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Chemical Ecology of the Red-Sided Garter Snake, *Thamnophis sirtalis parietalis*

Key Words

Chemical ecology
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Thamnophis sirtalis parietalis
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Methyl ketone
She-male

Abstract

The red-sided garter snake, *Thamnophis sirtalis parietalis* is an ideal model for the study of chemical or pheromonal communication in vertebrates. Results of long-term field and laboratory investigations of the chemical ecology of these unusual animals is summarized. These studies include a description of the characterization of the female attractiveness pheromone that serves to induce male courtship behavior. The male sex recognition pheromone system that identifies males as inappropriate individuals to court is also described. She-males, individual males that are courted as if they were females, seem to possess semiochemical components intermediate to both males and females. Finally, investigations of the species-specific nature of these pheromones indicate that chemical differences exist in the methyl ketones of all species examined to date.

Introduction

Recently, my collaborators and I have been investigating the chemical ecology of pheromonal communication in snakes. The Canadian red-sided garter snake, *Thamnophis sirtalis parietalis*, is an ideal model for studies of vertebrate pheromonal communication. Garter snakes possess a relatively simple suite of semiochemicals (natural products with signal function), and the behaviors monitored in the bioassay are robust, simple to quantify, and unequivocal in that they are only observed in a reproductive context in response to pheromonal cues [Noble, 1937; Garstka and Crews, 1981; Mason et al., 1989]. We are addressing chemical communication in this species at three levels: (1) the biochemical level, wherein the chemical nature of the semiochemicals used to mediate reproduction are characterized; (2) the physiological or organismal level, where we are addressing how the pheromone is produced and

expressed, including the possible evolutionary origins of these chemical signals and their associated behaviors and; (3) the population level, which addresses questions of species-specificity of chemical signals. Are garter snakes the only taxa of snakes that utilize integumental lipids as pheromones, or is this a trait shared by many species of snakes? If these pheromones function as premating isolating mechanisms, one would expect to find chemical differences in the signals between species.

Upon encountering a sexually attractive, unmated female, male garter snakes press their chins onto her dorsal skin and emit short, rapid tongue-flicks [Blanchard and Blanchard, 1942]. At the same time, the male traverses up and down the female, turning back at the head and tail. Eventually, the male comes to rest with his head behind the female's head. Once stationary, the male's body undulates in caudocephalic waves. Both chin-rubbing and caudocephalic waves are observed only in a mating context and are



Fig. 1. Male red-sided garter snakes, *Thamnophis sirtalis parietalis* in the field, exhibiting species-typical courtship behaviors in response to pheromonal components presented on a paper towel. Chin-rubbing behavior as seen here is observed only in a reproductive context in response to pheromonal cues.

not associated with any other behaviors [Camazine et al., 1980]. Both of these behaviors are generally true of most snake taxa and have been described as the most ubiquitous courtship acts observed in snakes [Gillingham, 1987]. Eventually, the female will gape her cloaca, the male will intromit one of his hemipenes, and mating will ensue.

Noble [1937] was the first to demonstrate that female garter snakes (*Thamnophis*) produce a pheromone which is sequestered in the skin of the dorsal surface. Sexually active males fail to court females following chemical manipulations of the pheromone on the female's back [Noble, 1937]. Other experiments eliminated auditory, visual, and tactile cues, which led Noble to suggest that the tongue delivers chemical cues to the vomeronasal organ in the roof of the mouth. Recent studies by Halpern and colleagues [Kubie et al., 1978; Halpern, 1983] demonstrated that chemical access to the vomeronasal system alone is sufficient for males to recognize females.

In studies of chemical signals, and especially pheromones, there are two major tasks: the development of a reliable bioassay and the chemical elucidation of the natural products. In chemical ecology studies, the development of the bioassay is crucial. Animals express a wide variety of behaviors that appear to be mediated by chemical cues. Trying to assign specific chemical compounds to these behaviors, however, is a tedious and often frustrating task. Without a truly robust and unequivocal bioassay, such a task frequently results in failure.

