



A Neotropical armored harvestman (Arachnida, Opiliones) uses proprioception and vision for homing

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Abstract

Animals use external and/or internal cues to navigate and can show flexibility in cue use if one type of cue is unavailable. We studied the homing ability of the harvestman *Heteromitobates discolor* (Arachnida, Opiliones) by moving egg-guarding females from their clutches. We tested the importance of vision, proprioception, and olfaction. We predicted that homing would be negatively affected in the absence of these cues, with success being measured by the return of females to their clutches. We restricted proprioception by not allowing females to walk, removed vision by painting the eyes, and removed the odours by removing the clutch and cleaning its surroundings. We found that vision is important for homing, and in the absence of visual cues, proprioception is important. Finally, we found increased homing when eggs were present, and that the time of the day also influenced homing. We highlight vision as a previously overlooked sensory modality in Opiliones.

Keywords

homing, navigation, chemical cues, visual cues, proprioceptive cues.

1. Introduction

Animals rely on navigation skills to find shelter, food, sexual partners, adequate sites for reproduction, or their own offspring (Papi, 1992), and can utilize internal information (i.e., proprioception) and/or external information such as environmental cues to direct movement (Able, 2000; Åkesson et al., 2014). A key use of navigation for many animals is to reliably return to a home site regardless of the distance travelled or displaced, a phenomenon known as homing (Reznikova, 2007; Åkesson et al., 2014). Studies across a wide range of taxa have shown that animals rely on a variety of sensory modalities to home, including the use of visual, chemical and proprioceptive cues. Both invertebrates and vertebrates are known to use visual cues such as the sun (Baker, 1987; Towne, 2008), polarized light (Marshall, 1999; Wehner & Müller, 2006; Muheim, 2011), moon light, beacons, landmarks (Collett et al., 2001; Biro et al., 2007; Reznikova, 2007), patterns of the stars and optic flow (Reznikova, 2007; Wittlinger & Wolf, 2013; Åkesson et al., 2014). Olfactory cues used for navigation, include atmospheric gases, odour plumes (Gagliardo et al., 2011; Gaudry et al., 2012; Svensson et al., 2014), or chemicals left on the substrate (Bonadonna et al., 2004; Sillam-Dussès, 2010; Wyatt, 2014). Finally, proprioception is important for animals to utilize internal information generated by their own movement as they walk away from a home site (Papi, 1992; Wehner, 1992; Müller & Wehner, 2010).

Manipulating the environment to remove certain cues, or experimentally reducing an animal's ability to gather specific cue information, can provide insight into the use of cues in navigation. For example, spiders trained to use coloured dowels as beacons become disorientated if this cue is removed or painted in a different colour (Hoefler & Jacob, 2006; Nørgaard et al., 2007). Sensory deprivation can similarly disrupt navigation: for example, spiders blocked from using vision and joint receptors (proprioceptors) (Seyfarth et al., 1982), amblypygids deprived of chemoreceptors (Hebets et al., 2014, 2015), and ants unable to generate an internal map of their navigation path by altering their ability to accurately count distance with the steps (Wittlinger et al., 2006) all have impaired navigation. However, manipulation of cue use can sometimes only minimally impair navigation. Cues in the environment can change or be unreliable, and so using multiple cues for navigation can maximize the chances that an animal finds its destination (Papi, 1992). Animals show a great deal of flexibility in cue use and are able to alternate between navigation strategies in the absence of a given cue. For example,

rodents and ants can use multiple cues (visual, olfactory, and proprioceptive) and can alternate among these depending on the information available in the habitat (Maaswinkel & Whishaw, 1999; Wittlinger & Wolf, 2013).

There are some key animals in which homing has been well-described (Reznikova, 2007), but homing has also been described in a growing number of lesser-known animals. Studying these lesser-known animals can not only add to our understanding of the wide range of navigation strategies employed but can also provide key insight into the sensory systems of those animals. Several species of harvestmen (Arachnida: Opiliones) have been observed to return to an original location, like a clutch or roosting site, after leaving to forage (Mora, 1990; Gnaspini, 1996; Machado et al., 2000; Willemart & Gnaspini, 2004a; Grether & Donaldson, 2007; Requena et al., 2009; Chelini & Machado, 2012), or after experimental displacement (Proud & Townsend Jr, 2008). A variety of other natural causes can lead to displacement from a site as well. For example, in species of Goniosomatinae, although females do not abandon their clutches to forage, heavy rain may cause them to look for shelter and leave the eggs unguarded (NFSS & GF Pagoti, personal observations, $N = 3$). Females may also leave following attacks by large predators such as marsupials or reduviid bugs (see Gnaspini, 1996; Silva & Willemart, 2015), or other disturbances (Gnaspini & Cavaleiro, 1998; Machado et al., 2000; Willemart & Gnaspini, 2004a). Despite the reason for displacement and distance, females of Goniosomatinae are still able to return to their clutches after minutes or hours (Ramires & Giarretta, 1994; RHW, G Machado, GF Pagoti & NFSS, personal observations), demonstrating consistent homing behaviour. Despite the growing number of examples of homing in harvestmen, there are no systematic studies on navigation in the whole order (more than 6500 species; Kury, 2015), and therefore also no information on the cues they use to navigate.

