Some like it humid: hygrotaxis in the striped bark scorpion.

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Fall 2017

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Summary

Scorpion tarsi contain organs that are physiologically responsive to increases in humidity. We hypothesize that scorpions may use these organs to sense the natural humidity gradient surrounding water to locate water sources for survival. We tested the response of striped bark scorpions, *Centruroides vittatus*, inside a specially designed rectangular chamber containing a humidity gradient. First, we dehydrated or hydrated groups of scorpions over a 48-hour pre-trial period. We then tested these animals individually in our humidity chamber during 55-minute behavioral trials, recording the amount of time they spent in four quadrants of the arena. We then reversed our groups so that dehydrated scorpions were hydrated and hydrated scorpions were dehydrated and repeated the behavioral trials. The scorpions would usually roam around the walls of the arena and make occasional forays across the middle. Some animals roamed the entire trial period while other eventually settled in one spot. While our behavioral chamber generated a measurable and consistent humidity gradient, our data did not reveal a consistent pattern in scorpion behavior.

Introduction

While scorpions typically survive on the water they acquire from their prey alone, they may need water from their surroundings when food is scarce (Polis 1990). For example, some species of scorpions may supplement their water intake by drinking from pools or chewing substrate and some even drink water from fog (Bradley 1998). However, how scorpions find water in their environment remains unclear. Scorpions are known to prefer higher levels of humidity, which may indicate the use of humidity-seeking behavior to find water.

Just as animals can sense light, temperature or sound, many have some means of sensing their environment's humidity (Filingeri 2015). Animals use sensors called hygroreceptors, which swell and constrict with the presence of water vapor in the air. Hygroreceptors are present in species ranging from worms to humans, and scorpions appear to have such receptors on their tarsi, since destroying the tarsi decreased scorpion reactivity to different levels of humidity (Abushama 1964). Furthermore, small organs on the dorsal side of the tarsi have been shown to respond electrophysiologically to moistened paper brought within centimeters of the organ (Gaffin et al. 1992). Scorpions react to contact with moistened substrate while these tarsal organ receptors are intact, though it is unknown if this is due to hygroreception or perhaps some other mechanism.

We examined how scorpions use humidity to orient themselves through the use of a humidity gradient, which was formed by placing desiccants in a container at the end of an arena while water was placed in a container on the other end. We hypothesized that if scorpions use their hygroreceptors to locate water sources across some distance and we placed dehydrated animals inside an arena containing varying levels of humidity due to desiccants placed underneath a mesh and water being placed on the opposite end of the arena, then they will spend more time in areas of higher humidity compared to hydrated animals. We found that there was no significant difference in the amount of time spent in each quadrant between hydrated and dehydrated animals.

Methods

Animal care

Our team used six male and six female striped bark scorpions, *Centruroides vittatus*, captured using black lights at Lake Thunderbird in Norman, Oklahoma on the evening of September 20th 2017. The scorpions were kept individually in either 0.95 L plastic containers or 3.8 L glass jars; each container included a small amount of Eco Earth coconut fiber and a shard of clay. We watered each scorpion once every two days and fed them each one cricket (Rainbow Mealworms Inc.) once every two weeks. We periodically cleaned out any mold that formed on the coconut fiber inside of the humid plastic containers. We kept the temperature and relative humidity constant, at room temperature (20-25°C) and humidity (~50% RH).

Animal preparation

We tested two groups of six animals on a rotating schedule. We dehydrated three scorpions of one group by placing them inside of a Gladware Soup & Salad plastic container (0.95 L) containing ~40 grams of silica gel from Dry & Dry within a 50 ml glass beaker placed in the middle of the container for two days before testing. We placed the other three scorpions in the same type of container alongside a 50 ml glass beaker containing 50 ml of water for 48 hours to minimize the dehydration of that group. After the first set of 6 trials, we put the remaining six scorpions under the same conditions, while the scorpions we experimented on were placed back into their containers. Then after all scorpions were tested, we repeated the setup, except scorpions that were previously hydrated were now dehydrated and scorpions that were dehydrated were now hydrated. We performed our experiments a week after the scorpions were meant to be fed so the scorpions were starved and to maximize dehydration, as scorpions obtain most of their hydration from their food. We sealed the containers with lids to prevent exchange of humidity with the environment.