To investigate the chemical nature of garter snake pheromones, we have developed a bioassay for testing mixtures and synthetic chemicals [Mason and Crews, 1985]. The bioassay is relatively simple in that it involves presenting chemical extracts and purified components on paper towels or filter paper to sexually active, courting males in the field.

At least three behaviors can be quantified routinely including: the mean number of tongue-flicks elicited by a sample; the rate of tongue-flicks elicited per minute; and the duration of courtship elicited by particular components [Mason et al., 1990]. In garter snakes, tongue-flicking has been shown to deliver odorant molecules to the vomeronasal organ [Halpern and Kubie, 1980]. The chemical components are presented in known quantities, dissolved in organic solvents. Controls consist of blank paper towels or filter papers, blank solvent controls, and chemical components present in the skin lipids but possessing no known semiochemical function. This particular bioassay appears to be robust, as evidenced by the response of the courting males to the solvent blanks. After examining the solvent blanks, male garter snakes react vigorously, usually retreating rapidly while briefly shaking their head. This behavior indicates a strong aversion to the solvent blanks. However, when pheromone samples are presented in the same solvent, the males immediately begin rapid tongue-flicking and begin to chin-rub the paper towel (fig.1). Thus, the robustness of this bioassay is demonstrated, not only by the

positive response of the snakes to the sample but additionally, by overcoming their apparent aversion to the solvent.

Previously, we discovered that the female sex attractiveness pheromones were readily extracted from the dorsal surface by means of nonpolar solvents such as hexanes or toluene [Mason and Crews, 1985; Mason et al., 1987]. We then fractionated these extracts and presented them to the courting males. Fractionations of the active material continued until only one set of active compounds remained. This identification strategy, which is guided by the response of the courting males to the isolated natural products, is called a 'response-guided strategy' [Albone, 1984]. That is, the progression of the identification of the compounds that warrant further elucidation is determined by the animals themselves and not by the investigators. Limitations to this type of strategy are that the behavioral response will frequently be extinguished long before the fractionation process reaches a point where individual components can be identified. In addition, most investigations of semiochemicals or pheromones require large quantities of starting material. The fractionation procedures are relatively inefficient in that much of the material may be lost during the analytical procedures. Thus, it is important that sufficient quantities of the putative semiochemical are available before undertaking the elucidation of natural products.

Through the use of analytical chemical techniques, including ultraviolet spectrometry (UV), infrared spectrometry (IR), gas chromatography-fourier transform IR (GC-FTIR), gas chromatography/mass spectrometry (GC/MS), ^1H - and ^{13}C -nuclear magnetic resonance (NMR) spectroscopy, and the formation of derivatives, we have been able to isolate, characterize, identify, and synthesize the sex attractiveness pheromones of the red-sided garter snake. The pheromone has been identified as a series of long-chain (C_{29} - C_{37}), saturated and monounsaturated methyl ketones [Mason et al., 1989, 1990]. Field testing of synthetically prepared samples induces species-typical courtship behavior from courting male garter snakes that is indistinguishable from that elicited by native material or from the response to unmated, attractive females [Mason et al., 1989; 1990]. It is noteworthy that these pheromone cues are essentially nonvolatile due to their molecular weights, which range from 394 to 532 daltons. Thus, the garter snake semiochemicals are a type of contact pheromone.

The pheromone system of the red-sided garter snake is relatively complex as compared to other vertebrates. In addition to the female sex attractiveness pheromones, these garter snakes also appear to possess a male sex recognition pheromone. During courtship behavior, male garter

