

CONSPECIFIC SCENT TRAILING BY GARTER SNAKES
(*Thamnophis sirtalis*) DURING AUTUMN
Further Evidence for Use of Pheromones in Den Location

JON P. COSTANZO

*Department of Zoology
Miami University
Oxford, Ohio 45056*

(Received August 15, 1988; accepted January 11, 1989)

Abstract—Adult garter snakes (*Thamnophis sirtalis*), collected in October near a traditional, communal hibernaculum in central Wisconsin, were acclimated to autumnal conditions and subjected to laboratory tests to determine whether they could follow scent trails of a conspecific. Graded responses were obtained, but 75% of the sample showed at least some inclination to follow scent trails. The results suggest that pheromone cues may be used by male and female garter snakes to locate traditional dens during autumnal migrations. Evidence from this and other studies suggests that pheromones are probably used in conjunction with other homing mechanisms and that the role of pheromones in den location may be more important in younger snakes and in populations inhabiting northern latitudes.

Key Words—Pheromone, scent trail, chemical cue, hibernation, denning, homing, garter snake, *Thamnophis sirtalis*, Colubridae.

INTRODUCTION

An increasing literature concerning the role of chemical signals in the socio-biology of snakes (see Ford, 1986, for a recent review) has shown that the vomeronasal sense of these organisms is important and highly developed. Most studies of snake pheromones have addressed reproductive behaviors; however, scent trailing of conspecifics has been implicated in the formation of nonsexual aggregations for the purpose of avoiding unfavorable environmental conditions and reducing predation (e.g., Dundee and Miller, 1968; Duvall et al., 1985; Graves et al., 1986; Noble and Clausen, 1936). Additionally, chemical cues

have long been suspected to play a part in the relocation of communal snake dens during autumn (Hirth, 1966; Klauber, 1956; Noble and Clausen, 1936), but few investigators have attempted rigorous tests of this hypothesis.

In northern climates, strong selection pressures have likely played a major role in the evolutionary development of mechanisms by which snakes relocate traditional dens during autumnal migrations from (often distant) summer habitats. Hibernacula with features that effectively protect snakes from severe winter conditions may be limited on a local basis; in fact, this may be a reason why snakes den communally (Gregory, 1984). Snakes unable to relocate "proven" dens are likely subject to greater risks of mortality during winter. Also, mating opportunities in northern snake populations are often restricted to the den site (Gregory, 1982, 1984); thus, any individual not in attendance may forfeit its chance to contribute to the genome. Not unexpectedly, long autumnal migrations (up to 17.7 km in *T. sirtalis*; Gregory, 1984) to communal dens are made by many species and, in numerous cases, individuals show remarkably high fidelity to particular hibernacula (Gregory, 1982, 1984).

Evidence that snakes are able to follow scent trails of conspecifics has been documented for 24 species representing five families (Ford, 1986). Few studies, however, have addressed this behavior in the context of den relocation (Brown and MacLean, 1983; Graves et al., 1986; Lawson, 1985). Accordingly, this study was undertaken to assess the ability of adult garter snakes, from a communally denning population in Wisconsin, to follow conspecific scent trails under simulated autumnal conditions.

METHODS AND MATERIALS

Eastern garter snakes (*Thamnophis sirtalis*) were collected during October 1986 as they gathered near a traditional, communal den in central Wisconsin (described by Costanzo, 1986). Twenty-eight snakes were housed, irrespective of body size or sex, in small groups. One additional snake, a large (94 g) female designated for use in forming scent trails, was maintained separately. Snakes were kept in plastic boxes and were exposed to autumnal circadian thermal and light regimes (20°C, 13 hr photophase; 5°C, 11 hr scotophase) for 14 days prior to testing. Snakes were provided with water, but food was withheld.

