

## RESEARCH ARTICLE

# Importance of the antenniform legs, but not vision, for homing by the neotropical whip spider *Paraphrynus laevisfrons*

Verner P. Bingman<sup>1,\*</sup>, Jacob M. Graving<sup>2</sup>, Eileen A. Hebets<sup>3</sup> and Daniel D. Wiegmann<sup>2</sup>

## ABSTRACT

Amblypygids, or whip spiders, are nocturnal, predatory arthropods that display a robust ability to navigate to their home refuge. Prior field observations and displacement studies in amblypygids demonstrated an ability to home from distances as far away as 10 m. In the current study, micro-transmitters were used to take morning position fixes of individual *Paraphrynus laevisfrons* following an experimental displacement of 10 m from their home refuge. The intention was to assess the relative importance of vision compared with sensory input acquired from the antenniform legs for navigation as well as other aspects of their spatial behavior. Displaced individuals were randomly assigned to three treatment groups: (i) control individuals; (ii) vision-deprived individuals, VD; and (iii) individuals with sensory input from the tips of their antenniform legs compromised, AD. Control and VD subjects were generally successful in returning home, and the direction of their movement on the first night following displacement was homeward oriented. By contrast, AD subjects experienced a complete loss of navigational ability, and movement on the first night indicated no hint of homeward orientation. The data strongly support the hypothesis that sensory input from the tips of the antenniform legs is necessary for successful homing in amblypygids following displacement to an unfamiliar location, and we hypothesize an essential role of olfaction for this navigational ability.

**KEY WORDS:** Amblypygid, Arthropod, Navigation, Olfaction, Radio telemetry

## INTRODUCTION

When one considers the remarkable navigational abilities of animals, what often comes first to mind is the long-distance migration of birds, sea turtles and salmon, or the homing behavior of pigeons. However, it is becoming increasingly apparent that despite their relatively small brains and generally shorter distances traveled (the monarch butterfly, *Danaus plexippus*, notwithstanding; Mouritsen et al., 2013), many arthropod species also display an impressive navigational ability (Cheng, 2012; Collett and Graham, 2004; Boles and Lohmann, 2003; Layne et al., 2003). Indeed, both honey bees (*Apis mellifera*; Menzel et al., 2005) and an Australian ant species (*Myrmecia croslandi*; Narendra et al., 2013) have been shown to be able to return to their nests even after experimental displacement to unfamiliar locations, suggesting a cognitively rich navigational capacity. Arachnids too

display impressive navigational abilities. For example, after searching for females, males of the Namib Desert spider *Leucorchestris arenicola* successfully return to their home burrows from as far away as 40 m (Henschel, 2002; Nørgaard, 2005).

Species of the Order Amblypygi (Class Arachnida), colloquially referred to as whip spiders or tailless whip scorpions, inhabit tropical and subtropical regions around the globe where they are often found in dense rain forest (Weygoldt, 2000). Beck and Görke (1974) were the first to report that tropical amblypygids are unexpectedly good at navigating to their home refuge shortly before dawn, having spent the night typically hunting on the vertical surfaces of tree trunks. Even after an artificial displacement of 10 m, one whip spider successfully returned to its home refuge. Building on the observations of Beck and Görke (1974), Hebets et al. (2014a) displaced individuals of *Phrynus pseudoparvulus* up to 4.5 m onto the opposite side of their home refuge tree and found that they were able to home successfully. They additionally used radio telemetry to track the navigational behavior of the amblypygid *Paraphrynus laevisfrons* (though this second species was not identified as such in Hebets et al., 2014a), and found that individuals routinely returned to their home tree after experimental displacements of up to 8 m. The routes that the displaced *P. laevisfrons* took were not necessarily straight and the journey home often took more than one night to be completed.

Amblypygids are excellent navigators, and although their homing success following displacement may not match the visually guided performance of diurnally active honey bees and some ants, one needs to consider that tropical amblypygids are nocturnally active and live in a structurally complex environment of a cluttered and uneven ground surface, with dense vegetation depriving the animals of any distal panorama of terrestrial stimuli and a dense canopy that offers little access to celestial cues or variation in light intensity. In other words, many of the sensory and behavioral mechanisms that guide the spatial behavior of bees, ants and even other studied nocturnal navigating arthropods (Warrant and Dacke, 2016) are likely to be inaccessible to navigating amblypygids. The fascinating question then is what enables amblypygids to be such successful navigators? The first order challenge is thus the identification of the sensory cues that guide their homing behavior.

Probably the most notable morphological feature of amblypygids is their elegantly articulating antenniform legs (hence ‘whip’ spiders). The antenniform legs, and in particular the distal tarsus, are covered with a rich array of sensory receptors (Santer and Hebets, 2011; Wiegmann et al., 2016). Among the numerous receptor types are multiporous sensilla that respond to olfactory cues (Hebets and Chapman, 2000). Unlike the other receptor types, these multiporous sensilla are only found on the distal tips of the antenniform legs (Foelix, 1975; Igelmund, 1987). Amblypygids also possess eight eyes composed of a pair of medial eyes and a bilateral set of three eyes positioned more laterally (Weygoldt,

<sup>1</sup>Department of Psychology and J.P. Scott Center for Neuroscience, Mind and Behavior, Bowling Green State University, Bowling Green, OH, USA. <sup>2</sup>Department of Biological Sciences and J.P. Scott Center for Neuroscience, Mind and Behavior, Bowling Green State University, Bowling Green, OH, USA. <sup>3</sup>School of Biological Sciences, University of Nebraska, Lincoln, NE, USA.

