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Multimodal sensory reliance in the nocturnal homing of the amblypygid *Phrynus pseudoparvulus* (Class Arachnida, Order Amblypygi)?

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Abstract

Like many other nocturnal arthropods, the amblypygid *Phrynus pseudoparvulus* is capable of homing. The environment through which these predators navigate is a dense and heterogeneous tropical forest understory and the mechanism(s) underlying their putatively complex navigational abilities are presently unknown. This study explores the sensory inputs that might facilitate nocturnal navigation in the amblypygid *P. pseudoparvulus*. Specifically, we use sensory system manipulations in conjunction with field displacements to examine the potential involvement of multimodal – olfactory and visual – stimuli in *P. pseudoparvulus*' homing behavior. In a first experiment, we deprived individuals of their olfactory capacity and displaced them to the opposite side of their home trees (<5 m). We found that olfaction-intact individuals were more likely to be re-sighted in their home refuges than olfaction-deprived individuals. In a second experiment, we independently manipulated both olfactory and visual sensory capacities in conjunction with longer-distance displacements (8 m) from home trees. We found that sensory-intact individuals tended to be re-sighted on their home tree more often than sensory-deprived individuals, with a stronger effect of olfactory deprivation than visual deprivation. Comparing across sensory modality manipulations, olfaction-manipulated individuals took longer to return to their home trees than vision-manipulated individuals. Together, our results indicate that olfaction is important in the nocturnal navigation of *P. pseudoparvulus* and suggest that vision may also play a more minor role.

Keywords: Mushroom body, Navigation, Olfaction, Spatial cognition, Spatial orientation

1. Introduction

Arthropods are known to rely both on idiothetic (internal) and allothetic (external) cues to navigate both short and long distances. For example, path integration, or the assimilation of information that an animal derives from its own movements with its memory of a past position (Mittelstaedt and Mittelstaedt, 1980, 1982), often abetted by visual cues, is known to be an important internal

mechanism in successful arthropod homing behavior (reviewed in Wehner and Srinivasan, 2003; Cheng, 2012). Such a mechanism is likely during “learning walks” in new environments, where animals locate landmarks and produce mental snapshots of their placement (Graham et al., 2010; Muller and Wehner, 2010). Navigation by path integration has been documented in ants (Cheng et al., 2009; Wehner and Srinivasan, 1981, 2003; Wehner and Wehner, 1986, 1990), bees (Von Frisch, 1967), spiders (Moller and

Gorner, 1994), roaches (Rivault and Durier, 2004), and fiddler crabs (Cheng, 2012; Zeil, 1998), and remains a primary focus of many studies of terrestrial arthropod navigation.

In combination with idiothetic cues, many arthropod taxa simultaneously rely on allothetic (e.g., visual, chemical) cues. For example, despite the assumed difficulty of vision in low light environments, numerous studies confirm the role of visual input in the navigation of nocturnally active arthropods (Cheng, 2012). In fact, Warrant and Dacke (2010) suggest that visual, landmark-based homing (the ability of an individual to return to its preferred retreat) is an essential mechanism of navigation in nocturnal environments (Warrant and Dacke, 2010). Even species with presumably poor eyesight have been shown to rely on visual cues for nocturnal homing. For example, the wandering spider, *Drassodes curpeus*, uses polarized moonlight to find its way back to a silk nest (Dacke et al., 1999) and nocturnal Dancing White Lady spiders in the Namibian desert, *Leucorchestris arenicola*, require only visual cues to return to home burrows (Norgaard, 2005; Norgaard et al., 2003, 2007, 2008). Similarly, African dung beetles, *Scarabaeus zambesianus*, navigate using celestial polarization and other night sky features, including the Milky Way (Byrne et al., 2003; Dacke et al., 2003a, b, 2004, 2011, 2013). Ultimately, reliance on a diverse set of visual cues is widespread among arthropods, even among those that navigate in low light conditions.

While visual cues play a well-characterized role in nocturnal arthropod navigation, the potential role of olfactory cues remains understudied. Past work has largely focused on the role of olfaction in tracking pheromone trails over short distances (Rönnegren, 1977; Beugnon and Fourcassie, 1988), but there is evidence that non-pheromonal olfactory cues can also facilitate navigation. For example, the desert ant, *Cataglyphis fortis*, which was previously thought to rely solely on path integration and visual cues to navigate and find their nests, was recently found to use odor landmarks as well (Steck et al., 2009, 2011). Additionally, German cockroaches, *Blattella germanica*, use path integration, visual landmarks, and olfactory cues to indicate the end of their path (Rivault and Durier, 2004). Examples of navigation based upon non-visual sensory modalities, such as olfaction, are nonetheless relatively uncommon. The relative paucity of multisensory navigation studies likely relates to the tradition of navigation research focusing predominantly on a small subset of taxa using modality-specific approaches. We suggest that advancing navigation research necessitates the integrated investigation of multimodality, complex navigation, and a diversity of taxa. Such an approach can not only advance our understanding of the mechanisms underlying navigation, but can contribute to our general understanding of the sensory control of complex behavior. Navigation is a tangible manifestation of complex behavior, and its study requires the identification of model species that successfully navigate in structurally complex environments. To that end, this study explores multimodal sensory reliance, olfaction and vision, during nocturnal navigation in the amblypygid *Phrynos pseudoparvulus* (previously misidentified as *P. parvulus*; see de Armas and Viquez, 2001).

