Temperature response in the striped bark scorpion, *Centruroides vittatus*.

A biology cornerstone manuscript by:
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Summary

To date, the only temperature-specific behavior published for scorpions is stilting, a stereotyped activity where animals raise their abdomens off the substrate in response to warmth. We wanted to characterize this behavior in the striped bark scorpion, *Centruroides vittatus*. To do this, we put individuals in a small, clear, circular enclosure in a dark environment while gradually raising and recording the temperature while observing their reactions. What we found was not stilting, but rather a new set of behaviors that were distinctively different from those described by previous research. Behaviors included increased activity, scratching the walls, extending their telsons, curling and uncurling the tail, sliding along the walls of the enclosure, and crouching. We compiled these behaviors into an ethogram and found that these behaviors occur in a certain order and within predictable temperature ranges. This demonstrates how *C. vittatus* reacts to extreme temperature and suggests that these reactions differ between species from different environments.

Introduction

Scorpions and other arachnids display thermal preference in response to their surroundings. Since there has been little research into their methods of heat detection, thermoreceptors in arachnids remain mostly unidentified. Ticks are one of the few arachnids with well-characterized heat receptors, which are located on the sensilla of their foreleg tarsi (Alexander & Ewer 1958). There is also some evidence that spiders are sensitive to internal and external temperature changes (Foelix 1996), and a temperature-sensitive cell among a field of humidity detectors on the cuticle of the wandering spider *Cupiennius salei* has been reported (Ehn & Tichy 1993). The presence of thermoreceptors in other arachnids adds to the likelihood of their presence in scorpions.

Scorpions show ample behavioral evidence for temperature detection. One such example is a desert scorpion's burrow structure, which creates a consistently cool and humid environment that contrasts the hot, arid climate of their ecosystem (Adams et al. 2016). Also, in response to heat, it has been reported that scorpions exhibit a behavior known as stilting: straightening the legs and raising the abdomen to regulate the circulating airflow, thereby lowering its internal body temperature (Warburg & Ben-Horin 1980, Root 1990). Above 28ºC, almost all scorpions tested stilt to some degree, but rarely do so below 18ºC (Alexander & Ewer 1958).

The study performed by Alexander and Ewer (1958) was on *Opisthophthalmus latimanus* Koch, a scorpion species from South Africa that lives in an arid climate. *Centruroides vittatus* are widespread in North America's temperate climates and diverse habitats. This may have led to alternate adaptations in behavior between species of scorpions, especially those from different environments.

Given the potential differences between the species, we tested *C. vittatus* to observe their response to temperature. We exposed *C. vittatus* to rapidly increasing temperature and observed the change in behavior. We found no evidence of stilting, as described by Alexander and Ewer. Instead, *C. vittatus* displayed a marked increase in motion, through predictable stages of behavior.

