

Low Temperature Dormancy Affects the Quantity and Quality of the Female Sexual Attractiveness Pheromone in Red-sided Garter Snakes

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Abstract Low temperature dormancy is a necessary requirement of the annual cycle of most nonmigratory, temperate vertebrates. The red-sided garter snake, *Thamnophis sirtalis parietalis*, overwinters in communal dens during its prolonged winter dormancy (8 mo), and upon emergence, reproductive behavior of both sexes is maximal. Previous work on this species showed that male courtship behavior is maximally induced after simulated low temperature dormancy. The purpose of this study was to determine whether low temperature dormancy affects the pheromone profiles of individual female red-sided garter snakes. We collected females in the fall at den sites in Manitoba, Canada, and extracted pheromones from individuals at three different time points: fall (field), winter (lab), and spring (lab). Total skin lipid and pheromone fraction masses increased from fall to spring, and pheromone profiles were distinctly different in the fall and spring. Pheromone profiles became dominated by the long-chain, unsaturated methyl ketone components of the blend by the time snakes emerged in the spring. Further, the amounts of both saturated and unsaturated components increased from fall to spring, suggesting significant sex pheromone synthesis was induced by low temperature dormancy.

Keywords Attractivity · Gas chromatography-mass spectrometry · Hibernation · Reproduction · Seasonality · Sex pheromone · *Thamnophis sirtalis parietalis*

Introduction

Temperate animals display seasonal regulation in sexual behavior and physiological states, which can be controlled or signaled by biotic (e.g., hormones) and abiotic (e.g., temperature, photoperiod) factors. In species that display seasonal reproductive patterns, both sexes show changes in their reproductive state that reflect the season. For instance, sexual signals (e.g., plumage, behavior, pheromones) are maximally expressed to overlap with the peak of reproductive effort, as is seen in the majority of avian taxa that display sexually dimorphic plumage almost exclusively within the breeding season (Andersson 1994). Many temperate vertebrates exhibit seasonal changes in hormones, such as melatonin, which can initiate reproductive activity and cause changes in external signals, such as pelage (e.g., hamsters, Hoffmann 1978; weasels, Rust and Meyer 1969). The production of sex-specific, external chemical signals (pheromones) primarily is controlled seasonally by biotic factors (hormones) in both invertebrate and vertebrate taxa (e.g., PBAN in moths, Raina et al. 1989; progesterins and androgens in newts, Moore 1978; Iwata et al. 2000a). The role of abiotic factors, such as temperature and photoperiod, is less well understood in the regulation of sex pheromone release and/or synthesis.

The effects of photoperiod on pheromone synthesis and expression have been studied more extensively than those induced by temperature, and most of the work has been done with insects, especially the Lepidoptera (e.g., Raina and Klun 1984; Choi et al. 1998; Foster 2000). In vertebrates, photoperiod affects at least the expression of pheromones in specific taxa (e.g., rabbits, *Oryctolagus cuniculus*, Hudson and Distel 1990). There is a paucity of studies on the effects of temperature on pheromone production and expression in animals, though examples of

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the lack of temperature effects on pheromone production can be found (e.g., Pandey and Pandey 1990). Certain insects exhibit pheromone-releasing behaviors that increase in frequency with increasing temperature, leading to periodicity in pheromone release that can be both circadian and seasonally controlled (e.g., Sower et al. 1971; Pope et al. 1982). In all vertebrates studied to date with described or putative pheromones, pheromone production and expression are thought to be controlled almost exclusively by hormones (e.g., amphibians, Yamamoto et al. 1996; Iwata et al. 2000a; Kikuyama et al. 2005; reptiles, Mendonça and Crews 1996; birds, Rajchard 2007; rodents, Bruce 1965; Thiessen et al. 1968; Mugford and Nowell 1971; primates, Michael 1975; goats, Iwata et al. 2000b). However, in at least one vertebrate, the red-bellied newt, *Cynops pyrrhogaster*, pheromone production may be promoted by low temperature (Iwata et al. 2000a; Takahashi et al. 2001).

