

Response of the eastern sand scorpion, *Paruroctonus utahensis*, to air movement from a moth analog

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Abstract. Arachnids have many setae that are used as sensory organs. Spiders have been shown to use trichobothria to sense air movements. Scorpions also have trichobothria, located solely on their pedipalps. In scorpions, these trichobothria have been used for taxonomic purposes, due to their systematic variations across taxa. In the lab, buthid scorpions respond to moth-like air vibrations by “hunting” a dummy prey, but scorpionids retreat. The eastern sand scorpion, *Paruroctonus utahensis* (Williams, 1968) is a member of the family Vaejovidae; by measuring its responses to air movements, we can begin to compare behavior of scorpion families. To determine the responses of *P. utahensis* to air stimulus, we created a piston-driven moth analog. To assess the trichobothria as candidate detectors of our air stimulus, we also monitored trichobothrial deflection in response to the moth analog. In behavioral trials, we ran this device for 10 seconds and recorded each scorpion’s immediate responses as negative, neutral, or positive. For a control, we ran the device without its piston. Scorpions experienced both conditions. We found a significant difference between scorpions’ responses under experimental and control conditions. On average, scorpions responded more negatively to experimental conditions as compared to control conditions. These data suggest that vaejovids, like scorpionids, retreat when they encounter a moth. We also found that our device was effective in stimulating appropriate trichobothrial deflection. Our moth analog could therefore be useful in further studies investigating the physiological mechanisms of prey detection.

Keywords: Trichobothria, sensory, orientation, Scorpiones, Vaejovidae

Scorpions hunt primarily by detecting ground vibrations (Brownell 1977). However, scorpions also catch prey such as moths and butterflies when they are in flight (Polis 1979). When the only stimulus is air movement, buthid scorpions exhibit prey-catching behaviors, whereas scorpionids react defensively (Krapf 1988). Other studies show that scorpions also orient themselves with a constant angle to a wind current (Linsenmair 1972).

To sense these air movements, scorpions appear to use thin, hair-like structures called trichobothria that are located on the pedipalps (Hoffman 1967). When researchers removed all the trichobothria from one side of a scorpion’s body and presented a stimulus, the scorpion responded by moving toward its intact side (Krapf 1988). The arrangement and number of these trichobothria vary greatly from species to species, making trichobothria useful in systematics (Fet et al. 2005). However, this array certainly has more importance than just as a way to determine relatedness. Scant information exists on the physiological relevance of the trichobothria or how their arrangement and patterns of deflections translate to a behavioral response. As a start, we have produced a simple mechanical device that simulates moth wing flapping movements to generate a relevant air stimulus. We used this moth analog to test the behavioral response of a vaejovid scorpion.

An ecological analysis of a scorpion species of the family Vaejovidae, *Smeringurus mesaensis* (Stahnke, 1957), shows that moths and butterflies make up around 3% of these animals’ diet (Polis 1979). Anecdotal field evidence suggests that *Paruroctonus utahensis* (Williams, 1968), another vaejovid, orients toward moths flying overhead. In light of this, we hypothesized that vaejovids would, like buthids, react to our moth analog with prey-catching behaviors. However, our experiment showed that the scorpions exhibited defensive behavior in response to the moth analog.

METHODS

Animals.—We used 20 female *P. utahensis*. Each scorpion was kept in a 3.8 L (14.0 cm diameter, 25.5 cm tall) glass jar containing sand to a depth of about 2.5–5.0 cm and a piece of clay pot. We moistened the sand with 5 mL of water three times weekly. We exposed the animals to a 14:10 hour light:dark cycle and shifted the cycle back one hour a day until the dark part of the cycle began at 1300. We began our trials between 1400 and 1500 and finished them no later than 1600 to take advantage of the time when the animals were most active (Polis 1980). We grouped our animals so half experienced the control condition a day before experiencing the experimental condition, and half vice versa. To control for scorpion hunger level affecting expression of hunting behavior, we deprived the animals of food for 5–7 days prior to testing.

Apparatus.—We ran our trials in a 20 cm x 20 cm x 20 cm wooden box, made of ¾ inch (1.9 cm) plywood (Fig. 1A). The wooden box had a large hole in the center of the top for an infrared camera (Nest Cam Indoor, Nest Labs) and a smaller hole for the piston holding the simulated moth. On two opposite sides of the box, a hole 14 cm above the bottom of the box and 8 cm from one side allowed for the crankshaft of the moth analog to go through the box. The interior of the box was dark. The box was set on top of “memory foam” (low-resilience polyurethane foam) to minimize any vibrations.