Based on what is known about their sensory biology, harvestmen could use a variety of cues to navigate. Harvestmen can detect contact chemicals and may bear chemoreceptors and mechanoreceptors on their legs I, II, and pedipalps (Willemart & Chelini, 2007; Willemart et al., 2009; Gainett et al., 2017). Furthermore, Santos et al. (2013) demonstrated that harvestmen can associate chemicals of the environment with their shelter, which may contribute to homing. Also, when walking, harvestmen rub several body parts (Donaldson & Grether, 2007; Willemart et al., 2007, 2009; Teng et al., 2012; Willemart & Hebets, 2012) or specific regions bearing glands (Fernandes &

Willemart, 2014; Murayama & Willemart, 2015) against the substrate. These behaviours could be related to chemical marking, which may potentially be used in navigation. Harvestmen also have organs called slit sensilla (Edgar, 1963; Shultz & Pinto-da-Rocha, 2007; Willemart et al., 2009), which are mechanoreceptors that measure the effects of stresses in the cuticle such as those produced, for example, by muscular activity and haemolymph pressure during movement (Barth, 2002). These slit sensilla are used in proprioceptive navigation in other arachnids by detecting body movement (Seyfarth et al., 1982), and may have the same function in harvestmen. Finally, while vision is thought to be fairly limited in harvestmen, they generally have a pair of simple eyes (i.e., median ocelli) (Shultz & Pinto-da-Rocha, 2007) that can distinguish black/white substrates, and are used for negative phototaxis in several species (Pabst, 1953; Shultz & Pinto-da-Rocha, 2007; Willemart et al., 2009). However, there are a few species thought to use visual cues because of their disproportionately large eyes (families Acropsopilionidae and Caddidae) (Shear, 1975; Groh & Giribet, 2014), and some species use vision to capture prey that emit light (Meyer-Rochow & Liddle, 1988, 2001), suggesting a potentially wider use of visual cues than typically assumed.

We studied the potential for homing in egg-guarding females of *Heterom Tobates discolor* Soerensen 1884 (Goniosomatinae). Female *H. discolor* lay their eggs on large boulders along river banks (Silva & Willemart, 2015), and care for their eggs and first instar offspring for extended periods (e.g., 20–60 days in species of the same subfamily; Gnaspini, 2007). Removing guarding females greatly increases egg predation in other species of the subfamily (Machado & Oliveira, 2002; Buzatto & Machado, 2009), thus, being able to utilize homing to relocate clutches after displacement could be a significant fitness-related behavior. Through a series of manipulations that ablated the use or availability of different internal and external sensory cues, we investigated the potential cues female *H. discolor* could use when returning to their clutches after displacement. We tested egocentric navigation (Wehner, 1992) by testing for the importance of the use of proprioceptive cues generated by the female's own movement. We also tested allocentric navigation (Wehner, 1992) by altering the presence/absence of olfactory cues (by removing potential chemical cues from the female's clutch and odors surrounding the clutch) and vision (by blocking eyesight). We predicted that homing — measured by return rates of displaced females to their clutches — would be negatively affected when one or more cues were removed.

2. Material and methods

2.1. Study species

We studied *H. discolor* females in a Neotropical forest, at the Parque Estadual da Serra do Mar, in the city of Ubatuba, state of São Paulo. Females of use granitic natural shelters by rivers as oviposition sites (Silva & Willemart, 2015), and we collected the data by four rivers that cross the highway Rio-Santos (SP-101): Corisco River (Casa da Farinha), River Cachoeira da Bacia, River Marimbondo (Cachoeira da Renata) and River Camburi (see Silva & Willemart, 2015).

