Responses of Python regius to animal chemosensory stimuli: Implications of foraging ecology

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RESPONSES OF PYTHON REGIUS TO ANIMAL CHEMOSENSORY STIMULI: IMPLICATIONS OF FORAGING ECOLOGY

by

Albert Klein
Bachelor of Science
Northern Arizona University
1994

A thesis submitted in partial fulfillment of the requirements for the

Master of Science Degree
Department of Biological Sciences
College of Science and Mathematics

Graduate College
University of Nevada, Las Vegas
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Master of Science

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ABSTRACT

Responses of *Python regius* to Animal Chemosensory Stimuli: Implications of Foraging Ecology

by

Albert Klein

Dr. Robert Winokur, Examination Committee Chair
Professor of Biological Sciences
University of Nevada, Las Vegas

*Python regius* is considered to be a sit-and-wait forager and has been reported to attempt capture of live and dead homeothermic prey. This preference for homeothermic prey may be innate and is not dependent on thermal cues. Chemosensory stimuli appear to be the only stimuli which may indicate to *P. regius* the metabolic group that a dead prey item at room temperature item may belong to. Snakes were exposed to novel odors from homeothermic and poikilothermic animals. Prey Searching Behavior (amount of locomotion) and Information Gathering Behavior (tongue flicking) were observed. Habituation to odors resulted in decreased behaviors. Odors of homeothermic animals elicited greater behavioral responses than did odors of poikilothermic animals. Particular characteristics of specific odor molecules may elicit neither, one, or both foraging behaviors. A relationship has been described here for the first time between Prey Searching Behavior and Information Gathering Behavior.
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CHAPTER 1

INTRODUCTION

*Python regius*

*Python regius* is a member of the family Boidae (boas and pythons) (Greene, 1997, Pough et al., 1998). Boidae is considered to be among the most primitive snake families (Greene, 1997; Pough et al., 1998). Evidence of this can be found in skeletal structure, including the presence of anal spurs, the last vestiges of legs in living snakes (Greene, 1997; Pough et al., 1998). Boas are distributed mostly in the New World, while pythons are distributed in the Old World (Greene, 1997; Pough et al., 1998). *P. regius* inhabits the savannas and steppe of western and central Africa (Mehrtens, 1987; Vosjoli et al., 1995; Schivre, 1972). Among Africa’s pythons, *P. regius* is the smallest, attaining an average length of one to one and a half meters (Mehrtens, 1987). It is a nocturnal, terrestrial snake that frequents burrows and cavities in the ground where it hunts for small mammals (Coborn, 1995). Observers of captive species predominantly report mice as the snake’s favored prey (Vosjoli et al., 1995). No studies were located that document the diet or feeding ecology of *P. regius* in its natural habitat. Few studies exist concerning such information for any boids (Slip and Shine, 1988), even though boid snakes are of interest from several points of view including their large size, constricting behavior, and ability to consume extremely large prey items (Slip and Shine, 1988). Although numerous snakes have been reported to feed on poikilothermic prey, no such literature exists on the ingestion of such prey by *Python regius*. Published data on the diets of other species of pythonine...
snakes are primarily anecdotal, but mammals seem to be a principal prey item of these snakes (Vosjoli et al., 1995; Slip and Shine, 1988).

Many owners of pet pythons such as *P. regius* choose to feed them dead prey (Vosjoli et al., 1995). An owner may purchase many dead, frozen prey. Thawed prey are then presented to the python. Often these prey are served at room temperature. Such prey are usually ingested. Many snake owners even report an unwillingness of their pets to capture live prey after being fed dead prey for some time (Vosjoli et al., 1995).

The American common name of *P. regius* is ball python (Vosjoli et al., 1995; Mehrten, 1987). This name was given to the snake due to its characteristic defensive behavior. When threatened, *P. regius* tightens its coils around its head. This results in a ball shape. The strength of the snake's coils in this shape often make it difficult for a human to uncoil the snake, and the snake can be rolled as a ball (Vosjoli et al., 1995; Mehrten, 1987).

**Snake foraging ecology**

The food that an animal eats, and the way it obtains that food, are central aspects of a species' ecology (Slip and Shine, 1988). Foraging behavior may determine rates of energy intake and survivorship and may have wide-ranging effects on the evolution of modified morphology, physiology, behavior, or reproductive biology (Slip and Shine, 1988).

Snakes move for a variety of reasons. Movement of snakes is associated with hunting and predator avoidance as well as the locating of mates and the finding of favorable conditions for inactivity, egg-laying, and thermophilicity (Greene, 1997). Pit vipers (Viperidae) have been observed trailing prey that has been envenomated (Melcer and Chiszar, 1989). In such studies, prey that were envenomated by any snake were more frequently trailed than were prey that were killed by other means. Movement of some
colubrid snakes (Colubridae) has been recorded in response to prey odors (Burghardt, 1970).

Snakes often are classified as being either sit-and-wait foragers or active foragers (Greene, 1997; Pough et al., 1998). Sit-and-wait foragers practice ambush predation, usually waiting near known animal trails or bird perches for prey to come within a reasonable distance for the predator to successfully capture the prey (Pough et al., 1998). Active foragers move throughout their range in a constant search for prey. Most research on snake foraging has focused on active foragers belonging to the families Elapidae and Colubridae (Greene, 1984). Sit-and-wait predation has only been described in detail for Viperidae (vipers and pit vipers) (Duvall et al., 1985) and the diamond python (*Morelia s. spilotra*) (Slip and Shine, 1988).

Locomotor specializations vary with the size of the organism and the needs of the organism to adapt to its environment (Greene, 1997). Large-bodied snakes, including pythons, are usually considered to be sit-and-wait foragers (Pough et al., 1998). Large-bodied snakes move relatively slowly on the ground due to their size (Greene, 1997), and typically ingest prey much larger than themselves (Pough et al., 1998). Although most snakes ingest large prey, it is most evident in boids (Pough et al., 1998). The ingestion of such large prey may require several weeks for digestion to occur, thus allowing the snake to engage in little movement during the digestion of such prey (Greene, 1997). Thus, many authors consider members of the subfamily Pythoninae (pythons) to be sit-and-wait foragers, despite the lack of scientific literature on these animals' ecology in natural settings.

The energy requirement for movement of large-bodied snakes is much greater than the energy requirement of smaller species. Thus, a sit-and-wait predation strategy is most common in such species, while smaller, more agile species' physiology allows for a more active foraging strategy (Greene, 1997; Pough et al., 1998). However, juvenile boids are more slender proportionally than are adults. Such young snakes are more agile than the
stocky adults and thus may utilize a more active foraging strategy than adults. This is true of juvenile reptiles in general (Pough et al., 1998).