Our focal species, the red-sided garter snake, *Thamnophis sirtalis parietalis*, is a reptilian model for understanding the relationship between seasonality, behavior, and sex pheromones. After a prolonged winter dormancy (8 mo, Gregory 1976), these animals emerge from limestone hibernacula by the thousands in the interlake region of Manitoba, Canada. At this time, males vigorously court and compete for females, leading to large mating balls of males courting single females (Aleksiuk and Gregory 1974). Courtship behavior in male red-sided garter snakes is affected by changes in temperature, with prolonged, low temperature dormancy (12 wk, 4°C) enabling robust, stereotypical courtship behavior in the laboratory after simulated emergence from hibernation (Hawley and Aleksiuk 1975; Garstka et al. 1982). Because the snakes hibernate several meters underground, there is no light, and photoperiodic cues have been shown to have no effect on the induction of spring mating behavior (Whittier et al. 1987; Lutterschmidt et al. 2006). Instead, the one seasonal signal, low temperature dormancy, causes changes in aromatase activity in the sexually dimorphic nuclei of the brain of males (hypothalamic pre-optic area, HPOA; Krohmer et al. 2002). The HPOA hypertrophies by the time of emergence, and is the neural center for the control of courtship behavior in this species (Krohmer et al. 2002). Laboratory-simulated hibernation conditions induce changes in the secretion patterns of melatonin in males, suggesting that this hormone may play a role in the regulation of courtship behavior as well (Lutterschmidt 2006). Thus, male red-sided garter snakes have evolved a behavioral response to a specific, relevant environmental cue: temperature (Crews and Moore 1986).

Female red-sided garter snakes exhibit seasonal changes in two components of female reproduction: receptivity and attractivity (Beach 1976). Sexual receptivity is controlled primarily by estrogen, with ovariectomy abolishing recep-

tivity and estrogen replacement in castrated females reinstating receptivity (Crews 1976). Attractivity is determined by using bioassays of male courtship behavior, and both the quantity and quality of the female sexual attractiveness pheromone blend can be determined easily with chemical analyses (Mason et al. 1989, 1990). The female sex pheromone of the red-sided garter snake is a series of nonvolatile, long-chain (C₂₉–C₃₇) saturated and monounsaturated methyl ketones ranging from 394 to 532 Da (Mason et al. 1989). Individual pheromone components, when presented singly, elicit much lower levels of courtship from males compared to the complete blend; however, the longest, unsaturated components can elicit significant courtship behavior when presented alone (Mason et al. 1989). More recent work has shown that the ratio of the abundances of unsaturated to saturated components relays information about female reproductive condition. Long and/or fat females produce more offspring and elicit more vigorous courtship from males when compared to females of lesser condition (LeMaster and Mason 2002; Shine et al. 2003). Sex pheromone blends from large females consist primarily of the long-chain, unsaturated components, specifically those with masses of 476, 504, and 532 Da, whereas sex pheromone blends from small females are composed equally of saturated and unsaturated methyl ketones that span the entire range (394–532 Da; LeMaster and Mason 2002). Males prefer isolated pheromone extracts from large females compared to small females, suggesting that they choose females based solely on differences in the composition of the pheromone, namely the ratio of unsaturated to saturated components (LeMaster and Mason 2002; Shine et al. 2003). Thus, the female sex pheromone is an honest signal that relays information about reproductive condition.