Arena trial design

We used the arena shown in figure 1 for our experiment. The arena consisted of a Plexiglas outer chamber (40 x 20 x 20 cm) and a Plexiglas inner chamber (39.5 x 19.5x 12 cm) that had a flange around its top edge and a 20 grade mesh secured using silicone caulk for its floor. The inner chamber rested inside the outer chamber so that the mesh floor was suspended 5 cm above the outer chamber floor. We placed containers with 100 g of silica gel (Dry & Dry) and 50 ml of water beneath the mesh on opposite ends of the outer chamber floor to generate a humidity gradient across the length of the arena. We covered the entire apparatus with a clear Plexiglas lid (40x20 cm) to prevent humidity leaks. We then drilled four holes 10 cm apart in the arena underneath the mesh to weave our wires through, and we taped four equally spaced DHT11 Arduino humidity sensors (+/- 5%) inside the walls of the outer chamber just below the mesh inner chamber floor to monitor relative humidity. We drew four equally spaced lines on the lid of the arena to mark off four quadrants (1-4) with different humidity levels.

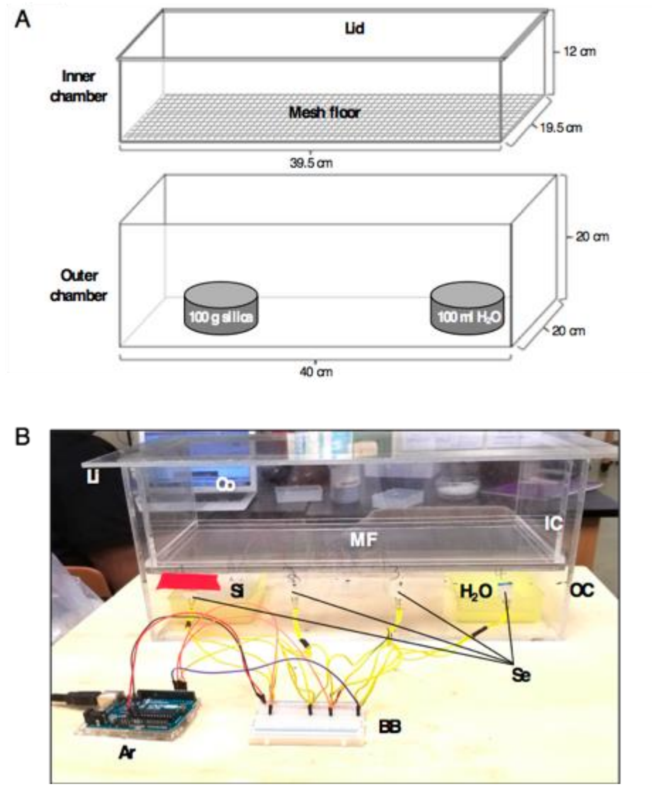

Fig. 1: Arena plan. A The floor of the inner chamber contained a screen mesh and silica gel and water were placed on opposite ends on the floor of the outer chamber, beneath the screen mesh. B The arena was equipped with four DHT11 sensors (se) connected through a breadboard (BB) to an Arduino (Ar) and laptop computer (Co). Sensors were taped to the inner walls of the outer chamber (OC), close to the mesh (Me) of the inner chamber (IC) to receive measurements of humidity consistent with what the scorpions experience. A plexiglass lid (Li) covered the arena and a Nest camera (not shown) monitored the arena from above.

Trial execution

First, we placed our 100 grams of silica gel and 50 ml of water in bowls at opposite ends underneath the mesh. Then we placed the scorpions in the middle of the mesh floor of the inner chamber and placed the lid on the container. We gave the scorpion five minutes to acclimate before we filmed them from above the container through the lid for 55 minutes using an infrared sensitive camera (Nest Inc.). We chose to film them from above the lid, as that allowed us to easily determine what section the scorpion would be in at all times. Afterwards, we reviewed the film and determined what percentage of that hour the scorpion spent in each section of the container. After performing each trial, we cleaned the arena using 75% ethanol. We allowed scorpions from the

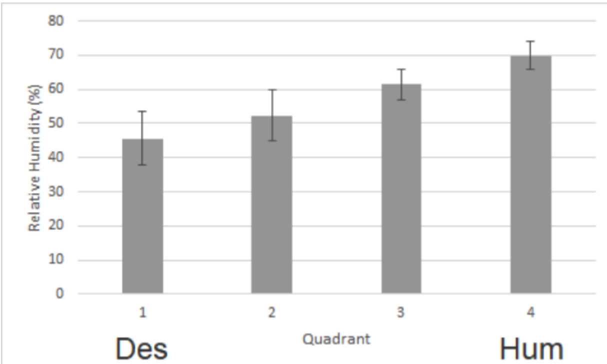

Fig. 2: Humidity readings from all 24 trials. Shown are averages (± standard deviation) of the sensor readings gathered at 3 second intervals over a 55 minute period. Quadrant 1 was above the desiccant while quadrant 4 was above the water.

first group to rest 48 hours while the other group was prepared for experimentation. Each scorpion received both desiccation and a controlled humidity treatment in a repeated measures design.