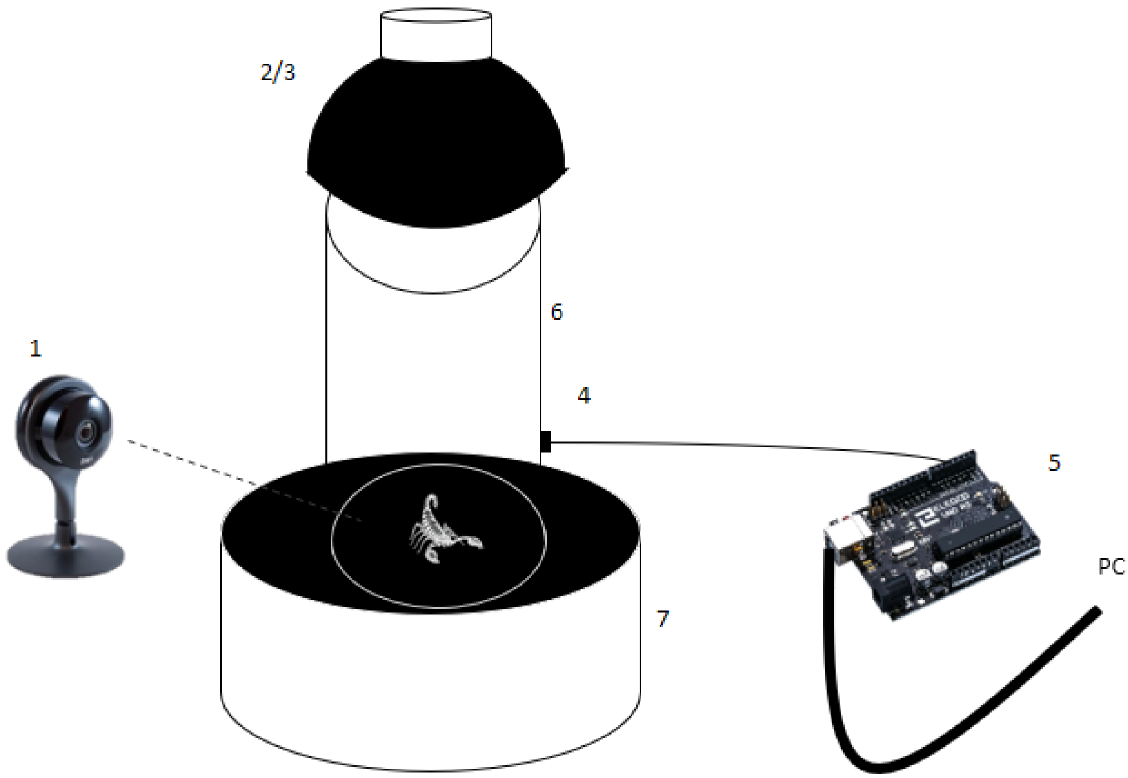

Fig. 1: Experimental apparatus. A Nest camera (1) recorded the experimental trials. A heat lamp (2) and the infrared ceramic heating bulb (3) heated the enclosure without producing light. A sensor (4) monitored and relayed temperature data to the microcontroller (5). The testing chamber was comprised of a clear polyurethane tube (6); a riser (7) was added to the set up to allow the camera to better record trials.

Methods

Scorpion care

We used six male and six female *Centruroides vittatus* captured on September 20, 2017 at Lake Thunderbird State Park, Norman, Oklahoma. The scorpions were kept in either plastic bowls (GLAD brand resealable bowls) or glass jars, containing approximately 2 cm of substrate (Eco Earth coconut fiber) and a small pottery shard. We fed them a live cricket once every two weeks (crickets and the food and hydration crystals to sustain them were sourced from Rainbow Mealworms Inc. Compton, CA), gave them 20-30 mL of water three times a week, and kept them in a light cycle of 11:13 day:night at room temperature.

Experimental design

We set up an isolated chamber by using a Petri dish and placing a polycarbonate tube with a 5 cm diameter set in the dish (Fig. 1). We put a piece of construction paper between the dish and the tube. Then, we increased the temperature of the chamber using a heat lamp (generic reptile clamp lamp) with an infrared ceramic bulb (Zoo Med ceramic heat emitter). There was a Pyrex cup beneath the petri dish, connected with modeling clay, to ensure the cameras were at the eye-level with the scorpion. We programmed an Uno R3 microcontroller (Elegoo EL-CB-001) with a temperature sensor (KOOKYE TMP36 Precision Linear Analog Output For Arduino) in the chamber to log the temperature and time of the experiment. We also used a Nest camera (NC1102ES), with electrical tape covering all but one of the infrared lights, positioned outside the chamber to video the experiment and match the timestamps of the videos to the recorded temperature results. Between trials, we washed the test chamber with tap water and changed the paper used for the base. Light in the room was turned off for all trials.

