

Species-isolating mechanisms in a mating system with male mate choice (garter snakes, *Thamnophis* spp.)

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Abstract: Reproductive isolation between sympatric taxa can be maintained by specific mate-recognition behaviours or by ecological divergence that reduces interspecific contact during reproduction. Common garter snakes, *Thamnophis sirtalis* (L., 1758), and plains garter snakes, *Thamnophis radix* (Baird and Girard, 1853), are sympatric over large areas, but morphological data suggest that the prezygotic isolation between these two species partially breaks down in a severely cold part of their joint range in Manitoba. Courtship trials show that male *T. radix* court intensely over a narrower range of (higher) ambient temperatures than do male *T. sirtalis*. Males selectively court females of their own species, but male *T. radix* are less choosy than male *T. sirtalis*. Hexane extracts of female skin lipids also elicited species-specific courtship. Although this male preference for species-specific pheromones contributes to species isolation, it is not strong enough to completely separate the two taxa. The absence of hybridization over most of the sympatric range may depend on the timing of mating (early spring, near the hibernation den). Differences between the species in hibernation-site selection and the timing of spring emergence break down in central Manitoba because severely cold winter temperatures force both species together into the few available hibernation (and thus, mating) sites, and the short warm season reduces temporal separation in emergence (and thus, mating) seasons.

Résumé : Les comportements de reconnaissance spécifique des partenaires et la divergence écologique qui réduit les contacts interspécifiques durant la reproduction peuvent maintenir l'isolement reproductif entre des taxons sympatriques. Les couleuvres rayées, *Thamnophis sirtalis* (L., 1758), et les couleuvres des plaines, *Thamnophis radix* (Baird et Girard, 1853), vivent en sympatrie sur un important territoire; des données morphologiques indiquent que l'isolement prézygotique entre les deux espèces est partiellement réduit dans une zone particulièrement froide de leur aire de répartition commune au Manitoba. Des expériences montrent que les mâles de *T. radix* ont des comportements de cour intenses sur une gamme plus étroite de températures ambiantes (plus élevées) que les mâles de *T. sirtalis*. Les mâles courtisent de façon sélective les femelles de leur espèce, mais les mâles de *T. radix* sont moins minutieux que ceux de *T. sirtalis*. Des extraits à l'hexane des lipides de la peau des femelles stimulent aussi des comportements de cour spécifiques à l'espèce. Bien que cette préférence des mâles pour les phéromones spécifiques contribue à l'isolement génétique, elle n'est pas assez forte pour isoler complètement les deux taxons. L'absence d'hybridation sur la majorité de l'aire de sympatrie peut s'expliquer par le moment de l'accouplement (tôt au printemps, près du terrier d'hibernation). Les différences de choix de site d'hibernation entre les deux espèces et de moment de l'émergence au printemps ne tiennent plus dans le centre du Manitoba parce que les températures très froides de l'hiver obligent les deux espèces à partager le petit nombre de sites d'hibernation (et donc de reproduction) disponibles et parce que la courte saison de chaleur réduit la séparation temporelle entre les périodes d'émergence (et donc d'accouplement).

[Traduit par la Rédaction]

Introduction

Although intense controversy surrounds the topic of species concepts, all biologists recognize that living organisms are (mostly) organized into a finite array of distinctive entities which we call species. Clarifying the nature of mechanisms that maintain species isolation has been a major theme

of evolutionary research (Mayr 1942; Panhuis et al. 2001; Turelli et al. 2001). Paradoxically, one of the best opportunities to understand such mechanisms comes from the situation where they break down. If two species hybridize in one part of their range but not in others, then we may be able to identify factors that affect the effectiveness of the isolating barriers. That is, incomplete barriers to species separation

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may be more informative about the nature of such barriers than are cases of total reproductive isolation (Mayr 1942; Dobzhansky 1951).

Many such cases have been explored, notably "ring species" (Mayr 1942; Dobzhansky 1951; Irwin et al. 2001). The work has provided many examples of the role of mate-recognition systems in preventing hybridization and clarified the complex selective forces operating on such systems (Panhuis et al. 2001; Via 2001). In almost all cases, however, such studies have focused on female preference for male traits. For example, female birds may select mates based on species-specific adornments and female frogs may respond only to males that give species-specific calls (Ryan and Wilczynski 1991; Ligon 1999). Reptiles have played little role in species-isolation studies (but see Losos 1985; Cooper and Vitt 1987; Shine et al. 2002), but they offer an opportunity to clarify these processes in very different systems. For example, male garter snakes (species of the genus *Thamnophis* Fitzinger, 1843) are highly selective courters (Mason 1993; Shine et al. 2001, 2003b; LeMaster and Mason 2002a, 2002b), and hence, species isolation might be maintained by choosy males rather than by choosy females. A diverse literature has explored topics such as asymmetries in species-isolating mechanisms under the assumption that females control mating (e.g., Kaneshiro and Giddings 1987; Wirtz 1999), but systems with male mate choice might produce very different outcomes or the same outcomes for different reasons (e.g., Ehmann and Wasserman 1987).

