EFFECT OF BIOGENIC AMINES ON DECISION-MAKING BEHAVIOR IN THE SOUTHERN DEVIL SCORPION (*VAEJOVIS CAROLINIANUS*)

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ABSTRACT

EFFECT OF BIOGENIC AMINES ON DECISION-MAKING BEHAVIOR IN THE SOUTHERN DEVIL SCORPION (*VAEJOVIS CAROLINIANUS*)

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Chemical cues play an important role in an organism's assessment of their environment and resulting decision-making behavior. Examples include kairomones, which prey species use to gather information about the predator species that produce them. *Vaejovis carolinianus*, the southern devil scorpion, occupies both secondary and tertiary trophic levels of its communities and, as with all members of its order, possesses dedicated chemosensory organs called pectines. *V. carolinianus* and members of its family exhibit radical sexual dimorphism in pectine size. Three biogenic amines, 2-phenylethylamine, tyramine and histamine, were selected as treatment. The aims of this study are to determine the effect, if any, of the tested amines on decision-making behavior and to assess if the difference in pectine morphology among the sexes conveys a chemosensory advantage to males outside of functions related to courtship and mate detection.

In order to assess the effect of three biogenic amines on decision-making behavior, twelve identical mazes were constructed. Treatment was deposited on a sand substrate in one arm of each trial, and 70 individuals were tested against each amine. Of the three test amines, two (2-phenylethylamine and tyramine) are trace amines belonging to the phenylethylamine class of neurotransmitters; of these, 2-phenylethylamine has been shown to function as a

kairomone used by rodents. Histamine, the third amine to be tested, is a monoamine and also functions as a neurotransmitter.

In each set of trials, females were shown to be more likely to avoid tested amines than males, although avoidance was significant only in trials concerning tyramine and histamine; 2-phenylethylamine did not produce a significant avoidance in either sex. Notably, males exhibited statistically significant avoidance of only tyramine, showing no discernable preference for histamine. The results of the study suggest that females use chemical information differently in matters of environmental assessment and decision-making behavior.

INTRODUCTION

Predation is likely to have constituted the driving force in the increase in size and complexity among Precambrian life, a process which eventually culminated in the apparent explosion of diversity upon the advent of the Phanerozoic eon, when complex, multi-trophic food webs emerged and evidence of antipredator adaptations such as biomineralization began to appear in the fossil record (Bengston 2002).

Since that time, predation pressure has influenced numerous forms and behaviors. These adaptations have benefitted predator and prey alike, permitting the former access to the latter and the latter recourse from the former. Of the two, however, the phenomenon of predation must inflict greater pressure on prey; for though a predator may survive a failed hunt, a successfully preyed-upon organism, by definition, will not, and will therefore lose any future potential for reproductive success. As a result, organisms inhabiting lower trophic levels must have evolved effective antipredator adaptations in order to remain extant. These adaptations may take the form of either morphological modifications or behavioral strategies.

These strategies, however successful, have costs as well as benefits; prey species must not only escape predation, but also acquire nourishment and reproduce. As any given individual has a finite amount of time and energy with which to accomplish these tasks, it follows that the use of predator avoidance strategies must detract from the individual's ability to obtain resources and improve their own reproductive fitness. However, because the cost of failure is so high with regard to predation risk, we may expect a given individual to prioritize predator avoidance behaviors, which increase the likelihood of survival, over behaviors such as foraging or reproduction.

These decisions are further complicated by the differing energy costs and degrees of effectiveness of various avoidance strategies such as fleeing or hiding behaviors, with regard to the environment in which they are undertaken; for example, an open habitat may encourage fleeing behavior whereas a more closed habitat might favor hiding (Camp et al. 2012). We can expect that the perceived evidence of a potential predator's prior presence must also inform decisions concerning foraging site selection and the ensuing acquisition of resources (Lima and Dill 1990). This information may be attained through an organism's use of both mechanosenses (informed by visual or tactile inputs) and chemosenses (informed by the detection of chemicals, as in gustation and olfaction).