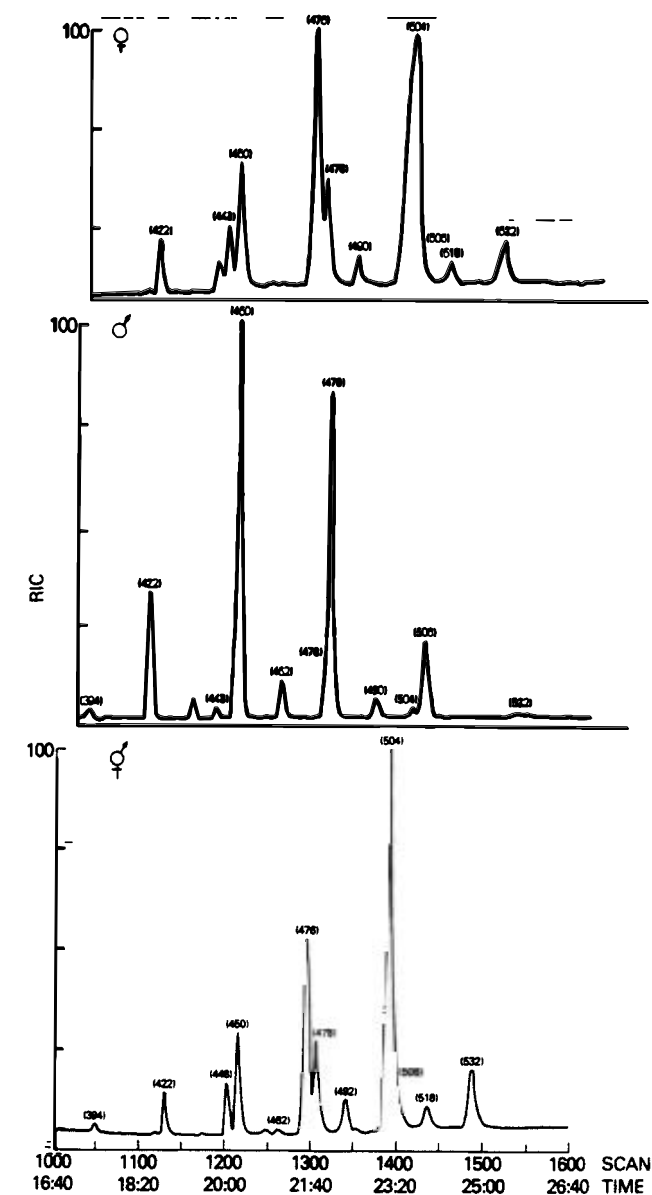


Fig. 2. Gas chromatograms of the methyl ketone fractions for females, males, and she-males obtained during the breeding season. The major chemical components, seen here as peaks, for females are predominantly unsaturated; those for males are primarily saturated, and those for she-males are intermediate, containing both saturated and unsaturated methyl ketones.

snakes will tongue-flick every individual with whom they come into contact. Males will normally ignore other males but will immediately begin courting a female after a brief investigation. One could hypothesize that those individuals that do not possess the female sex pheromones will be

ignored and not courted, and since males presumably do not possess the female pheromones, this could explain why they are not normally courted. Male garter snakes do possess methyl ketones in their skin lipids, however, although not in the same quantity or relative proportion as do females (fig. 2). Another hypothesis is that males possess a unique set of skin lipid semiochemicals which identifies them as males. Indeed, males do possess components in their skin lipids that will inhibit courtship in response to the female pheromone when both are placed on the same filter paper [Mason et al., 1989]. Subsequent chemical analyses have identified squalene as one of the components of this male sex recognition system [Mason et al., 1990]. Other components of the male sex recognition pheromone remain to be identified.

Another interesting facet to the pheromone system of the red-sided garter snakes is pheromone mimicry. A small percentage of males in several Manitoba populations are actively courted by other males as if they were females. These sexually attractive males, or she-males [Mason and Crews, 1985], are distinguishable from other males on the basis of their sexual attractiveness. She-males are morphologically and anatomically indistinguishable from other males. Behaviorally, she-males prefer to court and mate with females and male-male copulation has never been observed, but she-males seem to be a source of confusion for other males in the highly competitive mating aggregations commonly found in Manitoba, Canada [Mason and Crews, 1985]. When involved in a mating aggregation, she-males induce some of the males courting the female to pull off of the female's back and begin courting the she-male. Meanwhile, the she-male courts the female. By pulling off the female's back, the confused male has effectively removed itself from proximity to the female, and thus, reduced its chance of mating with the female. In staged arena trials, she-males are more than twice as successful in mating with females than are normal males [Mason and Crews, 1985]. Chemical analyses of the skin lipids of the she-males reveal that they possess methyl ketones that are intermediate between those of ordinary males and those of females [Mason et al., 1989] (fig. 2). In addition, they have little or no squalene in their skin lipids, having levels essentially the same as females [Mason et al., 1989].