The natricine snake lineage displays extraordinary species diversity in North America, especially considering its relatively recent invasion of this continent (de Quieroz and Lawson 1994; Rossman et al. 1996). Occasional breakdown of species isolation has been reported (Rossman et al. 1996), suggesting that the processes at work within such a situation might well clarify (i) the ways in which snake species retain their separate identities in sympatry and (ii) the role of male mate choice rather than female mate choice in this respect. Accordingly, we have examined mate-recognition systems in two sympatric species of garter snakes. Our preliminary studies suggested that prezygotic isolation between the two species involved (*Thamnophis radix* (Baird and Girard, 1853) and *Thamnophis sirtalis* (L., 1758)) might break down at a site near the northern (cold-climate) limit of their geographic distributions. Thus, we gathered extensive morphological data on a range of den populations of garter snakes in this region to see whether there was indeed evidence of hybridization and conducted arena trials to examine the cues that stimulate courtship by male garter snakes of both species.

Materials and methods

Study species

Red-sided garter snakes, *Thamnophis sirtalis parietalis* (Say in James, 1823), and western plains garter snakes, *Thamnophis radix haydeni* (Kennicott, 1860), are phylogenetically distant from each other within the North American natricine radiation; indeed, they belong to separate major clades within this lineage (de Quieroz and Lawson 1994). Nonetheless, these two taxa are broadly similar to each other in body sizes, colouration, behaviour, and ecology. Both

grow to approximately 1 m in body length, with females being larger than males (Rossman et al. 1996). Diets of the two species are geographically variable but broadly similar (often, frogs and worms), with only minor interspecific differences in habitat use (Hart 1979; D. Platt, personal observation, in Rossman et al. 1996). Both species return to communal hibernacula to overwinter and mate in early spring near those dens (Davis 1936; Aleksiuik and Gregory 1974; Hart 1979; Rossman et al. 1996). They generally select different dens but have been reported to use the same hibernacula in the Interlake region of Manitoba (Gregory 1977; Hart 1979).

Both species have extensive geographic distributions. The plains garter snake (*T. radix*) ranges from southern Alberta through to Colorado and from New Mexico and Oklahoma through the Great Plains to Indiana. The common garter snake (*T. sirtalis*) occupies an even larger range, throughout most of southern Canada and all of the USA except for the western deserts (Rossman et al. 1996). Despite this wide sympatry, one species is often much more abundant than the other in any particular region. For example, Lake Manitoba is a very long (190 km) and often wide (to 40 km) lake that runs north-south through south-central Manitoba. *Thamnophis radix* is much less common than *T. sirtalis* east of the lake (<1% vs. >99% of garter snakes at dens in the Inwood area; R. Shine, personal observation), whereas *T. radix* is common west of the lake (Gregory 1977; Hart 1979; Rossman et al. 1996; D. Karst, personal communication).

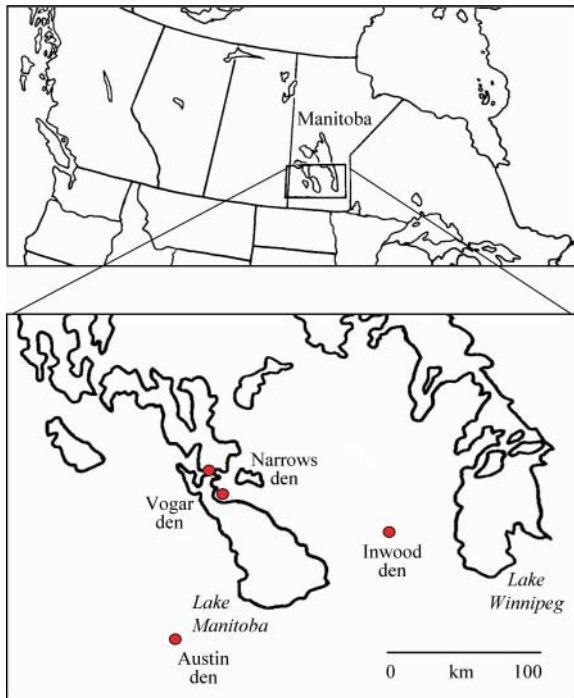
Study area

On 15 and 16 May 2001, we obtained samples of *T. radix* from southwestern Manitoba (Austin; 49°56'N, 98°56'W) and *T. sirtalis* from the Interlake of south-central Manitoba (Inwood; 50°32'N, 97°30'W; see Fig. 1). In neither location did we find the other species, although previous records indicated that both taxa occurred in both areas. Because *T. radix* is relatively common west but not east of Lake Manitoba, on 16 May we also looked for (and found) a den in the Narrows (51°10'N, 98°45'W), which is a small isthmus of land that connects the eastern and western shores of this lake about halfway down the lake's north-south length (Fig. 1). Although both species of garter snakes have been recorded at this site (D. Karst, personal communication), we found only *T. sirtalis*, perhaps because this species remains near the den for longer in spring than does *T. radix* (Gregory 1977). To evaluate the possibility of hybridization between the two species at this contact zone, we took a further sample of garter snakes from a den 10 km east of the Narrows (Vogar; 51°15'N, 98°30'W; Fig. 1), where only *T. sirtalis* had been observed (R. Shine, personal observation). The *T. sirtalis* dens at Vogar, Inwood, and the Narrows all comprised limestone outcrops with associated quarries and had large numbers of garter snakes (>10 000) emerging from very small areas (<0.2 ha). In contrast, the *T. radix* den at Austin involved a more diffuse system of burrows in sandy soil, with fewer garter snakes (<200) over a broader area (>2 ha).