During the trial, the scorpion being tested was contained within a circular glass bowl (11.3 cm diameter at widest point, 4.50 cm tall) containing sand to a depth of about 5 mm. The sides of the bowl curved out from the bottom and back in at the top. The bowl sat near one corner of the box, with the bowl’s outer edge 4 cm from either wall of the box. The bowl sat on top of a small Lazy Susan, for a combined height of 6.3 cm. The piston with the moth analog was centered over the bowl, with the piston’s tip 2 cm below the lip of the bowl.

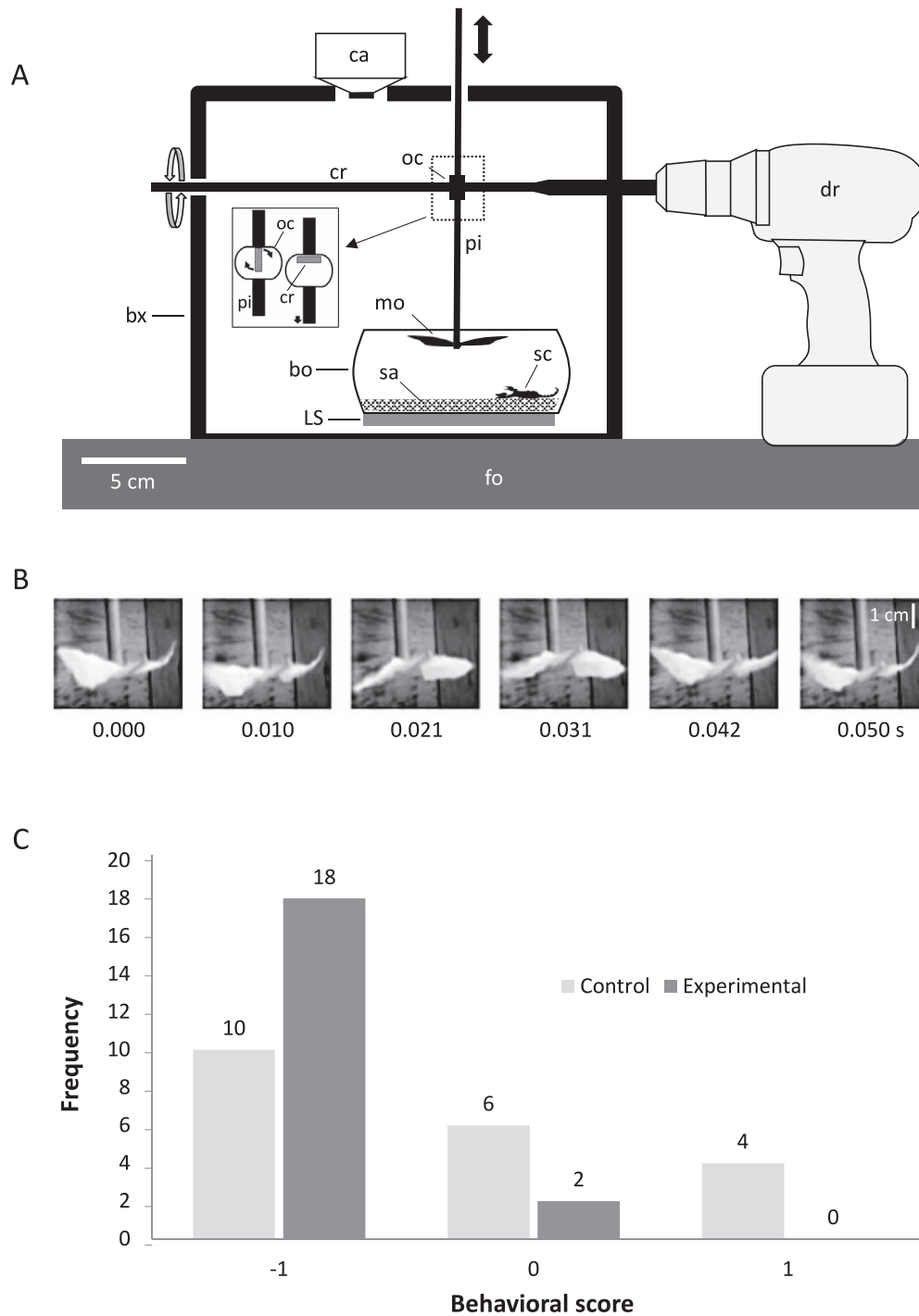


Figure 1.—Experimental apparatus and behavioral results. A: Diagram of the experimental apparatus. The wooden box (bx) covers the experiment and sits atop a 5-cm thick layer of memory foam (fo). An infrared camera (ca) is installed at the top of box for monitoring the trials. A drill (dr) powers the crankshaft (cr) that enters the box horizontally. The oblong cuff (oc) surrounds the crankshaft and is attached to the vertical piston (pi) which has the paper towel moth (mo) secured to its lower end. Under the moth is the bowl (bo) that sits on a Lazy Susan (LS) and contains sand (sa) and a test scorpion (sc). The inset shows an expanded, end-on view of the oblong cuff and the resulting vertical displacement of the piston during a quarter turn of the crankshaft. B: Moth wing movement. One flap of the paper towel moth's wings as the piston moves up and down. The wings flapped at a speed of about 20 times per second, and the range of the moth's vertical movement was about 2 cm. C: Frequency of behavioral scores in control and experimental trials. An experimental trial denotes the use of the moth analog, while a control trial denotes the use of the crankshaft without the piston and moth analog. A score of -1 represents movement away from the center of the bowl; 1 denotes movement toward the center of the bowl; 0 means no movement occurred. The mean behavioral scores were significantly different ($P = 0.0039$).

To create the air stimulus, we used a piston-driven moth analog (the “Blankenship device”; Fig. 1A). The piston was connected to the crankshaft by an oblong cuff, which allowed for rotation of the shaft while allowing for only vertical motion of the piston. To further decrease any horizontal movement, the piston came out of a small hole in the top of the box. A drill rotated the crankshaft, driving the piston at 1200 rpm or 1200 “flaps” of the moth’s wings each minute (Fig. 1B); this is roughly the same speed at which the geometer moth flaps its wings (Mountcastle & Daniel 2009). Attached to the piston were the two paper towel “wings;” paper towels have a density similar to a moth’s wings (Henningson & Bomphrey 2013). The wings, about 2 cm in length, were approximately the same dimensions as the wings of geometer moths, which are known to be eaten by *S. mesaensis* (Polis 1979). To simulate the wing angle of a real moth, the wings were affixed by Scotch Tape (3M Co., Maplewood, MN, USA) to one end of an unfolded paper clip at a 15-degree angle from horizontal. The other end of the paper clip was fastened to the piston by hot glue.

