



Article Investigating Path Integration Cues in Sand Scorpion Homing Behavior

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Abstract: Many sand scorpions are faithful to the burrows they dig; however, it is unknown how these animals get back home after hunting excursions. Of the many mechanisms of homing that exist, path integration (PI) is one of the more common tools used by arachnids. In PI, an animal integrates its distance and direction while leaving its home, enabling it to compute an approximate homebound vector for the return trip. The objective of our study was to test whether scorpions use PI to return home under absolute darkness in the lab. We first allowed the animals to establish burrows in homing arenas. Then, after they left their burrow, we recorded the scorpion's location in the homing arena before we transferred it to the center of the testing arena. We used overhead IR cameras to record its movements in the testing arena. If scorpions exhibited PI, we predicted they would follow a vector in the test arena that approximated the same angle and distance from the capture point to their burrow in their home arena. However, under the conditions of this experiment, we found no evidence that scorpions moved along such homebound vectors. We speculated that scorpions may need a reliable reference cue to accommodate path integration.

Keywords: navigation; behavior; proprioception; pectines; vision

1. Introduction

Desert sand scorpions (*Paruroctonus utahensis*) establish homes in self-dug burrows that they remain loyal to for most of their lives [1]. These scorpions hunt at night and may travel as far as five meters from their burrows [1,2]. Because of the dangers posed by predators and poor weather, selection should favor animals with efficient ways to return to their shelters [3]. It has been speculated that scorpions use such cues as prevailing winds or vision to return home [1]. However, more recent evidence suggests that scorpions may also use behaviors such as learning walks [4] and path integration [5] to find their burrows.

Path integration (PI) is the ability of an animal to integrate distance and direction while leaving its home to compute an approximate homebound vector [6,7]. PI has been documented in many animals, including, but not limited to ants [8], bees [9], some species of spiders [10–12], and may be possible in whip spiders [13]. Historically, PI has been tested using displacement experiments. For example, desert ants displaced after discovering a feeding station ran in a relatively straight vector towards where their home would have been had they not been displaced [6,14]. Wolf spiders (*Lycosa tarantula*) coaxed from their homes in a rectangular arena and transferred to the center of a circular arena also moved along a vector that correlated with where their home would have been had they not been displaced [11].

The lesser Asian scorpion, *Mesobuthus eupeus*, may also be using PI to return to a home location [5]. Scorpions were maintained in boxes that were placed in a circular arena of sand, and they were monitored as they ventured away on their own [5]. The scorpions (including those with coverings over their eyes) took non-linear paths to leave the box, but relatively direct sigmoid paths when returning to the box [5]. The authors suggested the



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Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). scorpions likely used proprioceptive information gathered during their outbound journeys to compute their homebound vectors. Since blinded animals returned to their shelters, the use of optic flow information was not supported. Furthermore, the animals did not have prior experience with the testing arena and did not use the outbound path to return to their shelter. This means the scorpions did not use self-generated chemical and/or textural cues (e.g., footprints) to retrace their outbound paths back to their shelter [5]. Even still, there remains the possibility the animals could monitor outbound distance and angular information based on the flow of surface texture across mechanosensitive elements in the peg sensilla of the pectines [15,16].

Recently, it has been hypothesized that scorpions navigate by comparing current glimpses of chemical and textural information acquired by dense matrices of peg sensilla on their mid-ventral pectines [17–19] to those acquired during previous homebound journeys [7,20,21]. While this hypothesis is intriguing, it raises a question: How do scorpions obtain their initial homebound information? Understanding behaviors involving path integration is key to resolving this question.

