparous snake and lizard species maintain higher and less variable body temperatures than do non-gravid conspecifics (Gier, Wallace & Ingerman 1989; Charland & Gregory 1990). In some species the effect is to increase mean body temperature, whereas in others it is to reduce it (Andrews, DelaCruz & SantaCruz 1997). In many cases, diel variance in body temperatures is lower in pregnant than in non-pregnant animals (Peterson *et al*. 1993). Laboratory studies keeping females under specific thermal conditions provide direct empirical support for the idea that maternal thermal regimes influence offspring attributes (Shine & Harlow 1993; Swain & Jones 2000; Wapstra 2000; Robert & Thompson 2001; Arnold & Peterson 2002; Caley & Schwarzkopf 2004). Correlations between annual variation in weather conditions *vs* offspring phenotypes in natural populations suggest that the same effects occur in nature (Lourdais *et al*. 2004). All these data are consistent with the

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adaptationist hypothesis that selection favours careful thermoregulation by pregnant female reptiles because this behaviour enhances offspring viability. However, evidence is lacking for one major plank of this adaptationist interpretation – the idea that exposure to suboptimal thermal regimes during embryogenesis significantly reduces offspring fitness.

The primary obstacle to quantifying a link between maternal thermoregulation and offspring fitness is a logistical one; it is difficult to follow the fate of a cohort of offspring under field conditions. However, a much easier alternative may be available: if we can identify a phenotypic trait induced by maternal thermal regimes, and especially one that distinguishes optimal from suboptimal developmental regimes, then we can simply examine the correlations between that trait and fitness among free-living animals. Uniquely, morphological asymmetry may offer such an opportunity. Such asymmetry can take three main forms or combinations thereof: fluctuating asymmetry (mean asymmetry of zero, but with individual animals deviating in either direction), directional asymmetry (mean asymmetry not zero) and antisymmetry (bimodal distribution of symmetry values, with a mean of zero). Both of the latter forms of asymmetry presumably reflect genetically coded adaptive traits, whereas fluctuating asymmetry is generated by developmental perturbations (Beardmore 1960; Van Valen 1962; Leamy 1984; Palmer & Strobeck 1986). Most attention has focused on fluctuating asymmetry, the slight deviations from bilateral symmetry that can be induced by disruptions to embryogenesis during early development (Møller & Swaddle 1997). Although some forms of asymmetry are continuous variables that may shift through ontogeny (e.g. relative limb length: Kellner & Alford 2003), others take discrete values that are set at birth and cannot change thereafter (e.g. number of ribs). It is this latter invariant category that offers the most powerful opportunity to examine long-term fitness effects of events in early development.

Viviparous reptiles provide ideal model organisms with which to implement this approach. First, viviparity has arisen from oviparity independently in more than 100 lineages of squamate reptiles (Blackburn 1985; Shine 1985), and parallel modifications of maternal thermoregulatory behaviour during pregnancy have been reported in species representing many independent evolutionary origins of viviparity (elapid snakes, Shine 1979; boid snakes, Dorcas & Peterson 1998; colubrid snakes, Gibson & Falls 1979; Rosen 1991; Tu & Hutchinson 1994; viperid snakes, Gier *et al.* 1989; Charland & Gregory 1990; gekkonid lizards, Rock, Andrews & Cree 2000; Rock, Cree & Andrews 2002; scincid lizards, Shine 1983; iguanid lizards, Beuchat 1986, 1988; Beuchat & Ellner 1987). Second, thermal regimes during embryogenesis have been reported to modify levels of scale asymmetry in a range of squamates (agamid lizards, Zhakarov 1989; Ji, Qui & Diong 2002; iguanid lizards, Qualls & Andrews 1999; colubrid snakes, Arnold & Peterson 2002). In the most detailed such

study, Arnold & Peterson (2002) maintained pregnant garter snakes (*Thamnophis elegans*) under a range of thermal regimes and documented effects on offspring phenotypes. These authors reported a significant effect of maternal temperatures on the level of asymmetry in ventral scalation, with asymmetry rare at optimal incubation temperatures (approx. 27 °C) but increasingly common at temperatures either higher or lower than this level. Although data are lacking, it also remains possible that the incidence of such asymmetries may be affected by genetic factors or other stressful events during embryogenesis. Importantly, this superficially minor scalation trait actually reflects a significant anatomical asymmetry: each incomplete ventral scale is associated with an unpaired rib (King 1959; Lindell, Forsman & Merila 1993).