Early laboratory studies on garter snakes have shown that males display increased interest in females during the time of shedding (as evidenced by increased tongue-flicking and chin-rubbing behavior), and this may be attributed to changes in the quantity of the pheromone produced by the skin (Noble 1937; Kubie et al. 1978). The quality of the pheromone differs between the breeding (spring) and nonbreeding (fall) seasons in the red-sided garter snake, suggesting that the pheromone relays information about season as well as sex (Mason et al. 1987; LeMaster and Mason 2001). However, pheromone profiles for this species are complex: there are at least 16 unique methyl ketone molecules comprising a single female pheromone profile (Mason et al. 1989). To fully understand this complexity, analyses are needed of pheromone profiles from individual red-sided garter snakes, however, this was not done in the previous two studies on pheromone seasonality in this species (Mason et al. 1987; LeMaster and Mason 2001).

The purpose of this study was to examine changes in the pheromone profiles from individual female garter snakes that may be affected by the process of hibernation. Initiation of sexual behavior in males is dependent on exposure to prolonged low temperature, and it may be that female attractivity also is affected by the low temperature dormancy that this species experiences annually. In the field, it is impossible to gather pheromone samples from females during hibernation because they are several meters below ground (Lutterschmidt et al. 2006). Thus, our laboratory study utilized simulated hibernation to determine how both the quantity and quality of the female sexual attractiveness pheromone may change during winter dormancy.

Methods and Materials

Animals Female red-sided garter snakes, *Thamnophis sirtalis parietalis*, ($N=24$) were captured at the hibernaculum in the fall of 2005 (Inwood, Manitoba, Canada). Pheromones were extracted from subsets of these snakes ($N=8$ each) at three different times: fall, winter (during hibernation), and spring. The fall sample was collected at the hibernaculum, whereas the remaining snakes were transported back to the laboratory at Oregon State University and placed into artificial hibernation approximating natural conditions (4°C; 0:24 h L:D; 85% RH). Pheromones were collected from the winter group after 12 wk in winter hibernation (January), and those for the spring group were collected 12 wk later (April) after 1 wk of simulated emergence (12°C, 10 h:6°C, 14 h, L:D). All procedures involving the use of live animals were approved by the Institutional Animal Care and Use Committee at Oregon State University (ACUP 3120) and were in compliance with guidelines established by the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The collection and use of these animals was approved by Manitoba Conservation (Manitoba Wildlife Scientific Permit WB02024).

Pheromone Collection and Isolation We followed previously published methods for collection and analysis of pheromone extracts (Mason et al. 1989). Snakes were sacrificed with a lethal overdose of brevitall sodium (6 mg/kg) prior to collection of skin lipids from individual snakes by immersion in hexane for 12 h. The snakes were then removed from the solvent, and snake mass (g), snout-vent length (SVL, cm), and midbody circumference (cm) were recorded. The volume of the skin lipid extracts was reduced under vacuum with a rotary evaporator, and the total skin lipid yield of the dry product was determined (mg) before fractionation. The pheromone was isolated by using alumina columns [activity III (Sigma-Aldrich, St. Louis, MO, USA); pooled fractions

4-6 (2% diethyl ether:98% hexane as mobile phase)]. The pooled fractions containing pheromone were reduced to dryness by using a rotary evaporator and weighed to determine the mass (mg; termed “pheromone fraction mass” hereafter). The combined pheromone fractions are composed almost solely of female sex pheromone methyl ketones (>99% of fraction mass) and have been demonstrated to elicit courtship behavior from males that is as intense as courtship directed to live females in the den in the spring (Mason et al. 1989). The pooled pheromone fractions were resuspended in a pheromone:hexane mixture (1 mg:1 ml) before analysis by gas chromatography-mass spectrometry.

Gas Chromatography-Mass Spectrometry Individual pheromone samples were analyzed with a Hewlett Packard 5890 Series II gas chromatograph fitted with a split injector (280°C) and an HP 5971 Series mass selective detector. Aliquots (1 μ l) of the 1:1 samples (1 mg pheromone:1 ml hexane) were injected onto the fused-silica capillary column (RTX-1; 15 m \times 0.25 mm i.d., 0.25 μ m film thickness; Restek Corporation, Bellefonte, PA, USA) with helium as the carrier gas (5 cm/sec). All injections were made in the splitless mode with the split valve closed for 60 sec. Oven temperature was held initially at 70°C for 1 min, increased to 210°C at 30°C/min, held at 210°C for 1 min, increased to 310°C at 5°C/min, and held at 310°C for 5 min. Individual compounds were identified by using mass spectral data and ion chromatograms comparing our spectra to published data and authentic standards (Mason et al. 1990). By using the peak integration function in ChemStation software (Agilent) interfaced with the GC-MS, we determined relative contributions of each component of the pheromone to the overall profile of each snake.