Additional experiments are needed to fully explore PI in scorpions. In this study, we used a displacement protocol modified from that of Ortega-Escobar to test whether sand scorpions demonstrated behavior consistent with idiothetic PI [11]. Specifically, we allowed scorpions to venture from self-dug burrows in circular home arenas before physically displacing them in complete darkness to larger featureless arenas where we tracked their subsequent movements. If the scorpions were capable of PI, we expected that their movement vectors after displacement would correspond with the general direction of the burrow before displacement occurred. Conversely, if the post-displacement movement vectors were unrelated to the burrow direction, there would be evidence that PI based on idiothetic information (such as proprioception or pecten-transduced textural flow) is insufficient for functional navigation in sand scorpions. We found no significant homeward directionality in the scorpion paths after displacement; as such, we speculated that additional reference cues may be necessary to facilitate PI in these animals.

2. Materials and Methods

2.1. Animals, Collection Details, Storage, and Maintenance

Our study used 18 adult female desert grassland scorpions (*Paruroctonus utahensis*), as females are known to be more loyal to their burrows than their male counterparts [1]. The scorpions were collected from a sandy area in the Chihuahuan Desert near Monahans, TX, USA. We used UV lights to find the animals at night during a period of the new Moon in early March 2022. The animals were maintained in 3.8 L glass jars with 100 mL of sand from their native habitat along with a 3 cm half PVC pipe for shelter. They were misted with water (approximately 2 mL) and fed one small cricket (*Acheta domesticus*) once a week. The jars were kept at a temperature of approximately 20 °C on shelves in a room inside the animal laboratory building on the University of Oklahoma campus. The room was windowless and set on a reverse day cycle, with the lights turning on at 20:00 and off at 06:00 to adjust the scorpions' normal nighttime activity period to times convenient for the researchers. The animals were maintained under the altered light regime for at least a week before testing began.

2.2. Pilot Studies

We ran several pilot tests to optimize parameters including room configuration, arena shapes, mound structure, scorpion transfer procedures, lighting and camera position, experimental protocol, etc. Descriptions of these can be found in the Supplementary Materials, including results of a pilot study (Figure S1) that revealed an initial room bias that was subsequently corrected by removing extraneous light sources.

2.3. Experimental Room Setup

The experimental room (also windowless) was adjacent to the animal storage room and was maintained at the same temperature and light cycle as the animal storage room. The experimental room contained four circular arenas: two smaller homing arenas and two larger testing arenas (Figure 1). The homing arenas were 70.5 cm in diameter, open at the top, and had 25.4 cm tall walls (visual angle to top of wall = 34.4° from arena center). These arenas were constructed from aluminum flashing wrapped around and attached to a circular plywood base. The testing arenas were open-top aluminum water heater drain pans (Camco Manufacturing, Greensboro, NC, USA; 77.5 cm diameter). An additional wall (25.4 cm tall) covered with black felt was placed around each testing arena (visual angle to top of wall = 33.3° from arena center). The four arenas sat atop rubber floor mats (Ottomanson Inc., Mahwah, NJ, USA; multi-purpose 61×61 cm exercise tile mat) to stifle any extraneous vibrations that could have disturbed the animals. All arenas were lined with 500 mL of leveled screened native sand. In the center of each homing arena, we poured a mound of sand (90 mL) over a 2.5 cm wide, 1 cm-tall three-legged plastic pizza saver, creating an internal cavity the scorpions could use as a retreat. We funneled the sand through a 5 mm hole in the bottom of a plastic yogurt cup (157 mL) to create the mound. One side of the pizza saver was left exposed to entice the scorpions to form a burrow (Figure S2). We sprayed 15 mL of water over the top of the mound to increase its stability.