\*Author for correspondence (vbimga@bgsu.edu)

 V.P.B., 0000-0002-5295-7096

2000). Given their nocturnal activity and the light-impooverished nature of the tropical amblypygid habitat, vision would seem to be an unlikely sensory channel to control navigation. Many nocturnal arthropods, however, are remarkably adept at using vision to guide their spatial behavior (Warrant and Dacke, 2016), opening up the real possibility that amblypygids could similarly rely on vision.

A preliminary, mark–recapture field investigation into the relative importance of the tips of the antenniform legs (and by inference olfaction) and vision in the amblypygid *P. pseudoparvulus* revealed that displaced animals deprived of sensory input from the tips of their antenniform legs were not able to relocate to their home tree (Hebets et al., 2014b); however, the behavior of the vision-deprived animals suggested a more modest loss of navigational ability. The goal of the current study was to expand on this previous work in a distinct species, *P. laevifrons*, using miniature radio transmitters to reconstruct nightly movements following an experimental displacement of 10 m from the home tree. In particular, we investigated more thoroughly the spatial behavior of amblypygids when visual information and sensory information from the distal tips of the antenniform legs were impaired, with the intention of reaching a better understanding of the sensory basis of their navigational ability.

## MATERIALS AND METHODS

### Animals

During the end of June and early July 2014 ( $N=19$ ) and 2015 ( $N=11$ ), 30 *Paraphrynus laevifrons* (Pocock 1894) were captured in second-growth rain forest at the La Suerte Biological Field Station (83°46'15"W, 10°26'30"N) near Cariari, Costa Rica, in the Caribbean lowlands. The animals were captured at night after they emerged from their home refuges and, with the exception of two control animals (see below), were fitted with Advanced Telemetry Systems A2414 radio transmitters. The transmitters were affixed with a cyanoacrylate adhesive to the posterior prosoma (cephalothorax) and transmitter antennae were trimmed to 2.5 cm. The widths of each individual's prosoma is given in Table 1 but, in general, they were large with typical widths of 15 mm or more. Spiders were then divided into three treatment groups (see below): (i) control individuals, (ii) vision-deprived individuals (VD) and (iii) individuals with compromised sensory input from the tips of the antenniform legs (antenniform leg-deprived, AD). We also made an attempt to sex each spider in the field, but because many of the individuals defied our best efforts to confidently sex them, we have chosen not to present our 'best guesses' on sex. No gravid females were used in the experiment.

### Sensory deprivation treatments and displacement

In 2014, control animals consisted of seven animals with attached transmitters and two animals that had colored markings placed on the dorsal surface of their prosomas behind the eyes using DecoColor paint markers. The two animals with colored markings were tested as mark–recaptures in the absence of any in-transit, positional data. To control for the application of black nail polish (424 Black Crème; Wet 'n' Wild, USA) to the SD animals (see below), all nine control amblypygids had the proximal segment of their fourth pair of walking legs covered with this nail polish. One control amblypygid's transmitter stopped transmitting after the first morning; its first night orientation and distance data are included in the results, but this animal was not included in the homing success analysis. The VD animals ( $N=5$ ) had all eight eyes painted over with the nail polish. AD animals ( $N=6$ ) had the distal tips of their antenniform legs painted with the nail polish, covering the entire

**Table 1. Summary of telemetry data for displaced individuals**

Animal	Prosoma width (mm)	Direction (deg)		Distance (m)			$N_{\text{home}}$
		Home	First night	First night	Total	Home	
C14-1	15.3	90	70	7.2	7.2	9.5	X
C14-2	18.7	315	95	7.7	20.7	0	3
C14-3	13.9	150	360	5.0	24.5	23.0	X
C14-4	14.3	360	270	2.5	12.7	0	2
C14-5	19.7	200	45	4.2	29.2	0	5
C14-6	16.7	360	60	3.2	–	–	–
C14-7	19.2	260	350	5.2	10.2	0	2
VD14-1	19.0	60	330	5.7	11.4	0	2
VD14-2	18.3	290	95	3.5	6.2	12.0	X
VD14-3	15.1	180	360	10.0	10.0	0	1
VD14-4	18.0	270	360	10.0	10.0	0	1
VD15-1	15.6	125	260	2.8	16.3	10.5	X
VD15-2	18.1	325	320	5.8	12.9	0	4
VD15-3	10.2	265	255	4.2	21.5	14.0	X
VD15-4	18.2	215	360	10.0	10.0	0	1
VD15-5	15.8	250	300	4.3	13.4	0	3
VD15-6	18.3	30	270	1.2	1.2	10.1	X
AD14(NP)-1	15.2	225	285	4.5	11.5	12.3	X
AD14(NP)-2	19.0	180	220	2.6	17.0	12.0	X
AD14(NP)-3	18.1	260	340	3.6	44.6	4.8	X
AD14(NP)-4	16.8	90	70	1.9	21.4	19.2	X
AD14(NP)-5	20.0	200	130	5.6	6.6	14.5	X
AD14(NP)-6	16.5	180	220	2.8	5.8	12.2	X
AD15(SC)-1	–	215	360	10.0	10.0	0	1
AD15(SC)-2	16.2	325	320	5.8	5.8	6.7	X
AD15(SC)-3	15.9	10	75	4.5	10.3	9.4	X
AD15(SC)-4	13.0	90	315	1.8	1.8	8.7	X
AD15(SC)-5	19.5	30	270	6.3	10.4	15.0	X