Although the classification of snakes into two foraging strategies allows for simple conclusions to be drawn, many authors have realized that the terms “sit and wait” and “active” foraging are misnomers; in reality there exists much more of a continuum, and closely related species may differ in their position on this continuum (Greene, 1997; Slip and Shine, 1988). In general, foraging mode is phylogenetically conservative in snakes (Slip and Shine, 1988). Viperid snakes, for instance, vary in the importance of mobility in their foraging behavior (Reinert, et al., 1984). Many colubrids and elapids may search actively for their prey (Greene, 1984; Shine and Lambeck, 1985).

Some snakes have specialized diets and feed on relatively few species of prey (Greene, 1997; Pough et al., 1998). Hognose snakes (Heterodon sp.) typically prey exclusively on anurans (Ernst and Zug, 1996; Greene, 1997). Some species of garter snakes (Thamnophis sp.) prefer fish or earthworms (Burghardt, 1970). Shivik (1997) reports that brown tree snakes (Boiga irregularis) will enter traps baited only with bird odors. Most species of rattlesnakes feed exclusively on rodents (Melcer and Chiszar, 1989) and exhibit traits that capitalize upon rodent morphology, physiology and behavior (Greene, 1983), such as waiting in ambush along trails habitually used by their prey. Chemical perception that is related to the feeding ecology of a species in newborn snakes is species-specific (Burghardt, 1967). Highly specific stimulus-response information is probably genetically coded in the organism and probably is expressed by an innate filtering mechanism at the level of the Jacobson’s organ or perhaps within the central nervous system (Burghardt, 1967).

**Chemosensation and odors**

Vertebrates are able to distinguish between thousands of different odorants (Freitag et al., 1998). The size and diversity of the olfactory receptor family is considered as basis
for the discriminative ability of the vertebrate olfactory system (Freitag et al., 1998). Snakes can recognize chemical cues at birth (Burghardt, 1990). The activation of different subsets of sensory neurons to different degrees is the basis for neural encoding and further processing of the odor information by higher centers in the olfactory pathway (Hildebrand and Shepherd, 1997). Chemosensory stimuli often are important for the organization of feeding, mating, and social behaviors, as well as for the processes of learning and memory that are associated with these behaviors (Hildebrand and Shepherd, 1997).

Neural mechanisms of odor discrimination begin with differential interactions of odor molecules with different types of receptors (Hildebrand and Shepherd, 1997). The odor molecule thus acts as a ligand (Hildebrand and Shepherd, 1997). In vertebrate odor receptor cells (ORCs), binding of odor ligands to the membrane receptors leads to gating of membrane conductances via activation of G-protein-coupled second-messenger pathways. The hypothesis that different second-messenger pathways are activated by different odors has been addressed by several investigators with mixed results (Hildebrand and Shepherd, 1997). Whether excitatory or inhibitory, ORC responses define the range of odors that can elicit a response in a given cell (Hildebrand and Shepherd, 1997). This profile has been termed the molecular receptive range (MRR) (Mori et al., 1992; Mori and Shepherd 1994). Thus, generalist ORCs have broad MRRs, whereas specialist ORCs have narrow MRRs.

Airborne chemicals influence animal behavior in many ways (Schab and Crowder, 1995). There has been a recent increase in the amount of scientific research devoted to understanding the behavioral effects that airborne chemicals have on animals (Schab and Crowder, 1995) and humans (Nef, 1998). Reptiles and especially snakes have not been excepted from this attention. Many recent studies have addressed the response of snakes to airborne chemicals (Austin and Gregory, 1998; Arnold, 1978; Cooper, 1991; Stone and Holtzman, 1996; Cooper, 1986; Halpern et. al. 1997).
Chemosensation is the most primitive of all senses (Doty, 1995; Shepherd, 1994). Chemical stimuli are important to a variety of organisms in obtaining information about their environment. In reptiles this sense is highly developed, especially in squamates (lizards, snakes and amphisbaenians) (Zug, 1993). It is perhaps developed to the greatest degree in snakes, more so than any other vertebrate (Greene, 1997; Ernst and Zug, 1986; Burghardt, 1993). Perhaps because snakes may lack a highly evolved auditory system they have evolved a highly sensitive chemosensory system. Many squamates detect volatile chemosensory stimuli via a bifurcated tongue that transfers stimuli from the environment to the Jacobson’s organ (Greene, 1997; Halpern and Kubie, 1980). The bifurcated tongue is extended from an opening in the mouth into the air and is flicked several times. The tongue is then retracted back into the mouth and inserted through two vomeronasal ducts and into the Jacobson’s organ where the cells detecting chemosensory stimuli are located. Relative concentrations on the right and left tines off the tongue indicate the proximity and direction of potential mates, enemies, and prey (Greene, 1997). Tongue-flicking has been used as a dependent measure of interest of a snake in chemical cues, and is based on the assumption that elevated tongue-flick rates and vomeronasal stimulation are functionally associated (Halpern, 1992). Tongue flicking may even be a species-specific behavior (Halpern and Kubie, 1980).

Memory of chemosensory stimuli has been documented in vertebrates (Schab and Crowder, 1995). Reactions to subsequent presentations of certain chemosensory stimuli elicit similar behaviors to the first presentation. Habituation to such stimuli has also been recorded in vertebrates (Schab and Crowder, 1995). In snakes, habituation to such stimuli such as that of a particular prey has resulted in increased, decreased, and equal responses to the same stimuli in subsequent presentations (Arnold, 1978). Food odor cues have been demonstrated to elicit associative behaviors in rats (Schab and Crowder, 1995). In humans, odor memory remains intact over time and is strongly resistant to change, even when new information about the odor is available (Engen, 1991).
Focus of this study

Research has addressed the foraging behavior of *P. regius* in response to familiar animal odors. Cooper (1991) exposed snakes that had eaten mice to mouse odor by presenting them with this odor on a cotton swab 1 cm. from their mouth. A pungency control (cologne) was also presented in this manner as was an odor control (distilled water). These pythons tongue flicked a great deal more when presented with mouse odor than when presented with either the odor control or the pungency control. Cooper (1991) suggested that *P. regius* can discriminate between prey chemicals and chemicals not derived from food sources. Anecdotal information suggests that *P. regius* will ingest dead birds and mammals but not live or dead poikilotherms (Vosjoli, 1995; Griehl, 1982; Klein, unpublished observations). Rattlesnakes (Viperidae) require both visual and thermal cues to elicit a predatory strike (Newman and Hartline, 1982) and to trail envenomated prey (Melcer and Chiszar, 1989). Is *P. regius* also constrained by such sensory requirements to elicit feeding behavior? If so, then why will it ingest dead rodents that are the same temperature as their environment? Visual cues may provide some information (Cooper, 1991).