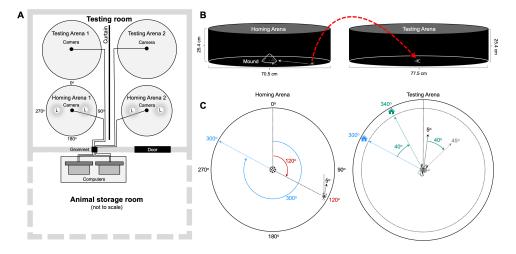


Figure 1. Experimental setup. (A) The experimental room contained two pairs of arenas, with each pair consisting of a homing arena and a testing arena. The pairs were separated by a hanging curtain that prevented the lights over each homing arena from casting shadows over the other homing arena. Overhead lights, a curtain, IR cameras, and lights (L) were suspended from an angle iron support structure. The cords from the cameras passed through a light excluding grommet to computers in the adjacent animal storage room. (B) Scorpions were allowed to burrow in a damp mound of sand in the center of a sand-lined homing arena (left part of (B)). Before each experimental run, the scorpion's position and orientation along the perimeter of the homing arena was calculated relative to the burrow using a 360-degree polar coordinate system; the wall perimeter at the top of the screen was assigned a coordinate of zero. The scorpion was then moved (red dashed arrow) to the center of the testing arena (right part of (B)), where its subsequent movements were recorded (see the text for the experimental details). (C) Shown is a depiction of the various coordinates and orientation adjustments we used in our analyses. The animal's position (120°) and orientation (5°) along the wall of the homing arena is noted as described above. After transferring to the middle of the testing arena, the position of the fictive burrow is noted both before (300°—shown in blue) and after correcting for a change in the animal's orientation after release (340°—shown in green). In this example, the animal's orientation changed from 5° when collected in the homing arena (black scorpion) to 45° after release in the testing arena (rotated gray scorpion). As such, the fictive burrow coordinate changed by 40°: from 300° to 340° .

We built an angle iron support structure to suspend lights and infrared cameras (IR) above the arenas (Figure 1). The IR cameras were mounted 145 cm above the floor of the testing arenas and 168 cm above the floor of the homing arenas, with one camera monitoring each arena. Each camera was connected to a desktop computer located outside of the experimental room through a small hole in the wall with a spacer to inhibit any extraneous light from entering the experimental room. Two lamps equipped with 75 W light bulbs (Duracell Ultra light bulbs, 1100 lumens) were suspended 165 cm over each homing arena. These lamps were connected to a timing switch which cycled the lights on and off according to the same night:day schedule as the main room lights. No lamps were suspended above the testing arenas. Because scorpions are attracted to shadows, a light-blocking curtain was strung between the homing arenas to prevent variations in light levels within the arenas due to light cast from the neighboring arena's lamps.

2.4. Procedure

Prior to testing, the animals were equipped with 5 mm dome rhinestones to reflect the infrared light emitted by the IR cameras. This allowed the scorpions to be detected by the IR cameras from all positions in the arenas. The crystals were about 7 percent of the animals' weight (crystal weight 0.034 g; animal weight 0.5 g) and were effectively used in a previous behavioral study [4]. To apply the rhinestones, each scorpion was placed in a plastic container and restrained using a small plastic sheet that contained a 7 mm hole in its middle. The plastic sheet was maneuvered to center its hole over the animal's dorsal mesosoma, allowing the rhinestone to be attached with double-sided tape to the scorpion.

Each Friday for nine weeks, one test scorpion was moved from its home jar to each of the two homing arenas and allowed four days to establish a burrow in the central mound (Figure 2). On the following Tuesday, we used a dim red light attached to a headlamp to check for signs of burrowing activity in the central mound. If a scorpion did not borrow over the course of four days, it was removed from the experiment and not used in the displacement tests. Due to low burrowing activity, at Week 5, we began placing the animals in the homing arena facing the center mound, a strategy that seemed to increase burrowing activity. Scorpions that showed burrowing activity were checked periodically on Tuesdays, Wednesdays, Thursdays, and Fridays; if it was found along the wall, we initiated a test run.



Figure 2. Weekly testing schedule. On Fridays, the homing arenas were reset by removing the mounds and thoroughly stirring the substrate sand. A fresh mound of sifted native sand was sprayed with 15 mL of water over a partially exposed pizza saver. Additionally, rhinestones were attached to the scorpions, and the scorpions were placed in the homing arena and allowed to form burrows through Monday. If the scorpions burrowed by Tuesday, runs were conducted Tuesday through Friday. Runs were only conducted if the animals had emerged from their burrows and were found along the wall.