Chemical cues in particular play an important role in the interaction of predator and prey species; a prey animal may rely on the recognition and avoidance of its predator's scent just as the success of a predator may depend on the detection of a social prey species' chemical signals (Cárdenas et al. 2012). In this way, habitats can be viewed as overlaid with a complex network of chemical stimuli, which in turn mediates the flow of information within predator-prey relationships (Dicke and Grostal 2001). For instance, Persons et al. (2001) found that the wolf spider *Pardosa milvina* will avoid feces and silk produced by the predatory wolf spider *Hogna helluo*. Evidence of nearby predators may also be conveyed by indirect chemical cues from conspecifics, as in Atlantic dogwhelk exhibiting a dramatic decrease in feeding and growth rates when exposed to the scent of damaged conspecifics as well as the excretia of predatory crabs (Palmer 1990). The recognition and subsequent avoidance of relevant chemicals, be they evidence of a damaged conspecific or simply compounds characteristic of a potential predator species, may thus provide important information concerning site selection.

As the above example of the dogwhelk and their reaction to predatory crab excretia shows, the phenomena of chemosensory-attained predator avoidance are not limited to the interactions of members of the same phylum; potentially predatory or otherwise harmful arthropods (such as spiders, aquatic insects, ants, parasitic flies, etc.) have been found to be repelled by certain compounds produced by various vertebrate species including amphibians, snakes, mammals and birds (Weldon and Carrol 2007). Arthropods will also avoid vertebrates perceived as predators, as Blake and Hart (1993) have shown that the crayfish *Pacifastacus leniusculus* will greatly reduce foraging behaviors, such as walking and climbing, and instead utilize shelters in the presence of chemical cues associated with predatory fish species such as perch and eels. It is therefore evident that the chemical evidence of predator species, as detected by prey items, has dictated and driven the foraging behaviors and decision-making processes of chemosensitive animals inhabiting all but the very highest trophic levels.

In this study, I have investigated the potential for chemosensory detection of predators by scorpions. Occupying both secondary and tertiary trophic levels of their communities, scorpions are primarily insectivores in habitats ranging from desert to forests both temperate and tropical. The order emerged from aquatic habitats in the Silurian period and achieved their modern, terrestrial form in the Devonian, over 300 mya; in the intervening years they have survived four mass extinctions and established themselves on every continent save for Antarctica while undergoing very little change in morphology (Kjellesvig-Waering 1986). Add to this that predation is the dominant mortality factor (Polis 1990) and continued study of scorpions can be viewed as of importance to anyone interested in the origins and evolution of modern predator-prey relationships.

In addition, scorpions are ideal subjects for laboratory studies, as they are large enough to be easily observed and manipulated, yet small enough to be kept comfortably in relatively little space. They are hardy animals, requiring a low level of upkeep relative to similarly sized vertebrate species, with which they share a somewhat analogous life history (maturity achieved early in life, followed by potential for several successful mating events) relative to other arthropod orders. They are easily identified and captured with the aid of UV illumination, and as ectothermic animals, their metabolism and activity is easily manipulated with changes in temperature. Specific traits such as viviparity, iteroparity, robust longevity and maternal care of young make them relatively unique research subjects among arthropods. Their primary sensory organs, pectines, are unique to the order, and so scorpions may be considered exceptional candidates for studies concerning the effect of a predator's prior presence on the behavior of small, mid-trophic terrestrial animals.

Pectines are antenna-like structures located perpendicular to the sternum in scorpions. These comb-shaped organs contain a number of "teeth," each covered in peg-shaped receptors called sensilla. Pectines have been shown to function as both mechano- and chemosensory receptors (Brusca and Brusca 2002), although chemosensory function has been shown to be limited to direct contact with substrate and stimuli (Steinmetz et al. 2004). Known functions include the mechanosensory detection of resources such as prey and water (Gaffin and Brownell 1992) and the chemosensory detection of mates as well as the assessment of familiarity among conspecifics (Brownell 1998; Mahsberg 2001). However, chemosensory pectinal function outside of pheromone recognition and courtship may not be limited to the recognition of sexually relevant conspecifics; Krapf (1986), in an early illustration of the organs' role in the acquisition of chemical information, found that the structures also play a role in the detection of dead or

motionless prey items, which could not be recognized by mechanosensory information alone. This suggests that scorpions are capable of recognizing chemical signals unrelated to courtship, and underlines the necessity of further work concerning the capacity of scorpions to make decisions concerning their environment based on acquired chemical information.