Questions presently under investigation center around the physiological mechanisms of production and expression of these skin lipid pheromones. Steroid hormones seem to play an integral role in at least the production and perhaps the expression of the sex attractiveness pheromone in garter snakes. Estrogen treatment has been shown to induce pheromone production in female garter snakes [Crews,

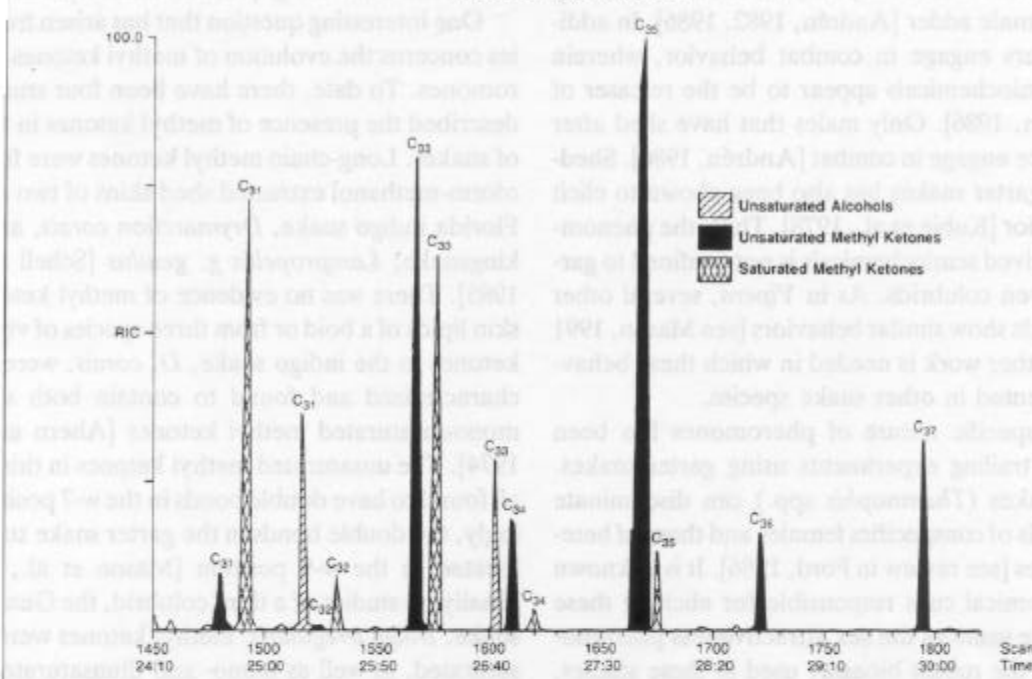
1976; Kubie et al., 1978; Garstka and Crews, 1981; Crews, 1985]. However, the specific mechanisms involved and the biochemical processes by which estrogen stimulates pheromone production remain to be elucidated. Previous work by Garstka and Crews [1981] suggested that the yolking protein vitellogenin might be responsible for eliciting male courtship. However, more recent work involving the transport, immunoreactivity, and field testing of vitellogenin were equivocal [Garstka and Crews, 1986]. Vitellogenin is a glycolipophosphoprotein that is produced in the liver and transported in the bloodstream. One working hypothesis is that the methyl ketones found at the surface of the skin could be a lipid component of the vitellogenin molecule. Definitive experiments must be conducted in order to determine if the methyl ketones are produced in the liver and transported to the skin, or, whether they are synthesized *de novo* in the dermal tissues of the skin itself.