To initiate a test run, we activated the IR cameras over both the homing and testing arenas for a 40 min recording session. This allowed us to video the entirety of the experiment without suffering from a lapse in recording. The scorpion was first videoed undisturbed for two minutes to record its initial location in the homing arena. Since the tests were all run in total darkness, we used the red headlamp to determine the animal's position on the wall relative to the central home burrow and the direction the animal was facing on screen. To capture the scorpion for displacement to the testing arena, we simultaneously covered it with an opaque plastic cap (4.5 cm diameter) and slid a fine mesh sieve (8 \times 12 cm piece of window screen with 1.6 mm mesh holes) under it. The scorpion remained under the cap for two additional minutes and was then lifted by the sieve and suspended for 30 s to

allow the sand to sift out. The contained scorpion was then displaced to the center of the testing arena. After another minute, the cap was removed, the direction the animal was facing on the screen was noted, and the IR camera over the testing arena monitored the subsequent movements of the scorpion until the 40 min recording session expired. We took great pains to ensure the animal was calm during transfer including resting times between the placement and removal of the cap. This ensured the animals were not in a defensive stance when released in the testing arena. After the run was complete, the animal was returned to its homing arena. Between each run, the sand in the testing arena was stirred and leveled.

Test runs occurred over four days (Tuesday through Friday), with a maximum of one run per day. Because test runs were only initiated for scorpions that were found along the wall, trials consisted of the one to four test runs taken by one scorpion; after four days, each scorpion was returned to its jar and placed back in the animal storage room. Between trials, the mounds in the homing arenas were removed, and the sand of both the homing and testing arenas was thoroughly stirred and re-leveled. The homing arenas were reset on Fridays with new mounds and new scorpions.

2.5. Video Recording, Data Processing, and Calculations

To quantify the scorpions' paths, we first established a decision line within the testing arena. Because the homing arena's diameter is 7 cm smaller than that of the testing area, we established a fictive decision line spaced 3.5 cm inside the wall of the testing arena. We then used a MATLAB script (The MathWorks INC., MATLAB version: 9.13.0 (R2022b), Natick, MA, USA) to review the recordings taken of each scorpion's run [22]. The run was considered complete once the scorpion crossed the fictive decision line. A run was omitted from the dataset if the scorpion did not cross the decision line within 30 min of its release in the testing arena. For the complete trials, we used a MATLAB script to analyze the video taken of the testing arena at one frame per second so we could hand plot the scorpion's movements. We also noted the location at which the scorpion crossed the fictive decision line, which correlated to a circular angle.

We entered the circular angle for each animal's runs into a MATLAB circular statistics script (Philipp Berens (2023) Circular Statistics Toolbox (Directional Statistics), MATLAB Central File Exchange) to find the averaged resultant vector for each trial. The resultant vectors were calculated from plots rendered three different ways (Figure 3). One plot displayed the raw path the scorpion took relative to true north. The second plot displayed the path the scorpion took relative to the position of its home burrow prior to displacement. The final plot displayed the path the scorpion took relative to be burrow prior to displacement. The final plot displayed the path the scorpion took relative to a field to how it was facing when released in the testing arena compared to how it was facing when captured in the homing arena. The averaged resultant vectors were calculated for all trials and displayed all three ways. We used Raleigh's Z test to determine the significance of the vectors (p < 0.05 alpha level).