Vaejovis carolinianus (Beauvois), the southern devil scorpion, is a member of the family Vaejovidae native to northern Georgia and North Carolina and is the only member of its family found on the eastern side of the continent (Shelley 1994a). This species shows severe pectinal sexual dimorphism, which is characteristic of the Vaejovidae. A significant amount of its range is now shared with the invasive bark scorpions *Centruroides vittatus* and *C. hentzi*, the ranges of which have expanded northward in the last decades (Shelley 1994b), although these are arboreal species and therefore are probably not in direct competition with the terrestrial *V. carolinianus*.

Given that this species exhibits sexual dimorphism in pectine size and chemosensory function outside of prey- and mate-detection is not well understood, *V. carolinianus* was selected as a test animal for this study, which aimed to determine the effect, if any, of chemical information on its decision-making and site-selection behavior. Although primarily known as neurotransmitters, biogenic amines were selected as treatment chemicals, as certain trace amines have been shown to also function as semiochemicals, or chemicals that convey information between two or more individuals: Ferrero et al. (2011) described an aversion in rodents to the trace amine 2-phenylethylamine, a compound commonly found in mammalian carnivore urine. They may also work to inhibit fitness in arthropods, as caged worker bees have been shown to exhibit delayed reaction times when fed dietary precursors of biogenic trace amines (Harris and Woodring1999).

It was hypothesized that the established kairomone 2-phenylethylamine would produce a consistent avoidance response, as would tyramine, a fellow member of the phenethylamine class of neurotransmitters, due to its similar function and structure. The monoamine neurotransmitter histamine was also chosen to provide a contrast to the selected trace amines, due to its distinct molecular structure relative to the tested phenethylamines. Little to no avoidance to histamine was expected. Should avoidance to any treatment be exhibited, males were expected to avoid contact with treatment in greater numbers than their female counterparts, as pectine size was hypothesized to correlate positively with increased chemosensory ability. It should also be noted that male scorpions are more likely to exhibit avoidance responses than females, due to their lower mass and increased mobility (Carlson et al. 2014), and so differences between male and female behaviors were also investigated.

METHODS

Seventy mature *Vaejovis carolinianus* individuals of mixed sex were collected from Devil's Fork, South Carolina in August and October 2014. Individuals were collected by hand at night with the aid of UV illumination, with the majority found exposed and foraging when discovered. Once collected, individuals were transferred to individual containers in which they were kept at approximately room temperature (25°C) and on a mixture of sand and soil from the site of their collection, with indoor lighting set to mimic a temperate day-night cycle. Trials were conducted under UV light. Gravid females were allowed to give birth prior to their use in trials, and the resulting scorplings were removed from the container upon dispersal from their mothers. Containers were periodically misted with DI water in order to maintain humidity, and individuals were fed commercially available juvenile crickets once a month in order to maintain capture weight.

Twelve identical y-mazes were constructed from PVC 3-way L fittings, each consisting of a single 1" fitting and two 3/4" fittings. Commercially obtained sand was used as substrate, as it provides a more even and less irregular surface than does forest soil; it has also been used in previous studies concerning the chemosensory capabilities of other Vaejovids (Gaffin et al. 1992). For each set of trials, a one molar (M) solution was prepared for one of three biogenic amines: 2-phenylethylamine, histamine and tyramine. Chemicals were provided by the Western Carolina University Chemistry department. At the start of each trial, a 5 mL portion of this solution was then added as treatment into one of the smaller fittings, each of which formed an arm of the maze; once the treatment had dried, individuals were loaded into the top of the larger fitting, which formed the base of the maze. The arm receiving treatment alternated between trials.

The duration of each trial ranged between 5 and 15 minutes. Trials were performed between the hours of 7:00 pm and midnight, in order to take advantage of the subjects' nocturnal habits. Individuals that remained stationary for more than five minutes were given a gentle tap on the telson with forceps, in order to stimulate movement and thus incite a decision-making event, and each individual was allowed to rest for a minimum of 24 hours between trials. Upon conclusion of every trial, sand was disposed of and the maze was washed with a 70% ethanol solution. Animals were returned to the site of capture upon completion of the study.

