# Use of a Y-maze to assess the response of the giant whipscorpion *Mastigoproctus giganteus* (Arachnida: Uropygi) to olfactory cues associated with a predator, *Hogna carolinensis* (Araneae: Lycosidae)

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## Summary

Studies were conducted to determine whether protonymphs of the whipscorpion *Mastigoproctus giganteus* could detect and respond to chemosensory (odour) cues associated with a naturally-occurring predator, the wolf spider *Hogna carolinensis*. Subjects were exposed to odour choice experiments in a Y-maze. They moved from a start box into the base arm of the maze which contained an unconditioned (no odour cues) substrate (vermiculite), and then to the choice point (intersection) leading to the two experimental arms. One of these contained vermiculite conditioned with the odour of a predator, *H. carolinensis*, while the other had substrate conditioned with the odour of conspecifics. Whipscorpions chose the arm containing vermiculite conditioned with chemosensory cues from conspecifics 23 out of 30 times (76.7%). There was no significant difference between the mean number of front-leg contacts (FLC) made by subjects in the base arm containing unconditioned vermiculite (mean: 36.8) as compared with the number made in the experimental arm containing odours from conspecifics (32.8). Subjects exhibited significantly more FLC (mean: 53.6) and spent significantly less time (mean: 156.3 s) in the arm containing vermiculite conditioned with the odour of *H. carolinensis* as compared with their responses in the arm containing conspecific odours (32.8 and 312.5 s, respectively). This ability to detect and respond to predator-associated odours appears to be innate because all subjects were captive-bred and had no previous experience with *H. carolinensis*.

## Introduction

The ability to detect the presence of potential predators is an important component of antipredator tactics used by prey organisms (see reviews by Lima & Dill, 1990; Kats & Dill, 1998). Prey may use multiple cues associated with predators (chemical, mechanical, visual) to assess the level of predation risk (Punzo & Kukoyi, 1997). Predator chemosensory cues derived from their integument and faeces are used by a wide variety of invertebrate and vertebrate prey organisms to detect and respond appropriately to their presence. Depending on the species, this ability of prey to respond to such cues may be innate or refined through experience (learning) (Barreto & Macdonald, 1999; Punzo, 2000a).

Most of the previous research has focused attention on aquatic and marine invertebrates as well as fish, larval amphibians, reptiles, and mammals (Chivers & Smith, 1998; Kats & Dill, 1998). Among terrestrial arthropods, most research has been conducted on insects and spiders. For example, many lepidopteran and coleopteran larvae alter foraging routes in the presence of chemosensory (odour) cues associated with naturally-occurring predators (Punzo, 2000a). Young wolf spiders (Lycosidae) reduce locomotor activity and avoid substrates when exposed to odours from faeces and silk of larger lycosid predators (Persons *et al.*, 2001).

In contrast, data are lacking on the ability of whipscorpions (Uropygi) to respond to predator-associated odour cues. The giant whipscorpion, *Mastigoproctus giganteus* (Lucas) (Thelyphonidae) is found in desert regions of southwestern USA and northern Mexico as well as in pine flatwoods and scrub habitats in south-central Florida (Weygoldt, 1971; Punzo, 2001). This arachnid is a generalist predator that feeds on a variety of arthropod prey (Punzo, 2000b). It locates prey by using its first pair of legs (modified as feelers and containing mechano- and chemoreceptors) to explore the ground surface as well as rock and bark crevices (Crawford, 1981). In turn, it is preyed upon by a variety of predators including other arthropods, small mammals, birds, and reptiles (Cloudsley-Thompson, 1991; Punzo, 2000a).

The purpose of this study was to determine whether *M. giganteus* can detect and respond to odour cues associated with the wolf spider *Hogna carolinensis* (Walckenaer) (Araneae: Lycosidae), a naturally-occurring predator, using a Y-maze to assess its behavioural responses. Adults of *H. carolinensis* are known to include whipscorpions in their diet (Punzo & Preshkar, 2002).

## Material and methods

I used 5-day-old protonymphs (first nymphal instars: 16–17 mm in body length) of *M. giganteus* in these experiments. The whipscorpions were captive-bred offspring from parents originally collected in pitfall traps on the Lake Wales Ridge (Highlands County, Florida) during the spring of 2002. They were "naive" in the sense that they had not previously encountered wolf spiders. Adult female wolf spiders (*H. carolinensis*) were collected from the same location. The whipscorpions and spiders were maintained individually in plastic cages ($20 \times 12 \times 10$ cm) at 22–23°C, 60–63% relative humidity, and on a 12L:12D photoperiod regime in Percival Model 85A environmental chambers (Boone, Iowa). They were provided with water *ad libitum*, and fed on a diet consisting of crickets, mealworms, cockroaches, and grasshoppers. Cages were provided with vermiculite as a substrate. Vermiculite from whipscorpion and spider cages (conditioned) was used as a source of odour cues (treatment condition) associated with conspecifics and spiders, respectively. Vermiculite for these treatment conditions was placed in cages housing spiders or whipscorpions for a period of one week before testing. Unconditioned (clean) vermiculite was used as the control condition (no odour).

