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SHORT COMMUNICATION

Self-derived chemical cues support home refuge recognition in the whip spider *Phrynus marginemaculatus* (Amblypygi: Phrynidae)

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Abstract. Whip spiders (Class Arachnida: Order Amblypygi) are hypothesized to use multimodal sensory inputs to navigate back to their diurnal shelter after a night of activity, and chemical cues are thought to be involved. In two experiments, we investigated whether self-deposited chemical cues on a substrate could be used for shelter recognition by *Phrynus marginemaculatus* C.L. Koch, 1840. When given a choice, individual *P. marginemaculatus* spent more time in a shelter that contained filter paper it had previously contacted compared to a shelter with filter paper never contacted. In the second experiment, subjects showed no significant preference for a shelter that contained filter paper they had contacted compared to a shelter with filter paper that had been contacted by a conspecific. The data support the hypothesis that during the last stage of nightly navigation to their refuge, detection of self-deposited chemical cues, likely mediated by contact chemoreceptors, may facilitate home refuge recognition by *P. marginemaculatus*.

Keywords: Homing, arthropod navigation, refuge recognition

Arthropods, as a group, are surprisingly skilled navigators (Cheng 2012; Perry et al. 2013; Ortega-Escobar & Ruiz 2014). For instance, tropical whip spiders (Class Arachnida: Order Amblypygi) navigate considerable distances at night in structurally dense rain forests, where, after a nighttime of activity, they consistently return to their daytime refuges located in tree crevices, cliff fissures or under rocks (reviews by Weygoldt 2000; Santer & Hebets 2011; Chapin & Hebets 2016). Displacement experiments in the field revealed that, unlike most studied invertebrates, whip spider navigation does not depend on path integration (Beck & Görke 1974; Hebets et al. 2014b; Bingman et al. 2017). Instead, whip spiders are hypothesized to rely on multimodal sensory cues to navigate successfully and olfaction is hypothesized to be involved (Wiegmann et al. 2016).

Field experiments suggest that whip spider navigation is supported by olfactory inputs to their specialized antenniform legs, which are covered with olfactory receptors and contact chemoreceptors (Foelix 1975; Beck et al. 1977; Weygoldt 2000; Foelix & Hebets 2001; Santer & Hebets 2011). Indeed, navigation after displacement is severely hindered when inputs to these receptors are prevented (Hebets et al. 2014a; Bingman et al. 2017). Exactly how chemical cues support navigation by whip spiders has not yet been elucidated and likely varies with the distance of an individual to its shelter. In the current study, we conducted two experiments to determine whether self-deposited chemical cues might be used at the end of a return journey home to facilitate identification of a shelter.

Test subjects were *Phrynus marginemaculatus* C.L. Koch, 1840 collected in Florida (Key Deer National Wildlife Refuge, Big Pine Key, Monroe County, FL) and lab-borne progeny of subjects collected in Florida. The sex of subjects was not recorded. In the laboratory*, P. marginemaculatus* establish rapid fidelity to artificial shelters and can be trained to associate plant-derived odors or tactile cues with access to a shelter (Santer & Hebets 2009; Graving et al. 2017; Wiegmann et al. 2019). Prior to our experiments, individuals were housed semi-communally in a 19 or 38 liter terrarium (30 cm x 19 cm x 20 cm or 50 cm x 25 cm x 30 cm, L x W x H) that contained a coconut fiber substrate and pieces of foam insulation for refuge (FOAMULAR, 1.5 cm thickness). Lab-reared sibs were housed in the same terrarium and were not paired together in either of the experiments. Field collected subjects were assumed to be non-sibs because they were collected remotely from each other. Individuals were fed crickets two times per week and had continual access to water. The room in which individuals were housed—the same room used for experiments—was lit by overhead broad-spectrum fluorescent lights (400–750 nm) set on a 12:12 h light:dark cycle (19:45–07:45 dark phase). Room humidity ranged between 25–65% and temperature ranged between 23–28 °C.

