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Are snakes right-handed? Asymmetry in hemipenis size and usage in gartersnakes (*Thamnophis sirtalis*)

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Male snakes possess paired reproductive systems (testes, efferent ducts, hemipenes and associated components of the kidneys), with an independent set on either side of the body. Our studies on gartersnakes (*Thamnophis sirtalis parietalis*) reveal significant morphological asymmetry in this system: testes, kidneys, and hemipenes on the right-hand-side of the body are larger than those on the left. Data from matings in the field, and in outdoor enclosures, suggest that this asymmetry has implications for reproductive behavior and, possibly, reproductive success. Copulations using the right hemipenis produced a larger gelatinous ''mating plug,'' and may thus more effectively delay remating by the female. Although the overall usage of the two hemipenes in field matings averaged close to 50/50, hemipenis usage was not random. Males tended to alternate hemipenis use in successive matings, perhaps because of depletion of plug material. Also, male gartersnakes preferentially used their larger (right) hemipenis when mating at high body temperatures, perhaps because they are more able to make subtle postural adjustments (and thus, select the better system) under these conditions. *Key words:* asymmetry, Colubridae, gartersnakes, handedness, lateralization, *Thamnophis sirtalis parietalis,* thermal effects. *[Behav Ecol 11:411–415 (2000)]*

Even in superficially symmetrical organisms, there is almost always some consistent deviation from absolute symmetry. Such deviations occur not only in anatomy, but also in behavior. When given the opportunity, many animals consistently use either their right or left hand (or foot, or paw) in preference to the equivalent appendage on the other side of their body (Bock and Marsh, 1991). At first sight, the concept of ''handedness'' is inapplicable to completely limbless animals, and thus it is difficult to imagine a right-handed snake. However, snakes (or at least, male snakes) do possess one set of paired appendages that might potentially display this kind of lateralization. Each male possesses two intromittent organs (hemipenes), one on either side of the body, only one of which is everted during a single copulation (Crews and Moore, 1986; Dowling and Savage, 1960; Murphy and Barker, 1980).

Asymmetry in the size and usage of hemipenes in squamate reptiles is particularly interesting because the reproductive organs on either side of the male's body are entirely separate, unlike many other asymmetric structures such as lungs. That is, sperm produced in the right testis can exit the body only via the right hemipenis (e.g., Dowling and Savage, 1960). Thus, any deviation from bilateral symmetry in the dimensions of male reproductive structures may directly influence the effectiveness of copulation—and by implication, the fitness benefit likely to accrue from inseminations with the left versus right hemipenis. A male that preferentially uses his larger hemipenis, attached to his larger testis, might thereby directly increase his genetic representation in the following generation.

METHODS

We studied red-sided gartersnakes (*Thamnophis sirtalis parietalis*) in central Manitoba, Canada (Chatfield Community Pasture; see Mason and Crews, 1985) during their mating seasons in May 1997 and May 1998. Snakes in this area mate immediately after emergence from large communal overwintering dens, so that courtship and mating often involve ''balls'' of many snakes (Mason and Crews, 1985). The present paper is based on two data sets: one concerning morphological asymmetry in the reproductive system, and the other concerning the relative usage of left versus right hemipenes in copulation.

Our morphological data are based on dissection of 90 adult male gartersnakes that were killed in a mass mortality event at one of the dens on 8 May 1997. Unusual weather conditions induced the snakes to form a single huge group on the floor of the den, and those underneath were suffocated by the mass of those lying above them. We found the snakes within a few hours of their deaths, stored their carcasses in a snowdrift overnight, and dissected them the next day. We recorded snout-vent length (SVL) and body mass, and then opened the snake body cavity with a midventral incision so that we could measure the length and width of the testes and kidneys on either side of the body. Part of the kidney (the sexual segment) is involved in producing secretions that are mixed with sperm (Devine, 1975; Saint Girons, 1957); because we could not clearly distinguish the sexual segment, we measured the entire kidney. The hemipenes were fully everted by squeezing the tail, and lengths and widths of these copulatory organs were recorded. Below, we use the term ''right-hand side'' from the snake's perspective; although this point may seem obvious, it is easy to become confused because snakes are typically laid on their dorsal surfaces for dissection; thus, the snake's right becomes the investigator's left.