P. pseudoparvulus are capable of nocturnal homing through complex tropical understory habitats (Hebets et al., 2014). These nocturnally active predators can travel more than 36 m in linear distance over the course of several weeks (Hebets, 2002). Prior field displacement studies demonstrate that established individuals can return home without the use of path integration and that they may take indirect return routes, which incorporate stop-overs at non-home trees during homeward journeys (Hebets et al., 2014). These previous studies paint a picture of a central place forager that retains some degree of site-fidelity, but also navigates around a home territory (of unknown dimensions) that may encompass multiple trees. The mechanism(s) of

their putatively complex navigational capacities are currently unknown, but a prior observation of a single displaced individual whose olfactory perception was compromised and did not return home hints towards a role of olfactory reliance (Beck and Gorke, 1974). Additionally, amblypygids possess unique sensory structures that can enable multisensory (including olfactory) perception as well as enlarged brain processing centers (i.e., mushroom bodies) that may provide neural substrates for complex behavior such as navigation.

Amblypygids possess extraordinary sensory appendages that earned them the common name “whip spider.” They walk on only six legs (as opposed to the typical eight), and their thin and elongate first pair of legs (frequently measuring 2.5 times the length of the walking legs or longer) are no longer used for walking (Igelmund, 1987). These “antenniform legs” are highly articulated and covered with thousands of sensory hairs that have mechanosensory and chemosensory functions (Igelmund, 1987; Beck et al., 1977; Foelix, 1975; Santer and Hebets, 2011). The multiporous sensilla, located on the distal ~1 cm of the antenniform legs (reviewed in Santer and Hebets, 2011; Weygoldt, 2000), are confirmed to have an olfactory function (Hebets and Chapman, 2000). The visual capacity of amblypygids, in contrast, is thought to be considerably less impressive, facilitated by eight relatively small, single-lens eyes (reviewed in Santer and Hebets, 2011). The sensory structures of amblypygids cast doubt on the importance of visual cues in nocturnal navigation, but raise the distinct possibility that olfaction may be crucial. Nonetheless, the predominance of vision in the navigation of other arthropods with purportedly poor sight makes this modality worthy of careful examination. Our goals here were to use the amblypygid *P. pseudoparvulus* to examine the roles of olfactory and visual input in nocturnal homing. We explored this in the field by displacing individuals with manipulated olfactory and visual capacities.

2. Materials and methods

2.1. Animals

P. pseudoparvulus were captured between 1800 and 2100 hours from the trunks of trees upon which they were residing. Given that most individuals were collected within 3 h of sunset, it is highly likely that they were collected on the tree possessing their prior night’s refuge — likely their home refuge (for details of site fidelity see Hebets, 2002). Individuals were immediately placed in a Ziplock bag to restrain their movements, and their cephalothorax widths (mm) were measured with digital calipers. Their cephalothoraxes (or sometimes abdomens) were then marked with a unique pattern of two (in one instance, three) colored paint dots using DecoColor paint markers (Uchida of America Corporation, USA; Fig. 1). The variation in the number and placement of paint dots was due to us exhausting potential unique color patterns based upon the paint markers available to us in the field. We determined and recorded each individual’s sex and developmental stage (male, female, or juvenile) and assigned it to a sensory manipulation treatment (sensory-intact vs. sensory-deprived) detailed in specific experiments. Following manipulations, individuals were transferred to a large, opaque plastic snap-cap vial (which precluded visual feed-back) for transport to displacement sites.

2.2. Experiment 1 – Olfaction manipulation (home tree displacement)

This experiment was conducted in the Arboretum at La Selva Biological Station in Heredia Province, Costa Rica on January 8–21, 2007.



Fig. 1. *Phrynus pseudoparvulus* individual with markings on its cephalothorax.

We conducted manipulations on a total of 28 individuals ($n = 28$). Half of the individuals ($n = 14$: 8 females and 6 males) were deprived of olfactory perception (olfaction-deprived) and half underwent a sham manipulation (olfaction-intact) ($n = 14$: 8 females and 6 males). To deprive individuals of olfactory perception, the distal 1 cm of the antenniform legs (distal tarsi) of olfaction-deprived individuals was clipped with scissors to remove the entirety of their olfactory sensilla. This procedure has been used in similar studies on other arthropods, such as crustaceans (Corotto et al., 1999; Kamio et al., 2005; Maruzzo et al., 2007) and insects (Roth and Barth, 2009; Vacha et al., 2008). Olfactory sensilla, and the capacity for olfaction, are restricted to this distal portion of the antenniform legs (Hebets, 2002). The distal 1 cm of the second pair of walking legs (distal tarsi) was likewise clipped as a procedural control, or sham, on individuals in the olfaction-intact treatment. When possible, we randomized the treatment for the 28 individuals making every attempt to pair sex and age groups across treatments.