The heat lamp was allowed a minimum of 10 minutes before the first trial of the day to heat up to maximum temperature and left on for all trials of each day. We allowed the setup to cool completely from the previous trial before beginning. We dropped each

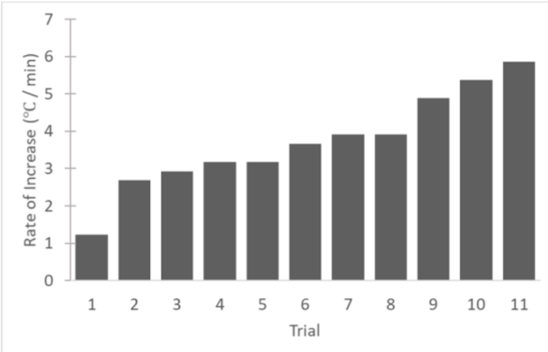

Fig 2: Rate of temperature increase for trials. The graph shows the difference in rate of temperature increase among the individual trials.

scorpion from the top of the tube into the enclosure. Starting at room temperature, we placed the enclosure under the preheated lamp and recorded the behavior and temperature. Our trials all lasted exactly two minutes, which was long enough for the temperature to reach desired levels in all but one trial. We conducted four trials the first night and eight on the s econd, finishing all our trials within those two days. Each scorpion was only tested once.

Analysis

This experiment recorded the behavior of *Centruroides vittatus* when exposed to higher, quicker-changing temperatures than their natural environment. To do so, we created an ethogram from the observed behaviors, which broke the behavior into seven separate stages. As a team, we viewed the footage from each trial and noted the exact time when scorpions entered the stages. Then, we paired those times to the temperatures in the microcontroller temperature log to create our data set. We used the temperature at the time of recordings to establish the actions of the behavior displayed. We had to remove one trial, due to a change in variables, which left us with footage from eleven trials to analyze.

Results

During the trials, we observed the scorpions did not exhibit stilting behavior. Instead, as the temperature increased, the behavior of the scorpions became frantic. We found that the actions consistently occurred in a chronological order as the temperature increased, even though slight variations in the arena placement caused differences in the rate of increase of the temperature (Figure 2). We categorized the observed behaviors into seven separate actions and placed them into an ethogram (Figure 3).

There was little variance between the order of behavioral stages as defined by our ethogram and the behavior during trials (Figure 4). Of the eleven scorpions, seven displayed every defined action listed in the ethogram. Another three scorpions displayed the first six actions, but not the seventh. Scorpion 1 displayed actions up through action four (tail curling) before the test ended; this could be due to the lower overall temperature it experienced compared to the other animals. Nine scorpions exhibited each action in the order shown on the ethogram. Scorpion 4 completed action 2 before action 1 and scorpion 8 completed action 3 before action 1. During individual trials, the scorpions would occasionally display up to two stages of behavior at the same time. Even with these divergences from the ethogram, the average starting temperature of each behavior occurred in a chronological order, which is shown by the linear increase of temperature (Figure 5).

Discussion

The scorpions appear to display specific behavior, categorized by rapid movement, as a response to increasing temperature. Though the behaviors may occur at different times and temperatures, they do so in a largely uniform and predictable manner.

To preface our interpretation of these data, we must discuss errors in our experimental design. The way the arena was placed under the lamp, as discussed in methods, resulted in different rates of temperature increase; the starting and ending temperatures were not the same across all trials. While these limited the accuracy of our results, the errors in placement led to beneficial data; the responses we saw to the varying temperatures supports the observed trend. In addition, our temperature sensor and system were not professional grade equipment and had a reported accuracy of ±1ºC. Nonetheless, the sensor indicated an increase in temperature that correlated with the observed behavioral responses.

Several potential biases also existed in our behavioral descriptions. Our team created the categorization for the ethogram from observed behavior. For this to be completely objective, it would be useful to recreate the experiment without bias. We saw that as the temperature increased, each scorpion's motions became much more rapid. If we could monitor some form of adrenaline or cardiovascular levels in the scorpions' bodies to get objective numbers or, alternatively, use heat or motion mapping, we could ensure that our bias did not influence our interpretation of the scorpion's motion. Nonetheless, we did see a relationship between temperature and behavior across all scorpions, which warrants attention.