Morphology

We scored traits that have been used to distinguish between *T. radix* and *T. sirtalis* in published literature (e.g.,

Fig. 1. Maps showing the locations of garter snake, *Thamnophis* spp., dens used in this study.



Rossman et al. 1996). These included number of midbody scale rows, number of dorsal scale rows (above the ventrals) that form the lower margin of the yellow lateral stripe, proportions of red colouration in skin between scales in the neck region, colour of middorsal stripe (yellow or orange), presence or absence of black bars anterior to the eye or on the loreal scale, and degree of fragmentation of the dark lateral line immediately above the ventrals. To maintain consistency in scoring, we retained a range of individual garter snakes as “standards” to typify each condition. We allocated individual specimens to species based upon these criteria and we also used the overall morphological data set to explore the possibility that garter snakes from the contact zone (Narrows’ population) included hybrids between *T. radix* and *T. sirtalis*. Statistical comparison using so many variables would create an unacceptably high incidence of artifactually “significant” results (Cabin and Mitchell 2000), so we reduced the number of variables by entering the morphological data into a principal components analysis (PCA). The variances in the data on proportions were normalized by a square-root arcsine transformation. The resulting data were subjected to factor analysis using StatView[®] version 5 (SAS Institute Inc. 1998). An orthogonal transformation of the initial factor solution was performed to produce an oblique solution reference structure. We retained roots >1 to identify principal components.

Courtship trials

On 17 May 2001, we erected 18 open-topped nylon arenas (1 m × 1 m × 1 m) on flat ground at our field laboratory near Chatfield, Manitoba. We placed four adult male garter snakes in each arena. Six of the arenas (randomly selected) contained male *T. sirtalis* from Inwood, another six contained male *T. sirtalis* from the Narrows, and the remaining

six arenas contained male *T. radix* from Austin. Within each of these groups, three arenas contained relatively small males (less than the mean size for the overall sample), whereas the other half contained larger animals. We included male body size as a factor in our design because previous work has shown that body size affects courtship selectivity in *T. sirtalis* (i.e., small males court small females, as well as large females, whereas large males court only large females; Shine et al. 2001). Apart from body size, males were collected and allocated to arenas randomly. Thus, the incidence of hybrids in our samples of courting males should be similar to the proportions in each den.

All males within each arena were colour-marked on their dorsal surfaces for individual recognition. We then took six adult females from each of the populations, taped their cloacal regions to prevent mating, and placed one female into each (randomly selected) arena at midday. We scored the intensity of courtship by each of the males to the “target” female on a four-point scale: 0 is no interest, 1 is tongue-flicking the female’s body, 2 is chin-pressing against the female, and 3 is body aligned with that of the female (Whittier et al. 1985). We recorded these behaviours three times, with 5 min between successive readings. After 15 min, each female was removed and transferred to an adjacent arena and the recording process repeated for this new combination of animals. Over the next few hours, we thus obtained data on the intensity of courtship directed by each male to each of the “target” females. All garter snakes were released unharmed at the conclusion of the trials. Our analyses used only the most intense courtship level recorded for each male to each female (i.e., the highest over the three readings per female). We treated the courtship score as a continuous variable to simplify the presentation of our results; the patterns were so strong that this approximation had no effect on our conclusions.

Additional trials in May 2004 were conducted to explore the effect of ambient temperatures on courtship intensity. Twenty groups of four (individually paint-marked) males plus one female of the same species (10 groups of *T. sirtalis* from Vogar, 10 groups of *T. radix* from Winnipeg) were set up in circular open-topped nylon arenas, 1 m in diameter, in a room with air temperature at 17 °C but with the floor temperature of each arena determined by subfloor heating pads (15–40 °C in the middle, grading to room temperature at the periphery). Temperatures remained constant (±1 °C) during each trial; temperature treatments were allocated randomly and each garter snake was used in only a single trial. At 5-min intervals for the next 50 min, we scored the numbers of males courting the female and the courtship intensity of each male (on a four-point scale; Whittier et al. 1985).

Response to skin lipids

In *T. sirtalis*, hexane-soluble skin lipids serve as sex pheromones (Mason 1993). To see whether such cues were responsible for species-specific courtship, we soaked paper towels in hexane and wiped each towel four times in an anterior-to-posterior direction along the dorsal surface of an adult female garter snake. The towel was then presented to an adult male garter snake and we recorded his response when he first encountered the stimulus. The same scoring system was used as for courtship to real females (above); al-

Table 1. Morphological traits of garter snakes from four populations in Manitoba.