Individuals for this experiment were collected close to dusk, and thus, close to when they were exiting their home refuge. As such, we were able to identify and mark home refuges. To displace individuals, they were taken to the exact opposite side of the home tree and placed at the same height above ground as their home refuge. In the days following displacements, we measured the linear distance between home refuge and displacement site. We took all distance measurements during the day so as to not disturb the focal individuals. Over the following four nights all home trees were searched at dusk and shortly after to see if displaced individuals had returned to their home refuges. If focal individuals were not re-sighted, the closest trees in each cardinal direction were searched for the presence of the marked amblypygids. Since this experiment was conducted in the Arboretum, which is relatively open compared to the surrounding primary forest, there were few trees to search.

2.3. Experiment 2 – Multimodal manipulations (olfaction and vision – off-tree displacements)

Our second experiment explored the role of both olfaction (using a different methodological approach) and vision in homing of individuals displaced longer distances – 8 m off their home tree. This experiment was also conducted in the Arboretum at La Selva, on January 4–16, 2014.

2.3.1. Olfaction manipulations

A total of 18 individuals were used in the olfactory manipulation experiment – 10 olfaction-intact (2 juveniles, 4 females, 4 males) and 8 olfaction-deprived (2 juveniles, 3 females, 3 males). In contrast to the clipping procedure used for olfactory deprivation in Experiment 1, for this experiment we used nail polish to fully cover the distal 1 cm of the tarsi of the antenniform legs of olfaction-deprived individuals. Specifically, following the successful measuring and marking of an individual, the distal tips of the antenniform legs were painted with black nail polish (424 Black Crème; Wet 'n' Wild, USA). We chose black so that we could clearly see whether the targeted portion of the leg was indeed fully covered, thus presumably making all multiporous sensilla non-functional (Hebets, 2002), and also to minimize the conspicuousness of manipulated individuals. Following painting, individuals were gently held for ~5 min while the nail polish dried, after which they were placed into a labeled and visually isolated snap-cap plastic vial and placed on the forest floor near their home tree. Individuals were held in their transport vial for 30–90 min prior to their displacement so that they could acclimate to their sensory manipulation. The procedure was exactly the same for olfaction-intact individuals, except that the distal portion of the femora of the antenniform legs, as opposed to tarsi, was painted with black nail polish. Although this section of the leg possesses sensory hairs, there are no olfactory (multiporous) sensilla on this leg segment (Hebets and Chapman, 2000; Santer and Hebets, 2011).

2.3.2. Vision manipulations

A total of 18 individuals were used in visual manipulations – 10 vision-intact (4 juveniles, 3 females, 3 males) and 8 vision-deprived (4 juveniles, 2 female, 2 males). Visual deprivation was accomplished by covering all three sets of eyes (i.e., all eight eyes; both primary and secondary) with a small amount of dental resin (Heritage 7, USA). Specifically, following the successful measuring and marking of individuals, they were restrained in the Ziplock bag and positioned such that the anterior region of their cephalothorax (the part of the prosoma where their eyes are located) was underneath an open hole in the bag. The dental resin was mixed to its quick-drying solid state and placed atop the three sets of eyes. The dental resin was white in color and thus, these individuals had an additional whitish marking on the cephalothorax. Vision-intact individuals were manipulated in exactly the same manner, except that instead of covering the eyes, a similar amount of dental resin was mixed onto the posterior portion of the cephalothorax (behind the eyes), again causing the same whitish color to be present. As with the olfactory manipulated individuals, vision-manipulated individuals were placed in their transfer vials on the forest floor near their home tree for later displacement, allowing the same acclimation period to adjust to their sensory manipulation.

In contrast to Experiment 1, in which individuals were placed on the opposite side of their home tree, individuals in this experiment were displaced to the forest floor 8 m from their home tree, always at a location beyond the catchment zone of the home tree's buttressing. On any given night, we assigned individuals a displacement direction (a cardinal direction: N, S, E, W) before release to randomize the direction towards home. The first displaced individual on any given night was assigned North, the second – South, the third – East, and so on. At their displacement

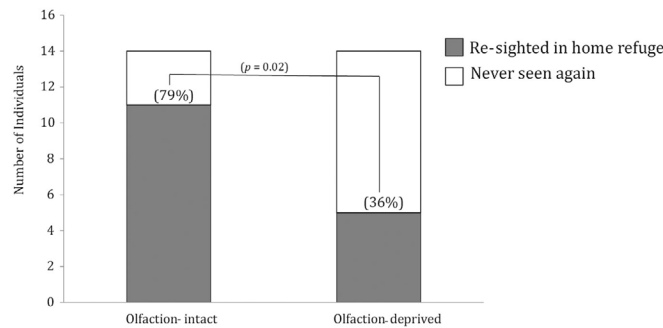


Fig. 2. Number of individuals either re-sighted in their home refuge or never seen again from olfactory-manipulations: home tree displacements (Exp. 1). Olfaction-intact individuals (those with second pair of walking legs clipped) were more likely to be re-sighted in their home refuge than olfaction-deprived individuals (those with the distal 1 cm of their antenniform legs clipped).

location, individuals were placed gently onto the forest floor and the displacement site was marked with a stick and labeled flagging.