Studies on the stimuli requirements of pythons to elicit prey searching behavior are lacking. Knowledge of the sensory mechanisms utilized to increase foraging efficiency is critical to our understanding of animal behavior and natural history (Austin and Gregory, 1998). The foraging behavior of snakes is of particular interest due to their many unique modes of locating, capturing and subduing prey (Slip and Shine, 1988). The response of boids such as *P. regius* to chemosensory stimuli has been observed (Cooper, 1991), but the majority of sensory studies of pythons has focused on the reception of infrared stimuli by the python’s infralabial pits (Austin and Gregory, 1998; Newman and Hartline, 1982). The role of the chemical senses in boa constrictors (*Boa constrictor*) is of particular interest since this species has the highest threshold of all heat-sensitive snakes examined (de Cock Buning, 1983), which suggests that these snakes may also rely on other sensory cues to
elicit feeding behavior (Stone and Holtzman, 1996). The use of visual, thermal and chemosensory cues may vary in boids during different phases of predatory behavior (de Cock Buning, 1983). Rattlesnakes (Viperidae: Crotalinae) require infrared as well as visual stimuli to elicit a strike on potential prey, and utilize infralabial pits to sense differences in temperature between potential prey and the environment (Newman and Hartline, 1982). This information is integrated with visual information in the optic tectum of these snakes (Hartline et al., 1978) and possibly as well in boid snakes (Molenaar, 1974).

Poikilothermic animals are thus rarely, if ever ingested by these snakes. Pythons utilize infralabial pits for prey detection as well (Greene, 1997; Newman and Hartline, 1982). The neural connections and neural integration of stimuli in the brain, however, are somewhat different from that of rattlesnakes. Sensory information from the infralabial pits of crotalids passes through an additional brain structure, the nucleus reticularis calor is, the function of which is not known (Newman and Hartline, 1982).

This study investigated the possibility that chemical cues which share certain characteristics such as might be found in mammalian and avian integument elicit greater foraging behavior responses than do other cues such as may be found in the integument of poikilotherms or in the absence of any animal cues. Although the study of molecular olfaction is relatively new (Shepherd, 1994), some speculation regarding the molecular nature of these odors may be possible. This study examines for the first time the responses of *P. regius* to novel, animal chemosensory stimuli, including stimuli from animals that have not been reported to be natural prey or preferred food (in captivity) of *P. regius*. Such animals are poikilothermic. An attempt will be made to determine if *P. regius* can discriminate between odors from poikilothermic and homeothermic animals, and will also attempt to determine if a relationship exists between prey searching behavior and tongue flicking behavior.

This study attempts to identify the locomotive patterns of *P. regius* in response to novel and familiar animal odors. Stimulation of chemosensory senses in snakes can
produce typical behaviors such as ambush positions or active foraging (Burghardt et al., 1988). An increase in tongue flick rate by a sit-and-wait forager is unusual among reptiles (Downes, 1999). Among lizards, only those that adopt an active foraging mode possess a vomeronasal system that is capable of discriminating between various prey cues (Cooper, 1994, 1997). Tongue flicking disrupts the crypticity required for successful ambush predation (Cooper and van Wyk, 1994). Thus, this study will attempt to determine the foraging mode of *P. regius*.

Observations of *P. regius*’ response to novel and familiar chemosensory stimuli was recorded. Stimuli consisted of integumentary odors of poikilothermic and homeothermic animals, including fish. Fish are not likely to be encountered by *P. regius*, a terrestrial species that often frequents burrows searching for mammals (Coborn, 1995). A pungency control (perfume) was also exposed to these pythons. Most studies that investigate responses in squamates to chemosensory stimuli employ use of a pungency control (Cooper, 1991; Cooper and Burghardt, 1990; Cooper, 1989b; Cooper, 1989a). Inclusion of the pungency control allows assessment of responses to a readily detectable but non-food stimulus (Cooper, 1997). Other controls will consist of an unscented odor conductor (paper ball) and the absence of experimental stimuli.

The unscented control (paper ball) was repeated several times consecutively to determine what the response of *P. regius* is to a repeated stimuli. This will be the first reporting of habituation of *P. regius* to a chemosensory stimuli.

Python behavior was observed also upon presentation of mouse odor, a familiar chemosensory stimuli. The results from this treatment may indicate the behavior of *P. regius* when presented with an odor the source of which has been previously observed, captured, and ingested.
CHAPTER 2

MATERIALS AND METHODS

Housing and care of pythons

Eight, captive-bred *Python regius* were purchased from a commercial dealer (Exotic Tropical Fish, Van Nuys, CA) in May of 1997. Pythons were approximately three months old at time of acquisition. Animals were cared for according to conditions accepted by the University of Nevada, Las Vegas Animal Care and Use Committee (Protocol #R701-0799-150) and the California State University, Northridge Office of Research and Sponsored Projects (Protocol #112497Klein). Pythons were housed together near Northridge, California in terraria of varying size utilizing an astroturf substrate. A hiding place was provided in one corner of the terrarium; a water bowl was provided in the opposite corner. Each terrarium was fitted with a screen cover. An incandescent light fixture fitted with a 40-watt bulb rested on top of the cover, and a photophase of 10 hours per day was maintained. Pythons were fed one live, juvenile, albino mouse each week. Water was provided *ad libitum*.

In August of 1997 each python was housed in its own thirty-eight liter terrarium with dimensions of 50 x 26 x 30 cm. in Northridge. All pythons were fed one live, adult, albino mouse every ten days. All other previously mentioned conditions were maintained.

In December of 1998, pythons were moved to Las Vegas, Nevada. All conditions maintained in California were maintained in Las Vegas.

Both trial sequences began seven days after a feeding; feeding was withheld throughout the duration of a trial sequence.
Odor presentation

Odor sources

Odor sources are listed in Table 1.

Table 1. Description of treatments and their odor sources. No object treatment included observations of python behavior with no experimental stimuli introduced into experimental chamber. Perfume treatment utilized an artificially-scented liquid designed to odorize indoor environments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Odor Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>No object</td>
<td>None</td>
</tr>
<tr>
<td>Blank (1, 2a, 2b, 2c, 2d)</td>
<td>Paper ball</td>
</tr>
<tr>
<td>Perfume</td>
<td>Artificial, lemon-scented liquid</td>
</tr>
<tr>
<td>Fish</td>
<td>Carassius sp.</td>
</tr>
<tr>
<td>Newt</td>
<td>Cynops sp.</td>
</tr>
<tr>
<td>Frog</td>
<td>Rana pipiens</td>
</tr>
<tr>
<td>Lizard</td>
<td>Anolis carolinensis</td>
</tr>
<tr>
<td>Snake</td>
<td>Lampropeltis getulus californiae</td>
</tr>
<tr>
<td>Bird</td>
<td>Erythrura sp.</td>
</tr>
<tr>
<td>Hamster</td>
<td>Cricetus cricetus</td>
</tr>
<tr>
<td>Rat</td>
<td>Rattus norvegicus</td>
</tr>
<tr>
<td>Mouse</td>
<td>Mus musculus</td>
</tr>
</tbody>
</table>

Odor-conducting material

Standard, white, 8 1/2” x 11” notebook paper was crumpled into a ball shape.