2.2. General experimental procedure

The general flow of data collection included: locating females on their clutches in the field, depriving them of one or more sensory cues through manipulations, and recording if they returned to their clutch. We conducted the research during 11 trips to the field, five days per trip, between February and June 2013 and January to April 2014.

We located females resting on or next to their clutch along the four rivers in the study site, with the legs flexed and the body close to the substrate and marked them individually with enamel on the femur of the legs III and IV. This procedure has been widely used in individual identification in the study of harvestmen behaviour (see, e.g., Gnaspini, 1996) because these regions are not used for sensory purposes. Next, we deprived females of one or more sensory cues — visual, proprioceptive, olfactory cues from eggs, and olfactory cues from the substrate (see details below). After the manipulation, we placed the females back on same location from which they were collected and allowed them to rest for three minutes while covered by a cylindrical bowl. We then released the female and poked its legs IV with a wood stick (25 cm long), causing the female to walk one meter away from her eggs. When proprioceptive cues were deprived, we instead carried the female in the bowl the one meter. The paths that females took away from their clutch were not typically a direct path to the end point. After displacement, we then covered the female again for 3 min with the same cylindrical bowl. To control for possible chemicals left on the substrate by the females while they walked away from the eggs, we cleaned that area over which they travelled by rubbing a sponge with 90% ethanol on the rock and dried the area with a portable fan to remove any potential volatiles.

After the three minutes resting period, we released the female, and used a time sampling recording rule (Martin & Bateson, 2010) to determine when they returned to their clutch. We sampled whether a female had returned to her clutch every 30 min for 3 h after displacement, with an additional sampling point at 24 h after displacement. We scored a successful return when the female was in contact with the eggs. No female was used more than once, and all trials were conducted during the day (8 AM–8 PM), because Goniomatinae species return to their shelter at dawn after foraging (Gnaspini, 1996; Willemart & Gnaspini, 2004a).

2.3. *Sensory manipulations*

2.3.1. *Deprivation of proprioceptive cues*

For an animal to use internal proprioceptive cues, it has to walk to estimate the distance and direction of displacement, which it can integrate to be able to return to its original location (Whishaw et al., 2001; Wehner, 2009). Therefore, we were able to prevent the use of internal proprioception in homing by manually displacing an animal, rather than allowing it to walk away from its clutch. To do so, we lifted a female by holding it by a leg II, taking care to not touch the prosoma or opisthosoma to minimize stress to the animal. Then, females were carried a distance of 1 m from their clutch and held under a bowl for 3 min before allowing them to navigate back to the clutch. The females did not release defensive secretions during this procedure.

2.3.2. *Deprivation of visual cues*

To deprive females of visual cues, we covered their eyes with a thick layer of black nail polish (Ella fashion), a method used in studies of other arachnids (Seyfarth & Barth, 1972). In order to control for an effect of painting, we also sham manipulated some females using a paint control for which we painted a small dot on the dorsum of the harvestmen, as well as a paintbrush control for which we rubbed a clean paintbrush on the harvestmen eyes and dorsum to control for the possible stress caused by the procedure.

2.3.3. *Deprivation of egg chemical cues*

To deprive females of chemical cues produced by eggs in her clutch, we manually removed the eggs from their original location and maintained them in a vial off site after we displaced the female. To control for possible chemicals from the eggs left on the substrate, we cleaned that area by rubbing a sponge with ethanol 90%. We then dried the area with a portable fan to remove volatiles.

2.3.4. Deprivation of contact chemical cues left by the female

To deprive females of chemical cues she might have left near her clutch, we removed cues left in a radius of 20 cm around the clutch after she was displaced. We cleaned that area by rubbing a sponge with ethanol 90%. We then dried the area with a portable fan to remove volatiles.

We conducted a total of 239 navigation trials split over two experimental periods. In the first experimental period (April–June 2013) we did not deprive the females of any chemical cues from the clutch and the 20 cm radius around the clutch site, and their eggs were left on site. Females during this period were deprived of: (i) only proprioceptive cues ($N = 40$, with 19 receiving paint on the dorsum), (ii) only visual cues ($N = 20$), (iii) both proprioceptive cues and visual cues ($N = 21$), or (iv) neither ($N = 40$, with 19 receiving paint on the dorsum).