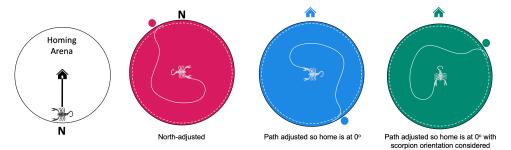


Figure 3. Tracking and analysis of scorpion movement. In the homing arena (white circle), we noted the animal's position on the wall relative to the central home burrow (indicated by the house) and the direction the animal was facing on the computer screen. After displacing the animal to the testing

arena, we used a MATLAB script to facilitate hand plotting of the scorpion's path (white line) until it moved past the decision line (dotted line) near the arena wall. Shown here are mock data to illustrate how we tested for the effects of arena orientation, home direction, and initial scorpion orientation. Plotting with the arenas adjusted to magnetic north tested for biases based on cues within the test room (pink arena). We then adjusted the paths so home would be at zero degrees relative to the animal's placement, not taking the scorpion's orientation into account (blue arena). Finally, we plotted the paths relative to the home direction considering the animal's orientation before capture in the homing arena and its orientation after the cap was lifted in the testing arena (green arena). The dots at the periphery of the arenas indicate the circular angle where the animal crossed the decision line.

3. Results

3.1. Summary of Runs and Trials

Of the 18 animals used in this study, 10 (55 percent) successfully burrowed in the time allotted. Trials were compiled of all animals that formed burrows and completed at least one successful run (n = 10). Our final sample size of 10 is comparable to path integration studies for other arachnids [5,11,23]. Each of the 10 animals completed from 1 to 4 runs (2.5 ± 0.43 , mean \pm SE).

We observed considerable variation in movement during the animals' runs. Some animals made meandering paths before crossing the decision line, while others had relatively direct paths. No matter the path, all animals walked with short movements punctuated by brief pauses and an overall velocity of approximately 3–5 cm per second. Using the hand-plotted frames, we determined the time it took for each animal to move at least one body length after the cap was lifted off it in the testing arena. The animals that completed runs took 62.2 \pm 23.9 s (mean \pm SE) to move after release and 34.3 \pm 6.1 s (mean \pm SE) to cross the decision line after their first movement (Figure S3).

3.2. Path Analysis

We plotted each of the animals' runs based on the three categories of adjustment (Figure 3) to visualize movement patterns (Figure 4). For trials that had more than one run, the patterns showed that the animals crossed the decision line at different circular angles for each run. Figure 4 also shows the averaged resultant vectors of the animals' runs for each of the trials and for each of the adjustment categories.

The resultant vectors for each trial were used to calculate the composite resultant vector for the three adjustment categories (Figure 5). The points on the perimeter of the circles in Figure 5 indicate the average circular angle at which the scorpion crossed the decision line for each trial, and the central arrows depict the overall composite vector based on Rayleigh's calculation. The plots show wide scattering of the trial vectors and no statistically significant directionality for any of the adjustment categories (p > 0.05; Rayleigh's Z test).

We also plotted all the scorpion runs, including those that were averaged in the above analysis. There were 24 runs in all, and Figure S4 shows the results of these runs adjusted so the fictive home is at 0° and adjusted so the fictive home is at 0° with the change in scorpion orientation during transfer factored. Again, no directionality was found for these plots (p > 0.05; Rayleigh's Z test). We also considered the possibility that the animals may express a directional choice early in their excursions with errors accumulating with distance. To examine this, we plotted the points of the 24 runs where the animals crossed a decision line that was one-third the distance from the released animal to the full arena decision line used above (Figure S4, inner dotted circles). There was no directionality for these plots either (p > 0.05; Rayleigh's Z test).