The first experiment involved $n = 17$ subjects. Each subject was given a choice between a shelter that contained a piece of filter paper with which it had extensive prior contact, referred to as *self-cued* filter paper, and a shelter that contained a piece of filter paper that was never in contact with a subject, referred to as *blank* filter paper. To prepare the self-cued filter paper a subject was housed for five consecutive nights—approximately 120 hours—in a small plastic deli cup (diameter 17.1 cm, height 10.8 cm) that contained a piece of 90 mm diameter filter paper (Whatman 1001-090 Qualitative Circles). A small plastic dish that held a water-laden cellulose sponge for water and humidity was also placed in the container. An identical deli cup, with similarly treated sponge, that contained no subject was simultaneously prepared to create the blank filter paper.

The day after its fifth night in a deli cup, one hour before lights off, a subject was transferred to a circular (diameter 12 cm, height 3 cm) opaque plastic shelter, which was placed in a rectangular acrylic arena with two identical shelters that a subject would later choose between (Fig. 1). Each shelter had a 3 cm x 1 cm (L x H) entrance and a removable lid. The shelters were positioned in an equilateral triangle configuration, with the unoccupied shelters in the middle of the arena such that the entrance of the shelter that held the subject (start shelter) was equidistant from the entrances of the two other shelters (choice shelters). The entrances of the two unoccupied choice shelters were separated by 15 cm, a distance that exceeds the span of the antenniform legs of *P. marginemaculatus* and, hence, prevented simultaneous contact with the two shelters (Graving et al. 2017). The self-cued filter paper was placed into the bottom of one of the unoccupied shelters and the blank filter paper into the other. The

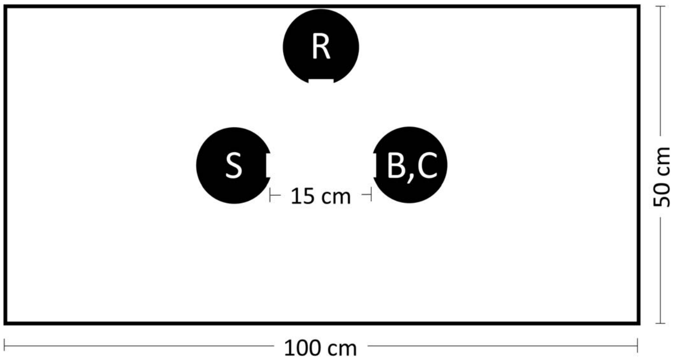

Figure 1.—Test arena. The height of the arena was 30 cm. Each shelter measured 12 cm x 3 cm (Diameter x H). Notches in the shelters indicate the shelter openings. B, C, blank or conspecific-cued filter paper in shelter; S, self-cued filter paper in shelter; R, occupied shelter used to introduce a subject to the arena. The right-left positions of S and B (or C) were determined randomly.

assignment of filter paper to the two unoccupied shelters was determined randomly.

A subject was given 45 minutes after lights-off to freely exit the occupied shelter and, after it exited, that shelter was removed from the arena. If a subject failed to exit the shelter in 45 minutes, we removed the shelter lid and gently coerced it to exit with a small paint brush and removed the shelter. The subject was then allowed to meander around the arena and its location at lights-on was recorded by visual inspection of the shelters. In addition, we video recorded the movements of a sample of $n = 12$ subjects throughout the night with Swann Alpha Series infrared cameras (model SWDVK-432502).

In this experiment we hypothesized that subjects would preferentially utilize the shelter that contained the self-cued filter paper. We used a one-tailed binomial test to determine whether subjects were more likely to be found at lights-on in the shelter that contained the self-cued filter paper. In laboratory situations where multiple shelters are available, as in this experiment, *P. marginemaculatus* regularly explore alternative shelters (Santer & Hebets 2009; Graving et al. 2017). Therefore, the digital records of movements of the video recorded subjects were inspected to determine the number of times a subject entered and switched between shelters before lights-on, as well as occupancy times in each of the shelters. We hypothesized that during the night subjects would spend more time in the shelter that contained the self-cued filter paper. We used a one-tailed binomial test to determine whether the majority of subjects spent more time in the self-cued shelter and a one-tailed, paired t test to compare individual differences in shelter occupancy times.