Data Processing and Statistical Analysis We tested for global differences in total skin lipid mass, pheromone fraction mass, pheromone concentration, unsaturated to saturated component ratio, individual unsaturated and saturated component mass, and low and high molecular weight contributions to profiles by using one-way and two-way ANOVAs (time, component type as factors) followed by pairwise comparisons (Tukey Tests; SigmaStat v.3.1). Total skin lipid mass and pheromone fraction mass were arcsine transformed after correcting for snake mass before analyses. Pheromone fraction mass was used to derive pheromone concentration (μ g/cm²) by using previously published methods and log-transformed before analysis (Mason et al. 1990; LeMaster and Mason 2002). Briefly, the circumference (cm) at midbody was measured and then multiplied by the snout-to-vent length (cm) to get total surface area (cm²), and pheromone fraction mass (mg) was converted to μ g and divided by total surface area to yield pheromone concentration (μ g/cm²). By using an internal

standard (methyl stearate, 10 $\mu\text{g/ml}$ hexane; LeMaster et al. 2008), we were able to derive individual component mass (μg) for all of the 16 methyl ketones comprising the pheromone. Global differences in pheromone structure were analyzed by using the Multi-Response Permutation Procedure in the vegan package for R (v.1.8-8; McCune et al. 2002). Pairwise comparisons for pheromone structure were run by using the same procedure but by excluding a new group each time. Coordinates for a non-metric multi-dimensional scaling plot to represent differences in individual pheromone profiles also were generated with the vegan package, and all graphics were created in SigmaPlot (v.8.0).

Results

Total skin lipid and pheromone fraction masses increased from fall to spring ($F_{2, 21}=95.471$, $P<0.001$; $F_{2, 21}=9.196$, $P=0.001$; Fig. 1). Total skin lipid mass was higher in both spring and winter than in fall ($q=17.893$, $P<0.001$; $q=15.750$, $P<0.001$, respectively). Pheromone fraction mass was higher in spring than in fall ($q=6.065$, $P=0.001$). No other differences were found in skin lipid or pheromone fraction masses. Pheromone concentration (μg pheromone/ cm^2 of skin) also increased over time ($H=14.791$, $P<0.001$), with the pheromone concentration being higher in spring and winter than in fall ($q=4.850$, $P=0.005$; $q=4.525$, $P=0.006$, respectively; Fig. 2).

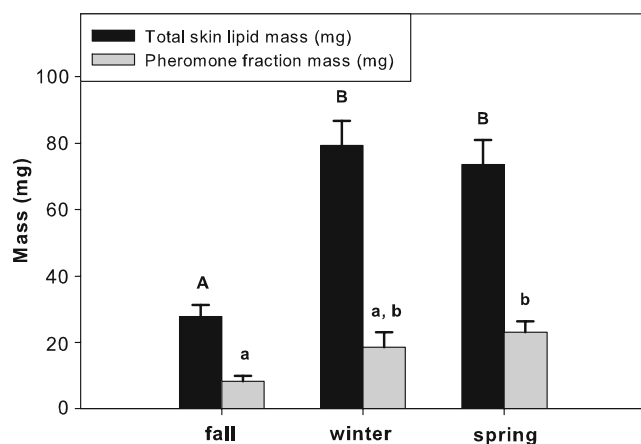


Fig. 1 Change in mass (mg; mean + s.e.; $N=8$ for each bar) of both total skin lipids (black bars) and pooled fractions containing only the nonvolatile methyl ketones that comprise the pheromone (pheromone fraction mass; gray bars) of female red-sided garter snakes. Pheromones were collected from individual females in the fall at the snake den, during winter in laboratory-simulated hibernation, and in the spring in the laboratory after simulated emergence. Different letters of the same case (e.g., “a” vs. “b”) represent significant differences ($P<0.05$) in mass for those sampling periods