Because this easy-to-score asymmetry reflects (at least in part) a significant thermally induced disruption to skeletal formation during embryogenesis, asymmetry in ventral scalation of snakes offers a powerful opportunity to examine the long-term effects of a mother's thermoregulation on the fitness of her offspring, as mediated via higher levels of scalation asymmetry in offspring that develop under suboptimal thermal regimes. Many such effects are likely to be subtle, so large sample sizes are essential for adequate statistical power. We took advantage of an unusual research opportunity in this respect: the massive aggregations of garter snakes that occur at dens on the Manitoba prairies.

Materials and methods

SPECIES AND AREA

Red-Sided Garter Snakes (*Thamnophis sirtalis parietalis*) are small (males average 45 cm snout–vent length (SVL), females 60 cm) non-venomous viviparous colubrid snakes. This species extends into higher-latitude, colder-climate areas than do most snakes, and is abundant in the prairies of south-central Canada (Rossman, Ford & Seigel 1996). The severely cold winters in this region force the adult snakes to aggregate in deep underground caverns to avoid freezing; thus, several thousand snakes may use the same burrow system. Many of these snakes travel many kilometres from their summer range to return to this communal den each autumn (Gregory 1974; Gregory & Stewart 1975; Larsen 1987). The snakes mate in early spring soon after emergence and prior to dispersal, providing a unique opportunity for research on reproductive biology (Mason 1993). Accordingly, the garter snakes of Manitoba are among the most intensively studied reptiles worldwide, providing extensive background data on their physiology, ecology and behaviour. We worked primarily at a communal den 1·5 km north of the town of Inwood, 250 m east of Highway 17 in central southern Manitoba (50°31·58′ N 97°29·71′ W), but also took samples from a smaller den 15 km further north on the Clematis Wildlife Refuge.

NATURE OF SCALATION ASYMMETRY

Our study focuses on one easily quantifiable type of asymmetry: ventral scales that extend only halfway across the snake's body instead of the entire distance. Because each ventral scale is associated with a pair of ribs, these anomalous scales reflect rib asymmetry; the 'extra' half-scale reflects the presence of an unpaired rib. Our dissections of Manitoba *T. sirtalis parietalis* confirm that scale asymmetries reflect underlying rib asymmetries in every case that we have examined (*n* = 35 individuals). Such asymmetries can occur at any point along the snake's body, but the vast majority (>95% in *T. s. parietalis* that we have examined) involve the two ventral scales immediately anterior to the vent (Plummer 1979; Merila, Forsman & Lindell 1992). The incidence of such asymmetric scales is higher when pregnant garter snakes (*T. elegans*) are kept at temperatures either higher or lower than the optimum for embryonic development (Arnold & Peterson 2002). Other researchers have shown reduced rates of scale asymmetry at closer-to-optimal incubation temperatures in a variety of other reptiles (Zhakarov 1989; Qualls & Andrews 1999; Ji *et al*. 2002), including our own study species (*T. sirtalis*, in Arnold & Peterson 2002).

METHODS

During fieldwork at snake dens in Manitoba in May 2003, we scored the incidence of 'half-scale' asymmetries within the first five ventral scales anterior to the vent. We also recorded each animal's sex (from tail shape and relative length: Shine *et al*. 1999), body length, mass and location. We individually marked males (using non-toxic paint) in the Inwood den on the day they emerged from their winter inactivity; their classification as newly emerged was based on two transitory characteristics (muddy appearance and pheromonal female mimicry) that disappear within a day or so of emergence (Shine *et al*. 2000a; Shine, O'Connor & Mason 2000b). We set up a 60-m drift fence in aspen woodland 100 m from the den, to intercept snakes as they dispersed back towards their summer ranges. Funnel-traps captured snakes attempting to cross the fence. Data from animals marked at the den and later recaptured at the drift fence revealed the amount of time a snake spent at the den prior to dispersing. Male snakes switch from courting to feeding as they leave the vicinity of the den (O'Donnell, Shine & Mason 2004), so snakes that leave sooner presumably spend less time in reproductive activities.

Additionally, we compared snakes with and without scale asymmetries in a series of trials to assess fitness-relevant behaviours.