Results of the experiment revealed an affinity of subjects for the shelter that held the self-cued filter paper. Thirteen of the 17 subjects were found in this shelter at lights-on and four were observed in the alternative shelter that contained the blank filter paper (one-tailed binomial test, $n = 17$, $\pi \leq 0.5$, $P = 0.0245$). The 12 video recorded subjects switched between shelters on average 6 ± 1.6 times in the course of the night and 10 of these subjects spent more time in the self-cued shelter (one-tailed binomial test, $n = 12$, $\pi \leq 0.5$, $P = 0.0193$). The time that these subjects spent in the two shelters was also concordant with our hypothesis. Individuals spent on average $\bar{X} \pm \mathrm{SE} = 358 \pm 59$ minutes in the shelter that held the self-cued filter paper before lights-on and just 137 ± 99 minutes in the alternative shelter that held the blank filter paper. The average difference of occupancy times between the two shelters was 222 ± 105 minutes, significantly higher than zero (Fig. 2, S-B). These results suggest that shelter recognition may be facilitated by self-deposited chemical cues.

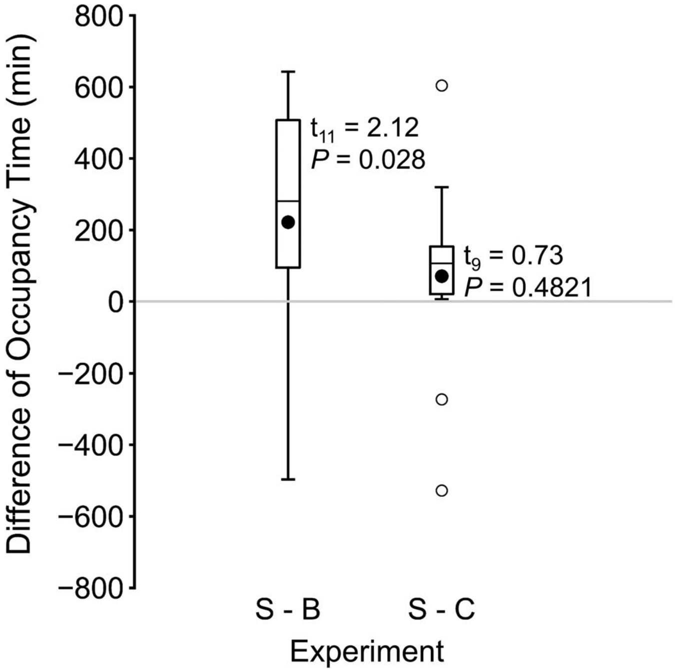

Figure 2.—Boxplots of differences of occupancy times between the self-cued shelter (S) and the blank-cued shelter (B) in the first experiment (S – B) and the self-cued shelter (S) and conspecific-cued shelter (C) in the second experiment (S – C). Mean differences (solid circles) and prospective outliers (open circles) are indicated. Results of paired, one-tailed t-tests are shown against the expected difference of zero (horizontal line). S, time in self-cued shelter; B, time in blank-cued shelter; C, time in conspecific-cued shelter.

In our second experiment subjects were given a choice between a shelter that contained a piece of self-cued filter paper and a shelter that contained a piece of filter paper with which a conspecific had contacted, referred to as *conspecific-cued* filter paper. Ten individuals from the first experiment were arbitrarily selected as test subjects and, as before, subjects were placed into deli cups to generate self-cued filter paper. To generate conspecific-cued filter paper we haphazardly sampled a conspecific from the terrarium in which a test subject was not housed and simultaneously placed it in a deli cup with a piece of filter paper for five nights. The procedures were otherwise identical to those already described. The nocturnal movements of all subjects in the arena were video recorded and we again hypothesized that subjects would preferentially utilize the shelter that contained the self-cued filter paper.