Trailing ability was tested (procedure modified after Ford, 1981) during the last week in October, under photophase conditions (overhead fluorescent lighting; 387 lux), in a rectangular, plywood arena (Figure 1). Seventeen nails spaced at 5.5-cm intervals protruded from the floor and formed a continuous series of gates through which a snake could pass. The nails were arranged in a slight arc so that all gates were located 200 cm from an entrance port in the front wall of the arena. The arena surface was covered with corrugated paper

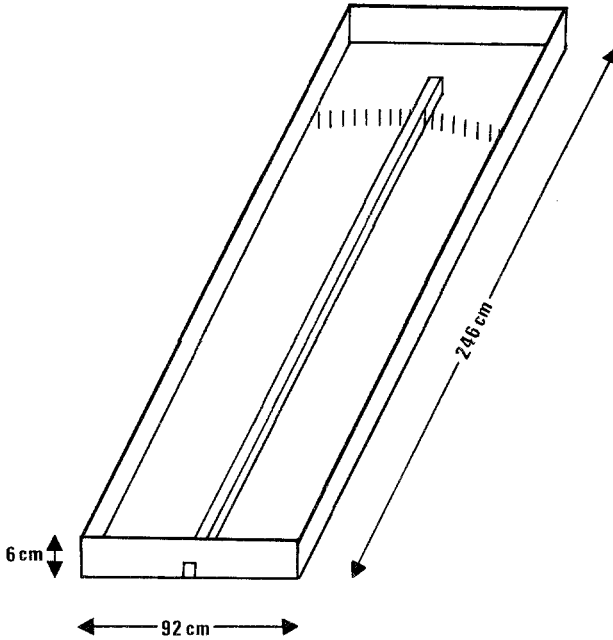


FIG. 1. Diagram of the testing arena used to measure the trail following ability of garter snakes during autumn. Scent trails were produced by guiding the movement of a stimulus snake, which was confined inside a bottomless, removable tunnel (shown), down the length of the arena and through a randomly chosen gate.

upon which 1200 ml of unscented cat litter (clay particles) was dispersed to provide traction. Nails were covered with cocktail straws to protect them from scent contamination. All coverings were replaced after each trailing and control test to ensure that residual odors were removed.

To promote stimulus uniformity, only one animal was used to create scent trails; fresh trails were produced within 30 min of testing each subject for trailing ability. This was done by guiding the movements of the large female snake, which was enclosed inside a 4.5-cm wide, bottomless tunnel, down the length of the arena and through a gate (Figure 1). The tunnel and snake were removed prior to introducing the experimental subject. Gates containing scent were chosen randomly, except that the three nearest each side wall were never used; this restriction was necessary since pilot studies and Ford (1986) indicated that non-trailing and control snakes had a strong affinity for arena walls.

Prior to testing, each subject was habituated for 20 min inside a holding box at the front of the arena. A pulley system was used to remove a partition between the holding box and the entrance port, thus allowing the snake to enter

the arena. Trials involving snakes not entering the arena within 15 min were terminated. Movements and gate choices of snakes were observed from behind a blind 2.5 m away. After a subject passed completely through a gate and reached the distal wall of the arena, it was replaced in its holding cage. Snakes were tested in control situations (no scent trail present) four to six days later and then sexed.

RESULTS

Twenty-four of 28 snakes completed the control trials. All but one individual (96%) passed through end gates (the two adjacent gates nearest each side wall), thus, "wall-seeking" was a prominent behavioral characteristic of non-trailing snakes. This necessitated statistical evaluation of the results using a binomial test (choice of four end gates vs. choice of 14 middle gates; Sokal and Rohlf, 1969). Frequencies recorded for control snakes were significantly different from probabilities based upon random gate choice (Table 1). Binomial probabilities calculated for experimental and control groups showed that the presence of conspecific scent in the arena significantly influenced snake behavior (Table 1).

Of the 28 snakes completing experimental (trials present) trails, 75% showed at least some inclination to follow scent trails. Trailing snakes demonstrated a behavior similar to the trail contact response (TCR) (Brown and MacLean, 1983), which involved slow and methodical sampling of the substrate, over which the trail-forming snake had passed, with its tongue tips. This behavior, which did not occur in control tests, allowed the observer to estimate (to within 10 cm) the length of the trail followed.

Garter snakes showed differential responses to the presence of conspecific

TABLE 1. EXPECTED AND OBSERVED BINOMIAL PROBABILITIES FOR GATE CHOICES OF GARTER SNAKES TESTED IN LABORATORY ARENA FOR ABILITY TO FOLLOW CONSPECIFIC SCENT TRAILS DURING AUTUMN

Test condition	Probability of end gate choice	Probability of middle gate choice	N	Significance (P)
Expected	0.222	0.778		
Control	0.958	0.042	24	<0.005 ^a
Experimental	0.607	0.393	28	<0.005 ^b

^aProbability that responses of snakes tested in the absence of scent trails do not differ from random gate selection.