The apparatus consisted of a standard Y-maze (Columbus Instruments, Model 0526M-D40, Columbus, Ohio). It contained a start box ($12 \times 12 \times 10$ cm) that had an opening at one end leading into the base arm of the maze (1.0 m in length, 9 cm in width, and 10 cm in height). A sliding aluminium panel door prevented the movement of subjects into the base arm when closed. At

its distal end (opposite the entrance to the arm) the base arm intersected with two side arms (experimental arms, 45° angle from base arm) with identical dimensions. The distal end of each experimental arm led into an opaque holding chamber ($12 \times 12 \times 10$ cm) containing unconditioned (clean) vermiculite. Unconditioned vermiculite was also placed in the base arm before the start of each trial (depth of 1 cm). Vermiculite conditioned with odour cues from *H. carolinensis* was placed in one experimental arm, and vermiculite conditioned with odour cues from *M. giganteus* was placed in the other experimental arm. After each trial, all vermiculite was removed, the Y-maze was cleaned thoroughly with a soapy sponge, and fresh substrates added. The position of the conditioned vermiculite containing spider or whipscorpion odours was reversed between each trial to avoid any position effect. All trials were recorded using a Panasonic video recorder.

A 75-watt lamp was placed at a height of 35 cm directly over the centre of the maze. These whipscorpions are negatively phototactic and exhibit pronounced locomotor activity when exposed to bright light (Howard, 1919). Light has been shown to act as an effective aversive and motivational stimulus in maze-learning experiments with *M. giganteus* (Punzo, unpubl. data).

Thirty whipscorpions were used in the Y-maze experiments. All subjects were deprived of food for 72 h before testing. For each trial, one whipscorpion was introduced into the start box facing the entrance into the base arm, and allowed to remain there for one min. The panel door was opened and the subject allowed to enter the base arm and move towards the experimental arms. I recorded the initial choice of experimental arm, the amount of time spent at the choice point (intersection) and in each experimental arm, and the number of times that each subject made contact with the substrate using its front legs (front leg contacts, FLC). A trial ended when the subject left the Y-maze and entered one of the holding chambers at the end of each experimental arm. Each subject was tested only once (total of 30 trials).

Methods for all statistical tests followed those outlined by Sokal & Rohlf (1995). Data were analysed using Chi-square ($\chi^2$) tests to determine the significance of response choice in the Y-maze experiments, and $t$-tests were used to analyse differences between mean times spent at various locations within the maze and number of front leg contacts with substrate.

## Results and discussion

All subjects moved out of the start box and into the base arm of the Y-maze in rapid fashion (within 12–21 s after panel door was opened) in response to the aversive light stimulus. The mean time spent at the choice point within the maze was 33.3 s ($\pm 8.7$ SD; range: 16–55). The whipscorpions chose the arm containing vermiculite conditioned with chemosensory cues from conspecifics 23 out of 30 times (76.7%; $\chi^2 = 8.09$, $p < 0.001$). There was no significant difference between the mean number of FLC made by subjects in the base arm containing

| Maze location | Choice of arms containing: | | $p$ |
|---|---|---|---|
| | Vermiculite from *M. giganteus* | Vermiculite from *H. carolinensis* | |
| **Experimental arm** | | | |
| Time (s) | 312.5 (15.7) | 156.3 (9.3) | <0.01 |
| FLC (number/trial) | 32.8 (5.9) | 53.6 (8.1) | <0.01 |

Table 1: Time spent in experimental arm of Y-maze and number of front leg contacts (FLC) by protonymphs of *Mastigoproctus giganteus* ($n=30$) per trial when tested in a Y-maze and given a choice between experimental arms containing vermiculite substrate conditioned with odours associated with *M. giganteus* or with the wolf spider *Hogna carolinensis*, a naturally-occurring predator. Base arm contained unconditioned vermiculite (no odour). Data expressed as means for all subjects: subjects received one trial each. Numbers in parentheses represent $\pm$ SD. Data analysed using $t$-tests.

unconditioned (no odours) vermiculite (mean: $36.8 \pm 7.7$ SD) as compared with the number made in the experimental arm containing odours from conspecifics (mean: $32.8 \pm 5.9$).

The amount of time spent in both experimental arms of the Y-maze and the frequency of FLC are shown in Table 1. Subjects exhibited significantly more FLC and spent significantly less time in the arm containing vermiculite conditioned with the odour of the predator, *H. carolinensis*, as compared with their responses in the arm containing odours from conspecifics.

These results are the first to indicate that whipscorpions (Uropygi) have the ability to detect and respond differentially to odours associated with a naturally-occurring predator. Furthermore, this ability appears to be innate because the protonymphs of *M. giganteus* had no previous experience with *H. carolinensis* before behavioural tests were conducted. When exposed to predator odours in the maze, they moved out of that arm and into the holding chamber more rapidly than when they were exposed to conspecific odours. This suggests that when substrates containing chemosensory cues from *H. carolinensis* are encountered in their natural habitats, these whipscorpions would relocate to an area devoid of these cues, thereby reducing predation risk. Concomitantly, substrates containing conspecific odours may be perceived as less threatening, and the tendency to spend more time on such substrates would reduce metabolic costs required by locomotor activities used in rapidly moving away from areas where predators have been detected.

Finally, these results show that a Y-maze can be used as a reliable behavioural assay to assess the ability of terrestrial arthropods to detect and respond to chemosensory cues associated with predators. Similar Y-maze experiments have been used successfully in studies assessing this ability in vertebrates such as the pine snake *Pituophis melanoleucas* (Burger *et al.*, 1991) and ferret *Mustela putorius* (Appfelbach, 1992).

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