Locomotor speed

Adult male snakes collected at the den were placed individually in numbered cloth bags, and brought to a range of temperatures from 7 to 35 °C (typical of those recorded in free-ranging snakes at these dens: Shine *et al*. 2000d,e) by placing the bags either inside a cooled, insulated container or exposing the bags to sunlight. We used a range of temperatures in case any effect of asymmetry on locomotor performance was manifested only over specific thermal ranges. Locomotor trials were run by placing the snake at one end of an open-topped (U-shaped) 1·4-m long wooden raceway. The rough wooden floor provided traction for snakes, and walls 12·5 cm high encouraged snakes to travel along the raceway rather than climbing out. Snakes were gently tapped on the tail with a leafy twig to keep them moving. We recorded cloacal temperatures prior to each trial, and the times (by electronic stopwatch) at which the snakes crossed marks made at 0, 40, 80 and 120 cm on the floor of the raceway. We also recorded any stops or reversals. Each snake was run five times in quick succession, then re-bagged for later measurement. Each snake was used for tests at only one temperature. Our statistical analyses were based on a single mean and maximum speed for each snake for the 40-cm segments (*n* = 15; three measures per trial) over which its speed was measured, as well as on the total number of stops or reversals that were exhibited during the trials.

Female tolerance of stress

Male garter snakes obtain matings by inducing hypoxic stress in females and, thus, inducing cloacal gaping (Shine, Langkilde & Mason 2003a). Males lie above a female with their bodies aligned with hers, and the males' anterior-directed caudocephalic waves impede female respiration by forcing anoxic air anteriorly from the female's saccular (avascular) lung across the respiratory surface of the vascular lung (Shine *et al*. 2003a). By mimicking those caudocephalic waves (stroking along the female's dorsal surface in a posterior-to-anterior direction), an observer can induce cloacal gaping in the female. To quantify female resistance to such harassment, we scored the numbers of strokes (up to a maximum of 20, after which we ceased the stimulus) required to elicit cloacal gaping. Females were held stretched out, and stroked (at a rate of one per s) from the tailbase up to 10 cm behind the head. Scores obtained using this method predict the duration of courtship that a female resists prior to mating (R. Shine, unpublished data), indicating that the method offers a valid index of female resistance to caudocephalic waving. In turn, such resistance might correlate with female fitness for two reasons: first, as a general index of vigour; and second, because a female that can resist hypoxic stress may be better-able to exert mate-choice.

Male mating success

We placed 20 newly collected males (10 symmetric, 10 asymmetric) into each of 12 open-topped nylon arenas (1 × 1 m², 0·8 m high) set up on an open grassy area near a den. Ten minutes later we added a single (symmetric)

adult female snake to each arena, and checked frequently thereafter for copulation. Copulating pairs were removed, and later measured and weighed, at which time we also recorded which hemipenis (right or left) was being used, whether or not the mating male had a scale asymmetry near the vent, and if so on which side of the body this asymmetry occurred. All remaining snakes were then removed from the arena, and a new set of animals added to run additional trials.

STATISTICAL ANALYSES

Assumptions of statistical tests (normality of distributions, homogeneity of variance) were tested prior to analysis; no transformations were necessary. However, we log-transformed SVL and mass to linearize their relationship and thus permit ANCOVA. All results from contingency-table analyses include Yate's corrections. The text reports means ± 1 SD.

Results

FREQUENCY OF ASYMMETRY WITH RESPECT TO SIDE OF THE BODY

Our inspections of a random sample of live snakes revealed 101 animals with an asymmetric ventral scale on the left-hand side of the body and only 55 animals in which the asymmetry occurred on the right (against a null of equal numbers on either side, $\chi^2 = 13 \cdot 56$, 1 df, $P < 0 \cdot 001$).

FREQUENCY OF ASYMMETRY WITH RESPECT TO SEX

Overall, asymmetric ventral scales were recorded in a higher proportion of female snakes at the Inwood den (203 of 1168, = 17·4%) than in conspecific males (482 of 3288, = 14·7%: $\chi^2 = 4 \cdot 70$, 1 df, $P < 0 \cdot 03$).