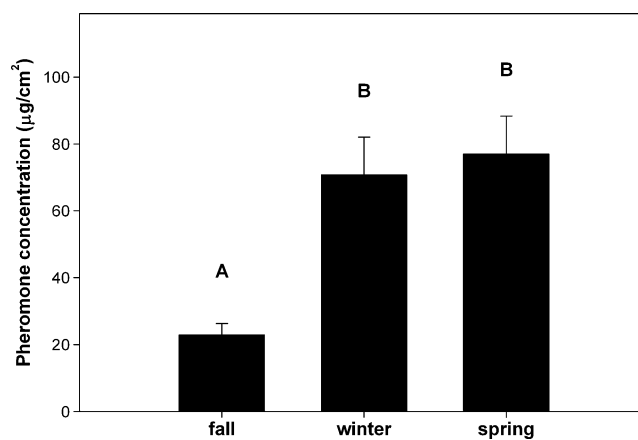
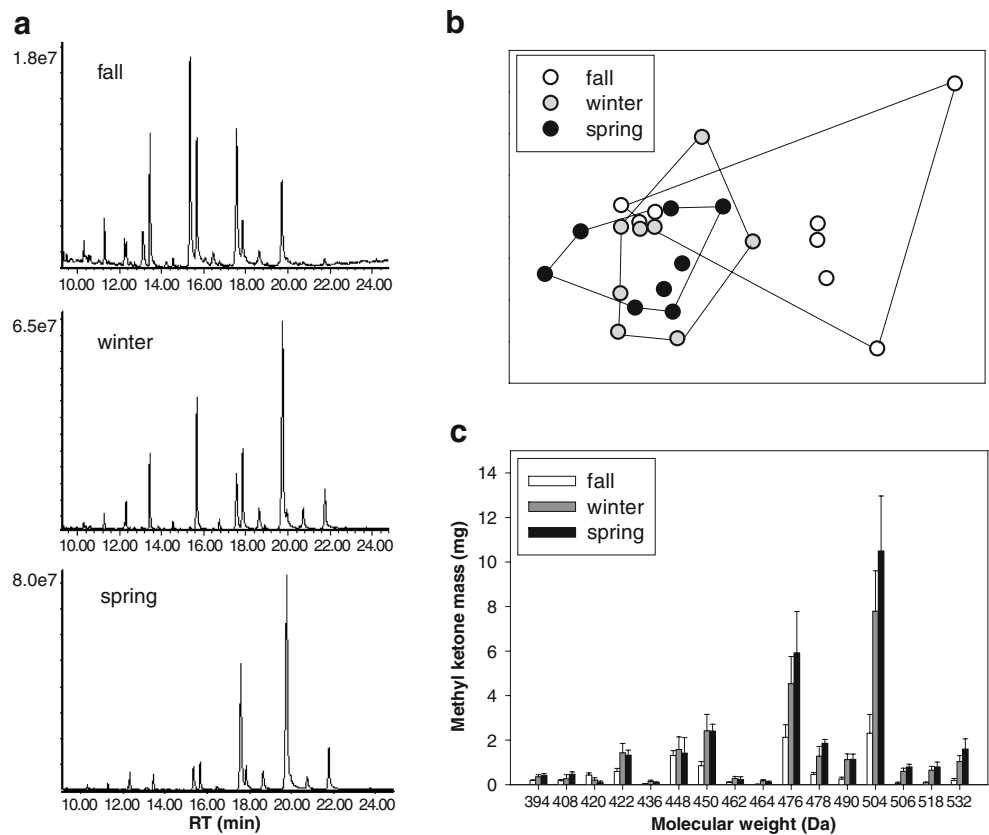