Each instance of avoidance of a treated arm was assigned a value of "1" for the purposes of statistical analysis. A binomial test (Snedecor and Cochran 1967) was performed in order to determine the probability of an individual's aversion to each of the three amines. A chi-square test of homogeneity was performed in order to assess the difference, if any, between the distribution of avoidance events in male and female population. Fisher's exact test was used to determine if the difference between male and female avoidance was statistically significant.

RESULTS

Observed avoidance of treated substrate varied between amines tested as well as the sex of the individual (Table 1). A binomial test suggested that although the established kairomone 2-phenylethylamine (PEOH) did not produce a meaningful number of avoidance reactions among either sex ($p = 0.139$ in males, $p = 0.102$ in females, $p = 0.074$ among all), both male and female individuals avoided areas treated with tyramine ($p = 0.049$ in males, $p = 0.002$ in females, $p = 0.001$ among all, Table 2). The monoamine histamine caused a significant avoidance rate in both sexes taken together ($p = 0.046$, Table 1) and in females alone ($p = 0.037$, Table 1) but not in males ($p = 0.149$).

A chi-square test (Table 3) showed that the specific amine used had an effect on the likelihood of avoidance when males and females were tested together ($df = 2$, $\chi^2 = 11.6$, $p = 0.003$, Table 3), which suggests the ability of individuals to discriminate between different amines. In addition, female individuals discriminated between the amines tested ($\chi^2 = 10.479$, $p = 0.005$, Table 3) while males did not ($\chi^2 = 2.428$, $p = 0.297$, Table 3). These results could be explained by sexual dimorphism, or may reflect a sexual difference in priorities concerning the interpretation of and reaction to chemical information. However, Fisher's exact test showed no statistically significant difference between the two populations' avoidance rates for PEOH ($p = 1$), tyramine ($p = 0.6.03$) or histamine ($p = .355$).

DISCUSSION

The results of the y-maze experiments do not support the hypothesis that 2-phenylethylamine functions as a kairomone to *Vaejovis carolinianus*. The only established kairomone tested in the study, 2-phenylethylamine produced the least significant aversion rates of the three amines tested. This suggests that scorpion pectines lack an equivalent to the mouse olfactory receptor described by Ferraro (2011), perhaps due to primary mammalivores exerting less predation pressure on arthropods than on rodents. It is also possible that scorpions use a different chemical cue for the purpose of large predator detection. While Berton et al. (1998) showed that mice in such studies were reacting to the byproducts of a carnivore metabolism, Persons et al. (2001) found no such connection between the diet of *Hogna helluo* spiders and the avoidance of their silk and feces by *Pardosa milvina* spiders. This contrast shows that predator-related chemical cues may take the form of compounds directly metabolized from the bodies of conspecifics, or they may be excretions specific to the predator species itself. A similar study, using compounds found in the excretion of scorpion-specific predators, might find evidence of a kairomone functioning in a similar capacity to a rodent's use of 2-phenylethylamine.

Tyramine, the second trace amine tested, did produce a statistically significant avoidance response by both male and female subjects. Tyramine can be an indicator of decay in organic tissues, as it is formed by the decarboxylation of the amino acid tyrosine (Marcobal et al. 2012). Tyramine may function as a semiochemical involved with discrimination between edible and toxic immobile prey items, as shown by Krapf (1986) in his work with *Androctonus australis* and *Buthus occitanus*, both members of the scorpion family Buthidae. It should be noted that these results could also be in part due to the relative insolubility of tyramine relative to the other tested amines, which might have contributed to a clumpier and thus more concentrated treatment

than other tested amines produced. However, tyramine was the only amine tested to produce a strong observable effect on the decision making of both males and females, and thus the only probable infochemical, if not kairomone, suggested by this study. Further investigation into the specific role of tyramine, and why scorpions might avoid it, may serve to further our understanding of the animals' interpretation of their environment and the resources therein.

Histamine, the only monoamine to be tested, produced a significant avoidance response in female subjects while males exhibited no discernable preference. Similar to the formation of tyramine from tyrosine, histamine may be produced by decarboxylation of the amino acid histidine by bacterial agents during the decomposition of animals (Morrow et al. 1991), and may also signal that a potential food item has decomposed past the point of providing nutrition. However, I did not observe a significantly high rate of avoidance to declare it an infochemical used by male *Vaejovis carolinianus* in decisions concerning path selection; that males reacted to histamine the least of all tested amines suggests that its role as an infochemical is at least unrelated to decisions concerning path finding and site selection. Females may react differently to the presence of histamine either because they possess some sexually dimorphic character absent in males, or because males simply prioritize the avoidance of histamine less than do females.