^bProbability that responses of snakes tested in the presence of scent trails do not differ from those obtained in control tests.

scent in the test arena. Strong trailing inclination was demonstrated by seven snakes (25%) that correctly chose the scented gate and four snakes (14%) that chose a gate immediately adjacent to the scented one. Ten snakes (36%) trailed initially (mean distance \pm SEM = 74 ± 15 cm), but eventually passed through end gates. Seven individuals (25%) showed no inclination to trail and also chose end gates. Snakes were classified, based on their inclination to trail, as exact trailers, near-exact trailers, partial trailers, or nontrailers (Table 2). Relative to males, a greater proportion of females (75%) showed partial and nontraining responses, but contingency analysis (Sokal and Rohlf, 1969) indicated that this difference was nonsignificant (chi square = 1.79, $df = 1$, $P > 0.20$).

DISCUSSION

The present study showed that garter snakes of both sexes, acclimated and tested under autumnal conditions, can follow the scent trails of a conspecific. This result concurs with the field observation that *T. sirtalis* followed common and well-defined "travel lanes" in their approach to the den (Costanzo, 1986, 1988) and further supports the hypothesis that pheromones are used for den location by snakes in nature.

The present study is the first to provide direct experimental evidence for trail following, in the context of den location, for any colubrid snake. However, Brown and MacLean (1983) convincingly demonstrated that neonate timber rattlesnakes (*Crotalus horridus*) from New York followed the trails of litter mates and adults of both sexes during autumn. This behavior apparently was not demonstrated by congeneric (*C. viridis*) adults from Wyoming, although details of testing protocol and specific results were not reported (King et al., 1983). Graves et al. (1986) later showed that *C. viridis* neonates were attracted to scents depos-

TABLE 2. PERFORMANCE, SEX RATIO, AND MASS DATA FOR GARTER SNAKES TESTED IN LABORATORY ARENA FOR ABILITY TO FOLLOW CONSPECIFIC SCENT TRAILS DURING AUTUMN

Performance group ^a	N	Males (N)	Females (N)	Mass ($\bar{X} \pm$ SEM)
Exact trailers	7	6	1	38.3 \pm 5.7
Near-exact trailers	4	2	2	26.3 \pm 6.6
Partial trailers	10	6	4	39.8 \pm 3.7
Nontrailers	7	2	5	62.9 \pm 10.0

^aSnakes were assigned to four groups on the basis of their inclination to follow scent trails. See text for classification details.

ited by adults and concluded that neonates follow knowledgeable conspecifics short distances to the den site.

Among snakes, pheromones facilitate aggregation for both sexual and non-sexual purposes. Nonsexual aggregations probably form when snakes follow conspecifics of both sexes (Burghardt, 1980; Dundee and Miller, 1968; Graves et al., 1986; Heller and Halpern, 1981); however, during the mating period, the trailing response is clearly sex-limited (i.e., males trail females but not other males, and females do not trail either sex; Ford, 1986). Thus, it is unclear whether separate chemical cues are involved. Although further study is needed to fully characterize these substances, the pheromone permitting *C. viridis* neonates to locate dens is likely a skin lipid (Graves et al., 1986).