Fig. 2 Sex pheromone concentration (μg pheromone/ cm^2 skin; mean + s.e.; $N=8$ for each bar) extracted from female red-sided garter snakes in the field and during laboratory-simulated hibernation and emergence. Different letters represent significant differences ($P<0.05$) in pheromone concentration for those sampling periods

Following the GC-MS analysis, we found that the composition of the pheromone blends changed over time ($A=0.07672$, $P=0.016$). Spring profiles were different than fall profiles ($A=0.1089$, $P=0.012$), but no other differences were significant (Fig. 3). We then tested the pheromone profile characteristics that may have contributed to these global differences. We found no difference in the ratios of the abundances of unsaturated to saturated components within the pheromone profiles over time ($F_{2, 18}=0.301$, $P>0.05$). Both main effects (season, component type [saturated, unsaturated]) were significant when we analyzed the masses of pheromone components ($F_{2, 47}=8.703$, $P<0.001$; $F_{1, 47}=18.329$, $P<0.001$, respectively). There was no season \times component type interaction ($F_{2, 47}=2.010$, $P=0.147$). Within season, the mass of unsaturated components was greater than the mass of saturated components for fall ($q=3.004$, $P=0.04$), winter ($q=3.772$, $P=0.011$), and spring ($q=3.218$, $P=0.028$; Fig. 4). Within component type, the mass of saturated components was greatest in spring compared to fall ($q=4.561$, $P=0.007$), but no other differences were detected. The mass of unsaturated components was greater in spring than in fall ($q=4.774$, $P=0.005$), and winter than in fall ($q=3.706$, $P=0.032$; Fig. 4). The compounds contributing most to the overall makeup of the pheromone blend were the 504 (unsaturated), 476 (unsaturated), 450 (saturated), and 448 (unsaturated) Da molecules (Fig. 3).

Components within each pheromone profile were arbitrarily split into two molecular weight classes: low molecular weight (LMW; <463 Da) and high molecular weight (HMW; >463 Da). Previous work has demonstrated that the attractivity of female red-sided garter snakes increases with increasing molecular weight and unsaturation of individual components of the pheromone, suggest-

Fig. 3 Changes in the female sexual attractiveness pheromone blend of red-sided garter snakes from three sampling periods (fall, winter, spring). **a** Representative total ion chromatograms of sex pheromone blends from individual females from the three sampling periods. **b** Non-metric multidimensional scaling plot showing individual pheromone profiles. Minimal convex polygons are drawn for each group to show how the area of similarity for pheromone profiles shifts and contracts from fall to spring, with individual profiles being most similar (most tightly clustered) in spring. The fall (white circles) and spring (black circles) were significantly different ($P < 0.05$). **c** Masses (mg; mean + s.e.; $N = 8$ for each bar) of prospective individual components (categorized by molecular mass; Da) of the female sex pheromone blend over the three sampling periods. The most abundant compounds were the 504 (unsaturated), 476 (unsaturated), 450 (saturated), and 448 (unsaturated) Da methyl ketones



ing that changes observed in the molecular weight of pheromone constituents over time have biological consequences in nature (Mason et al. 1989). Thus, we analyzed the ratio of HMW:LMW proportions over time (log-

transformed) and found a significant time effect ($F_{2, 21} = 5.362, P = 0.013$). Pheromone profiles were dominated by HMW compounds in spring compared to fall ($q = 4.365, P = 0.015$), but no other differences were significant (winter vs. fall, $q = 3.522, P > 0.05$).