To quantify patterns of usage of left versus right hemipenes, we recorded this information from two kinds of matings: those that occurred naturally (in the dens and their immediate vicinity) and those in mating arenas that we set up to control the numbers and types of mating males. For these trials, we placed freshly captured snakes (1, 2, 4, or 24 males, plus 1 unmated female) inside nylon mating arenas (1.0 × 1.2 m) near our field site and allowed them to mate. The varying numbers of males per female were used in an experiment to

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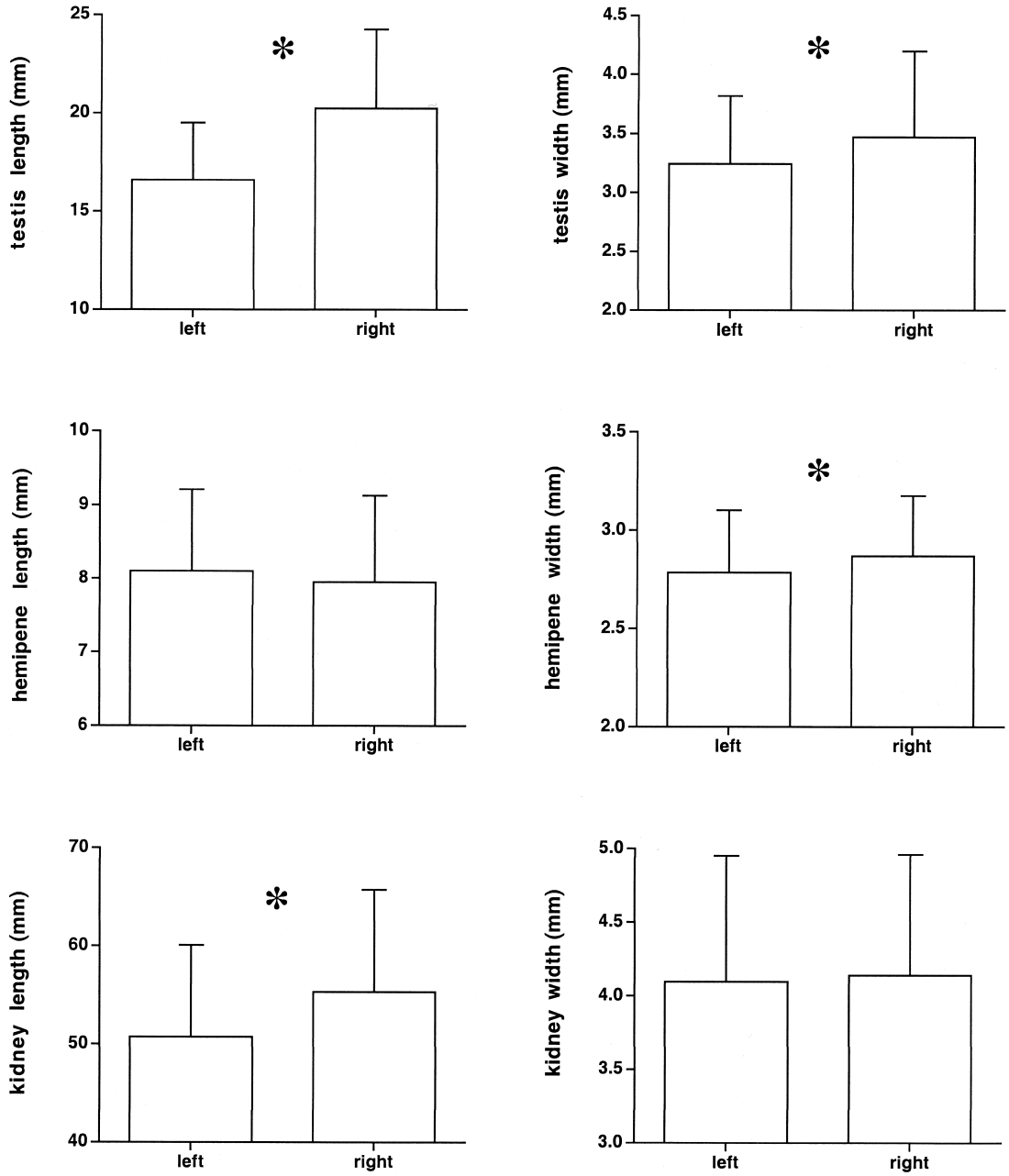