All home and surrounding trees were monitored for a minimum of 3 nights following displacements. On any given night, we collected, marked and displaced anywhere from zero to 15 individuals. Following our initial night ($n = 15$), we were not only looking for new individuals to mark and displace, but we were also monitoring home trees for the presence/absence of previously marked individuals. At each home tree, we extensively searched the tree as well as the closest trees in every direction. We continued this for the duration of the experiment, with the minimum number of nights that individuals could have been re-sighted being 3 nights total and the maximum number being 12 nights (see Supplementary Table 1; following the References).

3. Results

3.1. Experiment 1 – Olfaction manipulation (home tree displacement)

Eleven of the olfaction-intact individuals (four males and seven females, 79%) returned to the refuge from which they were collected, while only three of the olfaction-deprived individuals (two males and one female, 21%) returned (Fig. 2). These two return rates differ significantly (Chi Square Test, $X^2 = 5.40$, $p = 0.02$; effect size: ϕ (ϕ) = 0.439). No individuals were re-sighted anywhere other than at their home refuge. The cephalothorax widths of olfaction-intact individuals (mean \pm SD = 8.14 ± 1.66 mm) and olfaction-deprived individuals (mean \pm SD = 8.31 ± 2.36 mm) were similar (Two-sample t Test, $t = -0.21$, $p = 0.83$). Likewise, the displacement distances of olfaction-intact individuals (mean \pm SD = 202 ± 70 cm) and olfaction-deprived individuals (mean \pm SD = 250 ± 184 cm) did not differ (Two-sample t Test on ln-transformed distances with unequal variances, $t = -0.24$, $p = 0.81$). Thus, neither body size nor displacement distance can explain the differences we observed in the return rates of olfaction-intact vs. olfaction-deprived treatment groups.

3.2. Experiment 2: Multimodal manipulations (olfaction and vision – off-tree displacements)

3.2.1. Olfaction manipulation

Exactly one-half of the 10 olfaction-intact ($n = 5$) and one-half of the 8 olfaction-deprived ($n = 4$) individuals were re-sighted,

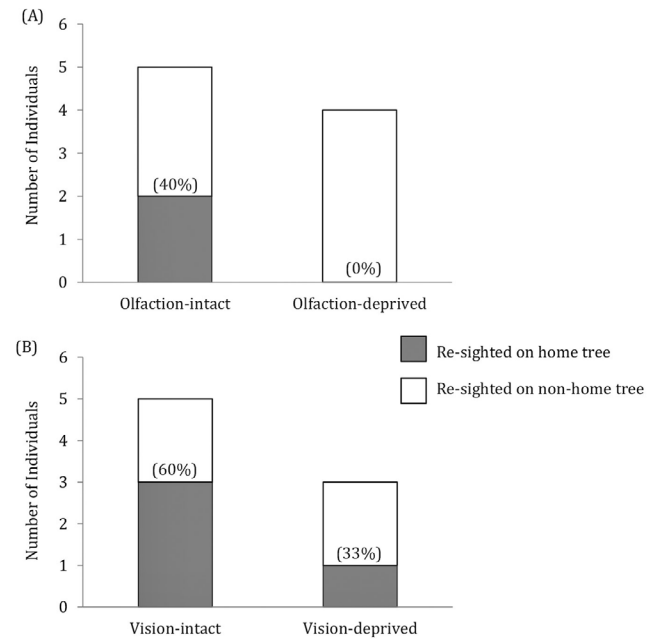


Fig. 3. Number of re-sighted individuals seen on their home tree vs. a non-home tree across olfactory and visual sensory manipulations following an 8 m displacement. (A) Olfaction-manipulated individuals (Exp. 2: total of 10 olfaction-intact and 8 olfaction-deprived individuals were marked). Although there is no statistical difference between the proportion that made it home between the two groups ($p = 0.09$), the effect size is large (ϕ (ϕ) = 0.56). (B) Vision-manipulated (Exp. 2: total of 10 vision-intact and 8 vision-deprived individuals were marked). There is no statistical difference between the proportion that made it home between the two groups ($p = 0.46$) and the effect size is moderate (ϕ (ϕ) = 0.26).