FREQUENCY OF ASYMMETRY WITH RESPECT TO LOCATION

The proportions of asymmetric snakes at the Inwood den were similar to those at the drift fence 50 m away (males – 109 of 732 at den, = 14·9%; 373 of 2556 at fence, = 14·6%; $\chi^2 = 0 \cdot 02$, 1 df, $P = 0 \cdot 89$; females – 85 of 427 at den, = 19·9%; 118 of 741 at fence, = 15·9%; $\chi^2 = 2 \cdot 72$, 1 df, $P = 0 \cdot 099$). In a smaller sample from a den in the Clematis Wildlife Refuge, 15 km from the Inwood den, we recorded asymmetric ventral scales in 14 of 79 males (17·7%), not significantly different from the incidence of asymmetry among males at Inwood ($\chi^2 = 0 \cdot 36$, 1 df, $P = 0 \cdot 55$).

FREQUENCY OF ASYMMETRY WITH RESPECT TO BODY SIZE

If scale asymmetry (or the developmental history that generates such asymmetry) affects a snake's growth rate or probability of survival, we expect to see differences in mean body size between symmetric and asymmetric animals. Analysis shows no such effects. In a logistic regression with SVL as the independent variable, the incidence of asymmetry was unaffected by body size in either sex (males – $\chi^2 = 0 \cdot 58$, 1 df, $P = 0 \cdot 45$; females – $\chi^2 = 1 \cdot 65$, 1 df, $P = 0 \cdot 20$).

FREQUENCY OF ASYMMETRY WITH RESPECT TO BODY CONDITION

To compare body condition of symmetric and asymmetric snakes, we used ANCOVA with ln mass as the dependent variable and ln SVL as the covariate, and asymmetry as the factor. Within males, mass relative to SVL was not affected by asymmetry ($F_{1,1595} = 0 \cdot 91$, $P = 0 \cdot 34$) nor by any interaction between asymmetry and SVL ($F_{1,1595} = 0 \cdot 86$, $P = 0 \cdot 35$). After deleting the interaction term and recalculating the intercepts effect, no significant difference was apparent between symmetric and asymmetric animals ($F_{1,1596} = 0 \cdot 70$, $P = 0 \cdot 43$). Similarly, asymmetry did not affect body condition in females (main asymmetry effect – $F_{1,265} = 0 \cdot 69$, $P = 0 \cdot 41$; interaction asymmetry * ln SVL – $F_{1,265} = 0 \cdot 73$, $P = 0 \cdot 39$; intercepts – $F_{1,266} = 0 \cdot 44$, $P = 0 \cdot 51$).

ASSOCIATION BETWEEN ASYMMETRY AND LOCOMOTOR SPEED

Unsurprisingly, all snakes were faster at higher body temperatures. In ANCOVA with body temperature as a covariate, asymmetry as the factor and speed measures as the dependent variables, we detected no significant differences between symmetric and asymmetric male snakes (for maximum speed, asymmetry effect – $F_{1,44} = 0 \cdot 20$, $P = 0 \cdot 66$; interaction asymmetry * temperature – $F_{1,44} = 0 \cdot 55$, $P = 0 \cdot 46$; for average speed, asymmetry effect – $F_{1,44} = 0 \cdot 25$, $P = 0 \cdot 62$; interaction asymmetry * temperature – $F_{1,44} = 0 \cdot 33$, $P = 0 \cdot 57$; for number of stops and reversals, asymmetry effect – $F_{1,44} = 0 \cdot 21$, $P = 0 \cdot 65$; interaction asymmetry * temperature – $F_{1,44} = 0 \cdot 96$, $P = 0 \cdot 33$).

ASSOCIATION BETWEEN ASYMMETRY AND DURATION OF RESIDENCY AT THE DEN PRIOR TO DISPERSAL

This variable provides an index of the duration of a snake's participation in mating aggregations, and hence may influence copulatory frequency in both sexes. Recapture rates of females were too low for analysis. However, asymmetric males left the den an average of only 5·44 days after emerging ($n = 16$, SD = 3·71) whereas symmetric males stayed longer (mean = 7·91 ± 3·62 days, $n = 79$; $F_{1,93} = 6 \cdot 16$, $P < 0 \cdot 02$).

ASSOCIATION BETWEEN ASYMMETRY AND FEMALE TOLERANCE TO STRESS

Regardless of whether or not SVL was included as a covariate in the analyses, symmetric and asymmetric

females did not differ significantly with respect to the number of strokes prior to cloacal gaping (means 4.03 ± 4.6 *vs* 4.72 ± 4.69; with SVL included as a covariate, $F_{1,170} = 0.83$, $P = 0.37$).