In the second experimental period (January–April 2014), all females were displaced manually and were thus deprived of proprioceptive cues, and the eggs were removed from all female clutches. Females during this period were deprived of: (v) proprioceptive cues, vision and eggs ($N = 19$), (vi) proprioceptive cues and eggs ($N = 40$, with 20 receiving paint on the dorsum), (vii) proprioception, vision, eggs and chemical cues within a 20 cm radius ($N = 20$), (viii) proprioception, eggs and chemicals, ($N = 39$, with 19 receiving paint on the dorsum). Throughout the trials, we conducted trials for 3 females from a given group at a time. No female was used in more than one trial, and we noted what time we removed the female from her clutch. Contrary to the first experimental period, we did not make the additional observation after 24 h for this second experimental period because we had removed the egg-batches. Therefore, females would have no motivation to stay where the batch used to be prior to the experiment.

2.4. Data analysis

We first analysed the entire data set together (all 239 navigation trials) to test the effects of all manipulations on the likelihood of females returning to their original site after 3 h. We used nominal logistic regressions with the response variable being whether or not the animal returned after 3 h. The independent variables were the presence or absence of proprioception, vision, paint on the dorsum, eggs and chemicals in the 20 cm radius around the eggs. We also included the following interaction terms: vision \times proprioception, vision \times chemical cues and vision \times egg presence. The interaction

terms allow us to determine if the utility of a sensory modality depends on the presence/absence of other sensory cues present. We also included as a covariate time displaced, measured as minutes elapsed after 8 AM to control for the time of day at which the females were removed from their clutches.

Next, we analysed data from the first experimental period only, in which eggs and chemical cues were present in all 121 navigation trials. We used a nominal logistic regression with whether the animal returned within three hours as the response variable. The independent variables were the presence/absence of: proprioception, vision, paint on dorsum, a vision \times proprioception interaction term, and time after 8 AM as a covariate. Again, the interaction term allowed us to determine if the use of proprioception or vision was dependent on the presence of the other cue.

Finally, we analysed data from the second experimental period only, in which animals had no proprioceptive or eggs cues for any of the 118 navigation trials. The response variable was whether the animal returned within three hours. The independent variables were the presence/absence of: vision, dorsum paint, chemicals in the 20 cm radius around the clutch, a vision \times chemical interaction terms, and time as a covariate. The interaction term allowed us to determine if the use of chemical cues or vision was dependent on the presence of the other cue.

We repeated all of the above analyses with whether or not the animal returned after 20–24 h as the response variable. We also repeated the above analyses with the time to return sampled every 30 min up to 3 h using a parametric survival analysis with a Weibull distribution, and data censored at 3 h. The results of these analyses were qualitatively similar to the main set of analyses and are included in the Appendix (Tables A1–A4).

3. Results

When considering all 239 trials, we found that females were more likely to return when eggs were present (Figure 1; Table 1). They were also more likely to return when vision was present, but vision was important only when eggs were present (Figure 1; Table 1: significant vision \times egg presence term). Females were also more likely to return when proprioception was present, but proprioception increased the likelihood of return only when vision was absent (Figure 2; Table 1: significant vision \times proprioception term).

For the first experimental period, when chemical cues and eggs were present for all females, females were more likely to return when they had

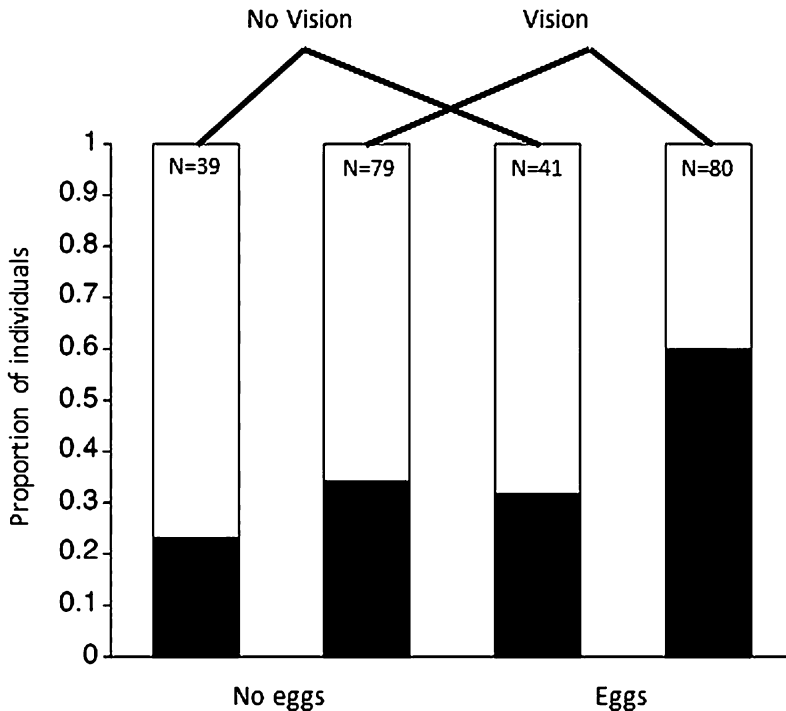


Figure 1. Proportion of females of the harvestman *Heteromitobates discolor* (Arachnida, Opiliones) that returned to the original site 3 h after experimental removal. Here we are considering both experimental periods, including females with and without eggs and chemical cues ($N = 239$). Black bars represent the individuals that returned successfully. See text for further details.