with no difference in the likelihood of re-sighting individuals from either group ($X^2 = 0.00$, $p = 1.0$; effect size: ϕ (ϕ) = 0). Of those that were re-sighted, 2 of the olfaction-intact individuals (both females) were re-sighted on their home tree while zero of the olfaction-deprived individuals accomplished this feat ($X^2 = 2.80$, $p = 0.09$; effect size: ϕ (ϕ) = 0.56; Fig. 3). There was no difference in the proportion of nights that individuals in the olfaction-intact vs. olfaction-deprived treatment were re-sighted (# nights re-sighted/total # of nights they could have been re-sighted; Wilcoxon Test: $X^2 = 0.13$, $p = 0.72$). There was also no difference in the number of nights it took to first re-sight individuals from either treatment group (olfaction-intact ranged from 1 to 6 nights; olfaction-deprived ranged from 2 to 7 nights; Wilcoxon Test: $X^2 = 0.14$, $p = 0.71$). We were not able to statistically compare the minimum movement distances of olfaction-deprived and olfaction-intact individuals because logistical complications prevented us from measuring distances for several re-sighted individuals (see Supplementary Table 1). However, the distances appeared similar based on a qualitative comparison only (olfaction-intact: mean \pm SD = 831.6 ± 1058.1 cm; olfaction-deprived: mean \pm SD = 430.5 ± 1.4 cm). As in Experiment 1, cephalothorax widths were similar between olfaction-intact individuals (mean \pm SD = 7.61 ± 2.54 mm) and olfaction-deprived individuals (mean \pm SD = 8.64 ± 2.002 mm; two-sample t Test, $t = 0.97$, $p = 0.35$).

3.2.2. Vision manipulation

One-half of the 10 vision-intact ($n = 5$) and three of the 10 vision-deprived individuals were re-sighted, with no difference in the likelihood to re-sight individuals from either group ($X^2 = 0.28$, $p = 0.60$; effect size: ϕ (ϕ) = 0.12). Of the individuals that were re-sighted, three of the vision-intact individuals (1 juvenile, 2 females) and one of the vision-deprived (juvenile) were re-sighted

at their home tree ($X^2 = 0.54$, $p = 0.46$; effect size: $\phi = 0.26$; Fig. 3). There was no difference in the proportion of nights that individuals in the vision-intact vs. vision-deprived treatment were re-sighted (Wilcoxon Test: $X^2 = 0.92$, $p = 0.34$). There was also no difference in the number of nights it took to first re-sight individuals from either treatment group (vision-intact ranged from 1 to 4 nights; vision-deprived ranged from 1 to 3 nights; Wilcoxon Test: $X^2 = 0.41$, $p = 0.52$). The minimum distance traveled of vision-intact (mean \pm SD = 684.5 ± 231.0 cm) and vision-deprived individuals (mean \pm SD = 419 ± 340 cm) did not differ (Mann-Whitney $U = 2.50$, $z = -1.23$, $p = 0.22$), and cephalothorax widths were similar for both vision-intact (mean \pm SD = 8.13 ± 1.99 mm) and vision-deprived individuals (mean \pm SD = 8.44 ± 1.52 mm; Wilcoxon Test, $W = 50$, $p = 0.65$).

3.2.3. Olfaction and vision combined

We ran a combined analysis comparing time to home between olfaction and vision manipulated groups in an effort to gain insight into the relative importance of each sensory system. When we combine all olfaction treatments (olfaction-intact plus olfaction-deprived) and all vision treatments (vision-intact plus vision-deprived), we find that displaced olfaction-manipulated individuals took longer to return home than vision-manipulated individuals ($X^2 = 4.47$, $p = 0.04$). We saw no differences between these two groups in terms of their likelihood to be re-sighted ($X^2 = 0.11$, $p = 0.74$) or in their likelihood of being re-sighted at their home tree, independent of their re-sighting rate (olfaction: 22%, vision: 44%; $X^2 = 1.0$, $p = 0.32$; $\phi = 0.24$). Additionally, the minimum distance traveled did not differ between olfactory manipulated (mean \pm SD = 717 ± 887.7 cm) and vision manipulated individuals (mean \pm SD = 570.7 ± 292.3 cm; Mann-Whitney $U = 20.0$, $z = -0.57$, $p = 0.57$). These results could indicate a greater reliance on olfactory perception for amblypygid navigation and/or that our olfactory manipulations compromised additional perceptual abilities (see Section 4).

3.2.4. Additional observations

Prior to Experiment 2 (in 2012), preliminary displacement studies on sensory-intact individuals were conducted along the CES trail at La Selva Biological Station. Twenty-seven individuals were marked with either DecoColor paint pens (Uchida of America Corporation, USA) or nail polish and were displaced 10 m from their home tree. A total of 8 individuals were re-sighted (30%), 5 of which (63%) were re-sighted on their home tree. We note that this preliminary experiment was conducted in an area of primary forest, as opposed to the more open area of the Arboretum, which was the location of the current experiments. The difference in habitat complexity, along with an increase in number of available trees in the primary forest, is likely responsible for the relatively low re-sighting rates in this preliminary experiment. These additional observations, however, are important for demonstrating the capacity of amblypygids to home even through the complexity of primary forest.

Nine days prior to performing Experiment 2, three sensory-deprived individuals (two olfaction-deprived and one vision-deprived) were manipulated and returned to their home refuge. This preliminary assay acted to insure that sensory manipulated individuals did not simply disappear due to an enhanced susceptibility to predation or increased wandering behavior. All three individuals were re-sighted 9 days following their marking. The two olfaction-deprived individuals were re-sighted at their home refuge while the vision-deprived individual was re-sighted on a nearby tree 7 m linear distance from its home tree.