	<i>Thamnophis sirtalis</i>							
	<i>Thamnophis radix</i> from Austin		Narrows		Vogar		Inwood	
	Male	Female	Male	Female	Male	Female	Male	Female
Sample size (<i>n</i>)	55	29	73	33	45	26	72	51
Snout-vent length (cm)	49.9±1.5	49.3±3.0	46.1±0.8	63.9±2.1	49.5±0.7	56.4±1.3	44.3±0.6	51.7±1.2
Residual tail length	0.68±0.15	-0.85±0.17	0.51±0.14	-0.52±0.21	0.66±0.14	-0.55±0.20	0.28±0.09	-1.39±0.09
Mid-body scale rows	20.9±0.1	20.8±0.1	19.0±0.0	19.0±0.0	19.0±0.0	19.0±0.0	19.0±0.0	19.0±0.0
Scale row for stripe	3.0±0.0	3.0±0.0	1.84±0.05	1.91±0.05	1.99±0.01	1.96±0.04	1.99±0.01	2.0±0.0
Proportion non-red skin	100±0	100±0	59.7±4.4	57.9±7.4	26.9±4.8	24.6±6.3	16.1±2.0	14.9±2.1
Proportion of snakes with								
yellow dorsal stripe	0.00	0.03	0.99	1.00	1.00	1.00	1.00	1.00
bar anterior to eye	0.11	0.21	0.01	0.00	0.00	0.00	0.00	0.00
bar on loreal	0.82	0.86	0.01	0.00	0.02	0.00	0.01	0.04
Degree of fragmentation of lateral line	76.7±3.2	86.6±3.1	39.6±3.0	25.5±3.9	19.3±1.9	23.9±3.6	20.8±1.6	25.5±2.5

Note: Values are means ± SE. See text for definition of variables. Residual tail length is the residual score from the linear regression of ln(tail length) versus ln(snout-vent length). Scale row for stripe is the number of lateral scales above ventrals containing yellow lateral stripe.

though body alignment was seen only rarely to the paper towels, chin-pressing was common. Each male was tested with the scent of females of both species, plus a control (hexane-only) treatment (presented in random order). In the case of *T. sirtalis*, we conducted the hexane trials on 180 free-ranging garter snakes at the Inwood den. This was done by clearing an area of courting males, lying a paper towel on the ground, and then recording the responses of the first 6 small (<45 cm snout-vent length (SVL)) and the first 6 large (>45 cm SVL) males who moved into positions so that their heads were directly over the stimulus. We used five replicate towels for each stimulus type (i.e., 5 female *T. sirtalis*, 5 female *T. radix*, 5 hexane). Given the huge numbers of males at this den (>10 000), inadvertent retesting of the same male more than once was unlikely to have occurred. We could not do this with male *T. radix* because of the diffuse nature of the Austin den, so we used a repeated-measures design instead and presented the paper towels to 6 male garter snakes in outdoor arenas. Order of presentation was randomized. Each male was exposed to each of the three stimuli three times and the analysis was based only on the most intense response to each stimulus.

The research was conducted under the authority of the Oregon State University Institutional Animal Care and Use Committee protocol No. LAR-1848B. All research was conducted in accordance with the US Public Health Service "Policy on Humane Care and Use of Laboratory Animals" and the National Institutes of Health "Guide to the Care and Use of Laboratory Animals".

Results

Morphology

Unsurprisingly, *T. radix* differed substantially from the three *T. sirtalis* populations that we sampled (Table 1). Body sizes were similar, but *T. radix* had more midbody scale rows, had its yellow lateral stripe on scale row 3 rather than on scale row 2, had no red skin between the scales on the neck, had an orange rather than yellow dorsal stripe, had black bars anterior to the eyes, and had dark lateral lines that were fragmented rather than continuous (Table 1). We en-

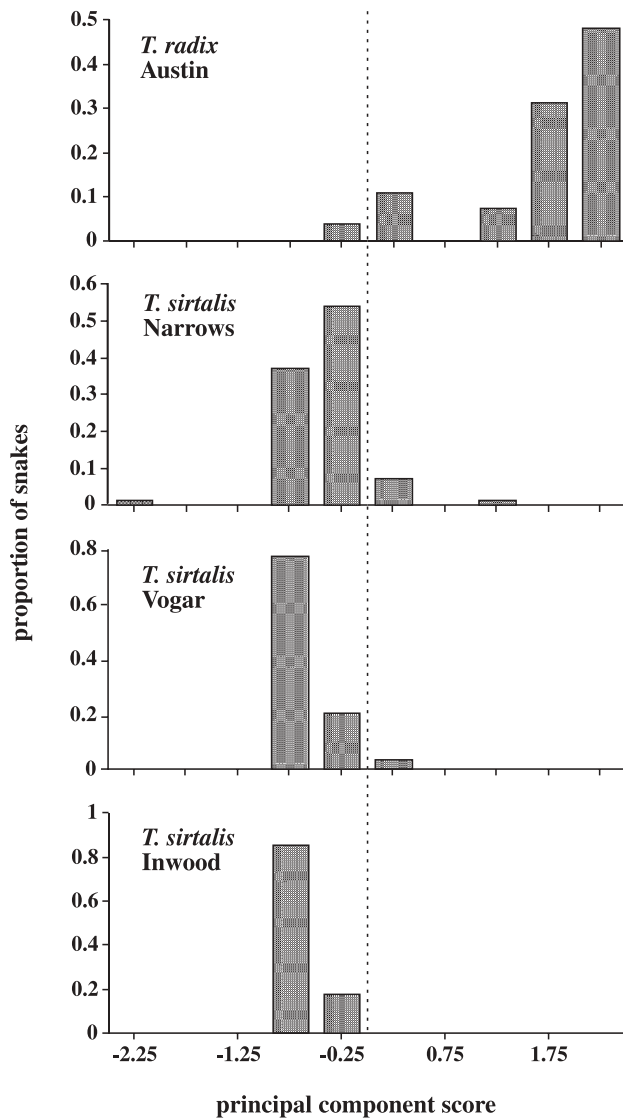
tered all of these traits into a PCA, and the first axis of variation, which explained 67% of the morphological variation within this data set, corresponded to this interspecific separation (Fig. 2). This first (interspecific) axis also separated the four populations clearly (ANOVA on PC axis 1, $F_{[3,380]} = 706.26$, $P < 0.0001$). Fishers' PLSD post hoc tests showed that all four populations differed significantly from each other on this axis ($P < 0.005$) except for Inwood vs. Vogar ($P = 0.62$).