The she-males present an interesting natural experimental in this regard. The only physiological difference that could be found between she-males, males, and females was the level of circulating androgens. She-males contained more than three times as much testosterone as did males. This seems rather counterintuitive in that the she-males are attractive like females, and it is known that estrogens induce attractiveness. We hypothesize that aromatase enzymes in the skin could be converting high levels of testosterone in the plasma to high levels of estrogen at the level of the target organ – in this case, the skin. Preliminary results of aromatase assays indicate that females possess the highest levels of aromatase in their skin, followed by she-males whose levels are significantly higher than males [R.T. Mason and Krohmer, unpubl. observ.].

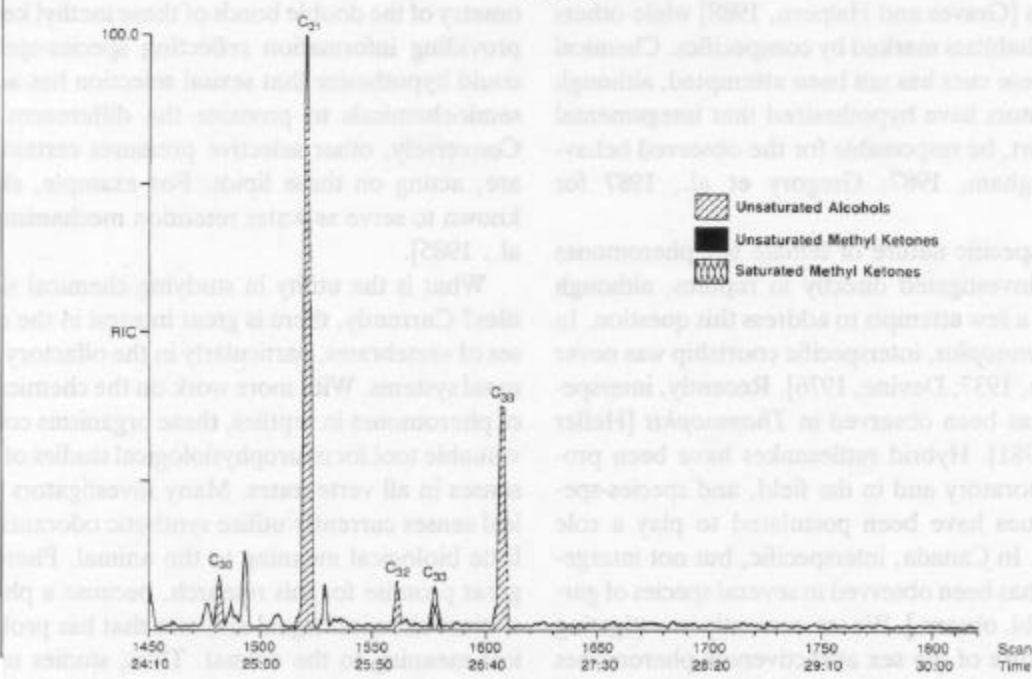
How common are skin lipid pheromones in snakes? The field of chemical ecology is still relatively young, and there has not yet been a concerted effort to isolate and identify pheromones in reptiles in general. However, there are scores of studies that document the use of pheromones in reptiles [see Mason, 1992 for review]. Perhaps most instructive are studies of the adder, *Vipera berus*. Behavioral observations in the laboratory and in the field indicate that

Fig. 3. Gas chromatograms of the methyl ketone and primary alcohol fractions for the red-sided garter snake, *Thamnophis sirtalis parietalis*, from Manitoba and the checkered garter snake, *Thamnophis marcianus*, from Texas. Although the two species share chemical constituents in common, their overall proportion of compounds is quite different. This may reflect species differences brought on by sexual selection, or it may reflect selective pressures due to environmental constraints.

Thamnophis sirtalis parietalis
Manitoba, Canada



Thamnophis marcianus
Texas



a pheromone, responsible for eliciting male courtship, is sequestered in or on the female's dorsal skin and that the vomeronasal system is necessary for induction of courtship behavior in the male adder [Andrén, 1982, 1986]. In addition, male adders engage in combat behavior, wherein skin-derived semiochemicals appear to be the releaser of combat [Andrén, 1986]. Only males that have shed after spring emergence engage in combat [Andrén, 1986]. Shedding in female garter snakes has also been shown to elicit courtship behavior [Kubie et al., 1978]. Thus, the phenomenon of skin-derived semiochemicals is not confined to garter snakes or even colubrids. As in *Vipera*, several other species of viperids show similar behaviors [see Mason, 1991 for review]. Further work is needed in which these behaviors are documented in other snake species.