Paper was chosen as odor-conducting material for several reasons: paper resisted mammalian physical activity such as chewing and clawing; paper demonstrated its capacity to hold odors in pre-experimental trials; and paper dried quickly, a characteristic that proved important with trials involving fish and amphibian whose moist skin altered other materials.

Scenting of paper balls

A nineteen-liter terrarium was used as an odor chamber. In most trials, two odor sources (animals) were placed inside the chamber along with ten paper balls for approximately thirty minutes. During this time the chamber was closed with a glass cover. Paper balls were then removed as needed during trials. A ball was usually rubbed on the
animal twice before the ball was removed from the chamber. The paper ball was disposed of after a trial, and the chamber was washed with bleach after each use. Exceptions to this procedure are noted below.

For the perfume odor treatment, an open vial of perfume was placed inside the odor chamber. Ten paper balls were placed inside the chamber. The lid was placed on the chamber. Fifteen minutes later, five drops of liquid were placed on each ball. The open vial of liquid was replaced in the chamber with the balls. The lid was replaced on the chamber. Fifteen minutes later, the trials began. Each ball received two drops of liquid a few minutes prior to each trial.

For the fish odor treatment, a nineteen-liter aquarium holding goldfish and fresh water was placed near the odor chamber. Before each trial, a fish was removed from the water and a paper ball was rubbed on the fish twice. The ball was then placed in the odor chamber for about five minutes to dry before being used in a trial.

Data collection

Experimental chamber

The bottom of a fifty-seven liter terrarium was marked with masking tape applied to the bottom of the terrarium into twelve sectors of equal area. The dimensions of the terrarium are 65 x 29 x 30 cm. Sectors were numbered one through twelve; those closest to the researcher were numbered seven through twelve; those furthest from the observer were numbered one through six (Fig.1).

Protocol

Latex gloves were worn to control avoid possible odor contamination. Before each trial began, the chamber was wiped with isopropyl alcohol and allowed to dry. A substrate of clear plastic sheeting cut to the dimensions of the floor of the chamber and wiped with...
isopropyl alcohol and dried was placed on the terrarium bottom. A scented (except in trials utilizing Blanks) paper ball was placed in sector five. The researcher disposed of the latex gloves being worn and wore a new pair of gloves. A python was obtained from the terrarium in which it was housed. The python’s body was placed on sector three and the python’s head was placed on sector two. The aquarium was fitted with a metal, closed cover. Pythons were allowed no more than thirty seconds to accommodate to the chamber.

Data recording

The following words were stated into a microcassette recorder to record python behavior.

1) The numbers one through twelve to indicate which sector the python’s head entered.

2) “Flick” to indicate a tongue flick.

Data were recorded for five minutes.
Setting of Trials

The following trials were performed at 1600h in the order listed on consecutive days commencing on July 2, 1998 in California.

Blank 1
Snake
Hamster
Lizard
Frog
Newt
Perfume
Bird
Fish
Rat

The following trials were performed at 1600h in the order listed on consecutive days commencing on March 21, 1999 in Las Vegas.

Blank 2a
Blank 2b
Blank 2c
Blank 2d
Mouse
No object

Data analysis

Statistical analyses utilized StatView software (Abacus Concepts, Inc., Berkeley, CA 1996). Specific tests are noted in results. Considerations of statistical significance were made at the p = 0.05 level.
CHAPTER 3

RESULTS AND ANALYSES

Responses to odors varied among different treatments and among test subjects. Total number of sectors entered ranged from 1 to 35. Total number of tongue flicks ranged from 0 to 173. (Fig. 2).

![Figure 2](image_url) Numbers of sectors entered and tongue flicks for nine novel odor treatments and one familiar (M) odor treatment. Treatments are ranked (1-10) from greatest to least response. Means + SE (n=8) are shown for each treatment. The x-axis shows odors in the order of presentation. (S = snake; H = hamster; L = lizard; F = frog; N = newt; P = perfume; B = bird; G = fish; R = rat; M = mouse).

**Effects of order of odor presentation**

Order of presentation was not correlated with either the number of sectors entered or the number of tongue flicks for novel odor treatments (Spearman rank correlation using mean and median response had the same results; \( p \gg 0.05 \) for both number of sectors entered and number of tongue flicks) (Fig. 2).
Inter-individual variation and the effects of python order

Pythons were highly variable in their responses (e.g., 0 to 158 tongue flicks for one treatment) and pythons that were tested earlier in a given trial tended to have more tongue flicks in response to both novel and familiar odors (Spearman rank correlation coefficient = 0.89 and 0.86, respectively; p = 0.02 for both). Python order was not significantly correlated with the number of tongue flicks among the blanks (unscented paper ball or no object), or with sectors entered in any of the treatments. Python order was the same in all treatments.

The high inter-individual variation, the small sample size (n=8) and the suggestion of an order effect dictated that nonparametric rank and sign tests be used for comparisons among treatments.

Accommodation to paper balls

Sectors Entered

The numbers of sectors entered decreased upon repeated, consecutive presentations of the blank treatment (Fig. 3). The greatest decrease was between the first and second blank treatments. The differences in responses elicited among second, third and fourth treatments were not significant (Kruskal-Wallis test; p >> 0.05). The response elicited by the first blank, B2a, was significantly greater than the response elicited by the fourth blank, B2d (Mann-Whitney U, p < 0.05).

Tongue Flicks

The numbers of tongue flicks also decreased upon consecutive, repeated presentations of the blank treatment (Fig. 4). This trend is most apparent between the first and second blank treatments. The first and second treatments are significantly different from each other (Mann Whitney U, p = 0.02). No significant differences were found among the second, third and fourth blanks (Kruskal-Wallis test; p >> 0.05).
Figure 3. Sectors Entered for sequential blank treatments. The number of sectors entered decreased after the first presentation. Means + SE (n=8) are shown for each treatment.

Figure 4. Tongue Flicks for sequential blank treatments. The number of tongue flicks decreased after the first presentation. Mean + SE (n=8) are shown for each treatment.

The fact that the expression of both behaviors dropped off sharply after the first presentation of the stimulus and did not significantly decay thereafter suggests that accommodation to novel stimuli occurs very rapidly under these test conditions, and that only the first presentation of a stimulus can be regarded as novel. Due to this rapid accommodation to the blanks (paper ball), a response above the level of the second blank was considered a response to the odor on the paper ball and not the paper ball itself. The
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Whitney U, \( p = 0.34 \)); or blank and snake treatments (Mann Whitney U, \( p = 0.71 \)) for numbers of sectors entered.