Figure 1
Deviations from bilateral symmetry in the reproductive systems of male gartersnakes, *Thamnophis sirtalis parietalis*. Compared to the equivalent organ on the left-hand side of the body, the right-hand side system comprises a wider hemipenis, a longer and wider testis, and a longer and wider kidney. Hemipenis lengths do not differ between the two sides of the body. *Comparisons are statistically significant ($p < .05$); see text for statistical tests.

test whether operational sex ratio influenced the determinants of male mating success (Shine et al., 2000). Operative temperatures were measured on Hobo-temp data-loggers, at 10-min intervals throughout the study, by means of thermocouples placed inside dead snakes (in May 1997) or in hollow copper tubes (in May 1998) adjacent to the enclosures. For the 1998 trials, we estimated operative snake temperatures from model temperatures, plus information on heating and cooling rates of snakes. Except for a small subset of repeated matings (which are analyzed separately from the other data sets), no individual male or female snake was represented in more than one mating. Thus, all matings comprise statistically independent data.

RESULTS

Asymmetry in size of the reproductive organs

Dissections of 90 adult male snakes revealed significant size differences between organs on either side of the body (Figure 1). The reproductive structures on the right-hand side averaged significantly larger than those on the left. Using paired t tests with 89 degrees of freedom, the right-hand side hemipenis was wider ($t = 2.82$, $p < .006$), and the right-hand testis was both longer ($t = 8.05$, $p < .0001$) and wider ($t = 2.46$, $p < .02$). Hemipenis lengths ($t = 1.63$, $p = .11$) did not differ significantly between the two sides of the body. Asymmetry was

also present in the kidney (larger on the right: length, $t = 4.88$, $p < .0001$; width, $t = 0.40$, $p = .69$).

Asymmetry in usage

Field matings

Within mated pairs found *in copulo* in the field, approximately equal numbers of males used their right versus left hemipenis. In May 1997 we recorded 161 such pairs, 74 of which (46%) involved the right hemipenis (against a null of 50%, binomial test, $z = 0.95$, $p = .34$). In May 1998 we recorded 44 pairs, exactly half of which (22 pairs) involved use of the right hemipenis.

We looked for any correlates of right versus left hemipenis use among these pairs, both in the combined data set and in each year separately, but found no significant differences between matings using either hemipene in terms of body sizes (SVLs, masses) of the male or female (one-factor ANOVAs, $p > .50$ for all traits). Our data also do not reveal any obvious spatial (habitat-associated) variation in hemipenis choice: for matings where we recorded locations of the pairs, usage patterns were similar inside the den (25 left/18 right) versus in the surrounding meadows (21 left/19 right).

Arena trials

In the arena matings recorded in 1997, the right hemipenis was used in 61% (52 of 85) of matings, an almost-significant deviation from the null expectation of 50% (binomial test, $z = 1.95$, $p = .051$). In contrast, the arena matings in 1998 produced almost equal usage of each hemipenis: we recorded a total of 80 matings using the left hemipenis and 79 using the right (49.7% right; against a null of 50%, $z = 0$, $p > .90$). The 2 years did not differ significantly in relative numbers of matings using right versus left hemipenes ($\chi^2 = 2.50$, 1 df, $p = .11$).

We examined these data in more detail to look for any correlates of hemipenis choice. As for the field matings, we detected no significant biases in hemipenis use with respect to male or female body sizes ($p > .50$). However, the more extensive data for the arena trials (since we knew when and where copulation commenced and concluded) allowed us to test additional variables in this way. The duration of copulation was similar in matings involving either hemipenis (means = 13.7 min, SD = 4.97 for the left hemipenis, versus 19.6 ± 11.1 minu for the right, Mann-Whitney $U = 24.0$, $z = 1.13$, $p = .26$). The male's choice of hemipenis did not depend on the numbers of other males with whom he was competing for courtship. The duration of courtship before intromission did not affect the male's choice of hemipenis; this duration varied among trials, but did not differ significantly between matings involving the left versus right hemipenis within any trial ($p > .40$ in all comparisons).

