III. USING CHEMICAL CUES TO CONTROL
TERRESTRIAL VERTEBRATES


Background--My training is in the chemical ecology of vertebrates, specifically reptiles. I did my graduate work in Zoology at the University of Texas at Austin under David Crews. There I worked on garter snake reproduction doing both field and laboratory studies of the Canadian red-sided garter snake. I was primarily interested in how pheromones drive reproductive behavior in these animals. After finishing at Texas, I moved to the National Institutes of Health in Bethesda, Maryland where I worked for four years in a natural products laboratory learning chemistry techniques. This lab specialized in natural products and especially pheromones, mostly of insects. At NIH we were able to isolate, identify, and synthesize the garter snake sex attractiveness pheromone. This was the first, and to this day, only pheromone identified in any reptile.

Currently, I am still working on projects involving the conservation of these garter snakes in Manitoba, Canada. There, the snakes are being killed off in large numbers during spring and fall migrations across highways. We are trying to use pheromone technology involving pheromonal attractants and repellents to build what I call "chemical fences" to control the movements of the snakes. My other primary research effort is on the control of the brown tree snake on Guam and other Pacific islands. Again, we are trying to exploit the same pheromone technology to try and alter the snakes behavior. For example, one scenario involves attracting snakes into traps by means of pheromones. Once in the trap, the snakes can be exposed to viruses or fungi, leave the trap, wherein they can then spread the pathogen through the rest of the population.

Submitted manuscript--The study of chemical communication, semiochemicals (chemicals with signal function), and pheromones is recognized as a small, but critical area of reptilian social behavior. Indeed, reptiles in general, and snakes in particular are well known to be exquisitely tuned to the reception and perception of chemical cues in the environment. Chemical cues are very efficient energetically in that they are cheap to produce, they relay messages long after the producer is gone, they work in the dark and over very great distances. Squamate reptiles are interesting in this regard in that they may arguably be more sensitive to chemical stimuli than any other vertebrate.

In the constant battle with pest species, new technologies for the control of vertebrate pests have given rise to the development of several methods that utilize chemicals that modify
behavior. These behavior-modifying chemicals are used to induce a variety of specific responses such as trailing, aggregation, mate selection, courtship, and repulsion. The extensive application of pesticides has not only upset the delicate biological balance of nature, but also poses a danger to human health and drastic, long-term alteration of the environment. As a result, the emerging "alternative agriculture" and a worldwide shift towards integrated pest management are bringing behavior-modifying chemicals, particularly pheromones, to the forefront of pest control (Shani, 1991). Pheromones are viewed as ecologically friendly, chemically safe, and efficient in terms of cost of production and the small quantities usually needed to produce an effect.

The prevailing approach in integrated pest management is an ecological approach to reducing pest damage by using all the available techniques to maintain pests below damaging levels (pesticidal). This strategy is based on the determination of the point at which a pest population approaches the level at which control is necessary to prevent excessive ecological damage to native fauna or excessive detrimental effects to human ecology, mainly in the form of bites.

Currently, a use of pheromones that has been gaining momentum in the fight against insect pests also shows considerable promise in application to snake pests. This growing technology is the use of pheromones as a means of monitoring pest populations, including their introduction into new, previously undisturbed environments. This is important on a smaller scale as well as on a larger scale in the introductions of snakes and lizards onto new islands in the Pacific and elsewhere.

The tropical colubrid brown tree snake, Boiga irregularis, is native to Australia, New Guinea, and the Solomon Islands. Sometime during or immediately after World War II, the brown tree snake became established on Guam probably due to accidental transport by aircraft and ships. During the ensuing years, its population density has increased to several thousand snakes (up to 13,000) per square mile. This snake has clearly established itself as a serious threat to native fauna and humans.

Boiga irregularis is nocturnal and arboreal. It is considered a generalist predator preferring roosting birds, nestlings, and even eggs. By the late 1960's and early 1970's, bird populations on Guam had begun to decline at an alarming rate. By 1986, nine species of native forest birds were considered extinct (Savidge, 1987). The few species of birds that remain are either too large for the snake to eat or are seriously threatened. Directly related to the decline of the bird populations is the potential threat to agricultural crops and native vegetation by insect pests previously kept in check by
avian predators. With the shrinking prey base caused by the mass extinctions of birds on Guam, the snake has broadened its prey base to include small mammals such as mice, rats, and shrews. Predation on the Marianas fruit bat has now caused populations of this valuable insectivore to be dangerously reduced (Wiles, 1987). The snake has even begun to seriously threaten populations of native lizards and skinks.