![Figure 5](image.png)

**Figure 5.** Tongue flick response to pungency control. The tongue flick response to the perfume odor treatment was significantly greater than the response to a blank treatment.

There was no significant trend toward increased or decreased response to number of sectors entered between blank and snake treatments (paired sign test, \( p = 0.99 \)); between blank and lizard treatments (paired sign test, \( p = 0.99 \)); between blank and frog treatments (paired sign test, \( p = 0.72 \)); or between blank and fish treatments (paired sign test, \( p = 0.29 \)).

The number of tongue flicks significantly differed between the blank and fish treatments (Mann Whitney U, \( p = 0.03 \)); blank and frog treatments (Mann Whitney U, \( p = 0.01 \)); and blank and lizard treatments (Mann Whitney U, \( p = 0.03 \)) (Fig. 7). No significant differences were found between blank and newt treatments (Mann Whitney U, \( p = 0.21 \)); or blank and snake treatments (Mann Whitney U, \( p = 0.19 \)) for number of tongue flicks.

There was a significant trend toward increased response to number of tongue flicks between blank and lizard treatments (paired sign test, \( p = 0.008 \)) and blank and frog.
Figure 6. Sectors entered response to poikilothermic odors. Fish and frog odors elicited a significantly greater number of sectors entered than did the mean blank.

Figure 7. Tongue flick response to poikilothermic odors. Fish, frog, and lizard treatments all elicited significantly greater number of tongue flicks than did the mean blank.
**Odors of homeotherms**

Significant differences were found between the blank and rat treatments (Mann Whitney U, \( p = 0.04 \)) for numbers of sectors entered (Fig. 8). No significant differences were found between the blank and bird treatments (Mann Whitney U, \( p = 0.07 \)) or blank and hamster treatments (Mann Whitney U, \( p = 0.30 \)) for numbers of sectors entered.

There was no significant trend toward increased or decreased response to number of sectors entered between blank and bird treatments (paired sign test, \( p = 0.29 \)); between blank and lizard treatments (paired sign test, \( p = 0.12 \)) or between blank and rat treatments (paired sign test, \( p = 0.07 \)).

![Figure 8. Sectors entered response to homeothermic odors. Rat odor elicited a significantly greater number of sectors entered than did the mean blank.](image)

Significant differences were found between the blank and bird treatments (Mann Whitney U, \( p = 0.05 \)) and blank and hamster treatments (Mann Whitney U, \( p = 0.05 \)) for number of tongue flicks (Fig. 9). No significant differences were found between blank and rat treatments (Mann Whitney U, \( p = 0.06 \)) for number of tongue flicks.

There was a significant trend toward increased response to number of tongue flicks between blank and bird treatments (paired sign test, \( p = 0.008 \)). There was no significant
trend toward increased or decreased response to number of tongue flicks between the blank and hamster treatments (paired sign test, \( p = 0.07 \)) or blank and rat treatments (paired sign test, \( p = 0.07 \)).

![Graph showing mean tongue flicks for bird and hamster treatments.](image)

**Figure 9.** Tongue flick response to homeothermic odors. Bird and hamster odors elicited a significantly greater number of tongue flicks than did the mean blank.

**Response to environmental stimuli only**

No significant differences were found between the blank and no object treatments. The number of tongue flicks did not differ (Mann Whitney U, \( p = 0.13 \)); nor did the number of sectors entered (Mann Whitney U, \( p = 0.24 \)). There was no significant trend toward increased or decreased response between the two treatments for either behavior (paired sign test; \( p >> 0.05 \)).

**Sectors entered / tongue flicks relationships**

The number of sectors entered was positively correlated with the number of tongue flicks for almost every treatment (\( r^2 = 0.359 \) to 0.923; \( p = 0.0005 \) to 0.05; Figs. 10 - 13). Correlations of fish, frog and hamster odors reflect this trend of positive correlation. The relationship between the number of sectors entered and number of tongue flicks differs
among treatments. Both slopes (range 2.3 to 8.6) and intercepts (2.3 to 67) vary substantially.

Figure 10. Linear regressions between numbers of tongue flicks and numbers of sectors entered for sequential blank treatments. Numbers of tongue flicks are positively correlated with numbers of sectors entered. Regression equations and correlation coefficients are shown for each treatment. (p < 0.05).

Summary of results

Responses to a familiar odor were not different from responses to the blank treatment. The pungency control elicited a greater number of tongue flicks but not a greater number of sectors entered. Odors of snake and newt did not elicit any significant response; frog odor elicited significantly greater responses for both numbers of tongue flicks and sectors entered. Lizard odor elicited a greater tongue flick response but not a greater sectors entered response. The number of tongue flicks for all novel, homeothermic odors was greater than the response to blank treatments. The number of sectors entered was greater
than the response to blank treatments for most novel, homeothermic odors. Responses to no object in the chamber were not significantly different from responses to the blank treatment. The number of sectors entered was correlated with the number of tongue flicks for almost all treatments.

![Figure 11. Linear regressions between numbers of tongue flicks and numbers of sectors entered for pungency control and no object treatments. Numbers of tongue flicks are positively correlated with numbers of sectors entered. Regression equations and correlation coefficients are shown for each treatment. (p < 0.05).](image)
Figure 13. Linear regressions between numbers of tongue flicks and numbers of sectors entered for all homeothermic odors. Numbers of tongue flicks are positively correlated with numbers of sectors entered for bird, rat, and mouse odor treatments (p < 0.05). Regression is insignificant for hamster odor (p > 0.05). Regression equations and correlation coefficients are shown for each treatment.
Figure 12. Linear regressions between numbers of tongue flicks and numbers of sectors entered for poikilothermic odors. Numbers of tongue flicks are positively correlated with numbers of sectors entered for newt, lizard, and snake odor treatments (p < 0.05). Regressions are insignificant for fish and frog odors (p > 0.05). Regression equations and correlation coefficients are shown for each treatment.
CHAPTER 4

DISCUSSION

Interpretation of behaviors

Prey searching behavior

Animals move for a variety of reasons, including foraging, defense, and sexual activity (Tinbergen, 1965). The age of the pythons in this study at time of experimentation was seventeen months. At this age *P. regius* is not capable of sexual activity (Greene, 1997; Mattison, 1986; Spellerberg, 1982; Vosjoli et al., 1995). Several species of snakes decrease locomotor activity and rely on crypsis for defense (Greene, 1997; Ernst and Zug, 1996) upon detection of a potential, predatory threat. *P. regius*’ defensive behaviors consist of cryptic and species-specific behaviors, both of which require decreased locomotor activity (Coborn, 1995; Mehrtens, 1987; Vosjoli et al., 1995). Increased locomotion in response to odors, therefore, is due to foraging efforts; specifically, Prey Searching Behavior (PSB). The total number of sectors entered indicates the python’s degree of locomotor activity, or PSB.