Trials.—For each trial, we placed the scorpion in the bowl under an empty film canister and gave it one minute to acclimate. After the acclimation time, we removed the canister, placed the box over the bowl, and allowed for 30 more seconds of acclimation. During this time, the scorpions usually moved from their original position to the edge of the bowl. After the second acclimation period, we began the trial by turning on the Blankenship device for 10 seconds. Our experimental and control trials were the same, except the piston and attached moth were removed in control trials. Between trials, we changed out the sand to eliminate the possibility of influence by the chemicals or footprint patterns left by the previous animal. Each animal underwent both control and experimental conditions; we randomly selected half to experience control conditions first, and the other half experienced experimental conditions first.

Scoring.—The infrared camera recorded each trial. After all trials were complete, we analyzed the recordings of the scorpions, assigning them a -1, 0, or 1 based on their behavior. The trial was given a score of -1 if the scorpion moved away from the stimulus in the first 2 seconds of the trial or if the scorpion had an immediate motion around the edge of the bowl. The trial was given a score of 1 if the scorpion moved towards the stimulus in the first 2 seconds of the trial or if the scorpion was located under the stimulus and rotated or moved its claws without moving away from the stimulus. If a scorpion exhibited no response to the stimulus, the trial was given a neutral score of 0. All movement directions were determined by measuring whether movement was toward or away from the center of the bowl, where the moth was centered. Animals that continued moving along the edge of the bowl were scored -1; the only way that an animal starting at the edge of the bowl could be scored a 1 is if it moved toward the center of the bowl. At first, four researchers scored each trial, but after determining that there was high inter-scorer reliability, two researchers (WC, IW) scored the remaining trials in near-real time. In the event of a scoring discrepancy, all four researchers reviewed the footage and resolved the score through a unanimous decision. A two-tailed, Wilcoxon matched pairs

test was used to determine whether treatment differences were statistically significant ($P < 0.05$).