As the prey base continues to shrink in inhabited areas of the island, the snakes are interacting more frequently with humans. Perhaps the most disturbing and costly problem is the increasing number of power outages and damage to electrical equipment of the Guam Power Authority and Naval Public works caused by snakes climbing the power poles, presumably to stalk roosting or nesting birds (Fritts, 1988). The resulting power losses have caused aggravation to local citizens and are a serious economic burden. Since 1984 there have been over 500 power outages due to snakes with resulting damages totalling in the millions of dollars.

Finally, the snake is now regarded as a potentially hazardous problem to humans. The brown tree snake is apparently quite comfortable living in close proximity to human activity and habitation. With this increased contact, the brown tree snake is now found raiding poultry houses and backyards where domestic pets, poultry and eggs are readily encountered and consumed. The snake also enters houses and snakebites are increasing dramatically in Guam (Fritts et al., 1990). Although the bites of B. irregularis have been described as harmless to humans (Cogger, 1975), the brown tree snake is a rear-fanged mildly venomous snake. The most serious encounters with the snakes are those involving small children. Indeed, four infants less than one year old were treated for snakebite recently on Guam (Fritts et al. 1990). The infants developed respiratory problems and exhibited symptoms indicative of some neurotoxins. All the infants recovered but serious injury or death could have resulted. The brown tree snake which can reach lengths of ten feet, can also be a threat if it should attempt to constrict a human infant.

This discussion will only consider three of several uses of behavior-modifying chemicals. Attraction and mating disruption have proven to be quite effective in selected examples of insect pest management (see Ridgway et al., 1990). These two experimental paradigms seem to hold the most promise for application in ophidian pest management. The third, repellents, has not received as much study. I will attempt to describe current efforts by researchers investigating pheromones in the brown tree snake. Finally, a discussion of the need for future research directions will be addressed. By taking stock of where the field is today, enlightened decisions can be made to direct
future efforts with the goal of effective, safe, and economical control of ophidian pest species.

Attraction and mating disruption are being focused on because they appear at this time to hold the most promise as biological control agents. They possess certain features that make them attractive candidates for further study including: behavioral responses are elicited at very low concentrations, they are highly specific, and they have potential for immediate use in pest management.

The use of pheromones as attractants and mating disruptants possesses several features that distinguish this approach from the use of chemical pesticides. Pheromones may indirectly kill snakes by luring them into traps, to toxicants or to pathogens, or they may alter normal reproductive or aggregative behavior. In some cases, such as mating behavior, pheromones will affect only one sex, in this case males. However, aggregation and trailing pheromones will affect both sexes equally. The field testing parameters including the location, number of replicates, size of the trap associated with the pheromones will vary and depend on the type of behavior being affected, the habits of the particular target species, environmental considerations, and estimated population densities of the target species.

Efficacy of the program would be defined as a lowered population density and could include quarantine maintenance, elimination of nuisance or noxious populations, or complete eradication. Efficacy must be demonstrated under field conditions. Significance of the treatment should normally be assessed by data from several experimental and control plots.

Another promising prospect for the control of ophidian pest species with pheromones is by use of a technique known as "male confusion." The basic premise is that when the environment is permeated with pheromone, the number of males locating females will be greatly reduced, and that this decrease in mating activity will result in lower population levels in subsequent generations (Birch and Haynes, 1982).

The physiological basis for mating disruption is not fully understood. Disruption could potentially result from competition between sexually attractive females and sources of synthetic pheromone. Here, a large number of sources releasing high concentrations of pheromones out compete the female. Permeation of the environment with pheromone trails and aggregation sites may lead to sensory adaptation or habituation to the pheromone. Both processes could decrease or eliminate behavioral responses to female pheromones and would decrease matings in the field.
To demonstrate mating disruption, small-scale test plots are established in which a trap baited with sexually attractive females or synthetic pheromones is surrounded by trails of synthetic pheromone. Alternatively, sexually attractive, unmated females are placed in the center of a plot to determine if males can find and mate with them. It is crucial that trails of pheromone lead to the female's location.

Historically, studies of pheromones in snakes are all extensions of earlier research conducted on a North American colubrid, the garter snake, *Thamnophis sirtalis* and the Swedish adder, *Vipera berus*. G. K. Noble (1937) was the first investigator to study the sex attraction pheromones that females use to attract males. He discovered that the source of the sex pheromones was the dorsal skin of the female and not the cloacal gland secretions as proposed by others (Baumann, 1929).

During the breeding season, male garter snakes and male adders, *Vipera berus* (Nilson, 1980; Andrén, 1982; 1986), initiate courtship behavior in response to a pheromone sequestered on the female's dorsum. Rapid tongue-flicking serves to deliver these cues to the male's vomeronasal organ. The vomeronasal organ is known to be the sole mediator of the reception and perception of pheromone cues in these snakes studied to date (Kubie et al., 1978; Kubie and Halpern, 1979; Halpern and Kubie, 1980; Andrén, 1982).