vision (Figure 3; Table 2), and less likely to return when they had paint on their dorsum (Table 2). Proprioception aided return, but only when vision was absent (Figure 3; Table 2: significant vision \times proprioception term). For the second experimental period, when proprioception and eggs were absent for all females, cue presence/absence did not influence the likelihood of return (Table 3).

For all analyses, time of day affected the likelihood of return: when females were displaced later in the day, they were more likely to return to their clutch (Tables 1–3). Finally, dorsum paint had a negative effect on the likelihood of return in all analyses (Tables 1–3).

Table 1.

Nominal logistic regression of predictors of female harvestmen *Heteromitobates discolor* returning to their clutch site 3 h after removal.

Variable	df	χ^2	<i>p</i>
Proprioception	1,9	0.0	0.9500
Vision	1,9	0.3	0.5929
Dorsum paint	1,9	4.9	0.0273*
Eggs	1,9	3.8	0.0499*
Chemicals	1,9	0.0	0.8969
Proprio × vision	1,9	11.6	0.0007***
Chemicals × vision	1,9	1.1	0.2901
Eggs × vision	1,9	3.9	0.0491*
Time released	1,9	48.8	<0.0001***

We deprived animals of proprioception by not allowing them to walk and of vision by painting the eyes (“dorsum paint” is a control of painting). We removed the eggs and their chemicals and chemicals of the egg guarding females by washing the site with 90% ethanol. “Time” considers the hour of the day when females were back to the clutch. Both experimental periods were considered here, see text ($N = 239$ navigation trials). Significant results are highlighted with asterisks.

4. Discussion

Through experimental manipulations in the field, we have shown that *H. discolor* uses more than one source of information for homing when displaced 1m away from its original location. Surprisingly, vision increases the likelihood of a successful return. Also, in the absence of vision, proprioception is important for successful homing. Finally, the presence of eggs increases the likelihood of return, and when eggs are not present vision does not matter, suggesting that eggs may provide a visual landmark of the female’s clutch site.

The effect of vision on homing in *H. discolor* was a surprising result, because harvestmen are not considered to be very visual animals (Willemart et al., 2009). This study therefore highlights an important use of a sensory modality previously thought to be restricted to a few visual specialists with large eyes in the Order Opiliones. How vision is used in homing is worth future research, as it could reveal important aspects of harvestmen sensory biology that have been previously overlooked. Other arthropods that use visual cues have to be able to recognize objects around the shelter before leaving it (Müller & Wehner, 2010). Egg-guarding females of *H. discolor* do not regularly leave their clutches, but they still could learn and memorize