C, control; VD, vision deprived; AD(NP), tips of antenniform legs coated with nail polish; AD(SC), tips of antenniform legs removed with scissors. The direction home from the release location is given with geographic north set to 360 deg; the direction moved during the first night after release is given with the home direction set to 360 deg. Distance traveled on the first night after release, total distance traveled across all recorded mornings and distance to the home refuge tree from the last recorded position are shown.  $N_{\text{home}}$ , number of nights taken to return home (X indicates the subject never returned home). Note, the transmitter for subject C14-6 stopped working after the first recorded morning position.

tarsus. We chose black so that we could clearly see whether the targeted portion of the leg was indeed fully covered, ensuring that all receptors were rendered dysfunctional.

In 2015, we did not test any control animals because the results from 2014 and our earlier studies revealed a consistent pattern of behavior of sensory-intact animals (Hebets et al., 2014a,b). The VD subjects ( $N=6$ ) were subjected to the same deprivation treatment as in 2014, having all eight eyes painted over with the nail polish. However, for AD animals ( $N=5$ ), instead of using nail polish, the ends of the antenniform legs were removed with small surgical scissors at a distance of about 11–12 mm from the tips. The different AD treatments of nail polish (2014) and scissor cut (2015) did not result in any detectable differences in behavior (see Table 1) and the data from all AD individuals were pooled for all analyses.

All captures and displacements occurred within 4 h of sunset after the amblypygids emerged from their diurnal refuges, which were located in the crevices of tree buttresses or under logs. Upon capture, an animal was held by hand, underwent nail polish or cutting treatment and then was carried to its release point. The displacement distance for all animals was 10 m, and the direction of displacement was haphazardly scattered across individuals (Table 1). We originally intended to carry out systematic displacements to the cardinal directions, but this proved

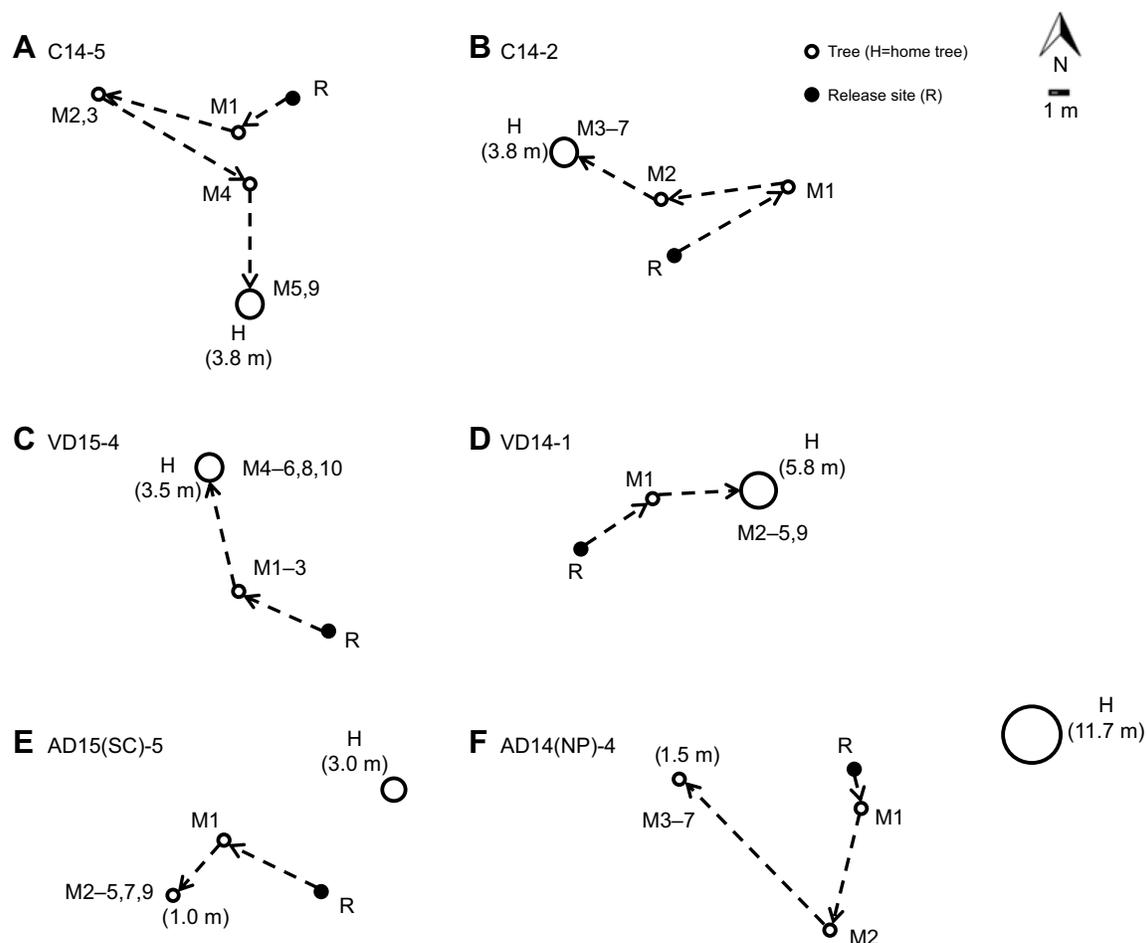
unfeasible as much of the understory vegetation was simply too thick to reliably move through in any predetermined direction. The 10 m displacements were far enough to prevent the use of home tree buttresses as navigational guides, i.e. beyond what one could call the ‘catchment zone’ of the home tree by which we mean that once an animal is within the enveloping buttresses of a tree, the geometry of the buttresses could literally funnel it toward the trunk and presumably closer to its home refuge.