The study performed by Alexander and Ewer (1958) was on *Opistophthalmus latimanus* Koch, a scorpion

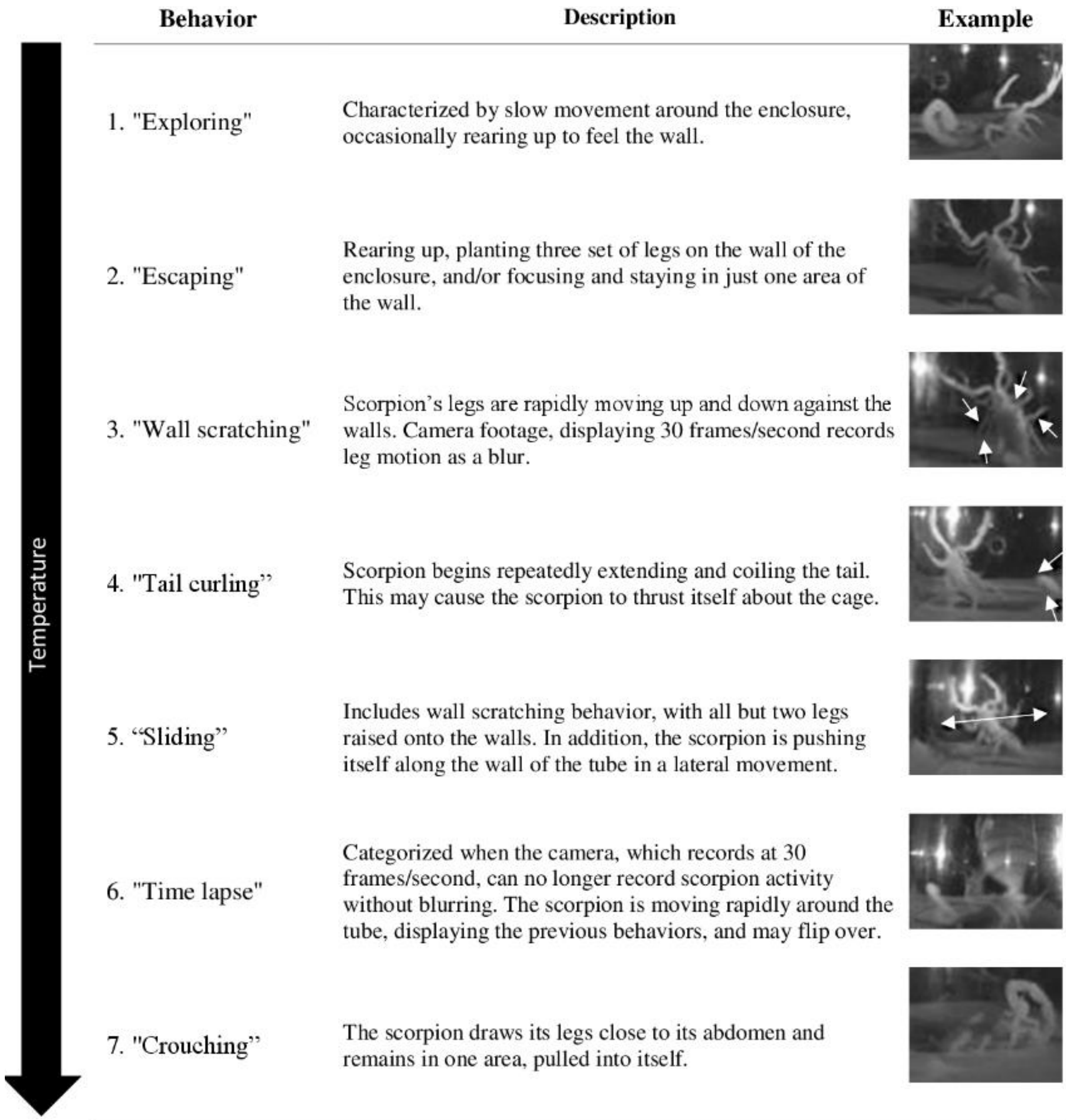

Fig 3: Ethogram. This chart labels and defines each behavioral action, as temperature increases, along with corresponding images.

species which in general lives in a more arid climate than that experienced by *C. vittatus*. *O. latimanus* exhibits stilting, a response to heat likely to maintain a proper body temperature. This species is often found at the entrances of their burrows during midday, to catch prey and they often stilt during this time. This species' habitat and hunting behaviors may be associated with their stilting behavior. If surroundings are uniformly hot, maintaining position and abating the heat gain may be the most efficient way to handle heat.