Results of our second experiment revealed no clear affinity of subjects for the shelter that held the self-cued filter paper. Six of the 10 subjects were found in the shelter that contained the self-cued filter paper at lights-on and four subjects were found in the shelter that contained the conspecific-cued filter paper (one-tailed binomial test, $n = 10$, $\pi \leq 0.5$, $P = 0.3770$). Video records revealed that subjects switched between the shelters 12 ± 1.7 times on average before lights-on and that the time subjects spent in the two shelters was similar. Individuals spent on average $\bar{X} \pm \mathrm{SE} = 71 \pm 97$ minutes longer in the shelter that held the self-cued filter paper before lights-on (270 ± 52) compared to the shelter that contained the conspecific-cued filter paper (200 ± 49; Fig. 2, S-C). Though not statistically significant, it is notable that eight of the 10 subjects spent more time in the self-cued shelter (one-tailed binomial test, $n = 10$, $\pi \leq 0.5$, $P = 0.0547$).

Displacement experiments with amblypygids in the field suggest that olfactory cues guide return routes from locations that are remote from the refuge site (Beck & Görke 1974; Hebets et al. 2014a,b;

Wiegmann et al. 2016; Bingman et al. 2017). The results of our first experiment suggest that self-deposited chemical cues could support shelter recognition at the end of a nocturnal homing journey, when an individual is in close proximity to its shelter, just as the desert ant *Cataglyphis fortis*, which uses path integration as its primary navigation strategy, exploits odors emitted from its nest to pinpoint the nest entrance (Steck et al. 2009, 2011). Whether the chemical cue that guided choices in our first experiment was olfactory or chemotactile, however, remains to be determined.

In our second experiment subjects switched repeatedly between shelters and were ultimately as likely to take refuge in the shelter that contained the conspecific-cued filter paper as in the shelter that contained the self-cued filter paper, which suggests *prima facie* that the chemical cue detected by subjects in the first experiment did not serve as a self-recognition signal. Indeed, a self-recognition cue may be unnecessary for refuge recognition if longer-distance navigation mechanisms are sufficiently accurate and precise because *P. marginemaculatus*, like other whip spiders, are largely solitary (Chapin & Hebets 2016). Results of earlier experiments with *P. marginemaculatus* revealed, however, that males are able to recognize rivals over an extended time period (Fowler-Finn & Hebets 2006). Likewise, immature *Damon diadema* (Simon, 1876) can discriminate kin from non-kin and, based solely on olfactory cues, recognize their mother (Walsh & Rayor 2008). These observations suggest that uncontrolled factors in our second experiment, like the sex of focal animals or the sexual receptivity of individuals used to create conspecific-cued filter paper, may have influenced shelter choices and movements between shelters. Further experiments will be necessary to establish whether self-derived chemical cues can be discriminated from conspecific-derived cues and the specific role of self-recognition signals in the identification of a shelter.

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LITERATURE CITED

Beck, L. & K. Görke. 1974. Tagesperiodik, Revierverhalten und Beutefang der Geisselspinne *Admetus pumilio* C. L. Koch im Freiland. Zeitschrift für Tierzüchtung und Züchtungsbiologie 35:173–186.

Beck, L., R. Foelix, E. Gödeke & R. Kaiser, R. 1977. Morphologie, Larvalentwicklung und Haarsensillen des Tastbeinpaares der Geißelspinne *Heterophrynus longicornis* Butler (Arach., Amblypygi). Zoomorphologie 88:259–276.

Bingman, V.P., J.M. Graving, E.A. Hebets & D.D. Wiegmann. 2017. Importance of the antenniform legs, but not vision, for homing by the neotropical whip spider *Paraphrynus laevifrons*. Journal of Experimental Biology 2017:885–890.

Chapin, K.J. & E.A. Hebets. 2016. The behavioral ecology of amblypygids. Journal of Arachnology 44:1–14.