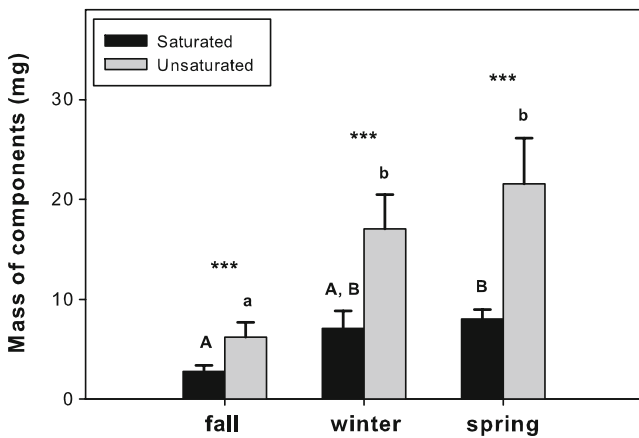


Fig. 4 Mass of components (mg; mean + s.e.; $N = 8$ for each bar) comprising the female sex pheromone blend of red-sided garter snakes from three sampling periods (fall, winter, spring) grouped by methyl ketone type (saturated, unsaturated). At all three sampling periods, unsaturated components were significantly more abundant ($P < 0.05$; asterisks) than saturated components, especially in the spring. Within each class, both types of components reached peak mass in the spring. Different letters of the same case represent significant differences ($P < 0.05$) within a class

Discussion

We have shown that the process of hibernation induces quantitative and qualitative changes in the female sexual attractiveness pheromone of the red-sided garter snake. Both total skin lipid mass and pheromone fraction mass increased from fall to spring as did the concentration of pheromone present on the skin. The quality of the pheromone also appeared to change concomitant with the changes in amount and concentration, with females producing pheromones dominated by unsaturated, high molecular weight (HMW) methyl ketones by the winter and into spring. Thus, hibernation is critical in the regulation of one of the two components of female reproduction in this species: attractivity.

Temperature has numerous effects on sexual signals, both in vertebrates and invertebrates. For instance, ambient temperature affects the length and color of the manes of lions, an information-rich sexual signal (West and Packer

2002). Vertebrates and invertebrates exhibit fluctuating asymmetry in sexually selected traits that reflect changes in ambient temperature and photoperiod (e.g., tail length in swallows, Møller and Szep 2005; sex combs in flies, Polak and Starmer 2005). Further, many animals use sex-specific sounds to attract and evaluate mates, and both the production and perception of these signals can be altered significantly by changes in temperature (e.g., flies, Ritchie et al. 2001; frogs, Gerhardt 1978; Shimizu and Barth 1996). Our results suggest that a powerful sexual signal, the female attractiveness pheromone of garter snakes, changes significantly as a result of low temperature dormancy (i.e., hibernation).

Sex pheromone production is known to be affected by temperature in red-bellied newts, *Cynops pyrrhogaster*, where low temperatures (8–12°C) induce increased synthesis of prolactin mRNA, and prolactin, in conjunction with androgens, can induce hypertrophy of the secretory capacity of the newt pheromone (sodefrin) gland and stimulate production of sodefrin (Toyoda et al. 1994; Yamamoto et al. 1996; Iwata et al. 2000a; Takahashi et al. 2001). Sodefrin is a peptide pheromone, thus the mechanisms controlling its synthesis and expression will differ from those acting in garter snakes to produce the relatively small methyl ketone molecules that act as pheromones in our system. However, both systems are responsive, at least in part, to changes in temperature, and they warrant further study. Our study is the first in a reptile to demonstrate the effects of hibernation on female attractivity at the level of pheromone production.