ASSOCIATION BETWEEN ASYMMETRY AND MALE MATING SUCCESS

Number of matings

Although our arenas contained equal numbers of symmetric and asymmetric males, of equal mean body sizes, 34 of the 49 matings that we recorded were by symmetric males and only 15 by asymmetric males ($\chi^2 = 7.37$, 1 df, against null of 50%, $P < 0.01$).

Body sizes of successful males

Because larger body size enhances male mating success in this population (Shine *et al.* 2000c), any disadvantage to asymmetry should also be apparent in the body sizes of mating snakes: we would expect that only the largest asymmetric males succeed in mating. As predicted, the mean body sizes of mated asymmetric males exceeded that of mated symmetric males (SVL – $F_{1,45} = 5.65$, $P < 0.025$; mass – $F_{1,45} = 4.09$, $P < 0.05$; see Fig. 1).
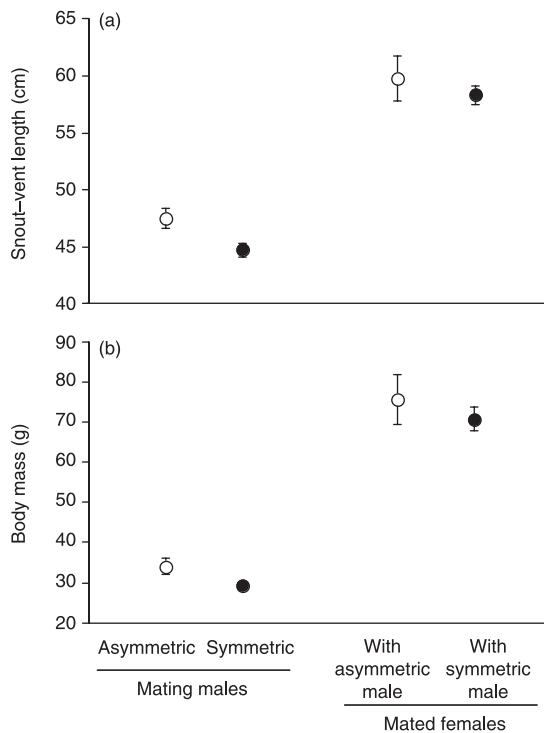

Fig. 1. Body sizes (snout–vent length and mass) of garter snakes (*Thamnophis sirtalis parietalis*) mating in outdoor enclosures, as a function of whether the male displayed or did not display an asymmetry in ventral scalation. Mating males are divided into those with asymmetry in this trait and those without such asymmetry; females are divided according to which type of male they mated with. Sample sizes (left to right) = 15, 34, 15, 34. Graph shows mean values and associated standard deviations.

However, there was no difference in the mean body sizes of females with which these two types of males obtained matings (SVL – $F_{1,45} = 0.69$, $P = 0.41$; mass – $F_{1,45} = 0.66$, $P = 0.43$; see Fig. 1).

Mating tactics

The most plausible mechanism to generate lowered mating success in asymmetric males (above) would be lowered ability to flexibly distort (bend) the critical body section near the vent, thus impeding the male in the frantic competition with his rivals (Shine, Langkilde & Mason 2003b). If so, we would expect to see a correlation between the side of the body on which the asymmetry occurs, and the hemipene that is used. Thus, snakes with an extra scale on the right-hand side of the body could bend more easily to the left, and might thus use the left hemipene; the reverse would be true for asymmetries on the left-hand side of the body. As predicted, hemipene usage matched asymmetry. Snakes with asymmetries on their left-hand side used the right hemipene in 7 out of 11 matings, whereas those with asymmetries on the right-hand side used the left hemipene in 4 out of 4 matings (i.e. 11 of 15 results in predicted direction, $\chi^2 = 3.27$, 1 df, one-tailed $P < 0.05$).