That the skin is the source of sex pheromones in many snakes has been known for some time by zoo workers (Radcliffe and Murphy, 1983). Indeed this was confirmed by a series of experiments in which skin tubes from male and female garter snakes were used to induce courtship from courting males (Gillingham and Dickinson, 1980). Treatment with exogenous estrogen causes intact, reproductively inactive, and ovariectomized female *Thamnophis s. sirtalis* to become attractive to and elicit courtship from sexually active males (Crews, 1976). Thus, it appears that ovarian steroids positively affect the production and/or expression of sexual attractiveness in female garter snakes. Estrogen treatment in conjunction with shedding appears to be the most effective means of eliciting pheromone production (Kubie et al., 1978). In an attempt to characterize the sex pheromones of the garter snake, Garstka and Crews (1981, 1986) used plasma from estrogen-injected females and males to induce courtship from sexually active males. They originally hypothesized that the yolking protein vitellogenin or some lipidaceous subunit of vitellogenin was acting as the sex pheromone (Garstka and Crews, 1981). However, further investigations lead these authors to conclude that vitellogenin was not responsible for eliciting courtship behavior (Garstka and Crews, 1986).
The sex attraction pheromones of the red-sided garter snake, *Thamnophis sirtalis parietalis*, have now been isolated, identified and synthesized (Mason et al., 1989; 1990; 1993). The pheromones, a novel series of long-chain saturated and monounsaturated methyl ketones have been shown to elicit courtship behavior from sexually active males in the field. To date, this is the only pheromone yet identified in a reptile.

The most recent work on snake pheromones has concentrated on the skin lipids as the source of the sex attractiveness pheromones. Thus, the more recent work on the brown tree snake has concentrated on the skin lipids of the female as a source for the sex attractiveness pheromones. It was hypothesized that because the garter snakes and brown tree snakes are both members of the family Colubridae, their sex pheromones might be related chemically as well. Hexane extracts of female garter snakes were fractionated and yielded one active fraction that contained a novel series of saturated and monounsaturated methyl ketones ranging in molecular weigh from 422 to 534 daltons. Using the same experimental paradigm, Murata et al. (1991) isolated a similar series of saturated and monounsaturated methyl ketones. However, in addition, the main components were a novel series of methyl ketodienes. Ketodienes have never been detected in garter snake skin lipids. Behavioral trials with brown tree snakes using ketodienes have been ambiguous. The major problem is that reliable sex behavior is difficult to induce in both male and female brown tree snakes. However, recent observations of breeding behavior of brown tree snakes in Australia may provide information of value to these investigations.

There are two other behaviors in snakes mediated by pheromones. Trailing studies have focused primarily on three behaviors: detection and location of conspecifics during the breeding season, aggregation, and migration to winter hibernacula. Only the first two are relevant to this discussion. At least 10 species in five families of snakes are known to utilize pheromone trails (see Ford, 1986; Mason, 1992 for review). Most reports of trailing in snakes concern reports of trailing during the breeding season. It is generally the case that male snakes preferentially follow female trails. Females in general tend not to trail males or females during the breeding season.

Another important behavior mediated by pheromones in snakes is aggregation. Gravid female snakes have been reported to aggregate, presumably for some benefit in gestating offspring or eggs (see review in Gillingham, 1987; Gregory et al., 1987). It is not clear what proximate factors are responsible for aggregation in the field. In the laboratory, temperature, humidity, and stress have been demonstrated to be directly related to the incidence of aggregation (see Mason, 1992 for
review). However, whether any or all of these are major factors operating in nature has not been tested directly.

In most investigations of aggregation in snakes, stimulus animals leave chemical cues on filter papers that can then be used as bedding under shelters. Snakes prefer to reside under shelters in laboratory conditions and probably in the field as well. In a number of experimental procedures using a variety of different species of snakes, investigators have repeatedly found that when groups of snakes are offered the chance, they choose to aggregate as opposed to remaining alone. That this tendency to aggregate is based on chemical cues left on the substrate has been demonstrated in many studies (see Mason, 1992 for review). The source of the chemical cues differs among species but the two most effective sources are skin lipids and cloacal gland secretions. Aggregation behavior has also been described in the brown tree snake. An aggregation of fourteen brown tree snakes was reported in a hollow tree on Guadalcanal (Pendleton, 1947). Experimental investigations of aggregation behavior in the brown tree snake have not been attempted.

All snakes described have paired cloacal scent glands located in the tail dorsal to the hemipenes in males and in the corresponding position in females. The glands are holocrine in nature and produce primarily lipids (Oldak, 1976). The function of these glands has been the subject of much controversy over the past century. Two of the proposed functions can be attributed to defensive aspects of ophidian behavior. The effects of the cloacal gland secretions have usually been described as repellent and/or alarming.