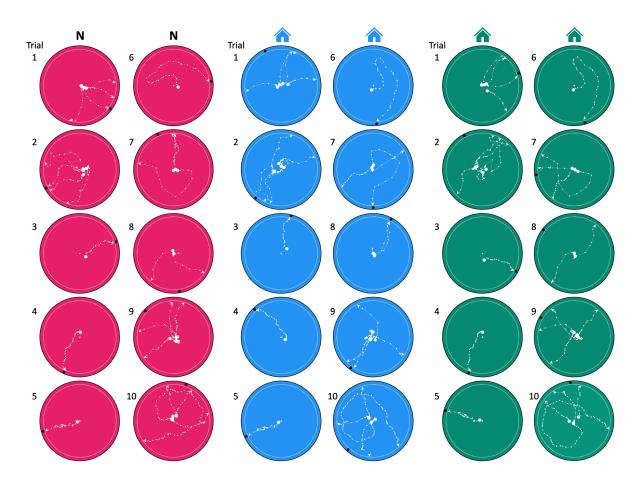


Figure 4. Scorpion path analysis. The movements of all animals in our study are shown based on the adjustments described in Figure 3. The animal's superimposed paths from all runs are plotted at 1 s intervals (small white dots; the larger white dots indicate the animals' starting points after transfer to the testing arenas). The resultant angle based on averaging all runs for each animal are indicated by a black dot at the perimeter of each circle. The white dashed circles indicate the decision lines.

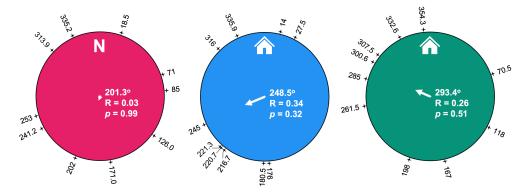


Figure 5. Mean composite vectors for each adjustment category. The composite vectors are shown at the center of each circle along with the mean angle and radial vector length (R). The individual resultant angles for each trial are indicated at the periphery of each circle, with values expressed as tenths indicating averaged vectors for trials with multiple runs. The *p*-values indicate statistical significance based on the Rayleigh test for uniformity.

4. Discussion

Some have speculated that internal (idiothetic) mechanisms, such as information gathered by proprioceptors near leg joints [10] or by mechanoreceptors on the pectines [7], could aid the homeward return of scorpions via PI [15]. Proprioception has been shown to

be important for PI in other arachnids. For example, in the banana leaf spider (*Cupiennius salei*), displacement experiments showed that vision-occluded animals used lyriform organs on their legs to estimate the distance and angle moved from a prey item [24]. The funnel web spider (*Agelena labyrinthica*) uses both proprioceptors and the plane of polarized light to return home [25–28], and proprioception has been shown to contribute distance information to angular information gathered via vision in the wolf spider, *Lycosa tarantula* [29]. However, our study found no significant mean direction of displaced scorpions towards a fictive home burrow when visual information was minimized.

Animals use a variety of external (allothetic) reference mechanisms to aid in PI. Various invertebrates are known to use magnetoreception, celestial positions, landmark orientations, and prevailing winds to return home [7]. Insects such as ants and bees, as well as some arachnids use polarized light in navigation. Ants can use both polarized light (e-vectors) and light wavelength information for navigation, even in the absence of direct sunlight [30]. More closely related to scorpions, the wolf spider (*Lycosa tarantula*) uses its anterior medial eyes to navigate using polarized light patterns [23]. In the absence of polarized light, the spider uses its anterior medial eyes to track outbound rotational information to determine and update its angular position for return trips to its burrow [12].

Our results may at first appear at odds with the evidence for path integration described by Prévost and Stemme for the lesser Asian scorpion [5]. However, we do not feel this is the case. First, many methodological differences exist between the studies. Our study allowed animals to create their own home burrows in the middle of a homing arena with orientational memory being assayed after displacement to a novel testing arena environment. The Prévost and Stemme study used animals acclimated to "home" containers; the animals were then monitored as they ventured from these containers into a novel surrounding arena. The return of the animals (including those with eyes covered) back to the container under various lighting conditions provided strong evidence for path integration information gathered during the animals' outbound journeys [5]. Of course, there could also be behavioral nuances between the species used (Mesobuthus eupeus vs. Paruroctonus utahensis), which represent different clades (clade Buthida, family Buthidae vs. clade Iurida, family Vaejovidae) estimated to have diverged in the Carboniferous-Permian (300 Mya) [31]. Furthermore, a recent study provided evidence that *P. utahensis* scorpions engage in looping forays (putatively learning walks) away from and back to their burrows immediately after their initial digging [4]. This strongly implies that these animals path-integrate information during their outbound movements to get back to their burrows. The current study excluded visual cues to test if idiothetic information (e.g., proprioceptive information or textural flow across mechanosensory elements of the pectines [15,16]) is retained and expressed in a displacement experimental model. It would be interesting to expand on the Prévost and Stemme study by displacing animals captured at the wall in their paradigm to a novel arena to see if path integration information is still expressed. This could motivate additional ablation treatments to deduce the salient cues used for path-integrative homing.