The most important result from this analysis is that garter snakes from the Narrows were intermediate between *T. radix* and "pure" *T. sirtalis* (i.e., the Interlake samples from Inwood and Vogar; see Fig. 1) and differed significantly from both these groups. Garter snakes from the latter dens were very similar morphologically, despite the fact that Vogar was much closer to the Narrows than to Inwood. These data thus suggest that *T. sirtalis* and *T. radix* hybridize to a limited degree when they come into contact in the Narrows. Nonetheless, only a single animal in our samples (a male from the Narrows) was an obvious intermediate (= hybrid?), which failed to key out clearly to either species using available keys (Rossman et al. 1996). This animal (male with a 42.4-cm SVL) had several features characteristic of *T. radix* but not seen in our extensive sample of Interlake *T. sirtalis*. These included an orange middorsal stripe (rather than yellow as in *T. sirtalis*), a black bar on the loreal scale (absent in *T. sirtalis*), a highly fragmented dark lateral line immediately above the ventrals (a score of 8 on a 10-point scale vs. a score of <3 for most *T. sirtalis*), a yellow stripe on lateral scale row 3 (vs. on lateral scale row 2 for *T. sirtalis*), and yellow skin between the scales of the neck (rather than red as in *T. sirtalis*). Nonetheless, other major features of this animal resembled *T. sirtalis* rather than *T. radix* (19 midbody scale rows, no black bar immediately anterior to the eye). Other multivariate tests applied to the same morphological data generated the same conclusions; e.g., cluster analysis placed two of the Narrows garter snakes with the *T. radix* (Austin) sample rather than with other *T. sirtalis* samples.

Courtship trials

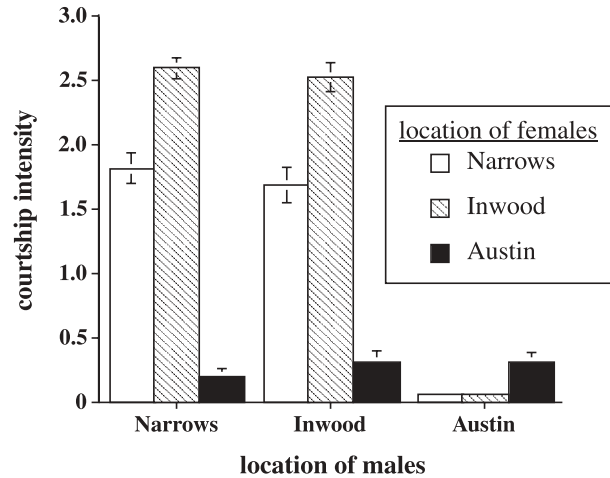
Garter snakes in the arenas displayed intense courtship,

Fig. 2. Scores on the first principal component of the morphological variation for garter snakes from four populations in Manitoba. See text for description of methods used to collect and analyze statistically these data. See Table 1 for sample sizes (n).



with male *T. sirtalis* being more vigorous in this respect than were male *T. radix* (Fig. 3). Male *T. sirtalis* from both Inwood (virtual allopatry) and the Narrows (contact zone) strongly courted female *T. sirtalis* from both of these locations but virtually ignored female *T. radix* (Fig. 3). To avoid pseudoreplication, we analyzed these data using a nested ANOVA with female identification number nested within female source location (Inwood, Narrows, or Austin), and we calculated and used mean values for each group of males in each enclosure rather than treating the behaviour of each male as an independent datum point. All effects and interactions were tested against the nested term (variation in courtship intensity to females within each location) rather than the residual error term. ANOVA with source of males and females as the factors revealed a significant interaction term ($F_{[4,15]} = 30.37$, $P < 0.0001$). Male *T. radix* were less selective courters than were male *T. sirtalis* (Figs. 3, 4). To eval-

Fig. 3. Intensity of courtship by male garter snakes in trials in outdoor arenas. Data are shown separately for male *Thamnophis sirtalis* from dens at Inwood and the Narrows and for male *Thamnophis radix* from Austin, and indicate mean courtship levels to females from each of these three populations. The histograms show mean scores for courtship intensity and the bars show 1 SE on either side of the mean. Sample size is 12 males for each category; see text for statistical analysis.