Two of the individuals that were marked on the abdomen in Experiment 2 were re-sighted in the field, deceased at the base of their tree. These individuals were re-sighted no sooner than 4 days

following painting and we believe that the paint markers (which contain xylene) that we used were able to penetrate the softer cuticle of the abdomen (opisthosoma) and were toxic to these individuals. None of our animals marked on the cephalothorax were found dead and multiple individuals (~25) marked on both their cephalothorax and/or abdomen have remained alive for more than two and half months with no obvious aberrant affects (and are currently still alive). A total of nine additional manipulated individuals were similarly marked on their abdomens during this displacement experiment — none of which were re-sighted. We have included these individuals in the above analyses as, even if they had been intoxicated, they would have had at least 4 nights to successfully return to their home tree, or to be re-sighted on an alternate tree (of the two re-sighted individuals with abdominal markings, one made it home, one was re-sighted on a different tree). We note this observation as a warning to other researchers attempting similar techniques.

4. Discussion

Results of two independent experiments, using different experimental methods and different displacement distances, suggest that olfactory input facilitates successful nocturnal homing in the amblypygid *P. pseudoparvulus*. Our results hint to a more minor role of vision as well. In a first experiment, which employed the removal of olfactory sensilla, olfaction-intact individuals were more likely to be re-sighted at their home refuge as compared to olfaction-deprived individuals. In a second experiment, which involved covering the olfactory sensilla with nail polish to ablate olfaction in conjunction with displacements off of the home tree, results also suggest that olfaction-intact individuals are more likely to successfully home as compared to olfaction-deprived individuals. This second experiment failed to find statistical significance ($p = 0.09$), but this is likely due to the small sample sizes of re-sighted individuals in each of our treatments during our available observation period (5 olfaction-intact re-sighted; 4 olfaction-deprived re-sighted; 50% of total animals in each treatment). Our effect test reveals a large ϕ (0.56), supporting the biological relevance of our findings. In addition to a role of olfactory input in nocturnal homing, we also found preliminary evidence for the importance of visual input, as 60% of our vision-intact individuals that were re-sighted successfully homed compared to only 33% (1 individual) of the vision-deprived individuals. Again, this finding was not significant, but our effect size estimate for the role of vision was moderate (ϕ of 0.26). Our power to infer treatment effects was limited by small sample sizes for re-sighted individuals (5 vision-intact re-sighted, 50%; 3 vision-deprived re-sighted, 38%). Interestingly, the one vision-deprived individual that successfully homed did so from a distance of 8 m, despite being displaced 2.6 m from another tree. The successful homing of this individual indicates that while navigation may be supported by vision, it is not contingent upon it since a vision-deprived animal was able to successfully home. Together, our experiments provide fairly strong evidence that olfaction plays a role in amblypygid nocturnal navigation and hint that vision might as well, implying multimodal sensory reliance.

In addition to ablating olfactory perception, both of our olfactory manipulations (Experiments 1 and 2) likely impacted additional sensory systems. The tips of the antenniform legs possess rod sensilla and bristles (mechanosensory and contact chemosensory capabilities) as well as olfactory club sensilla (reviewed in Santer and Hebets, 2011). Thus, clipping this section of the antenniform legs (Experiment 1) presumably compromised perception through other sensory modalities. These same sensory systems are likely also compromised in our manipulation involving the application of nail polish (Experiment 2). However, although

other sensory systems may have been affected during our antenniform leg manipulations, olfaction was the sole sense completely ablated, making it the most likely driver of behavioral differences between amblypygids differing in olfaction treatment. Furthermore, the distal portion of the femur, our control treatment in Experiment 2, possesses tactile sensory structures. Thus, even our olfaction-intact treatment likely influenced sensory perception in other modalities. This sensory compromise may be at least partly responsible for the slower homing rates of olfactory-manipulated vs. vision-manipulated treatments. Regardless, our results are highly suggestive of olfactory and visual-based homing in amblypygids and we suspect that future tracking studies using telemetry on sensory-deprived individuals (olfaction and vision) in combination with tightly controlled laboratory studies focusing on modality-specific cues will confirm the validity of our findings.