Some authors have attributed an alarm function to the cloacal gland secretions of disturbed snakes. Anecdotal reports frequently relate descriptions of snakes becoming very agitated and disturbed when exposed to the cloacal scent gland secretions expressed by a conspecific individual. Experimental studies have demonstrated that animals exposed to conspecific scent gland secretions react more intensely to stressful conditions than do animals that were not conditioned by the scent gland secretions (Graves and Duvall, 1988). A common response of a snake to these scent gland secretions is to flee. The current hypothesis is that since cloacal scent gland secretions are expressed under stressful conditions such as predation, it would benefit a conspecific to be able to interpret these semiochemicals as an indicator of imminent and proximate danger and leave the area at once. The exploitation of these scent gland secretions as a tool for modifying snake behavior is obvious. To date, no studies have been conducted on the repellency of brown tree snake cloacal gland secretions. Studies on the chemical constituents that comprise the cloacal gland secretions have been conducted (Mason, unpublished). Further, behavioral tests are planned in order to
determine what behavioral response, if any, the brown tree snake will have to its cloacal gland secretions.

The role of pheromonal agents in integrated pest management strategies as applied to reptilian pests is still in its infancy. However, it is recognized that aspects of social behavior in the brown tree snake may be amenable to exploitation by pheromones. I have concentrated here on reviewing the research to date on four behavioral paradigms that seem to hold the most promise for use as control agents: sex behavior, trailing behavior, aggregation behavior, and alarm or repellent behavior. Much more work is needed before these pheromones can be brought to bear as one of the multifaceted methods utilized in integrated pest management strategies.

A novel strategy that has not been discussed in regard to integrated pest management of reptiles warrants inclusion here. One system that has been attempted in insect control strategies involves the use of pheromones in conjunction with naturally occurring pathogens. For example, one paradigm is to use pheromone-baited traps to attract snakes. The trap is contaminated with a virus or other pathogen and the snake allowed to escape. During aggregations or mating behavior, the pathogen is passed on to additional individuals which in turn pass it on yet again. This strategy would necessitate the investigation of potential contagious pathogens in reptiles. Published accounts of virulent pathogens affecting exclusively snakes are becoming more common as experts knowledgeable in their identification and their concomitant research have proliferated (Frye, 1981; Cooper and Jackson, 1981; Hoff et al., 1984). Indeed, research into potential viral pathogens of brown tree snakes is currently underway (D. Nichols, personal communication).

One indicator that pheromones are becoming an important part of integrated pest management systems is the recent involvement of the agricultural and chemical industries. It has been suggested that the fact that pheromone success in monitoring or even controlling pest populations will reduce pesticide sales is not conducive to the involvement of industry, particularly since the quantities of pheromones needed are relatively so small and must be specifically formulated for each species (Birch and Haynes, 1982). However, as humankind becomes more reluctant to add synthetic substances of broad and lasting effectiveness to the environment, the impact of pheromone technology on industry and agriculture will continue to assume revolutionary significance. Only by integrating our knowledge of the basic biology, behavior, reproduction, and chemical ecology of ophidian pest species into an integrated pest management strategy will meaningful and significant advances be made toward controlling snake populations.
REFERENCES


III.2. **Sulfur-containing Semiochemicals Attract Predators and Repel Prey** (Russell Mason).

**Background**--Dr. Russ Mason is a psychologist who received his M.A. and Ph.D. degrees from Clark University. Following post-doctoral training at Brown University and the Monell Chemical Senses Center, Russ became an Assistant Member on the regular Monell staff. In 1986, he accepted a position with the Denver Wildlife Research Center. At present, Dr. Mason is a project leader for the Wildlife Research Center, and an Associate Member at Monell. He also has faculty appointments at the University of Pennsylvania (Biology) and Rutgers University (Animal Sciences).

**Submitted manuscript**--Regardless of vertebrate class, carnivores and omnivores are attracted by sulfurous odors and herbivores are repelled by them. This is especially true in feeding contexts. The present manuscript seeks to provide a plausible explanation for this dichotomy. As a background for the explanation, several illustrative examples are provided below.

When faced with a choice among feeding sites, Norway rats prefer locations that conspecifics are exploiting (Galef and Clark 1971, Galef and Heiber 1976). When faced with a choice among several novel foods, naive rats choose the types of novel foods that have previously been ingested by conspecifics with whom they have interacted (e.g., Strupp and Levitsky 1984). This socially mediated transfer of food preference is semiochemically mediated (Galef and Wigmore 1983, Galef and Stein 1985).