Trichobothrial response.—To determine if the moth analog produced an effective air stimulus for deflecting the trichobothria, we used foam rubber to restrain a live scorpion and position its right pedipalp about 1 cm from one of the moth’s wings (Fig. 2A). Since the microscope precluded use of the wooden box support, we assembled ring stands and various clamps and sleeves to hold the shafts of the crankshaft and piston. We used a 40x dissecting microscope and an adaptor to mount a cell phone to view and video trichobothrial deflection both with and without the drill activated.

RESULTS

Before the drill was activated at the start of every experimental and control trial, the scorpions tended to move away from their original position in the middle of the bowl. They would often spend a lot of time around the rim of the bowl, trying to climb out. In the experimental trials, the scorpions would often start moving more quickly when the stimulus began, whereas in control conditions they typically continued moving in the same way as they had been moving before. Only one scorpion remained motionless for both its experimental and control trial.

The mean behavioral score was significantly different for the experimental trials as compared to the control trials (experimental: -0.9 ± 0.18 SE, control: -0.3 ± 0.07 SE; $P = 0.0039$, paired two-tailed Wilcoxon test, $n = 20$). The frequency of behavioral scores in control and experimental trials is shown in Fig. 1C. While many scorpions in both trials responded negatively, there were many more positive responses in the control trials than in the experimental trials.

The trichobothrial response to movement of the moth analog is shown in Fig. 2. We monitored the movement of individual trichobothria while the moth analog was on (drill activated) and during the intervening no-stimulus periods (Fig. 2B). A plot of the hair deflections shows slow wafting movements during no-stimulus periods and quick side-to-side deflections consistent with the 1200 wing flaps per minute while the stimulus was on (Fig. 2C).

DISCUSSION

Other species in the vaejovid family, such as *S. mesaensis*, are known to eat moths occasionally (Polis 1979) and individuals of *P. utahensis* have been seen orienting toward moths in the field (B. Brayfield, pers. comm.). The animals in this study, however, consistently retreated under experimental conditions. Our result was surprising, as it suggests that when exposed to a moth in the field, *P. utahensis* may initially retreat.

There were some limitations to our experiment. Due to their availability, we tested only females’ responses. Additionally, since we don’t have specific information on the typical diet of *P. utahensis*, our moth analog may not be similar to the kinds of moths these scorpions may encounter and eat in the field. Ideally, we would have exposed the scorpions to air movements from the wings of an actual moth, but it was not feasible to control for potential olfactory or auditory sensory influences when using a real moth.