An Advanced Telemetry Systems R410 receiver with a Yagi three-element antenna was used to locate individuals. Each morning following displacement, the position of each individual was recorded as the distance and direction from the release site for the first morning or its last known position on subsequent mornings (if not back at the home tree). The data were collected during daylight hours, while individuals were sheltering in their tree or log refuges, to ensure that our activities did not interfere with their behavior. We are confident that our ability to locate animals with the telemetry was accurate as the positions of some subjects were visually verified during the day and every marked animal we saw at night was at the location indicated by telemetry the following morning. With few exceptions, animals were located for at least five consecutive mornings following displacement and all animals were located at

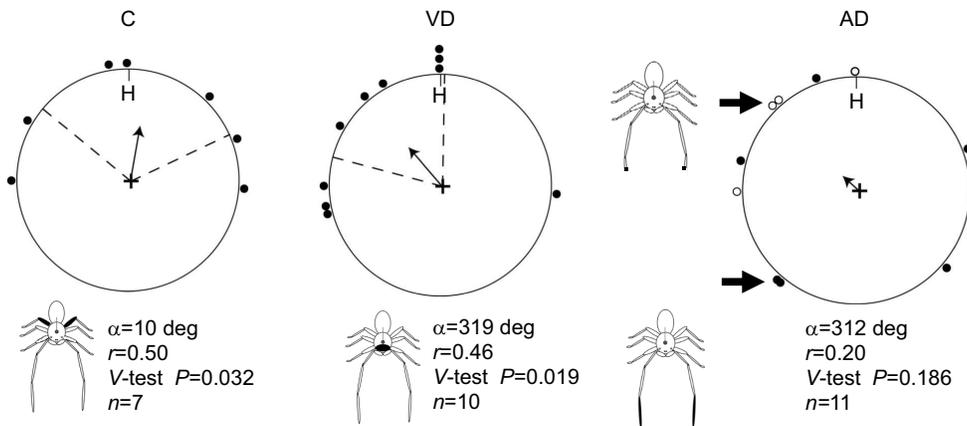
least three sampled mornings in a row at the same location, i.e. position fixes of individuals at their morning locations were taken until they seemed to cease to move from their current refuge. Some animals were located for as many as 10 mornings following displacement. Importantly, all distances reported are the beeline distances from where an animal was found on a given morning and where it was on the previous morning (or its displacement location). Thus, distances reported are minimum distances, and if animals took indirect paths during nightly movements, the actual distances covered would have been longer than those reported.

### Statistical analysis

The first-order dependent measure was homing success, which was the proportion of animals that eventually returned to the home refuge within each of the VD, AD and control groups. Of additional interest was the direction of movement on the first night in relation to the refuge tree, the distance traveled on the first night, the total distance a subject traveled and the distance to the home tree from the last recorded position for each of the subjects. A Fisher exact test, modified for three groups, was used to test for group differences in homing success, followed by pair-wise tests (Freeman and Halton, 1951); circular statistics were used to analyze the orientation of the animals on



**Fig. 1. Homing tracks of *Paraphrynus laevisfrons* deprived of visual input or sensory input from the tips of their antenniform legs.** Representative tracks of two control (C) individuals (A,B), two vision-deprived (VD) individuals (C,D) and two individuals following removal or covering of the tips of the antenniform legs (AD; E,F). The release site (R) of an individual and the location of the home tree (H; circumference in parentheses) are shown (not to scale). M (open circles) indicates the locations of the animals on the mornings following release; the associated numbers identify the number of mornings after release. (Because weather conditions did not permit us to search for subjects every day, the daily locations are incomplete for some subjects.) For AD animals in E and F, the circumference of the tree they eventually adopted as their new home is given in parentheses at their last recorded location. All displacements were 10 m.