Discussion

Measures of morphological asymmetry have the potential to provide simple, unambiguous indices of the degree of disruption experienced by an organism during its development. Whether or not this potential is realized has been a subject of considerable research and heated debate. Many authors who have looked for correlations between fluctuating asymmetry and various fitness measures (or their surrogates) have found no such effects (e.g. Breuker & Brakefield 2002; Goncalves *et al.* 2002; Martin & Hosken 2002; Rivera, Perez & Andres 2002; Siikamaki, Lammi & Mustajarvi 2002; Kolliker-Ott, Blows & Hoffmann 2003; Kruuk *et al.* 2003). Nonetheless, many other studies report significant links between levels of fluctuating asymmetry and attributes such as increased vulnerability to predation (Bergstrom & Reimchen 2003), abiotic stress (Frechette, Goulletquer & Daigle 2003) or reduced reproductive output in females (Hendrickx, Maelfait & Lens 2003) or males (Mallard & Barnard 2003). There is thus considerable empirical support for the idea that asymmetry sometimes is correlated with an organism's fitness either directly (i.e. asymmetry influences performance) or indirectly (i.e. asymmetry provides an index of developmental history, with the factors that generate asymmetry also modifying fitness). Such associations between asymmetry and fitness may occur via processes as diverse as female choice (Mazzi, Kunzler & Bakker 2003), growth rates (MøIler & Manning 2003) or energy storage (Blanckenhorn, Kraushaar & Reim 2003). Most directly comparable to results from the present study, recent studies have reported significant correlations

between fluctuating asymmetry and male sexual behaviours and/or mating success in a wide range of taxa including spiders (Ahtiainen *et al.* 2003), anisopterans (Szallassy *et al.* 2003) and damselflies (Beck & Pruett-Jones 2002).

Importantly, the asymmetry that we found in ventral scalation of garter snakes was not 'true' fluctuating asymmetry, because abnormal ventral scales occurred more often on one side of the body than the other. This kind of directional asymmetry (population-wide mean value is not zero) usually is attributed to adaptation rather than developmental 'noise' (e.g. heart position in mammals: Van Valen 1962; Palmer & Strobeck 1986). The nature of the process generating an asymmetry is the critical issue for its biological interpretation, not whether or not the mean value is zero. The experimental evidence of Arnold & Peterson (2002) shows that asymmetry in the ventral scales of garter snakes is caused by developmental peturbation, the factor generally implicated as a cause of fluctuating rather than directional asymmetry. Many traits show both directional and fluctuating asymmetry in this manner (Beardmore 1960; Van Valen 1962; Leamy 1984). Previous studies suggest a possible adaptive function for the directional asymmetry that we observed. Male garter snakes have separate reproductive systems on either side of the body, and the testis and hemipenis on the right-hand side of a male's body average larger than the corresponding structures on his left-hand side (Shine *et al.* 2000f). Accordingly, males may benefit by using the right-hand side system, and mating trials confirm that they do so if conditions make this possible (Shine *et al.* 2000f). Hence, a ventral-scale abnormality on the left-hand side of the body (allowing the male to twist to his right and, thus, use the right hemipene) likely will confer less fitness disadvantage than would an equivalent abnormality on the right-hand side. Thus, if suboptimal thermal conditions during pregnancy generate a ventral asymmetry in the offspring, selection should act to direct such developmental errors to the left-hand side of the body.

Although asymmetry in ventral scales (and thus, asymmetry in rib numbers) was relatively common (16%) in the snake population that we studied, even higher levels have been reported in other snake species: for example, Merila *et al.*'s (1992) review reported values of 15·4–48·9% of asymmetric individuals. Despite our large sample sizes (>4000 snakes) and consequently, high statistical power, we did not detect significant correlations between asymmetry and either body length or body condition. This result suggests that asymmetry (or the developmental conditions that generate asymmetry) did not affect either growth rates or survival of snakes in our population. The same conclusion was reached in studies of European Adders, *Vipera berus* (Merila *et al.* 1992; Lindell *et al.* 1993), but Arnold (1988) reported that asymmetric garter snakes (*Thamnophis elegans*) grew more slowly than did symmetric animals in the same population. Research on another

garter snake species (*T. radix*) also found that asymmetry was associated with reduced locomotor speeds, based on a large sample of same-aged (neonatal) snakes (Arnold & Bennett 1988). The smaller sample sizes and greater body-size range used in our own study may have masked any weak relationship between asymmetry and locomotor ability in *T. sirtalis*.