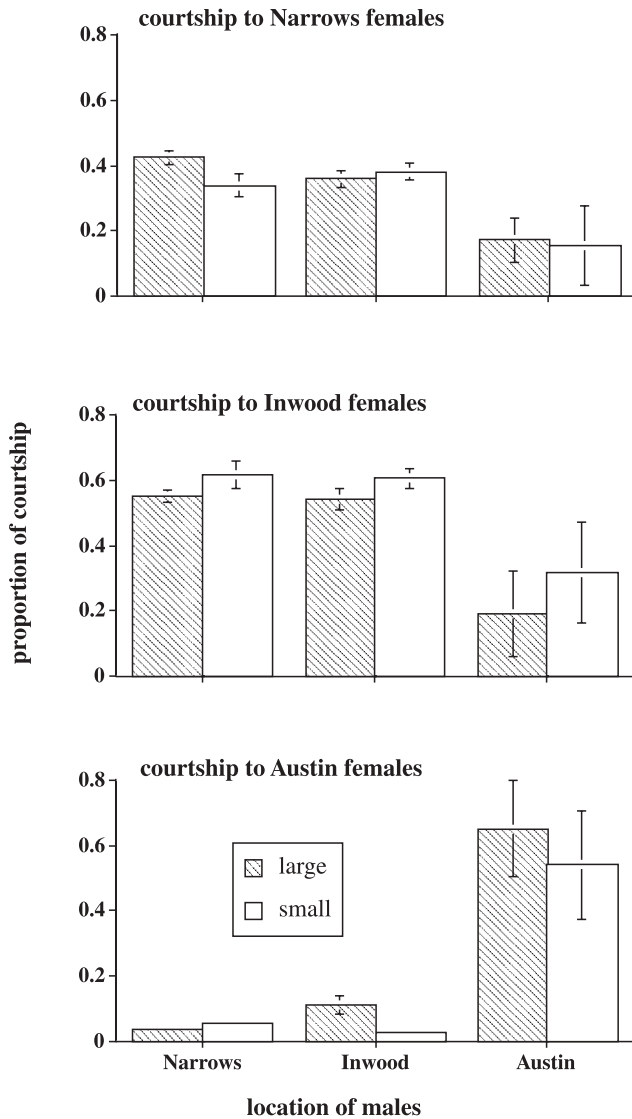
uate the statistical strength of this result, we repeated the nested ANOVA with two factors: male species and whether or not the female was of the same species as the male. This ANOVA generated a highly significant interaction term ($F_{[1,16]} = 121.09$, $P < 0.0001$), confirming that males of the two species differed in their relative courtship intensity to females of their own species versus those of the congeneric taxon. Including male body size as a dichotomous variable in the above nested ANOVA showed a nonsignificant trend for large males to court more actively than small males ($F_{[1,15]} = 3.73$, $P = 0.07$) and no interaction between male body size and any other factor (all $P > 0.33$). Male garter snakes of both taxa and both size classes directed more intense courtship to females of their own species than to heterospecific females (Fig. 4).

Male *T. sirtalis* courted intensely at every thermal regime that we tested, but male *T. radix* courted actively only at higher temperatures (Fig. 5). Thus, ANCOVA with species as the factor and floor temperature as the covariate showed significant differences between the two species in the relationship between courtship intensity and ambient temperature (for the number of males seen courting, $F_{[1,16]} = 12.49$, $P < 0.003$; for the proportion of observations when courtship was seen, $F_{[1,16]} = 18.11$, $P < 0.0001$; for the total number of courtship records, $F_{[1,16]} = 8.76$, $P < 0.01$; for the maximum intensity of courtship recorded per trial, $F_{[1,16]} = 4.60$, $P < 0.05$).

Response to skin lipids

Trials with hexane-soaked paper towels also revealed species-specific courtship by male garter snakes. The hexane control attracted little attention, whereas lipids from conspecific females were more effective (Fig. 6). Lipids from heterospecific females attracted an intermediate level of

Fig. 4. Proportional intensity of courtship by large (>45 cm snout-vent length (SVL)) and small (<45 cm SVL) male garter snakes in outdoor arenas to female garter snakes from three locations. Data are shown separately for male *T. sirtalis* from dens at Inwood and the Narrows and for male *T. radix* from Austin, and indicate the proportion of overall courtship (i.e., proportion of the summed total of all courtship scores in all trials by that group of males) to females from each of these three populations. The histograms show mean scores for proportional courtship and the bars show 1 SE on either side of the mean. Sample size is 24 males for each category; see text for statistical analysis.



courtship (Fig. 6; ANOVA on courtship intensity: for male *T. sirtalis*, $F_{[2,177]} = 114.67$, $P < 0.0001$, post hoc tests show that all stimuli differ at $P < 0.001$; for male *T. radix*, repeated measure $F_{[2,10]} = 131.36$, $P < 0.0001$, post hoc $T. sirtalis = \text{hexane}$; others differ at $P < 0.001$). For *T. sirtalis*, the inclusion of male body size as a dichotomous factor in the analyses did not explain significant additional variation in male responses (main effect of size, $F_{[1,174]} = 0.38$, $P = 0.54$; interaction, size \times stimulus, $F_{[2,174]} = 0.02$, $P = 0.98$).