**Fig. 2. Orientation of the first night movement across the three treatment groups.** Dots on the outside of each circle identify the direction of movement of one individual (for the AD animals, filled dots are subjects painted with nail polish and open dots are scissor-clipped subjects). Arrows at the center of each circle identify the group's mean vector, the length of which is proportional to the mean vector length (with an arrow equal to the radius of the circle indicating a mean vector length of 1.0). Directions are plotted with respect to home (H) located at the top of each diagram.  $\alpha$ , mean direction;  $r$ , mean vector length.

the first night (Batschelet, 1981); and one-way ANOVA was used for all other between-group comparisons (Sokal and Rohlf, 2011).

## RESULTS

The transmitter of one control subject stopped transmitting after the first morning and only its orientation and distance data for the night on which it was displaced could be utilized for analyses. All the individual data used in the analyses are summarized in Table 1.

### Homing success

Fig. 1 shows sample paths recorded from animals in the three treatment groups. With respect to homing success, application of the Fisher exact test revealed a significant difference across the three treatment groups ( $P=0.006$ ): whereas 6/8 control animals and 6/10 VD animals successfully homed (no group difference,  $P=0.64$ ), only 1/11 AD amblypygids homed, a performance deficit that significantly differed from both the VD ( $P=0.02$ ) and control (0.006) groups.

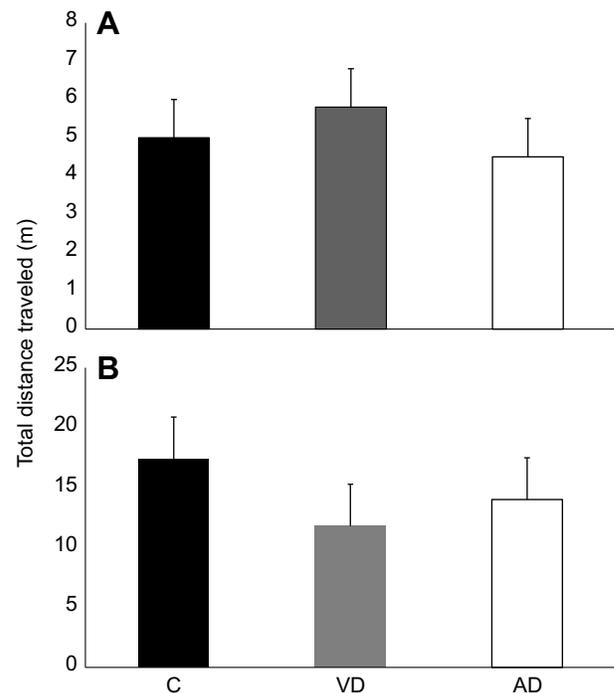
Associated with the homing success data were some noteworthy behavioral observations. First, it was not unusual for successfully homing animals to initially move farther away from their home refuge, and then return to eventually reach their refuge (Fig. 1). The four transmitter-carrying control animals that homed took between 2 and 5 nights to return to their refuge, and two of those four traveled a minimum distance of more than 20 m before reaching home. The six VD animals that homed took between 1 and 4 nights to return to their refuge, with three of the animals returning on the morning following displacement. In summary, depriving whip spiders of sensory input from the distal tips of their antenniform legs completely disrupted their navigational ability.

### Initial orientation

Summarized in Fig. 2 is the orientation of movement of the subjects on the night of displacement. Using the direction to the refuge as the predicted direction, both the control ( $N=7$ , mean 10 deg, mean vector length 0.50,  $V=0.49$ ,  $P=0.03$ ) and VD ( $N=10$ , mean 319 deg, mean vector length 0.46,  $V=0.46$ ,  $P=0.02$ ) animals were significantly oriented with 95% confidence intervals of the mean direction that included the direction to the home refuge. By contrast, the AD amblypygids were disoriented ( $N=11$ , mean vector length 0.20,  $V=0.19$ ,  $P=0.19$ ). Despite the presumed conflicting motivations of escaping from capture and returning to the home refuge, both the control and VD animals already displayed an ability to orient homeward on the first night. No such ability was detected in the AD animals.

### Distance traveled

The interpretation of the AD animals' inability to return home as a failure to navigate is confounded by the possibility that disrupting the tips of the antenniform legs eliminated the motivation to return. To partially untangle a navigational from a motivational effect, we were particularly interested in how far the animals moved after they were displaced (Fig. 1). Fig. 3 summarizes the minimum distance moved on the first night after displacement and the minimum total distance traveled across all nights among the animals in the three treatment groups. No between-group differences were found in either the distance traveled on the first night ( $F_{2,5,2}=0.62$ ,  $P=0.56$ ; mean $\pm$ s.e.m. control 5.0 $\pm$ 0.73 m, VD 5.8 $\pm$ 1.02 m, AD 4.5 $\pm$ 0.72 m) or the cumulative distance traveled across nights ( $F_{2,4,2}=0.71$ ,  $P=0.50$ ; control 17.4 $\pm$ 3.55 m, VD 11.9 $\pm$ 1.63 m, AD 14.0 $\pm$ 3.48 m). A curiosity was one AD subject that covered a minimum total distance of 44.6 m during its nightly wanderings. Despite moving as