The species-specific nature of pheromones has been well studied in trailing experiments using garter snakes. Male garter snakes (*Thamnophis* spp.) can discriminate between the trails of conspecifics females and those of heterospecific females [see review in Ford, 1986]. It is unknown whether the chemical cues responsible for eliciting these behaviors are the same as the sex attractiveness pheromones. Because of the robust bioassay used in these studies, this phenomenon is a good candidate for biochemical elucidation of the semiochemicals involved.

Aggregation behavior has also been studied in regard to the species-specificity of chemical cues [see Mason, 1991 for review]. Results of behavioral studies have been somewhat equivocal, with some species preferring to aggregate with conspecifics [Graves and Halpern, 1988] while others choose to avoid habitats marked by conspecifics. Chemical elucidation of these cues has not been attempted, although several investigators have hypothesized that integumental lipids may, in part, be responsible for the observed behaviors [see Gillingham, 1987; Gregory et al., 1987 for reviews].

The species-specific nature of female sex pheromones have not been investigated directly in reptiles, although there have been a few attempts to address this question. In studies with *Thamnophis*, interspecific courtship was never observed [Noble, 1937; Devine, 1976]. Recently, interspecific courtship has been observed in *Thamnophis* [Heller and Halpern, 1981]. Hybrid rattlesnakes have been produced in the laboratory and in the field, and species-specific chemical cues have been postulated to play a role [Klauber, 1956]. In Canada, interspecific, but not intergeneric, courtship has been observed in several species of garter snakes [unpubl. observ.]. We are currently investigating the chemical nature of the sex attractiveness pheromones in several species of garter snakes. Preliminary results indi-

cate that chemical differences could serve in species recognition (fig. 3). Why males would choose to court heterospecifics is an interesting question that requires further study.

One interesting question that has arisen from these studies concerns the evolution of methyl ketones as snake pheromones. To date, there have been four studies that have described the presence of methyl ketones in the skin lipids of snakes. Long-chain methyl ketones were found in chloroform-methanol extracted shed skins of two colubrids, the Florida indigo snake, *Drymarchon corais*, and an eastern kingsnake, *Lampropeltis g. getulus* [Schell and Weldon, 1985]. There was no evidence of methyl ketones from the skin lipids of a boid or from three species of viperid. Methyl ketones in the indigo snake, *D. corais*, were isolated and characterized and found to contain both saturated and monounsaturated methyl ketones [Ahern and Downing, 1974]. The unsaturated methyl ketones in this species were all found to have double bonds in the *w*-7 position. Interestingly, the double bonds in the garter snake studies were all located in the *w*-9 position [Mason et al., 1989, 1990]. Finally, in studies of a third colubrid, the Guam brown tree snake, *Boiga irregularis*, methyl ketones were found to be saturated, as well as mono- and diunsaturated [Murata et al., 1991]. These double bonds were more complex and their position different than those in the other two colubrid species. Thus, at this point, it appears that methyl ketones are limited to colubrid snakes. It is interesting to speculate that, because of their demonstrated semiochemical properties, variation in the chain length and the position and geometry of the double bonds of these methyl ketones may be providing information reflecting species-specificity. One could hypothesize that sexual selection has acted on these semiochemicals to promote the differences noted here. Conversely, other selective pressures certainly have, and are, acting on these lipids. For example, skin lipids are known to serve as water retention mechanisms [Burken et al., 1985].

What is the utility in studying chemical signals in reptiles? Currently, there is great interest in the chemical senses of vertebrates, particularly in the olfactory and vomeronasal systems. With more work on the chemical elucidation of pheromones in reptiles, these organisms could provide a valuable tool for neurophysiological studies of the chemical senses in all vertebrates. Many investigators of the chemical senses currently utilize synthetic odorants, which have little biological meaning to the animal. Pheromones hold great promise for this research, because a pheromone is a distinct chemical signal and one that has profound biological meaning to the animal. Thus, studies using reptilian pheromones are an ideal approach to investigating the rela-

tively poorly understood physiological basis of olfaction in vertebrates.