In contrast, *C. vittatus* lives in more temperate and waterlogged areas of North America. Water absorbs and releases heat, acting as a thermoregulator for the area around it, allowing convection, which causes flow of hot and cool air. Lack thereof causes the vast differences in temperature in arid climates. However, these air flows may allow for a simpler way to

Scorpion	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 6	Stage 7
1	22.8°C	24.2°C	24.7°C	24.7°C	N/A	N/A	N/A
2	26.2°C	26.7°C	27.1°C	27.6°C	27.6°C	28.1°C	N/A
3	24.2°C	27.6°C	27.6°C	28.6°C	28.6°C	29.6°C	N/A
4	24.7°C	24.2°C	27.6°C	28.1°C	28.6°C	28.6°C	29.6°C
5	22.8°C	25.2°C	26.7°C	26.7°C	26.7°C	27.1°C	28.2°C
6	23.7°C	24.7°C	24.7°C	24.7°C	24.7°C	29.6°C	30.1°C
7	24.7°C	24.7°C	27.6°C	28.6°C	31.5°C	32.0°C	32.5°C
8	24.2°C	27.1°C	23.7°C	28.1°C	28.1°C	29.1°C	30.6°C
9	23.2°C	26.7°C	29.1°C	30.1°C	31.5°C	31.5°C	32.5°C
10	23.2°C	31.5°C	32.0°C	32.5°C	33.0°C	33.0°C	N/A
11	23.7°C	29.1°C	30.1°C	30.6°C	31.5°C	32.5°C	33.0°C

Fig 4: Behavioral response to temperature change. The table shows the temperature at which each scorpion displayed the actions defined in the ethogram.

maintain body temperature -- simply moving to a different area may be a more efficient way to cool the scorpions' bodies. The behavior we observed for *C. vittatus* may be the scorpion attempting to leave the hot area. *C. vittatus* has been shown to have a behavioral preference in microclimate based on air temperature (McReynolds 2008). They prefer open ground, which may be due to more air currents. Similarly, stilting cools the body of the scorpion through air currents (Alexander & Ewer).

We also noticed a strong correlation between the temperature and behavioral actions. This suggests that the stages are based on temperature intensity rather than the time spent in each temperature. Trial one's overall temperature was much lower than the rest of the trials and its animal was the only one not to exhibit past stage 4. Two of the other scorpions that did not reach stage 7 ended at comparatively lower temperatures, which supports that the behavior is temperature dependent.

Future studies should develop a more objective method of describing the behavior. This could involve

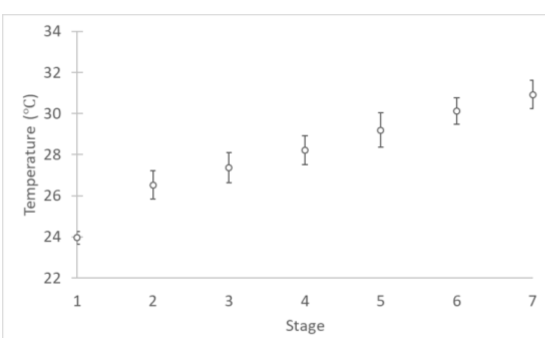

Fig 5: Temperature vs observed action of reaction. The graph shows the average temperature at which each action occurred, ± standard error.

using motion capture or measuring internal factors, such as chemical signals. More trials should use a consistent setup with variable rate increase, as this would allow a distinction between the scorpions' reaction to temperature and rate of temperature increase.

We are also interested in how the scorpions detect temperature differences. A potential sensory organ, called the constellation array, has been found on the pedipalps (Fet et al. 2006). Initially, we attempted to test if this organ is responsible for temperature detection. Due to the difference in behavior between the species in this study and the species used by Alexander and Ewer, we had to first examine *C. vittatus'* response to temperature. If the behaviors described here are consistent in this species of scorpion, potential external temperature sensors could be tested by blocking sensors and measuring differences in behavior.

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