We found two major correlates of asymmetry, both related to sexual selection on male snakes. First, asymmetric males moved from the den to the surrounding aspen woodlands (where they encountered the drift fence) sooner after their initial emergence than did symmetric males. Most males captured at the fence presumably had abandoned reproductive activity and were dispersing towards their summer ranges (Shine *et al.* 2001; O'Donnell *et al.* 2004). Given that the duration of male residency at the den is driven by factors such as body size and condition (Shine *et al.* 2004b), which were not associated with asymmetry in our study, the relationship between asymmetry and duration of residency may be indirect. That is, male behaviour may have been modified by the thermal conditions during pregnancy that also generated scalation asymmetry, rather than by asymmetry *per se*.

The second major correlate of asymmetry was reduced competitive ability in courting males, and this may have been a direct effect rather than an indirect one. Asymmetric males performed less well than symmetric males in obtaining matings in our outdoor arenas. The most likely mechanism for asymmetry to reduce mating success involves a reduced flexibility of the posterior body, immediately above the vent, because of the presence of an unpaired rib. To achieve intromission, a male garter snake must wrap his posterior body around the female's vent, cling tightly to withstand attempts by rivals to push his tail out of the way, and respond rapidly when the female gapes her cloaca open (Shine *et al.* 2003b; Shine, Langkilde & Mason 2004a). Thus, asymmetry in the posterior body may disadvantage a male in these frenetic pursuits. In keeping with this hypothesis, asymmetric males disproportionately obtained copulations with the hemipenis that was on the opposite side of the body from the 'extra' (unpaired) rib. The trend for successfully mating asymmetric males to be larger than successful symmetric males fits well with earlier studies on Manitoba garter snakes, that have revealed a significant mating advantage to larger body size (Shine *et al.* 2000c). Presumably, larger size helps to overcome any disadvantage due to asymmetry.

Although our work clarifies the fitness correlates and consequences of asymmetry, we rely upon the elegant field and laboratory work of Arnold & Peterson (2002) to identify maternal thermal regimes during pregnancy as the proximate cause of asymmetry. Although their study concerned *Thamnophis elegans* not *T. sirtalis*, they note that they have found similar effects on *T. sirtalis* in unpublished work. Further support for thermal causation of asymmetry comes from research on other reptiles (Zhakarov 1989; Qualls & Andrews 1999; Ji

et al. 2002) as well as more distantly related organisms such as squid (Gowland, Boyle & Noble 2003). More generally, many features of the morphology of hatchling and neonatal snakes are highly sensitive to incubation temperatures (e.g. Vinegar 1973, 1974; Osgood 1978; see reviews by Deeming 2004; Shine 2004). Levels of asymmetry can be affected also by other factors during embryogenesis, for example corticosterone levels (in birds: Eriksen *et al.* 2003), but the experimental data of Arnold & Peterson (2002) directly demonstrate a role for maternal thermoregulatory behaviour in viviparous snakes. Nonetheless, we reiterate that this earlier work was based on a different species from our own, so that the putative link between maternal thermoregulation and offspring asymmetry has no empirical support within our specific study population.

Why did the frequency of asymmetry differ (albeit by only a few per cent) in male *vs* female snakes within our study population? One possibility is strong sex-specific selection against asymmetry, generating survival differences early in life (Dunn 1942). Alternatively, the sexes may differ in their reaction norms against temperature, as found for *T. elegans* by Arnold & Peterson (2002), so that at the same maternal thermal regime, sons and daughters show different incidence of asymmetry. A third possibility is that some maternal trait such as body size influences both offspring sex ratios and body temperatures during pregnancy (Dunlap & Lang 1990; Seebacher, Grigg & Beard 1999), such that sons and daughters differ in asymmetry because they have different thermal experiences (on average) during embryogenesis.

Despite an enormous theoretical and empirical literature on thermoregulatory biology, evidence that more precise thermoregulation actually enhances organismal fitness is almost always indirect, and generally based upon plausibility arguments about links between phenotypes and fitness. Although perforce we have made some critical assumptions in the current study (e.g. we used mating success rather than paternity analyses to document fitness differentials related to asymmetry), our data on garter snakes provide more robust support for a causal link between maternal thermoregulation and offspring fitness than has previously been available. Careful thermoregulation by a pregnant female garter snake may confer a fitness benefit via an unexpected route: sexual selection on her sons. More direct experimental studies to further test this hypothesis would be of great interest.

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