Information gathering behavior

The flicking of a snake’s tongue enables the snake to retrieve molecules in the air and obtain information about its environment (Cooper, 1991; Gillingham and Clark, 1981; Greene, 1997). The total number of tongue flicks indicate the quantity of molecular information obtained and is often utilized as a measure of foraging interest based on the assumption that elevated tongue flick rates and vomeronasal stimulation are functionally

Habituation

The initial response to an odor stimulus is typically followed by a period of reduced responsiveness, termed sensory adaptation, evident in reduced response to repeated stimuli (Hildebrand and Shepherd, 1997). Several mechanisms that affect the nervous system may contribute to such an adaptation in vertebrates (Hildebrand and Shepherd, 1997). One such mechanism is the reduction of single-channel open probability due to internal Ca2+ acting through calcium-calmodulin (Zufall et al., 1991). Another possibility is that kinases may act on the second-messenger components to reduce their activity (Breer, 1994). A third possibility is that low concentrations of CO increase cyclic nucleotide-gated membrane conductance (Leinders-Zufall et al. 1995).

In this experiment, exposures to an unscented paper ball resulted in subsequently decreased responses to the ball. These results suggest that repeated exposures to an odor from a source which is not ingested result in decreased future responses to that odor. A stimuli whose source is neither prey nor predator is likely to be responded to less in subsequent exposures because the source of the odor apparently has little consequence for the animal. Habituation allows animals to not respond to insignificant stimuli (Maier, 1998). Reacting less vigorously to an insignificant stimulus may result in a significant saving of time and energy (Maier, 1998).

Prey searching behavior (PSB)

Responses of *P. regius* to repeated exposures to blanks indicate that subsequent exposures to the same odor result in decreased PSB when compared to the original odor presentation. Therefore, any deviation from this pattern of response can be attributed to the
odors themselves. This suggests that responses to other novel odors are due to the odors themselves and are independent of order of presentation.

**Information gathering behavior (IGB)**

IGB of *P. regius* when exposed to repeated exposures of blanks decreased as did PSB. These behaviors also indicate that subsequent exposures to the same novel odor result in decreased IGB when compared to the original odor presentation. This suggests that responses to other novel odors are due to the odors themselves and are independent of order of presentation.

**Familiar odors**

Pythons responded to mouse odor with neither increased PSB nor increased IGB. These pythons were fed mice exclusively for over a year before any trials commenced. They were fed in the following manner. With few exceptions, prior to feeding pythons were underneath their hiding places in their respective terraria as feeding usually occurred in the daytime. A live mouse was placed inside the subject’s terrarium. Mice will invariably investigate a dark area such as these hiding places within a few minutes (Griehl, 1982). Upon investigation of the hiding place, these pythons usually captured the mouse and later ingested it.

Learning in snakes has been documented, including garter snakes that learned their way through mazes (Kubie and Halpern, 1978; Kubie and Halpern, 1979). Learning prey behavior after repeated interactions with such prey is widely documented among all animals (Alcock, 1998). Rattlesnakes wait by small mammal trails in an attempt to ambush such prey (Greene, 1997; Mehrtens, 1987); green tree pythons (*Chondropython viridis*) wait near frequented perches for avian prey (Greene, 1997; Mehrtens, 1987). A captive snake whose natural behavior is to enter dark, underground tunnels in search of rodent prey (Vosjoli et al. 1995; Coborn, 1995) is likely to learn its only prey’s behavior, especially if
such prey invariably engages in such behavior repeatedly. Garter snakes associate chemosensory cues with prey movement during their first experience with live fish, and in subsequent encounters chemoreception alone is sufficient to elicit attack (Arnold, 1978). However, some studies have shown that feeding experience does not always enhance chemical prey preference (Arnold, 1978).

Knowledge of the behavior of mice may allow for *P. regius* to remain in its hiding place and not search as it knows that the mouse will invariably investigate a dark hiding place and will not ever move greater than a 40 cm. radius (maximum distance that a mouse can move away from a python in this study in the python's own terrarium) away from the snake. The python therefore can expend less energy by remaining in its hiding place and either waiting for the mouse to approach or even very slightly moving in the direction of the mouse odor. Increased IGB is not necessary as the odor source can be readily identified, and knowledge of the prey’s behavior eliminates a need for increased PSB.

**Novel homeothermic odors**

*Prey searching behavior*

Snakes move for a variety of reasons. Since the snakes in this study were not capable of reproductive activity and do not increase locomotion as a defensive behavior, it is likely that an increase in locomotor activity of *P. regius* is the result of detection by the snake of potential prey and subsequent interest by the snake in capturing such prey quickly.

The *P. regius* in this study responded to the odor of a rat by engaging in PSB at a significantly greater rate than they did towards blanks. Although the difference between the response to bird odor and the blank was not significant (Mann Whitney U, p = 0.07), the results suggest a trend that might become significant with a larger number of observations (n = 8).

Hamster odor did not elicit greater PSB; rat odor did elicit greater PSB. Mammals secrete many odors, primarily for social reasons (Brown, 1985; Hurst, 1993). Rodents use
odor cues to detect and avoid potential competitors (Daly et al., 1980) and mammalian predators (Dickman, 1992). Hamsters and mice are more alike in their odor secretions than are either of those animals to rat odor (Brown, 1985). Males of all three rodents secrete social odors from their oral lips. Female mice and hamsters also secrete social odors from their oral lips. Female rats do not secrete social odors from oral lips (Brown, 1985). These odors secreted by hamsters and by mice are postulated to be involved in sex identification and/or dominance or rut identification. Although gender of treatment rats were not recorded at the time, recent research into the rats used from the university vivarium strongly suggests that female rats were utilized for this study. It is likely that female hamsters and female mice secrete a very similar perhaps even identical odor from these lips that female rats do not.

Reception of these odors by pythons that are aware of mouse behavior after having experienced their behavior and subsequent ingestion may elicit the same PSB for hamsters as for mice upon reception of such an odor. Thus, *P. regius* may interpret the source of hamster odor to be a mouse, and therefore a large amount of investigation is unnecessary as the behavior of mice is known to *P. regius*. However, some differences may exist between integumentary odors that may elicit greater IGB. One of the functions of hamster odor that is not reported to be a function of mouse odor is that of territory marking (Brown, 1985). Hamsters possess flank glands and they flank-mark, a gland and behavior not known to be utilized by mice or rats (Brown, 1985). The odor(s) from these glands may elicit the heightened IGB observed in this study. These odors were definitely novel, and are not characteristic of mice. Apparently, the odors from these glands do not elicit greater PSB.