**Fig. 3. Mean ( $\pm$ s.e.m.) distance moved by each treatment group.** (A) Distance moved on the first night after release. (B) Cumulative distance moved over all nights.

far as the control and VD amblypygids, the mean ( $\pm$ s.e.m.) distance from the home refuge of the AD animals at the end of sampling was  $10\pm 1.5$  m; as a group, the AD animals made no progress toward their home refuge following displacement. Sensory deprivation to the tips of the antenniform legs did not inhibit the AD animals from moving around (cutting the tips of the antenniform legs similarly does not interfere with the tendency to move in *Phrynus marginemaculatus* tested in a laboratory arena; V.P.B., J.M.G. and D.D.W., personal observation), rendering it more probable that the behavioral effects of antenniform leg disruption were principally the result of a navigational rather than a motivational impairment.

## DISCUSSION

The present study demonstrates that, despite their nocturnal behavior and the structurally complex and light-diminished nature of their natural habitat, individual *P. laevifrons* are remarkably capable navigators that routinely return to their home refuge following experimental displacements of 10 m (see also Hebets et al., 2014a). Indeed, displacements carried out in other subjects, which were not part of the current experiment, yielded evidence for successful navigation from distances as far as 25 m (D.D.W., V.P.B. and E.A.H., in prep). Amblypygids are not the only arthropods that have been shown to be successful nocturnal navigators (Warrant and Dacke, 2016), and their homing ability may not be as good as in, for example, bull ants (*Myrmecia pyriformis*; Reid et al., 2011). However, most of the documented examples of successful navigation in nocturnal arthropods are in species that live in relatively open spaces with easily accessible visual information and where retinal adaptations that increase the gain of a visual signal have evolved (Warrant and Dacke, 2016). What is remarkable about *P. laevifrons* is not so much that they are nocturnal but that they are able to successfully navigate in an environment of seemingly overwhelming structural complexity and minimal reliable sensory guide posts.

Animals rarely organize their movements in space randomly, but the apex of spatial ability is generally considered to be the capacity to navigate to a goal following experimental (or any) displacement to an unfamiliar location (Wiener et al., 2011). In arthropods, this capacity to re-orient following displacement has been documented in a number of species including honey bees (Menzel et al., 2005), a species of diurnal ant (Narendra et al., 2013), the nocturnal bull ant (Reid et al., 2011) and spiny lobsters (*Panulirus argus*; Boles and Lohmann, 2003). Amblypygids can now be confidently added to this list. However, we acknowledge that we cannot be certain that every displaced individual was unfamiliar with its release site given that amblypygids, particularly larger, older ones like those used in the current study, move considerable distances and change home trees (Hebets, 2002). However, the general success of the control and VD subjects in returning to their home refuge and, more importantly, the fact that they were released on the ground indicates that amblypygids can characteristically return to their home refuge following experimental displacement to unfamiliar locations. Finally, it is worth noting that despite the impressive distance of their migration, monarch butterflies do not count among the arthropod species that can re-orient following displacement, at least during migration (Mouritsen et al., 2013).

The principal goal of the present study was to determine the extent to which vision and sensory information from the tips of the antenniform legs are necessary for successful navigation. The results reveal a complete loss of navigational ability following disruption of the antenniform leg tips while there was no detectable effect of depriving the animals of visual input – results consistent with the preliminary mark–recapture findings of Beck and Görke

(1974) and Hebets et al. (2014b), who studied *Heterophrynus batesii* and *P. pseudoparvulus*, respectively. However, one cautionary note is warranted regarding vision. Our sample sizes were of the order of 10 animals per group, and therefore we acknowledge the possibility that a larger sample size could have revealed a (modest) navigational deficit following visual deprivation. However, the impression we had in the field is that, if anything, the VD animals were perhaps even better at homing than the control animals (but see the *P. pseudoparvulus* of Hebets et al., 2014b), and clearly any eventual effect of visual deprivation would be modest compared with the devastating effect on homing of the loss of information derived from the tips of the antenniform legs.

Depriving whip spiders of sensory input from the distal tips of their antenniform legs completely disrupted their navigational ability. It is noteworthy, however, that one did return home and did so in one night. However, that one animal was displaced and released at close to the same time and location as a VD animal, which was captured from the same tree and also homed that same night. This admittedly anecdotal observation strikes us as extraordinary and, in our view, raises the real possibility that social interactions between animals can occur during nightly forays, which may have navigational consequences even in animals deprived of sensory inputs to their antenniform leg tips.