Snake locomotion is well suited to the deposition of a continuous scent trail (Ford, 1986), yet the hypothesis of pheromone use for den location is not without conceptual problems. One criticism is that snakes would have an even chance of following a scent trail in the wrong direction. Ford and Lowe (1984), however, cleverly demonstrated that snakes gain directionality information from the differential placement of scent on objects contacted by the trail-forming individual. Nevertheless, the first snake to arrive at a den would not have the benefit of these cues. It is probable that older, experienced snakes may relocate traditional dens using other means, e.g., solar and celestial orientational cues (Landreth, 1973; Newcomer et al., 1974), topographic landmarks (Parker and Brown, 1980), and polarized light (Lawson, 1985). Naive snakes, such as neonates and juveniles (in the case where snakes hibernate apart from adults their first winter; Gregory, 1982, 1984), may rely more heavily on pheromones for den location than do adults. This contention is consonant with the finding in the present study that nontrailing *T. sirtalis* were significantly (Kruskal-Wallis, $P = 0.02$) larger (older?) than trailing snakes; it is also supported by the relatively late arrival of smaller snakes at some communal dens (Costanzo, 1986; Gregory, 1982, 1984; Parker and Brown, 1980), and by Lawson's (1985) suggestion that solar orientational abilities are less well developed in younger *T. radix*. Since many hibernacula are inconspicuous landscape features, orientational cues alone seem insufficient to precisely locate den sites. A more parsimonious explanation is that a combination of orientational, landmark, and pheromonal cues are involved (Brown and MacLean, 1983; Gregory et al., 1987; Hirth, 1966; Lawson, 1985; Parker and Brown, 1980).

It is not surprising that snakes inhabiting high latitudes show remarkable abilities to relocate traditional dens during lengthy autumnal migrations. The original northward expansion of species' ranges doubtless depended upon the evolution of ecophysiological adaptations to colder environments and the use of winter refuges that enhanced survival. Although den fidelity typically is high, it is also imperfect (Gregory, 1984). Accordingly, individuals that located (perhaps during summer forays) and used suitable hibernacula likely prospered and

established new communal dens. The progressive northward expansion of snake populations probably was associated with increasing selection pressures for the evolution of effective mechanisms (including pheromones) for den relocation and possibly relied on traditional, communal dens as "stepping stones." This may partly explain why communal denning behavior is more prominent in snake populations near the northern extent of the species' range (Gregory, 1982, 1984). Garter snakes inhabiting milder climates (with presumably less stringent requirements for overwintering sites and consequently a greater abundance of suitable dens) show reduced inclination to trail conspecifics outside of the courtship period (Ford, 1986). The role of pheromones in den location may therefore be of greatest importance in populations inhabiting the harsh environment characteristic of northern latitudes.

Most trail-following studies are performed under idealized conditions, thus their results are only suggestive of what may occur in nature. Precipitation decreases the arrival frequency of snakes at communal dens (Costanzo, 1986; Parker and Brown, 1980), perhaps by interfering with pheromone detection. Tongue flicking, a critical element in the pheromone detection process, is slowed dramatically in *T. elegans* at low body temperatures (Stevenson et al., 1985). Preliminary work in the present investigation showed that, at body temperatures of 5 and 12°C, *T. sirtalis* were largely unresponsive to the scent trails of conspecifics. Future studies should address the influence of environmental factors (e.g., temperature, precipitation, humidity, substrate texture, wind velocity, etc.) on the trail-following ability of snakes, as well as the taxonomic and geographical limits of pheromone use for den location.

Acknowledgments—I thank Dr. Dennis L. Claussen, Dr. Douglas H. Taylor, Debbie Holland, and two anonymous reviewers for commenting on this manuscript. This work is a portion of a Ph.D dissertation submitted to the faculty of Miami University, Oxford, Ohio.

REFERENCES

- BROWN, W., and MACLEAN, F. 1983. Conspecific scent-trailing by newborn timber rattlesnakes (*Crotalus horridus*). *Herpetologica* 39:430-435.
- BURGHARDT, G.M. 1980. Behavioral and stimulus correlates of vomeronasal functioning in reptiles: Feeding, grouping, sex and tongue use, pp. 275-301, in D. Muller-Schwarze and R. Silverstein (eds.) *Chemical Signals: Vertebrates and Aquatic Invertebrates*. Plenum, New York.
- COSTANZO, J. 1986. Influence of hibernaculum microenvironment on the winter life history of the garter snake (*Thamnophis sirtalis*). *Ohio J. Sci.* 86:199-204.
- COSTANZO, J. 1988. Eco-physiological adaptations to overwintering in the eastern garter snake, *Thamnophis sirtalis sirtalis*. PhD dissertation. Miami University, Oxford, Ohio.
- DUNDEE, H., and MILLER, M., III. 1968. Aggregative behavior and habitat conditioning by prairie ringneck snakes, *Diadophis punctatus arnyi*. *Tulane Stud. Zool.* 15:41-58.
- DUVALL, D., KING, M., and GUTZWILLER, K. 1985. Behavioral ecology and ethology of the prairie rattlesnake. *Nat. Geogr. Res.* 1:80-112.