Female rats may not release this chemical and do not release any chemical from their oral lips (Brown, 1985). In the absence of such stimuli and in the presence of other integumentary stimuli that is perhaps characteristic of homeotherms, *P. regius* may be likely to investigate the source of the stimulus as it is unsure of the behavior of this odor source. A highly mobile, less investigative animal such as a rat or perhaps a bird would require quick identification and attack. The strong PSB response for rat and bird support

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this hypothesis. An alternate explanation is that the odors which are secreted from male rodents may inhibit, at least to some extent, PSB. The odors of a hamster may be different enough from the odor of a mouse to elicit greater IGB than a mouse odor does, but may not be different enough to elicit greater PSB behavior because the python may associate the hamster odor with mouse odor, and thus associate hamster behavior with mouse behavior. Further investigation into other characteristics of these odors may also help to identify molecular as well as other differences in the odors of these animals.

Another hypothesis for the explanation of the disparity in results among rodent odors in this study may be the acquisition of chemical cues from diets in rodents. Melcer and Chiszar (1989) suggested that this possible acquisition of chemical cues may vary among rodents. Rattlesnakes were believed to have detected this information and were able to successfully trail envenomated prey with this information; this was confirmed in another study as well (Lavin-Murcio and Kardong, 1995). Although the rodents utilized in this study consumed similar diets, their varied physiology may have allowed for the acquisition of particular chemical cues in one species and not another.

Downes (1999) also observed non-elevated numbers of tongue flicks of juvenile broadheaded snakes (Hoplolcephalus bungaroides) when it was exposed to odors of mice, a major food item of adults of this species. She suggests that snake’s may respond only to scents of prey that they can physically ingest (Downes, 1999). Thus, snake’s ability to detect mouse scent may be turned on later in life when the animal undergoes a shift in diet and habitat use (Mushinsky and Lotz, 1980).

Information gathering behavior

P. regius in this study also responded to odors of most homeothermic animals with significantly greater IGB than they did to blanks. Although the difference between the response to rat odor and the blank was not significant (Mann Whitney U, p = 0.06), the
results suggest a trend that might become significant with a larger number of observations (n = 8).

Odor detection is a critical sense for all species of snakes (Greene, 1997). It is used to find food and mates, to detect predators, to stimulate courtship, and for other activities (Ernst and Zug, 1996). The age of the pythons utilized in this study was not sufficient to presume that any responses were due to sexual behavior. Increased IGB is therefore due to either defensive or foraging behavior. Although the possibility of increased IGB being a response to odors perceived to be a threat has not been investigated, several authors have found increased IGB in response to known and unknown prey odors (Cooper, 1991; Cooper, 1986; Halpern, 1982; Arnold, 1978). It thus seems likely that increased IGB is a response that is engaged in due to increased interest in potential prey. Many authors report small mammals as being the presumed natural prey of *P. regius*, although there exists no scientific literature which addresses this (Vosjoli et al., 1995).

**Exceptional poikilothermic odors**

The response to the odors of newt and snake were not different for either behavior. These results support anecdotal information about the preferred prey of *P. regius*. The PSB response to lizard odor also reflects this presumed preference. However, responses to other poikilothermic treatments were greater than expected. These exceptional odors are discussed below.

*Lizard odor*

Comparisons of lizard odor treatments to blank treatments resulted in significantly greater IGB but not significantly greater PSB for lizard odor than for blank. Lizard odor, a poikilothermic odor, would be expected to elicit a response similar to an unscented paper ball. Instead, this odor elicited a greater response.
Perfume odor also elicited greater IGB but not significantly greater PSB when compared to blank. That floral perfumes can be registered and remembered by rattlesnakes implies that ophidian chemosensation is not restricted to cues associated with natural prey, mates or enemies (Melcer and Chiszar, 1989). Some olfactory receptor cells, or ORCs (Hildebrand and Shepherd, 1997) and associated central nervous system processes must transduce, encode, and retain novel chemical information (Arnold, 1981). Lizard and perfume odors may exhibit a similarity at the molecular level. Odor quality is also affected by molecular properties such as electrical charge and chemical reactivity (Cain, 1988).

(Burghardt et al., 1988) observed that responses of garter snakes to various prey extracts was possibly due to different observed molecular weights of the molecules constituting the odors. Thus, odors with molecular characteristics such as found in lizard and perfume odor may elicit increased IGB in response to information which may indicate the existence of predators. The absence of locomotor movement in response to these stimuli appears to support this hypothesis; crypsis requires decreased movement. Snake tongues are about 55 mm. long and 3 mm. wide; detection of a snake tongue by a predator large enough to be dangerous to *P. regius* seems unlikely. *P. regius* can flick its tongue and continue to obtain information about threatening stimuli while maintaining cryptic behavior consisting of decreased locomotion. Although tongue flicking disrupts the crypticity required for successful ambush predation (Cooper and van Wyk, 1994), potential prey are unlikely to observe tongue extrusion by an ambushing snake hidden within a retreat site (Downes, 1999). This is likely to be true as well for predators. Individual snakes can show a diversity of antipredator responses including crypsis, flight and a variety of stereotyped responses to a predator at close range (Arnold and Bennet, 1984). Rattlesnakes utilize motionlessness as a defensive behavior, and this may reflect the snake’s modest capabilities of rapid locomotion (Schieffelin and Queiroz, 1991). This may be what odors such as lizard and perfume elicit: curiosity (increased IGB) and caution (decreased or equal PSB). These odors may have properties which are interpreted by *P. regius* as being dangerous.
The snakes utilized in this study were less than 50 cm. in length. Although the ecological significance of perfume to *P. regius* is obviously not applicable, there exist in western Africa several families of lizards, such as Varanidae and Gerrhosauridae (Pough et al., 1998; Welch, 1982) that are potential predators of juvenile pythons of this size. Innate cryptic behaviors that are engaged when such an odor is detected surely would be advantageous to such pythons. IGB risks little in the way of detection; therefore, utilization of this behavior may aid the python in determining if the odor actually is a source of danger, and if so what behaviors may be engaged in to minimize the risk to itself.