Nonetheless, hemipenis usage in the arena trials was not random: the magnitude of the bias toward "right-handedness" depended on thermal conditions. Males used their left and right hemipenes in approximately equal proportions when the weather was relatively cool, but showed a strong right bias under warmer conditions (Figure 2). This result is most easily seen from a logistic regression with ambient (operative) temperature as the independent variable and right versus left hemipenis usage as the dependent variable. The log-likelihood ratio test for the influence of temperature on hemipenis choice yielded a chi-square value of 6.92 (1 df, $p = .0085$). The pattern was apparent within both years of our study.

The choice of hemipenis use may also depend upon a male's prior mating history. In one set of trials, we allowed males to mate repeatedly with a series of females, so that we could record which hemipene was used in successive matings. The males in these trials showed a strong tendency to alternate hemipenis use when they mated more than once within a single day (alternated in 7 out of 8 cases; against a null of 50/50, $\chi^2 = 4.50$, 1 df, $p < .05$). However, in successive matings that were separated by > 12 h (i.e., on successive days), the same hemipenis was used in two of four cases. Thus, males may alternate hemipenis use primarily when they mate in rapid succession. We also removed mating plugs (Devine, 1975) from these females after each mating, so that we could compare the sizes (masses) of plugs produced from each hemipene. Plugs from the right hemipenis averaged significantly larger (mean = 0.08 g, SD = 0.03, $n = 14$) than those from the left (mean = 0.06 g, SD = 0.03, $n = 11$; $F_{1,23} = 4.62$, $p < .05$). However, mating plugs from field matings did not differ significantly in mass depending on which hemipenis was used ($F_{1,41} = 1.22$, $p = .28$).

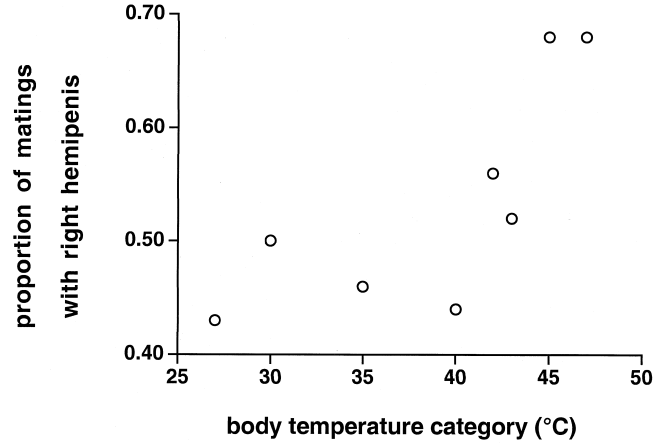

Figure 2
The proportion of gartersnake matings using right versus left hemipenes, as a function of the thermal conditions at the time of coitus. The data on hemipenis use were taken from mating trials in outdoor arenas; the ambient thermal environment was quantified by temperatures inside physical (copper) models placed in full sunlight, a few meters from the arenas. Body-temperature categories were defined so as to include approximately similar sample sizes (number of matings) where possible; thus some temperature intervals are wider than others. Sample sizes for each temperature group (starting with the lowest temperature) were 7, 16, 35, 43, 32, 25, 31, and 28. Spearman rank correlation for these data: $r_s = .81$, $p = .047$.

DISCUSSION

It has been known for a long time that the internal anatomy of snakes displays considerable asymmetry, and this is true for the position (anterior–posterior) as well as for the size of many internal organs such as the testes and kidneys (e.g., Bergmann, 1951, 1958a,b; Wallach, 1991). Our data show that this asymmetry extends to the hemipenis as well, at least in *Thamnophis sirtalis parietalis,* and that it may be associated with nonrandom choice of hemipenis use in this species.