- FORD, N. 1981. Seasonality of pheromone trailing behavior in two species of garter snake, *Thamnophis* (Colubridae). *Southwest. Nat.* 26:385-388.
- FORD, N. 1986. The role of pheromone trails in the sociobiology of snakes, pp. 261-278, in D. Duvall, D. Muller-Schwarze, and R. Silverstein (eds.). *Chemical Signals in Vertebrates, IV*. Plenum, New York.
- FORD, N., and LOWE, J., Jr. 1984. Sex pheromone source location by garter snakes: a mechanism for detection of direction in nonvolatile trails. *J. Chem. Ecol.* 10:1193-1199.
- GRAVES, B., DUVALL, D., KING, M., LINSTEDT, S., and GERN, W. 1986. Initial den location by neonatal prairie rattlesnakes: functions, causes, and natural history in chemical ecology, pp. 285-304, in D. Duvall, D. Muller-Schwarze, and R. Silverstein (eds.). *Chemical Signals in Vertebrates, IV*. Plenum, New York.
- GREGORY, P. 1982. Reptilian hibernation, pp. 53-154, in C. Gans and F. Pough (eds.). *Biology of the Reptilia, XIII*. Academic Press, New York.
- GREGORY, P. 1984. Communal denning in snakes, pp. 57-75, in R. Seigel, L. Hunt, J. Knight, L. Malaret, and N. Zuschlag (eds.). *Vertebrate Ecology and Systematics—A Tribute to Henry S. Fitch*. University of Kansas, Lawrence, Kansas.
- GREGORY, P., MACARTNEY, J., and LARSEN, K. 1987. Spatial patterns and movements, pp. 366-395, in R. Seigel, J. Collins, and S. Novak (eds.). *Snakes: Ecology and Evolutionary Biology*. Macmillan, New York.
- HELLER, S., and HALPERN, M. 1981. Laboratory observations on conspecific and congeneric scent trailing in garter snakes (*Thamnophis*). *Behav. Neurol. Biol.* 33:372-377.
- HIRTH, H. 1966. The ability of two species of snakes to return to a hibernaculum after displacement. *Southwest. Nat.* 11:49-53.
- KING, M., MCCARRON, D., DUVALL, D., BAXTER, G., and GERN, W. 1983. Group avoidance of conspecific but not interspecific chemical cues by prairie rattlesnakes (*Crotalus viridis*). *J. Herpetol.* 17:196-198.
- KLAUBER, L. 1956. *Rattlesnakes. Their Habits, Life Histories, and Influence on Mankind*, (2 vols.) University of California, Los Angeles.
- LANDRETH, H. 1973. Orientation and behavior of the rattlesnake, *Crotalus atrox*. *Copeia* 1973:26-31.
- LAWSON, P. 1985. Preliminary investigations into the roles of visual and pheromonal stimuli on aspects of behavior of the western plains garter snake, *Thamnophis radix haydeni*. MS thesis. University of Regina, Saskatchewan, Canada.
- NEWCOMER, R., TAYLOR, D., and GUTTMAN, S. 1974. Celestial orientation in two species of water snakes (*Natrix sipedon* and *Regina septemvittata*). *Herpetologica* 30:194-204.
- NOBLE, G., and CLAUSEN, H. 1936. The aggregation behavior of *Storeria dekayi* and other snakes, with especial reference to the sense organs involved. *Ecol. Monogr.* 6:271-316.
- PARKER, W., and BROWN, W. 1980. Comparative ecology of two colubrid snakes, *Masticophis t. taeniatus* and *Pituophis melanoleucus deserticola*, in northern Utah. Milwaukee Publ. Mus. Publ. Biol. Geol. No. 7:1-104.
- SOKAL, R., and ROHLF, F. 1969. *Biometry*. W.H. Freeman, San Francisco.
- STEVENSON, R., PETERSON, C., and TSUJI, J. 1985. The thermal dependence of locomotion, tongue flicking, digestion, and oxygen consumption in the wandering garter snake. *Physiol. Zool.* 58:46-57.