Given the necessary role of the antenniform leg tips in enabling navigation in *P. laevifrons*, the important question that arises is which stimulus or combination of stimuli detected by the antenniform legs is essential in supporting navigation? The antenniform legs are elegantly articulated and possess chemosensory, mechanosensory and possibly humidity-sensing sensilla (Santer and Hebets, 2011; Wiegmann et al., 2016). However, whereas mechanosensory, contact chemosensory and humidity-sensing receptors are found along most of the entire length of the antenniform leg, multiporous olfactory sensilla are limited to the distal tarsus. Therefore, by disrupting only the tips of the antenniform legs of our experimental subjects, it logically follows that the detection of olfactory cues would be most seriously impaired. This consideration lends strong support to the hypothesis that much of the navigational behavior of *P. laevifrons* is based on olfactory information (see also Beck and Görke, 1974, and Hebets et al., 2014b). Indeed, olfaction as a navigational guide is not unusual in arthropods, particularly ants (Steck, 2012; Buehlmann et al., 2015).

In the context of the proposed olfactory hypothesis, there are three considerations worthy of elaboration. First, several of the AD animals were later seen again after treatment and one of those animals had dropped both its entire antenniform leg tarsi, presumably as a result of our experimental manipulation. In the lab, cutting the tip of the antenniform legs in *P. marginemaculatus* results in the dropping of an entire antenniform leg tarsus in about 10% of cases (V.P.B., J.M.G. and D.D.W., personal observation). We acknowledge that more than one of the AD subjects may have dropped the entirety of both of its antenniform leg tarsi, consequently losing much more sensory ability than just olfaction. However, only one of the AD animals observed after treatment dropped an entire antenniform leg tarsus and we remind the reader that just one of the AD subjects returned to its home refuge, and that one animal likely followed a VD subject.

Second, following the olfactory hypothesis, the temptation is to assume that each home refuge was characterized by some unique olfactory signature that could be detected by the displaced animals. However, it is noteworthy that the vast majority of the home refuges were associated with *Pentaclethra* sp. trees. Whatever olfactory information the animals may have used to discriminate their home refuge from alternative sites, it is unlikely to be based on a signal

unique to the species of tree associated with the home refuge. It is important to point out here that the successful navigation of the control and VD animals also could not have resulted from the simple strategy of ‘find a *Pentaclethra* tree’. Many of the animals that successfully homed were closer to alternative *Pentaclethra* trees than to their home refuge when released. Also, in one anecdotal case, we displaced a marked, unmanipulated subject from one *Pentaclethra* tree on to the trunk of a nearby (5 m) *Pentaclethra* only to see the animal on its home, capture tree a few nights later.

Finally, probably what was most striking about the successful homing of *P. laevifrons* is that it would routinely take more than one night for the animals to return to their home refuge, with the subjects taking routes that often deviated substantially from the beeline route between release site and home refuge (an observation that also speaks against the unlikely use of path integration by the displaced subjects). Indeed, subjects that eventually homed could even increase their distance to the home refuge on the night of displacement (see also Hebets et al., 2014a). If the olfactory hypothesis can explain, at least in part, the successful navigation of amblypygids following displacement, then the typically multiple-night, less-direct paths taken by *P. laevifrons* could provide hints as to the chemical nature of the signals, how those signals are distributed in the environment and how the olfactory information is processed and represented in the brains of *P. laevifrons*. For example, at night a few individuals were observed on the trunks of non-home trees prior to eventually returning to the home tree, suggesting the possibility that climbing trees could be a behavioral mechanism that facilitates detection of olfactory cues hypothetically useful for navigation.

In our view, the challenge now is to experimentally test the validity of the olfactory hypothesis and investigate the behavioral and neural implementation of an eventual ‘olfactory map’ under controlled laboratory conditions while remaining cognizant that olfaction may be only one element in the navigational tool box of amblypygids that enables their impressive homing ability (Wiegmann et al., 2016).

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#### Competing interests

The authors declare no competing or financial interests.

#### Author contributions

V.P.B., J.M.G., E.A.H. and D.D.W. designed the study, V.P.B., J.M.G. and D.D.W. conducted the experiments, V.P.B. and D.D.W. analyzed the data, and V.P.B. wrote the manuscript with contributions from E.A.H. and D.D.W.

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#### References

**Batschelet, E.** (1981). *Circular Statistics in Biology*. London; New York: Academic Press.

**Beck, L. and Görke, K.** (1974). Tagesperiodik, Revierverhalten und Beutefang der Geißelspinne *Admetus pumilio* C. L. Koch im Freiland. *Z. Tierpsychol.* **35**, 173–186.

**Boles, L. C. and Lohmann, K. J.** (2003). True navigation and magnetic maps in spiny lobsters. *Nature* **421**, 60–63.

**Buehlmann, C., Graham, P., Hansson, B. S. and Knaden, M.** (2015). Desert ants use olfactory scenes for navigation. *Anim. Behav.* **106**, 99–105.

**Cheng, K.** (2012). Arthropod navigation: ants, bees, crabs finding their way. In *The Oxford Handbook of Comparative Cognition* (ed. T. R. Zentall and E. Wasserman), pp. 347–365. Oxford: Oxford University Press.

**Collett, T. S. and Graham, P.** (2004). Animal navigation: path integration, visual landmarks and cognitive maps. *Curr. Biol.* **14**, R475–R477.