Although studies of vertebrate chemical ecology are still relatively new, reptiles, especially snakes, offer good models for the elucidation of semiochemicals. In many cases, rigorous bioassays exist for behaviors that are conducive to investigation of the chemical cues responsible. It now seems apparent that several behaviors in snakes may rely on integumental lipids as a source of the semiochemical. Investigations of the chemical ecology of these behaviors should be forthcoming. On a more specific level, future work will be needed to address the biosynthesis of pheromones. How are they synthesized? Are the chemical constituents novel natural products, or are they exaptations of skin-derived, water-retention lipids as postulated by Maderson [1986] and Graves et al., [1986]? What is the nature of the pheromone receptor? In chemical communication systems, what are the dynamics involved in the co-evolution of pheromones and the pheromone receptors? Is

this a major framework upon which the formation of new species occurs? These and other questions will foster exciting research in the future.

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References

- Ahern, D.G., and D.T. Downing (1974) Skin lipids of the Florida indigo snake. *Lipids*, 9: 8-14.
- Albone, E.S. (1984) *Mammalian Semiochemistry*. John Wiley and Sons, Ltd., Chichester, England.
- Andr n, C. (1982) The role of the vomeronasal organs in the reproductive behavior of the adder, *Vipera berus*. *Copeia*, 1982: 148-157.
- Andr n, C. (1986) Courtship, mating and agonistic behaviour in a free-living population of adders, *Vipera berus* (L.). *Amphibia-Reptilia*, 7: 353-383.
- Blanchard, F.N., and F.C. Blanchard (1942) Mating of the garter snake *Thamnophis sirtalis sirtalis*. *Pap. Mich. Acad. Sci., Arts and Letters*, 27: 215-234.
- Burken, R.R., Wertz, P.W., and D.T. Downing (1985) The effects of lipids on transepidermal water permeation in snakes. *Comp. Biochem. Physiol.*, 81A: 213-216.
- Camazine, B., W. Garstka, R. Tokarz, and D. Crews (1980) Effects of castration and androgen replacement on male courtship behavior in the red-sided garter snake (*Thamnophis sirtalis parietalis*). *Horm. Behav.*, 14: 358-372.
- Crews, D. (1976) Hormonal control of male courtship behavior and female attractivity in the garter snake (*Thamnophis sirtalis parietalis*). *Horm. Behav.*, 7: 451-460.
- Crews, D. (1985) Effects of early sex steroid hormone treatment on courtship behavior and sexual attractivity in the red-sided garter snake, *Thamnophis sirtalis parietalis*. *Physiol. Behav.*, 35: 569-575.
- Devine, M.C. (1976) Species discrimination in mate selection by free-living male garter snakes and experimental evidence for the role of pheromones. *Herp. Rev.*, 7: 79.
- Ford, N.B. (1986) The role of pheromone trails in the sociobiology of snakes. *In* *Chemical Signals in Vertebrates 4: Ecology, Evolution, and Comparative Biology* (ed. by D. Duvall, D. M ller-Schwarze, and R.M. Silverstein). Plenum Press, New York, pp. 261-278.
- Garstka, W., and D. Crews (1981) Female sex pheromone in the skin and circulation of a garter snake. *Science*, 214: 681-683.
- Garstka, W., and D. Crews (1986) Pheromones and reproduction in garter snakes. *In* *Chemical Signals in Vertebrates. IV. Ecology, Evolution, and Comparative Biology* (ed. by D. Duvall, D. M ller-Schwarze, and R.M. Silverstein). Plenum Press, New York, pp. 243-260.
- Gillingham, J.C. (1987) Social behavior. *In* *Snakes: Ecology and Evolutionary Biology* (ed. by R.A. Seigel, J.T. Collins, and S.S. Novak), McGraw-Hill, New York, pp. 184-209.
- Graves, B.M., D. Duvall, M.B. King, S.L. Lindstedt, and W.A. Gern (1986) Initial den location by neonatal prairie rattlesnakes: functions, causes, and natural history in chemical ecology. *In* *Chemical Signals in Vertebrates 4: Ecology, Evolution, and Comparative Biology* (ed. by D. Duvall, D. M ller-Schwarze, and R.M. Silverstein), Plenum Press, New York, pp. 285-304.
- Graves, B.M., and M. Halpern (1988) Neonate plains garter snakes (*Thamnophis radix*) are attracted to conspecific skin extracts. *J. Comp. Psychol.*, 102: 251-253.
- Gregory, P.T., J.M. Macartney, and K.W. Larsen (1987) Spatial patterns and movements. *In* *Snakes: Ecology and Evolutionary Biology* (ed. by R.A. Seigel, J.T. Collins, and S.S. Novak). McGraw-Hill, New York, pp. 366-395.
- Halpern, M. (1983) Nasal chemical senses in snakes. *In* *Advances in Vertebrate Neuroethology* (ed. by J.P. Ewert, R.R. Capranica, and D.J. Ingle), Plenum Press, New York, pp. 141-176.
- Halpern, M., and J.L. Kubie (1980) Chemical access to the vomeronasal organs of garter snakes. *Physiol. Behav.*, 24: 367-371.
- Heller, S.B., and M. Halpern (1981) Laboratory observations on conspecific and congenic scent trailing in garter snakes (*Thamnophis*). *Behav. Neural Biol.*, 33: 372-377.
- Klauber, L.M. (1956) *Rattlesnakes: Their Habits, Life Histories, and Influence on Mankind*. University of California Press, Berkeley.
- Kubie, J.L., A. Vagvolgyi, and M. Halpern (1978) The roles of the vomeronasal and olfactory systems in the courtship behavior of male snakes. *J. Comp. Physiol. Psychol.*, 92: 627-641.
- Maderson, P.F.A. (1986) The tetrapod epidermis: A system protoadapted as a semiochemical source. *In* *Chemical Signals in Vertebrates 4: Ecology, Evolution, and Comparative Biology* (ed. by D. Duvall, D. M ller-Schwarze, and R.M. Silverstein). Plenum Press, New York, pp. 13-26.