Previous work on these topics has been limited (especially for field matings), but results are consistent with our study. For example, overall usage of the two hemipenes in field matings approximated 50/50, as reported in an earlier study of the same species (Blanchard and Blanchard, 1941); this may be the general rule among squamates (Crews, 1978; Secor, 1987; Tokarz and Slowinski, 1990; Zweifel, 1980, 1997). Despite the overall equal usage of the hemipenes, however, it is clear that the male's selection of his left versus right hemi-

penis for copulation is not random. Previous authors have speculated that male snakes simply use whichever hemipenis is closest to the female's cloaca (e.g., Murphy and Barker, 1980; Secor, 1987). This may often be true, but other factors also play a role.

The most straightforward influence on hemipenis use is the male's prior mating history. Our study suggests that males alternate between usage of their two hemipenes in some circumstances but not in others, depending on the time interval since previous copulation. A tendency for males to alternate use of the two hemipenes, at least when multiple matings are available in quick succession, has been reported in other squamate species (e.g., Olsson and Madsen, 1998; Tokarz and Slowinski, 1990; Zweifel, 1981, 1987). The ultimate selective advantage for this alternation may involve sperm depletion for many species (Tokarz and Slowinski, 1990; Zweifel, 1981, 1987), but this argument cannot be applied to gartersnakes. Male *Thamnophis sirtalis parietalis* produce their sperm several months before mating, and their testes are inactive during the mating season (e.g., Crews and Moore, 1986). However, gartersnakes also transfer kidney secretions (to form the mating plug) when they copulate (Devine, 1975). Hence, there is a substance other than spermatozoa that might potentially be difficult to generate quickly enough for two matings in rapid succession. In keeping with this hypothesis, data from our repeated matings trials (see above) suggest that the mass of the mating plug may be lower from second copulations than from first copulations. Plug mass declined from the first to the second mating in nine males, remained the same in two, and did not increase in any (using the binomial test, $p = .03$). Similarly, the number of sperm transferred per copulation fell rapidly in another squamate species (an anoline lizard) when males were forced to use the same hemipenis for multiple matings in quick succession (Tokarz and Slowinski, 1990).

More surprisingly, hemipenis use was strongly associated with the ambient thermal environment, suggesting that male snakes facultatively adjust their use of hemipenes in relation to thermal influences. We interpret this pattern to mean that snakes prefer to use the larger (right-hand side) hemipenis if they are able to do so without compromising mating opportunities. High body temperatures enhance gross locomotor ability in this species (Heckrotte, 1967) and plausibly also improve the male's ability to make subtle adjustments of posture (such as shifting his tailbase from one side of the female to the other), without losing his position in competition with other males.

Male gartersnakes that use their right hemipenis under such circumstances may be advantaged because the hemipenis is wider and is connected to a larger source of sperm (the testis) and accessory secretions (the kidney). However, the benefits are likely to be modest, especially in relation to the fitness penalty associated with foregoing a favorable position by realigning the body during courtship. Hence, males often use their left hemipenis, especially when they are relatively cold (and thus less able to carry out complex mating maneuvers), or when they have recently expended semen and plug material from the right-hand-side copulatory system.

Although our data set is the most extensive available on asymmetries in structure and function of the male reproductive system in squamates, many puzzles remain, and some of our results were inconsistent among samples. For example, plug mass was higher from right-hemipenis matings in the arenas but not in the field. We do not know why this difference exists. One possibility is that a small number of males obtain a high proportion of matings under field conditions; if so, many of our field-mated males may have already used the same hemipenis in a prior mating. Thus, these males would be likely to produce a smaller mating plug the second time around. If males tend to use the right hemipenis for their first mating, such a trend would decrease any apparent difference in plug masses from matings that relied on right versus left hemipenes. However, this possibility is entirely speculative.

More importantly, our inferences about the fitness benefits of matings using either the right- or left-hand system also remain speculative. We have no direct evidence to show that a larger hemipenis (and associated testis, kidney, and mating plug) is actually a more effective copulatory device than a smaller unit. The nonrandom patterns in hemipenis usage suggest that the asymmetry is not a trivial one, however, and further work to clarify these issues would be of great interest. Indeed, the potentially direct link between handedness and reproductive success in this system (a link that is much weaker with most other examples of lateralization in usage) suggests that this phenomenon may offer a valuable model system with which to investigate the fitness consequences of deviations from bilateral symmetry.

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