Despite their ubiquitous presence in other arthropod groups such as insects, olfactory (multiporous) sensilla are not common within arachnids. Amblypygids, however, possess a number of such multiporous sensilla that serve an olfactory function (Santer and Hebets, 2011; Hebets and Chapman, 2000), indicating the capacity for utilizing olfactory stimuli. In addition to these external morphologies that implicate the importance of olfaction (i.e., the presence of multiporous sensilla), amblypygids possess the largest mushroom bodies (relative to their body size) documented in arthropods (Strausfeld et al., 1998). These higher-order processing centers have traditionally been ascribed roles in olfactory discrimination, as well as olfactory learning and memory, but their roles may not be limited to olfaction (Akalal et al., 2006; Heisenberg, 1998, 2003; Perez-Orive et al., 2002; Strausfeld et al., 2009). In insects, mushroom bodies are known to be involved in contextual information processing, learning, and memory—including spatial memory (de Belle and Heisenberg, 1994; Heisenberg, 2003; Mizunami et al., 1998; Pascual and Preat, 2001; Zars et al., 2000). In fact, the size of arthropod mushroom bodies has recently been proposed to reflect the complexity of spatial navigation strategies (Jacobs, 2012). This “spatial orientation” hypothesis is consistent with our current knowledge of amblypygid neuroanatomy (i.e., large mushroom bodies) and their seemingly complex navigational capacities (present study). This hypothesis warrants future research, with subsequent studies explicitly exploring the role of multisensory inputs in navigation.

Our data are admittedly limited in terms of sample size, a lack of knowledge regarding individual's past experiences (e.g., navigation patterns and potential knowledge of surroundings), and a lack of knowledge of individual movement patterns. For example, we cannot know for sure if sensory-deprived individuals were (a) less likely to find their way back home due to their sensory deprivation (our presumption here), (b) less motivated to move, (c) displaced to an area outside their previously experienced range, or (d) more likely to succumb to predation or some other source of death. Nonetheless, we find the first explanation most likely. Increased likelihood to succumb to predation seems unlikely since we found no difference in the likelihood to re-sight sensory-intact vs. sensory-deprived individuals and our re-sighting rates are similar to those reported from a larger mark-recapture study of this same species (males: 40% re-sighted; females: 75% re-sighted) where individuals were not displaced, but simply monitored over time (Hebets, 2002). Furthermore, all individuals were randomly assigned a displacement direction, making it highly unlikely, though not impossible, that a larger subset of the sensory-intact individuals happened to be released in areas where they had previously traveled, facilitating their homeward travel. Finally, we believe that our consistent re-sighting rate (olfactory treatment 5:4 and vision 5:3, intact:deprived respectively) combined with the similar minimum distances traveled (olfactory 831.6 ± 1058.1 cm:430.5 \pm 1.4 cm and vision 684.5 \pm 231.0 cm:419

\pm 340 cm, intact:deprived respectively) suggests that sensory deprived individuals were not less likely to move compared to sensory intact individuals.

Our experimental design was predicated on the assumption that the trees on which the amblypygids were initially located were their home trees. This assumption follows from our protocol in which amblypygids were initially sighted close to dusk, and thus were likely to be emerging from their over-day refuge. Previous work has shown relatively high sight fidelity in this species (Hebets, 2002). However, it is possible that amblypygids seek refuge on alternate trees throughout their home range while still maintaining a dominant home tree. Any comparison of a field-collected amblypygid's ability to navigate back to a home tree after displacement must consider this possibility. In fact, we consistently relocated individuals on a tree not initially deemed to be their “home” tree, and the possibility exists that we collected them on an alternate tree and they did indeed succeed in traveling “home.” Although this scenario is certainly plausible, it is likely pertinent to only a small subset of individuals, yet still may have contributed to our weak statistical values, exacerbated by the small sample size.

Though our results provide preliminary evidence of multimodal sensory reliance during amblypygid navigation, we cannot yet say anything about *how* or *what* cues are being used. Amblypygids do not follow the same outbound and inbound path (unpublished data, EAH), yet there remains the possibility of chemical marking. Their reliance on olfactory stimuli suggests a capacity for olfactory-learning and prior studies have already established their capacity for tactile-learning (Santer and Hebets, 2009). Our vision-deprived results also indicate a more substantial role of visual input than previously anticipated, making future work exploring their visual capabilities now essential. Finally, our displacements involved already established individuals, many of whom were adults. While these displacement experiments clearly demonstrate that path integration is non-essential for successful homing in established individuals, it may indeed be essential in the initial stages of learning environmental cues. For example, many arthropods, including ants (Nicholson et al., 1999; Wehner et al., 2004) and spiders (Norgaard et al., 2012), engage in “learning walks” to facilitate the learning of visual landmark information and these walks are likely controlled by path integration (Graham et al., 2010; Muller and Wehner, 2010). Learning walks facilitated by path integration and associated with olfactory and visual snapshots remain a possibility in amblypygids. Ultimately, we are at the inaugural stages of navigation research in this unique system and the number of future research directions is plentiful.

While field studies of sensory reliance during navigation are a crucial piece of the puzzle that ultimately facilitates our understanding of the mechanisms underlying success navigation through complex environments, they are insufficient on their own and pose unique challenges. The unavoidable challenges of this study, for example, are (1) the necessary use of already established individuals and (2) our low re-sighting rates which unavoidably result in small sample sizes. Given our early stage of understanding of amblypygid navigation, initial studies require us to first establish their capacity for nocturnal homing and second to provide hints towards their sensory reliance. These goals were both achieved in this study, paving the way for more controlled and elaborate field and laboratory studies which hone in on specific sensory system reliance and their potential integration. With respect to our low re-sighting rates, preliminary studies of non-manipulated individuals displaced at 10 m from the home tree revealed even lower re-sighting rates than in our sensory manipulation studies. We suggest that our low re-sighting rates reflect the natural history of these long-lived arthropods (suggested

lifespans of more than 5 years in the field) that appear to regularly travel across large areas of tropical forest understory, utilizing multiple trees and logs along the way as diurnal refuges. Given their putatively comprehensive knowledge of their local environment, their motivation to return to a specific refuge may not be strong, making field studies such as ours challenging, yet nonetheless essential.