Some aspects of the experimental procedure in our study may have affected the "normal" behavior of scorpions. For example, the laboratory setting and the circular test arenas may not properly simulate the natural environment of *P. utahensis*, which lives in an arid desert climate. Additionally, the abrupt relocation of the scorpions from the homing arena to the testing arena may have erased any path integration information previously stored by the animal.

One assumption underlying this experiment was that the displacement of the animal would be enough to motivate a homeward bound journey. The question of motivation is difficult to assess, but a post hoc analysis of the trajectories of the 25 successful runs in the testing arenas suggested that most animals were expressing some directionality in their movements. Even though this directionality was not necessarily home-directed, it could be interpreted as "intentionality", as opposed to random, exploratory movements. We assessed both the average displacement distance and the angle of departure relative to

the straight path from the animal's starting point in the testing arena to where it crossed the decision line (see the procedures in Prévost and Stemme [5]). We found the average distance of displacement to be a useful measure for distinguishing paths that were directed (putatively motivated) from those that appeared exploratory. In all, only 2 of the 25 runs appeared to be exploratory (Figure S5), which gives us additional confidence that most animals in our study were expressing some form of directed orientation. Going forward, however, we think it is important to develop an improved assay to test whether scorpions "intend" to move toward home following a displacement event or other disturbance.

Future investigations on PI in scorpions are needed to determine if scorpions use allothetic information or require contextual cues to integrate their homebound paths [32]. Magnetoreception seems unlikely since the animals in our study had access to the Earth's magnetic field. While Earth's magnetic field can be distorted in laboratory situations, we found consistent magnetic north compass inclinations around all four arenas. Scorpions have been shown to be behaviorally responsive to polarized light [15,31,33]. Their median eyes contain birefringent lenses that alter polarized light into a cross-like pattern [7,15,34], which could project intensity differences to the underlying retina cells that relay directional information to higher brain centers. We suggest that future tests should add cues such as polarized light and/or distant light sources while also using animals with their eyes intact or covered. As a first step, repeating our displacement experiment outdoors would introduce polarized light relative to the Moon or the setting Sun, which could provide scorpions a global reference that may be necessary for deducing the direction of movement for PI.

5. Conclusions

We tested for cues that could affect home-directed navigation in sand scorpions via path integration. Using a displacement protocol adapted from PI studies of spiders [11], we allowed animals to establish burrows in homing arenas and then transferred them to a larger featureless arena to test for the expression of PI homing vectors. Our tests excluded light to determine whether other cues gathered during outbound paths (e.g., proprioception, magnetoreception, chemo-textural information via pectines) could be used to provide homeward directional information. We assessed the animals' paths in the testing arenas under various scenarios: relative to magnetic north, relative to changes in animal orientation during transfer, and at different distances from the release point in the testing arena. We found no indication of homebound directionality in any of our scenarios. This result led us to speculate that the expression of path integration may require allothetic information that was unavailable within our laboratory setting.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/ 10.3390/arthropoda1020008/s1, Figure S1: Pilot study movements and patterns, Figure S2: Pizza saver aid in burrowing, Figure S3: Movement and burrowing data for all animals used in the study, Figure S4: Analysis of all runs and runs restricted to a closer decision line, Figure S5: Analysis of scorpion path trajectories in testing arenas.

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