Analysis

We reviewed the film using the video software VLC to determine how long the scorpion spent in each quadrant of the arena, and compared the differences in percentage of time spent in each quadrant after the desiccation treatment and the controlled humidity treatment. We focused primarily on the scorpions movements to the extreme ends of the arena, as those were the areas right above the water and silica gel, and therefore experienced the most extreme humidity levels. We excluded any trials where the scorpion didn't move. We used a Wilcoxon matched-pairs signed rank test to test for significant differences in the percentage of time spent in the most desiccated quadrant (1) compared to the most hydrated quadrant (4) of the apparatus.

Results

The arena in our experiment maintained a gradient throughout our trials, as the desiccated end was always drier than the section above water (Fig. 2). However, over the course of the experiment the silica gel lost some efficacy as indicated by the increased error for the desiccated quadrants. The differences between the extreme edges of the arena stayed from 40%-50% with an average of 25% difference, but the differences between adjacent quadrants were less stable.

We placed our scorpions inside of our arena, and allowed them to acclimate for 5 minutes. Afterwards, we filmed them for 55 minutes and recorded which section of the arena they stayed in. After that acclimation period, some scorpions remained in the same position and quadrant for the entire 55 minutes, while others would run around the edges of the arena for some time before coming to rest in a quadrant (Fig. 3). The amount of time the hydrated scorpions spent in quadrant 1 versus quadrant 4 was not significantly different (P=0.3594). Using the same test on our desiccated scorpions, we also did not see a significant difference between the time spent in quadrant 1 versus quadrant 4 (P=0.6406).

Discussion

While our experiment did not yield any conclusive data, a majority of the scorpions spent more time in quadrants 1 and 2, which were the drier quadrants of the arena (Fig. 4). It appears that the quadrant preference is

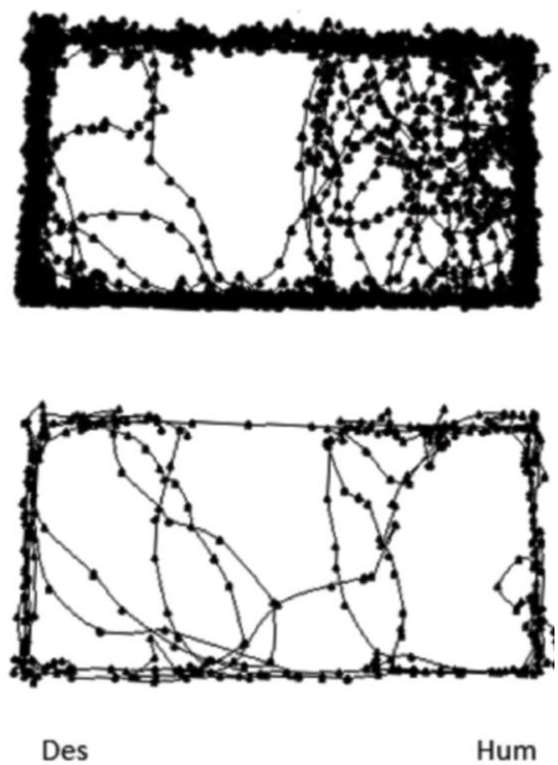

Fig. 3: LED fiber optic connection. A breadboard with two UV (395 nm) LEDs and two cyan-green (505 nm) LEDs connected were controlled using an Arduino computer to standardize irradiance. The breadboard sat above the testing chambers on a platform, allowing the attached fiber Variability of scorpion movements during two sample trials. Shown are tracks for two hydrated scorpions plotted at 3.6 second intervals over the 55 minute trials. Both scorpions spent most of their time along the arena walls. The scorpion in the upper plot was active throughout the trial and made several forays over the water end. The scorpion in the lower plot made fewer forays away from the wall and came to rest above the desiccant.