Fig. 5. Courtship intensity of male garter snakes to females of their own species as a function of ambient thermal conditions (floor temperature). Courtship intensity was scored as the proportion of 10 observation sessions (5 min apart) in which active courtship was seen in each group of four male garter snakes plus a conspecific female. The figure shows one point for each group of garter snakes and the lines show least squares regressions of best fit. Total sample size is 40 males and 10 females of each species (4 males plus 1 female per datum point; 2 points obscured).

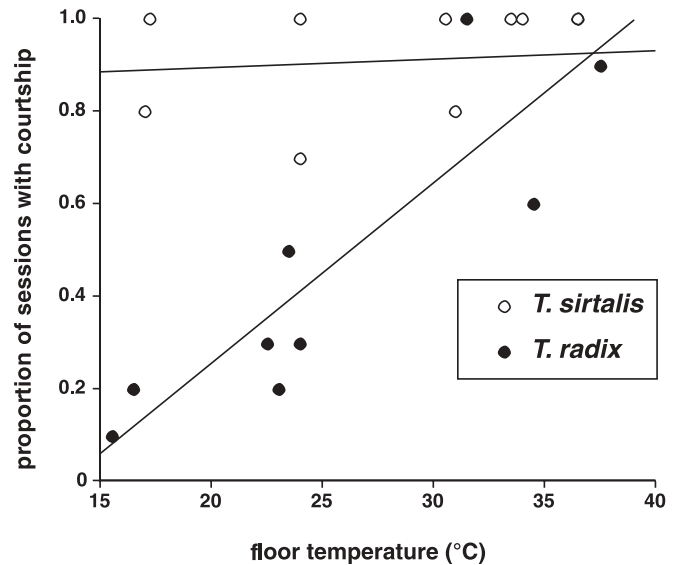
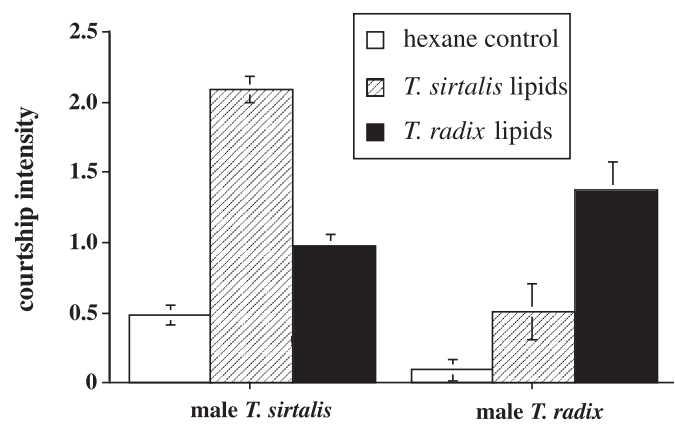


Fig. 6. Courtship intensity of two species of male garter snakes, *T. radix* and *T. sirtalis*, directed towards paper towels brushed with hexane (as a control) or with hexane-containing skin lipids of females of either species. The histograms show mean scores for courtship intensity and the bars show 1 SE on either side of the mean. Sample size is 180 male *T. sirtalis* tested while free-ranging in the Inwood den and 6 male *T. radix* tested in outdoor arenas; see text for statistical analyses.



Discussion

Our morphological data suggest that *T. radix* and *T. sirtalis* interbreed to a limited degree in a contact zone in central Manitoba, although the two taxa are sympatric without any

reports of hybridization over a very large area in other parts of their range. Nonetheless, *T. radix* has been reported to hybridize with *Thamnophis marcianus* (Baird and Girard, 1853) in Kansas (Smith 1946) and with *Thamnophis butleri* (Cope, 1889) in Wisconsin (Rossman et al. 1996; G. Burghardt, personal communication), so the situation in Manitoba may not be unusual. An additional behavioural trait supports the hypothesis that *T. radix* genes have penetrated the Narrows *T. sirtalis* population: the antipredator tactics of these garter snakes are more like those of *T. radix* than is the case for Interlake *T. sirtalis* populations (Shine et al. 2003a).

Mate-recognition systems differ between the two taxa, with the species-specificity of courtship by males of both species providing an obvious barrier to hybridization. This divergence in male responses presumably tracks a phylogenetic divergence in female sex pheromones (skin lipids) between *T. radix* and *T. sirtalis*; in sea snakes, such divergence maintains reproductive isolation between closely related sympatric species (Shine et al. 2002). Nonetheless, the behavioural barrier between *T. radix* and *T. sirtalis* is insufficient to reproductively isolate these two taxa, because males respond (albeit at a low intensity) to females of the “wrong” species (Figs. 3, 4). This is particularly true of male *T. radix*, which perhaps explains why there are more reports of hybridization involving *T. radix* than *T. sirtalis* in spite of the much greater geographic range of the latter taxon (see above). Nonetheless, male *T. sirtalis* in our study courted female *T. radix* and skin lipids from female *T. radix* (Figs. 4, 6). Field observations by Gregory (1977) were crucial in this respect, as he reported two instances of female *T. radix* being actively courted by male *T. sirtalis* at a communal den. One of us (R.T.M.) has seen the same phenomenon in Interlake dens.