In summary, the amblypygid, *P. pseudoparvulus*, appears to rely on olfactory inputs for successful navigation and likely, though to a lesser degree, visual inputs as well. Currently, the nature of the olfactory or visual stimuli remains unknown and the degree to which successful homing relies on one or both sensory stimuli is inconclusive. Interestingly, work by Steck et al. (2011) have demonstrated that desert ants (*C. fortis*) also use both olfactory and visual cues to guide their return routes to the nest. Following training with independent or combined cues, they found that ants learned the location of their nest more quickly with multimodal cues (Steck et al., 2011). Similar cross-modal influences on learning have been discussed in non-navigation contexts (e.g., signaling and communication; mate choice; warning coloration) and we suggest that navigation research may benefit from incorporating existing theoretical frameworks for studying multimodality (Candolin, 2003; Hebets and Papaj, 2005; Partan and Marler, 1999, 2005; Rowe, 1999; Rowe and Halpin, 2013).

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Supplementary Table 1. Table summarizing all raw data for *Experiment 2: Multimodal Manipulations (Olfaction and Vision – Off-Tree Displacements)*. Individuals ID: * symbolizes individuals marked on the abdomen, while all other individuals were marked on the cephalothorax; all the dates belong to 2014; Treatments: O+ olfactory-intact individuals, O- olfactory-deprived individuals, V+ vision-intact individuals, V- vision-deprived individuals. CW: cephalothorax width.

Individual ID	Date Marked	Treatment	Tree #	Sex/Age	CW (mm)	Displacement Direction	Resighted	Homed	Minimum distance moved (m)
Green, Pink	04-Jan	O+	2	juvenile	6.17	East	X		26.6
Blue, Green ₁	04-Jan	O+	7	juvenile	5.97	East			
Pink, Pink	04-Jan	O+	5	female	10.02	North	X	X	4.3
Pink, Green *	07-Jan	O+	15	female w/eggs	9.68	South	X	X	8
Green, Pink *	09-Jan	O+	17	female	7.84	East	X		1.3
Pink, Red *	09-Jan	O+	10	female	7.03	South			
Green, Green *	09-Jan	O+	11	male	11.12	West			
Blue, Blue	04-Jan	O+	7	male	10.93	North	X		1.38
Blue, Purple ₂	04-Jan	O+	8	male	7.16	South			
Pink, Purple *	09-Jan	O+	2	male	7.95	West			
Pink, Green	04-Jan	O-	2	juvenile	8.58	South			
Blue, Red	04-Jan	O-	7	juvenile	5.12	West			
Green, Blue	04-Jan	O-	1	female	10.79	East			
Pink, Pink *	07-Jan	O-	5	female	9.05	West	X		3.31
Green, Red *	13-Jan	O-	13	female w/eggs	9.17	North			
Blue, Silver	04-Jan	O-	6	male	11.5	North	X		5.3
Silver, Red	05-Jan	O-	12	male	8.44	East	X		NA
Silver, Purple	05-Jan	O-	14	male	9.38	West	X		NA
Green, Red	04-Jan	V+	1	juvenile	6.26	West	X	X	8
Purple, Green	05-Jan	V+	9	juvenile	4.32	East			
Purple, Purple	05-Jan	V+	10	juvenile	6.91	West			
Green *	11-Jan	V+	10	juvenile	4.69	South			
Blue, Green ₂	04-Jan	V+	8	female	9.63	East	X	X	8
Pink, Pink, Pink	06-Jan	V+	9	female	9.6	North	X		NA
Green, Purple	13-Jan	V+	3	female	8.87	South			

* w/eggs									
Green, Purple	04-Jan	V+	1	male	10.4	North	X	X	8
Pink, Blue *	07-Jan	V+	16	male	8.51	East			
Green, Blue *	13-Jan	V+	9	male	10.23	West	X		3.38
Green, Green	04-Jan	V-	4	juvenile	6.66	West			
Purple, Pink	05-Jan	V-	9	juvenile	4.89	South			
Silver, Green	05-Jan	V-	13	juvenile	5.39	South			
Pink *	11-Jan	V-	10	juvenile	4.88	North	X	X	8
Blue, Purple₁	04-Jan	V-	7	female	8.25	South	X		1.45
w/eggs									
Silver, Silver	05-Jan	V-	11	female	9.12	North			
Blue, Pink	04-Jan	V-	5	male	10.3	South	X		3.12
Pink, Red *	13-Jan	V-	11	male	9.17	East			