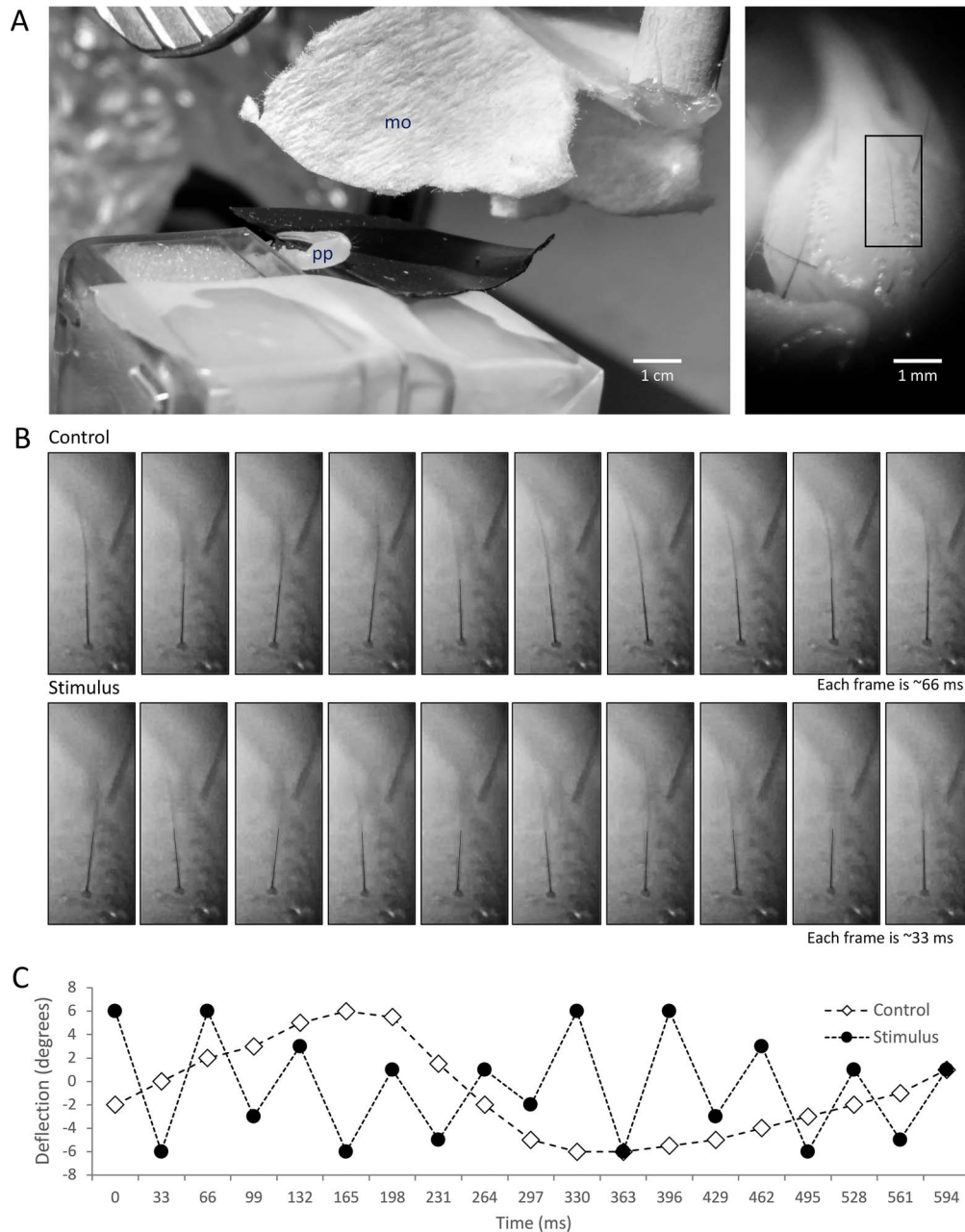


Figure 2.—Trichobothrium response to moth analog. A: A live female *P. utahensis* is restrained within foam inside of a plastic chamber with its right pedipalp (pp) exposed. A set of ring stands and supports allowed operation of the moth analog (mo) within a few cm of the pedipalp. Right: expanded view of pedipalp with the focal trichobothrium highlighted in box. B: Movements of trichobothrium before and during moth stimulation. The upper set of images show normal, baseline swaying of trichobothrium while the moth was stationary (66 ms between each frame). The lower set of images shows a segment of activity while the moth was activated (33 ms between each frame). Highlighting has been added to the lower portion of the trichobothrium shaft to help visualize deflection. C: Comparison of trichobothrial deflection change during stimulus and control situations. The deflection in degrees of a trichobothrium is plotted for both a non-stimulated control section of record across its full breadth of angular movement and for a section of movement during stimulation by the moth analog.

This research could take many future paths. For example, studying the responses of scorpion families other than buthids or vaejovids could uncover a pattern in, or an explanation of, behavioral differences between species. Other studies could focus on perfecting the moth analog; by conducting ecological analyses on different species of

scorpions, we could match the analog's size and speed to that of a moth that a scorpion species is known to eat in the field. Additionally, varying the size, wing speed, duration of wing movement, or distance of the moth stimulus to the scorpion would contribute to a better understanding of the kinetics of the scorpions' behavioral responses. A longer

stimulus exposure could allow scorpions to show a changing response, such as an initial retreat followed by a return to the area of stimulus.

The mocked-up test we used to visualize trichobothrial deflection showed that the moth analog did in fact cause commensurate deflections of trichobothria. Here we report the deflection of a single trichobothrium; however, when panned out we saw that several trichobothria reacted to the stimulus. Each trichobothrium has its own plane of oscillation, which means that it takes two or more trichobothria working in concert to provide the scorpion with directional information about the source of a movement (Hoffmann 1967). While the trichobothria remain candidate receptors, additional behavioral tests involving animals with their trichobothria removed or covered are necessary to determine if these sensilla are responsible for sensing the air movements of the moth analog or if another sensory organ may be involved, such as the constellation array on the fixed finger of the scorpion (Fet et al. 2006).

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