Female receptivity and male courtship behavior in both amphibians and reptiles are known to be affected by changes in temperature. Low temperatures are critical for either initiating or maintaining expression of both male courtship and female receptivity in a number of salamanders, newts, and frogs (Duellman and Trueb 1994). In reptiles, the effects of temperature on sexual behavior in males have been studied more extensively than in females (Whittier and Tokarz 1992). Male red-sided garter snakes do not express typical courtship behavior in the spring unless they experience an extended (8–17 wk) low temperature dormancy (4°C, 0 h:24 h L:D; Camazine et al. 1980; Garstka et al. 1982). Female receptivity in garter snakes is influenced by low temperature dormancy, but hormonal priming (estrogen) before hibernation exerts a much stronger effect, as evidenced by abolished receptivity in ovariectomized females and only reduced receptivity resulting from exposure to warmer temperatures during winter dormancy (Bona-Gallo and Licht 1983; Mendonça and Crews 1996). Our results suggest that female attractivity is optimized by low temperature dormancy, though our study did not include a warm hibernation control group due to the high rate of female mortality demonstrated in previous studies (16%, Whittier et al. 1987).

Collectively, research on this system has shown that maximal, coordinated sexual behavior in the red-sided garter snake (male courtship and female attractivity and receptivity) is contingent upon sustained, low temperature dormancy. This species overwinters in large, communal dens, and snakes emerge en masse in the tens of thousands to engage in spectacular displays of scramble mating as males search for and court singly-emerging females (Aleksiuk and Gregory 1974; Gregory 1976). In an evolutionary sense, this species has adapted to maximally express sexual characteristics (behavior, pheromones) at a time when population densities are highest and precision in identifying and selecting mates is critical.

The role of skin and gland-derived compounds as pheromones in sauropsids (birds and reptiles) may have arisen from the modification of phospholipids originally derived for waterproofing the integument (adapted from Maderson 1986). In another sauropsid (mallard, *Anas platyrhynchos*), the waterproofing gland (uropygial) produces sex pheromones, and pheromone synthesis occurs from lysosomal modification (lengthening, esterification) of monoester waxes, and ultimately is regulated by female sex steroid hormones (Jacob et al. 1979; Kolattukudy and Rogers 1987; Bohnet et al. 1991). Thus, skin waxes generated primarily for waterproofing have been co-opted into a secondary role as sexual signals in this species. The pheromone of red-sided garter snakes may be similarly derived. First, the skin may increase its lipid production to retard transcutaneous water loss during low temperature dormancy, which is a common process in sauropsids during cold acclimation (e.g., turtles, Willard et al. 2000; pigeons, Peltonen et al. 2000). Second, as is the case in mallards, the lipids would be modified by enzymes that are activated by cold temperatures. The data presented in this paper show that the pheromone of female red-sided garter snakes becomes dominated by longer, unsaturated molecules, the most sexually attractive components of the female pheromone, as a result of low temperature dormancy (Mason et al. 1989; LeMaster and Mason 2002; Shine et al. 2003). Several enzymes are activated by cold temperatures in vertebrates that either synthesize or lengthen long-chain lipids (C16 or greater; Jakobsson et al. 2005, 2006). Other enzymes, such as desaturases, play a critical role in maintenance of the phospholipid bilayer by desaturating lipid chains to increase membrane fluidity and prevent damage from freezing (reviewed in Hazel and Williams 1990; Tiku et al. 1996). Such temperature-induced mechanisms (elongation, desaturation) may exist in the pheromone synthesis of red-sided garter snakes and warrant further study.

We have shown that female pheromone content is altered during hibernation in this species, and that the pheromone present at emergence is composed primarily of long-chain,

unsaturated methyl ketones, which in other studies have been shown to elicit the strongest behavioral response from males (Mason et al. 1989; LeMaster and Mason 2002; Shine et al. 2003). The red-sided garter snake serves as a robust model for testing hypotheses on the seasonality of sexual signal production, and it also represents a point of evolutionary divergence in vertebrate sexual signals. Rather than plumage and pelage to reflect sex, condition and season, the skin lipids of the female red-sided garter snake are modified to signal condition and fecundity so as to be optimally honest at a time when mate density is highest and the odds of mating are virtually certain. In the future, we plan to examine skin lipid production by male garter snakes to determine if they may also display the same seasonal pattern we have shown in females of this species.

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