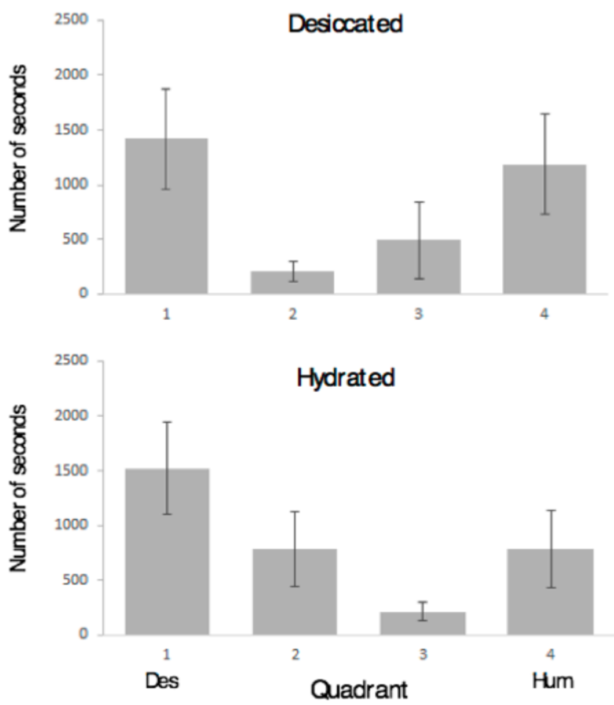

Fig. 4: Average quadrant occupancy of hydrated and desiccated scorpions across all trials. Shown is the average time in seconds (± std dev) the animals spent in each quadrant for all hydrated (upper) and desiccated (lower) animals. We removed one hydrated trial and three desiccated trials due to a lack of movement. Quadrant one was above the desiccants while quadrant four was above the water.

somewhat influenced by the hydration of the scorpion, as hydrated scorpions showed a preference for the drier ends. While the data from hydrated scorpions does correspond with our hypothesis, the desiccated scorpion's results did not, as there was not a preference for the hydrated quadrant.

Should another experiment related to hygroreception in scorpions be conducted, certain factors need to be considered, such as the time of the experiment. It is important to take into account seasonal influences on the animal's behavior. Also, scorpions are nocturnal animals and, as such, demonstrate more behavior at night than during the day. Therefore, it would be best to keep the timing of the trials consistent to minimize these effects.

The dehydration of animals could also be improved. We placed the scorpions being tested inside of our hydrated and dry containers for 48 hours before our trials. However, this treatment didn't appear very effective. We weighed six of our animals pre and post treatment, and there was not a consistent weight loss or weight gain in these scorpions (Fig. 5). Should this experiment be repeated, it would be better to dehydrate the scorpions for longer than 48 hours, but it is important to not dehydrate the scorpion too much, as one of our scorpions died after 72 hours of desiccation in one of our pilot studies.

The humidity gradient inside of our arena was crucial to our experiment. It would be interesting to explore different methods for producing humidity gradients. We used silica gel as our desiccant, resulting in a decent humidity gradient, but the gradient lost efficacy over time as indicated by the increased error seen in the desiccated quadrants (Fig. 2). A potential means of establishing a more stable humidity gradient could be through the use of saturated salt solutions, such as NaCl, as was done in a study of humidity preferences in cockroach nymphs (Dambach & Goehlen 2007).

Another interesting topic to study would be if scorpions from different regions have different humidity preferences. For example, scorpions from more humid areas of the world might show a greater preference for humid areas than scorpions from Oklahoma, which did not show a preference in the conditions of our study. Researching water detection in scorpions is crucial, as it would suggest further research into how arthropods find water. With that information, we may be able to discover a more efficient repellant for arthropods, as many arthropods are considered pests. The research might also promote research in other fields of water detection for other pests that we have little information on.

Acknowledgments

We thank Liz Cooley for her assistance in providing materials for our experiment, as well as George Martin for the development and creation of our arena. Ellen Welti volunteered use of her electronic scale. We would also like to thank the University of Oklahoma Honors College

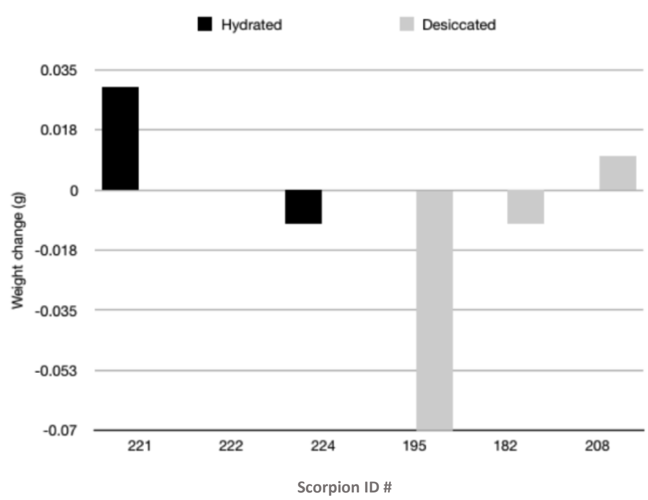

Fig. 5: Scorpion weight change during pre-trial treatments. Plotted are the changes in weight of three hydrated and three desiccated scorpions after their 48 hour treatments.

for funding our project, as well as Dr. Gaffin, Bradley Brayfield and Mallory McMahon for their invaluable help in setting up our experiment and evaluating our results.

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