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

**Freeman, G. H. and Halton, J. H.** (1951). Note on an exact treatment of contingency, goodness of fit and other problems of significance. *Biometrika* **38**, 141–149.

**Hebets, E. A.** (2002). Relating the unique sensory system of amblypygids to the ecology and behavior of *Phrynus parvulus* from Costa Rica (Arachnida, Amblypygi). *Can. J. Zool.* **80**, 286–295.

**Hebets, E. A. and Chapman, R. F.** (2000). Electrophysiological studies of olfaction in the whip spider *Phrynus parvulus* (Arachnida, Amblypygi). *J. Insect Physiol.* **46**, 1441–1448.

**Hebets, E. A., Gering, E. J., Bingman, V. P. and Wiegmann, D. D.** (2014a). Nocturnal homing in the tropical amblypygid *Phrynus pseudoparvulus* (Class Arachnida, Order Amblypygi). *Anim. Cogn.* **17**, 1013–1018.

**Hebets, E. A., Aceves-Aparicio, A., Aguilar-Argüello, S., Bingman, V. P., Escalante, I., Gering, E. J., Nelsen, D. R., Rivera, J., Sánchez-Ruiz, J. A., Segura-Hernández, L. et al.** (2014b). Multimodal sensory reliance in the nocturnal homing of the amblypygid *Phrynus pseudoparvulus* (Class Arachnida, Order Amblypygi). *Behav. Processes* **108**, 123–130.

**Henschel, J. R.** (2002). Long-distance wandering and mating by the dancing white lady spider (*Leucorchestris arenicola*) (Araneae, Sparassidae) across Namib dunes. *J. Arachnol.* **30**, 321–330.

**Igelmund, P.** (1987). Morphology, sense organs, and regeneration of the forelegs (whips) of the whip spider *Heterophrynus elaphus* (Arachnida, Amblypygi). *J. Morphol.* **193**, 75–89.

**Layne, J. E., Barnes, W. J. P. and Duncan, L. M. J.** (2003). Mechanisms of homing in the fiddler crab (*Uca rapax*): information sources and frame of reference for a path integration system. *J. Exp. Biol.* **206**, 4425–4442.

**Menzel, R., Greggers, U., Smith, A., Berger, S., Brandt, R., Brunke, S., Bundrock, G., Hulse, S., Plumpe, T., Schaupp, F. et al.** (2005). Honey bees navigate according to a map-like spatial memory. *Proc. Natl. Acad. Sci. USA* **102**, 3040–3045.

**Mouritsen, H., Derbyshire, R., Stalleicken, J., Mouritsen, O. Ø., Frost, B. J. and Norris, D. R.** (2013). An experimental displacement and over 50 years of tag-recoveries show that monarch butterflies are not true navigators. *Proc. Natl. Acad. Sci. USA* **110**, 7348–7353.

**Narendra, A., Gourmaud, S. and Zeil, J.** (2013). Mapping the navigational knowledge of individually foraging ants, *Myrmecia croslandi*. *Proc. R. Soc. B Biol. Sci.* **280**, 20130683.

**Norgaard, T.** (2005). Nocturnal navigation in *Leucorchestris arenicola* (Araneae, Sparassidae). *J. Arachnol.* **33**, 533–540.

**Reid, S. F., Narendra, A., Hemmi, J. M. and Zeil, J.** (2011). Polarised skylight and the landmark panorama provide night-active bull ants with compass information during route following. *J. Exp. Biol.* **214**, 363–370.

**Santer, R. D. and Hebets, E. A.** (2011). The sensory and behavioural biology of whip spiders (Arachnida, Amblypygi). *Adv. Insect Physiol.* **41**, 1–64.

**Sokal, R. R. and Rohlf, F. J.** (2011). *Biometry: The Principles and Practices of Statistics in Biological Research (fourth edition)*. New York: W.H. Freeman and Company.

**Steck, K.** (2012). Just follow your nose: homing by olfactory cues in ants. *Curr. Opin. Neurobiol.* **22**, 231–235.

**Warrant, E. and Dacke, M.** (2016). Visual navigation in nocturnal insects. *Physiology* **31**, 182–192.

**Weygoldt, P.** (2000). *Whip spiders (Chelicerata, Amblypygi)*. Vester Skerninge, Denmark: Apollo Books.

**Wiegmann, D. D., Hebets, E. A., Gronenberg, W., Graving, J. M. and Bingman, V. P.** (2016). Amblypygids: model organisms for the study of arthropod navigation mechanisms in complex environments? *Front. Behav. Neurosci.* **10**, 47.

**Wiener, J., Shettleworth, S., Bingman, V. P., Cheng, K., Healy, S., Jacobs, L. F., Jeffery, K. J., Mallot, H. A., Menzel, R. and Newcombe, N. S.** (2011). Animal navigation: a synthesis. In *Animal Thinking: Contemporary Issues in Comparative Cognition* (R. Menzel and J. Fischer), pp. 51–76. Cambridge, MA: MIT Press.