- Mason, R.T. (1992) Reptilian pheromones. *In* Biology of the Reptilia. Vol. 18, Behavioral Physiology (ed. by D. Crews and C. Gans), University of Chicago Press, pp. 114-228.
- Mason, R.T., and D. Crews (1985) Female mimicry in garter snakes. *Nature*, 316: 59-60.
- Mason, R.T., J.W. Chinn, and D. Crews (1987) Sex and seasonal differences in the skin lipids of garter snakes. *Comp. Biochem. Physiol.*, 87B: 999-1003.
- Mason, R.T., H.M. Fales, T.H. Jones, L.K. Pannell, J.W. Chinn, and D. Crews (1989) Sex pheromones in snakes. *Science*, 245: 290-293.
- Mason, R.T., T.H. Jones, H.M. Fales, L.K. Pannell, and D. Crews (1990) Characterization, synthesis, and behavioral responses to sex attractiveness pheromones of red-sided garter snakes (*Thamnophis sirtalis parietalis*). *J. Chem. Ecol.*, 16: 2353-2369.
- Murata, Y., H.J.C. Yeh, L.K. Pannell, T.H. Jones, H.M. Fales, and R.T. Mason (1991) New ketodienes from the integumental lipids of the Guam brown tree snake, *Boiga irregularis*. *J. Natural Products*, 54: 233-240.
- Noble, G.K. (1937) The sense organs involved in the courtship of *Storeria*, *Thamnophis*, and other snakes. *Bull. Am. Mus. Nat. Hist.*, 73: 673-725.
- Schell, F.M., and P.J. Weldon (1985) ¹³C-NMR analysis of snake skin lipids. *Agric. Biol. Chem.*, 49: 3597-3600.