*Fish and frog odors*

Frog odor and fish odor both elicit greater PSB and IGB. Both odors are similar in many respects. Many frogs are known to have noxious odors (Myers et al., 1991). Both odors utilized in this study were noxious and easily discernible by humans. The chemosensory system of snakes is among the most highly developed of any animal (Greene, 1997); therefore, it is likely that an odor easily detectable by humans is detected by snakes. Both odor sources are animals whose epidermis is characteristically moist, and this was the case of the animals used at the time of paper-ball scenting. The chemical identification of the prey substances to which snakes respond has resulted in the identification of molecular weights of what may be odor ligands from the moist integument of some animals. Burghardt (1990) reports that he has identified effective fractions of both low and high molecular weight from a fish and earthworm, two animals with moist skin. Both experienced and naive snakes responded to these fractions. Sheffield et al. (1968) showed that garter snakes (*Thamnophis sirtalis*) responded to surface substances of earthworms (*Lumbricus terrestris*) which were non-volatile, stable, water-soluble macromolecules. Halpern (et al., 1986) concluded that a large molecule, perhaps of 400,000 daltons was involved in these substances that appeared to be collagen derived from the cuticle of the earthworm.
P. regius is not known to capture or ingest fish or frogs. The results of these treatments therefore may allow us to identify which characteristics of odors elicit PSB and IGB. Other characteristics, perhaps not found in either fish nor frog integument, but found in homeothermic integument may elicit capture behavior. Some snakes require visual or other prey cues to attack prey even after prey odors have been identified (Cooper, 1991). The intermediate frequency of biting by P. regius in Cooper (1991) may suggest the importance of alternate cues in some circumstances. Its heat-seeking pits may be important in its behavior of frequently occupying rodent burrows (Mehrtens, 1987).

The presence of novel, homeothermic odors alone may elicit increased PSB, increased IGB, and perhaps capture behavior. Other odors, such as those from frogs or lizards, may elicit increased PSB and/or increased IGB but not prey capture behavior if the odor source is in the absence of differences in temperatures between the potential prey item and its surrounding environment. Future research, perhaps utilizing the methods described in this research and adding infrared components to the odor sources may help to answer these questions.

Evidence suggests a biological cut-off, presumably based on molecular size, across series of various chemicals to produce threshold pungency (irritation) in the human nose (Cometto-Muniz et al., 1998). Two proposed mechanisms for this cut-off include a physical mechanism whereby the maximum available quantity of stimulus in the vapor phase falls below the threshold, and a biological mechanism whereby the stimulus lacks a key property to trigger transduction (Cometto-Muniz et al., 1998). A molecule, could, for example, exceed the size that allows it to interact effectively with a target site or to fit into a binding pocket in a receptive macromolecule (Cometto-Muniz et al., 1998). Such a cut-off may exist in the molecular nature of animal chemoreception, such that certain odors elicit behaviors due to either their quantity or their characteristic shape while others do not possess the correct quantity or shape to elicit a particular response.
Responses to the absence of experimental stimuli

The responses of *P. regius* to no object in the chamber are not significantly different from the responses to any of the blanks. Odors from the chamber’s surrounding environment which were present at other trials may have been weaker than those odors which were used to scent the paper balls as well as odors of the paper balls themselves. In the absence of these experimental odors, other environmental odors may have elicited behaviors of *P. regius* in response to these environmental odors as if these odors were novel. The no object trial needs to be repeated several times before a solid hypothesis about *P. regius* behavior in the absence of specific, experimental stimuli can be proposed.

Correlation of Prey Searching Behavior and Information Gathering Behavior

Responses to odors are significantly correlated between PSB and IGB. These behaviors are also correlated in garter snakes (Burghardt et al., 1988) and iguanid lizards in captivity as well as in the field (Burghardt et al. 1986). A desire to locate a prey item may elicit these and other behaviors at the same rate in a coordinated effort to locate prey. Even the responses to no object being in the chamber as well as the responses to repeated blanks are correlated. Thus, Prey Searching Behavior and Information Gathering Behavior are correlated whether or not novel, chemosensory stimuli are present. This correlation may only be true for juveniles of this species that are characteristically more active than the adults, or this correlation may exist throughout all behaviors, such as defensive and reproductive behaviors.

Conclusions and future studies

Intraspecific variation exists in responses of *P. regius* to chemosensory stimuli. Burghardt (1975) tested snakes repeatedly with the same fish and worm extracts and statistically significant differences in preference were shown for individuals. As snakes
grow older or larger their prey preferences can change (Burghardt, 1990). Thus, innate and genetically based intraspecific variation in chemical prey preference exist (Arnold, 1981).

*P. regius* do habituate to chemosensory stimuli and engage in decreased foraging behaviors when presented with repeated exposures to familiar stimuli. Odors of previously ingested prey (mice) do not elicit increased foraging behavior presumably due to the snake’s knowledge of this animal’s behavior. Rodent odors may elicit different intensities of foraging behaviors depending on the species of rodent. This variability in response to this taxon may be due to a variety of factors such as different social odors and different anatomical odor sources, or may be due to different physiology or ecology of these animals with respect to feeding and diet. Other odors of homeothermic animals (birds) elicit increased foraging behavior.

Odors of some poikilothermic animals do not elicit increased foraging behavior. Some odors of poikilothermic animals do elicit increased foraging behavior. These results may reflect dietary preferences of *P. regius* previously undocumented, or may simply identify characteristics of odors that elicit increased investigative behavior but not capture behavior.

Juvenile *P. regius* do not appear to engage in sit-and-wait predation. Known prey (homeotherms) elicited increased Prey Searching Behavior. The size of these juvenile snakes may dictate that active foraging be employed as its smaller prey is likely to also be quite mobile and more difficult to identify than the larger prey of adult *P. regius* may be.

Foraging behaviors are correlated with each other. This correlation exists in the presence of all stimuli presented and in the absence of stimuli.

Examining the possibility of capture of poikilothermic prey by *P. regius* under several different conditions would yield tremendous insight into the results obtained in this study. Capture and ingestion of poikilotherms would indicate a prey preference that has been until now discounted. More likely, prey capture would not result. This would allow...
for a stronger hypothesis to exist regarding the specific, molecular nature of odors eliciting varied responses among *P. regius*.

Repeating these experiments utilizing various rodent species and identifying such species odor emanation may also yield insight into the nature of odors that elicit various responses in *P. regius*.

The ability to detect prey odors is widespread among squamates and ubiquitous among snakes studied (Cooper, 1991). Examining the possibility of foraging behavior correlations in other squamate species may allow for hypotheses to be drawn regarding the evolution of tongue flicking and prey searching behavior in these animals.

It is important to consider these results and interpretations within the context of the study. Many animals, when kept in captivity and deprived of the various stimuli they would encounter in a natural setting, may respond differently if not captive (Shivik, 1997). One author suggests that all future analyses of chemical perceptual mechanisms utilized for foraging consider evolution and ecology (Burghardt, 1967). Studies focusing on the ecology of *P. regius* in its natural habitat would benefit this research a great deal.

Knowledge of foraging activities and prey preferences if they exist would greatly contribute to the knowledge thus far accumulated about this species.

Burghardt (1993) suggests that any attempt to typologically study the snake chemosensory system by looking at only one convenient species is to "place self-imposed binders on oneself", conceptually and empirically, or even risk being seriously misled. Further studies such as this one that utilize other squamate species undoubtedly would allow for hypotheses to be formulated regarding the evolution and ecology of these behaviors in Squamata. A more complete understanding as well of *P. regius*’ interactions with its ecological community may result from such research.
LITERATURE CITED


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