The results also fail to support the hypothesis that the larger male pectinal size grants an advantage in terms of non-sexual semiochemical detection. In fact, when assessed independently, female subjects exhibited greater aversion rates than did males across all treatments. This may suggest that the difference in pectinal size observed between males and females of the family is accounted for by its contribution to increased sexual function in males, via mate detection, alone. This emphasis on mate detection capability may even carry a cost in terms of the ability of males

to detect dangerous compounds. Sexual dimorphism in the peg sensilla count and density may only apply to specific pectinal regions, and not extend to all aspects of an organ's sensory function, as in the ladybird beetle *Semiadalia undecimnotata*: the antennae of males of this species exhibit 40 sensilla and two taste receptors not found on the antennae of females, while females exhibit one more antennal mechanoreceptor than do males with no dimorphism evident in regions related to olfaction (Jourdan et al. 1995). The specific sensory region subject to sexual dimorphism varies among species: in *Periplaneta* cockroaches, the antennae of males exhibit almost twice as many olfactory sensilla than those of females do, although the presence of sexually dimorphic differences in the number of contact chemoreceptors varies among species and strain (Schafer and Sanchez 1976). Male and female scorpions may also be sensitive to different semiochemicals as related to sex-dictated priorities; mature males are primarily concerned with the location of potential mates whereas females may prioritize burrow selection due to their lesser mobility relative to males (Carlson et al. 2014).

Yet predation remains the dominant mortality factor of scorpions (Polis 1990), and we could expect to find aspects of the pectine to exhibit sensitivity to some kairomone, if not biogenic amines produced by the metabolic processes of a predator species. It is important to consider the limitations of the experimental methods. The y-mazes used may result in aversion or escape responses simply because they do not allow for other behaviors (Kats and Dill 1998), and so I cannot regard a lack of aversion as a lack of detection. Because histamine and tyramine can both result naturally as byproducts of the decomposition of animal matter, they may provide information about the environment that is helpful although unrelated to predator detection or avoidance.

In addition to additional studies using y-mazes to assess potential semiochemicals used by scorpions, future investigations could use an arena set up, in which one of four quadrants is treated with a suspected infochemical. Recorded movements over a set time would indicate preference for certain treatments and avoidance of others, which would allow a broader view of the effect tested chemicals may have on decision making behavior concerning path finding and site selection. Similar to the design employed by Gaffin and Brownell (1992) in their investigation into the recognition of female prior presence by male conspecifics, this setup could also serve as the basis for studies concerning the identification of chemical indicators of prey matter decomposition.

In summation, of the three amines tested, only tyramine produced a significant avoidance reaction in both sexes; histamine produced a mild response in females but not males. As these chemicals are produced by decarboxylation of amino acids by microbes during the decomposition of animal matter they may function as semiochemicals to scorpions, assisting them in determining if a non-mobile prey animal is consumable. Although there is good reason to expect that scorpions do indeed use chemical information to assess predator threat, the results of this study do no suggest that 2-phenylethylamine ranks among them. Further investigation is necessary in order to identify semiochemicals relevant to scorpions and assess their functionality.

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TABLES

Table 1. Avoidances and non-avoidances of tested amines by sex.

2-Phenylethylamine		
	Avoid	Non-Avoid
Male	15	13
Female	23	19

Tyramine		
	Avoid	Non-Avoid
Male	18	10
Female	30	12

Histamine		
	Avoid	Non-Avoid
Male	14	14
Female	26	16

Table 2. P-values for binomial distribution of sex compared to avoidance of tested amines.

Sex	PEOH	Histamine	Tyramine
Female	0.102	0.038	0.003
Male	0.140	0.150	0.049
All	0.074	0.047	0.001

Table 3. Chi-square test of homogeneity results by sex.

Sex	df	χ2	p-value
FEMALE	2	10.279	0.005
MALE	2	2.428	0.297
ALL	2	11.6	0.003