Cheng, K. 2012. Arthropod navigation: ants, bees, crabs, spiders finding their way. Pp. 347–365. *In* The Oxford Handbook of Comparative Cognition, (T.R. Zentall & E.A. Wasserman, eds.). Oxford University Press, New York NY.

Foelix, R.F. 1975. Occurrence of synapses in peripheral sensory nerves of arachnids. Nature 254:146–148.

Foelix, R.F. & E. Hebets. 2001. Sensory biology of whip spiders (Arachnida, Amblypygi). Andrias 15:129–140.

Fowler-Finn, K.D. & E.A. Hebets. 2006. An examination of agonistic interactions in the whip spider *Phrynus marginemaculatus* (Arachnida, Amblypygi). Journal of Arachnology 34:62–76.

Graving, J.M., V.P. Bingman, E.A. Hebets & D.D. Wiegmann. 2017. Development of site fidelity in the nocturnal amblypygid, *Phrynus marginemaculatus*. Journal of Comparative Physiology A 203:313–328.

Hebets, E.A., A. Aceves-Aparicio, S. Aguilar-Argüello, V.P. Bingman, I. Escalante, E.J. Gering et al. 2014a. Multimodal sensory reliance in the nocturnal homing of the amblypygid *Phrynus pseudoparvulus* (Class Arachnida, Order Amblypygi). Behavioural Processes 108:123–130.

Hebets, E.A., E.J. Gering, V.P. Bingman & D.D. Wiegmann. 2014b. Nocturnal homing in the tropical amblypygid *Phrynus pseudoparvulus* (class Arachnida, order Amblypygi). Animal Cognition 17:1013–1018.

Ortega-Escobar, J. & M.A. Ruiz. 2014. Visual odometry in the wolf spider *Lycosa tarantula* (Araneae: Lycosidae). Journal of Experimental Biology 217:395–401.

Perry, C.J., A.B. Barron & K. Cheng. 2013. Invertebrate learning and cognition: relating phenomena to neural substrate. Wiley Interdisciplinary Reviews: Cognitive Science 4:561–582.

Santer, R.D. & E.A. Hebets. 2009. Tactile learning by a whip spider, *Phrynus marginemaculatus* C. L. Koch (Arachnida, Amblypygi). Journal of Comparative Physiology A 195:393–399.

Santer, R.D. & E.A. Hebets. 2011. The sensory and behavioural biology of whip spiders (Arachnida, Amblypygi). Pp 1–64. *In* Advances in Insect Physiology (Vol. 41) (S.J. Simpson & J. Casas, eds.). Academic Press, Burlington MA.

Steck, K., B.S. Hansson & M. Knaden. 2009. Smells like home: Desert ants, *Cataglyphis fortis*, use olfactory landmarks to pinpoint the nest. Frontiers in Zoology 6(5).

Steck, K., B.S. Hansson & M. Knaden. 2011. Desert ants benefit from combining visual and olfactory landmarks. Journal of Experimental Biology 214:1307–1312.

Walsh, R.E. & L.S. Rayor. 2008. Kin discrimination in the amblypygid, *Damon diadema*. Journal of Arachnology 36:336–343.

Weygoldt, P. 2000. Whip Spiders (Chelicerata: Amblypygi): Their Biology, Morphology and Systematics. Stenstrup, Denmark: Apollo Books.

Wiegmann, D.D., E.A. Hebets, W. Gronenberg, J.M. Graving & V.P. Bingman. 2016. Amblypygids: model organisms for the study of arthropod navigation mechanisms in complex environments? Frontiers in Behavioral Neuroscience 10:47.

Wiegmann, D.D., C.H. Moore, N.R. Flesher, E.D. Harper, K.R. Keto, E.A. Hebets et al. 2019. Nocturnal navigation by whip spiders: antenniform legs mediate near-distance olfactory localization of a shelter. Animal Behaviour 149:45–54.

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