Interspecific matings may be rendered more, not less, likely by species differences in the thermal conditions required to elicit courtship. In arena trials, male *T. sirtalis* courted actively even at low substrate temperatures, whereas male *T. radix* courted actively only when they were hot (Fig. 5). At first sight, this difference might seem likely to reduce interspecific courtship, and thus, mating. However, the primary effect will be that under some thermal conditions most or all courtship will be by males of one or the other species, even when both are present; our observations in outdoor arenas showed a decrease in *T. sirtalis* courtship at very high temperatures, although no such decrease was evident over the thermal range used in our indoor experiments. Because female cooperation is not essential for mating (Shine et al. 2003c), matings may ensue despite the level of female receptivity. This thermally driven divergence in the times and places for courtship may reduce the intensity of competition between males of the two species. Because larger males generally achieve more matings (Shine et al. 2001), the (relatively small) male *T. sirtalis* would be unlikely to defeat the (larger) male *T. radix* in head-to-head courtship. However, any female *T. radix* emerging in cold weather likely would be subject to vigorous courtship by male *T. sirtalis* but not by conspecific male *T. radix*. Similarly, in hot weather a female *T. sirtalis* likely would be courted more vigorously by male *T. radix* than by male *T. sirtalis*, even though under ideal conditions males preferentially court females of their own species.

Why then is hybridization not more common? Female choice cannot explain this paradox, because garter snake mating involves not only mate choice by males rather than females, but also coercion by males (Pfrender et al. 2001; Shine et al. 2001, 2003c). Hence, interspecific matings could result even if females are reluctant to copulate. A high incidence of interspecific hybridization in ducks and geese (Anseriformes) relative to other avian lineages (Grant and Grant 1992) may result from the high frequency of forcible insemination in these birds (Seymour 1990; Wirtz 1999). The unusual mating system of garter snakes also is important. These garter snakes gather in communal dens to overwinter and mate immediately after spring emergence before leaving the vicinity of the den (Gregory 1974; Gregory and Stewart 1975). Over most of the joint range of *T. sirtalis* and *T. radix*, winters are sufficiently mild that garter snakes do not have to penetrate deep underground to avoid freezing temperatures. A wide range of suitable sites is thus available and the two species appear to select different attributes in this regard. For example, our observations of *T. radix* at Austin (above) support Gregory’s (1977) report that *T. radix* rarely use large communal hibernacula like those of *T. sirtalis*. Additionally, Gregory (1977) found that where *T. radix* and *T. sirtalis* do share communal hibernacula in the Interlake, *T. radix* disperse from the den before *T. sirtalis*. Hence, reproductive animals of these two taxa may be separated both spatially and temporally over most of their joint distribution.

The severely cold winters of central Manitoba greatly reduce the numbers of hibernacula that are deep enough to permit overwinter survival, resulting in garter snakes from large areas congregating in the few suitable sites (Aleksiuk and Gregory 1974; Gregory 1974; Gregory and Stewart 1975). Hence, both species can be found in the same den within the Interlake region (Gregory 1977). The Manitoba climate also reduces the possibility of any thermally driven temporal separation in emergence times between the taxa. Air temperatures rise very rapidly in spring (both minimum and maximum temperatures are typically >5 °C higher on 30 May than on 1 May; Environment Canada weather data for Lundar, Manitoba). Thus, both spatial and temporal overlap between reproductive activities of *T. sirtalis* and *T. radix* will be greater in Manitoba than elsewhere. Although *T. radix* disperse from the den earlier than *T. sirtalis* (Fitch 1965; Rossman et al. 1996), male *T. sirtalis* emerge even earlier (Rossman et al. 1996), and then remain near the den to intercept emerging females (Gregory 1974; Gregory and Stewart 1975). Thus, a female *T. radix* using a common den site with male *T. sirtalis* would attract courtship as she emerged, although not as much as would a female *T. sirtalis* in the same situation.

Our study thus suggests that the effective species isolation between these two garter snake species over most of their range is a function of two factors. First, species-specific sex pheromones result in most but not all courtship being directed towards conspecific rather than heterospecific females. The unusual feature here is that it is males rather than females that are the choosy sex. In turn, this may reflect the male’s ability to copulate (and transfer an effective mating plug, etc.) only a limited number of times within a season (Shine et al. 2001). It remains possible that females also ex-

ert choice in this respect, although it is difficult to see how a female submerged within a writhing mating ball of >100 conspecific plus heterospecific males could control the identity of the male with which she copulated. Our study also suggests an important role for weather-mediated spatial and temporal separation of reproductive activities in the two taxa. It is only in the extreme cold of Manitoba that the scarcity of suitable winter refugia and the steep rise in temperatures during early spring bring reproductive animals together at the same place and the same time. In combination, these factors apparently lead to a partial breakdown in prezygotic isolation between the two species.

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