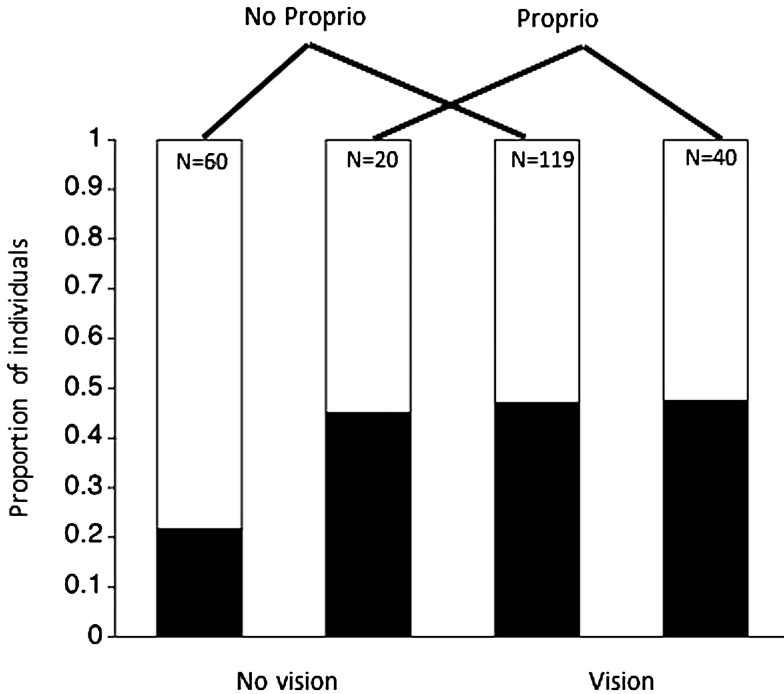


Figure 2. Proportion of females of the harvestman *Heteromitobates discolor* (Arachnida, Opiliones) that returned to the original site 3 h after experimental removal. Here we are considering both experimental periods, including females with and without eggs and chemical cues ($N = 239$). Black bars represent the individuals that returned successfully. See text for further details. Proprio = proprioception.

physical features around their clutch, as harvestmen are capable of associative learning (Santos et al., 2013). Landmark features the harvestmen could use include dark/light patterning on the rocks, irregularities on boulders, sun light shining on the water or contrast between trees and the sky (Warrant & Dacke, 2010; Rodrigues & Oliveira, 2014). Additionally, they could use polarized light (Wehner, 1992; Wehner & Müller, 2006). While any of these visual cues could be used in homing, the effect of vision only in the presence of eggs suggests that eggs could represent an important visual cue the harvestmen use in homing. Females could potentially be identifying clutch sites by using eggs as beacons. Eggs are whitish in the initial phases of development, darkening as age (Gnaspini, 2007), and could stand out against dark areas of the boulders when younger, and whiter areas of the boulders when they darken. If vision is also used by other harvestmen species, it could help

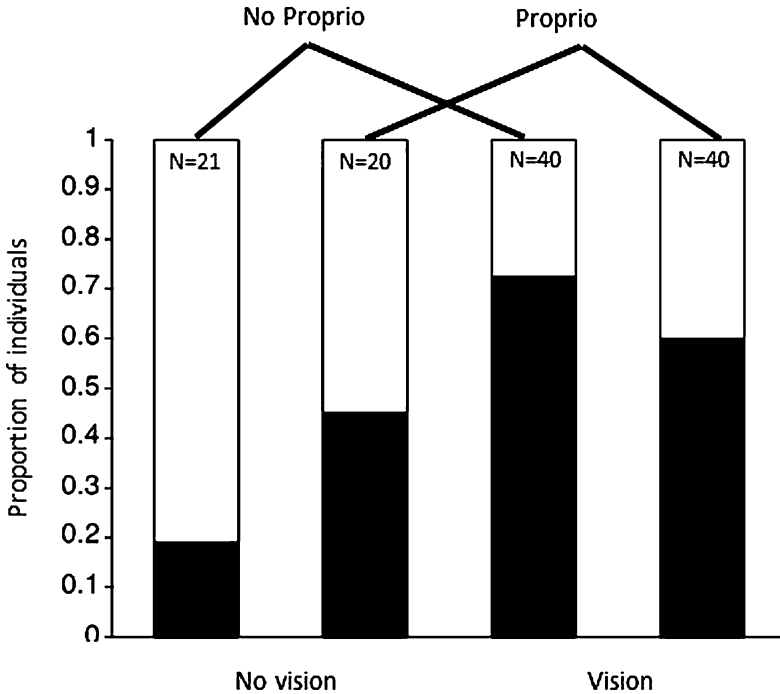


Figure 3. Proportion of females of the harvestman *Heteromitobates discolor* (Arachnida, Opiliones) that returned to the original site 3 h after experimental removal. Here only the first experimental period is considered, when chemical cues and eggs were present for all females ($N = 121$). Black bars represent the individuals that returned successfully. See text for further details. Proprio = proprioception.

explain homing behavior in species in which males or females temporarily desert their egg batches (e.g., Mora, 1990; Hara et al., 2003; Machado et al., 2004; Proud et al., 2011; Chelini & Machado, 2012) and philopatric species that leave caves to forage (Gnaspini, 1996; Machado et al., 2000; Willemart & Gnaspini, 2004b).

In addition to being a potential visual beacon, eggs may also provide olfactory cues that can be used in homing. Harvestmen are well equipped with olfactory hairs (Gainett et al., 2017) and are known to use olfaction to find food (Costa & Willemart, 2013; Costa et al., 2016). Interestingly, we found no effect on removing chemical cues surrounding the eggs, but the eggs themselves could have a chemical signature that females use in homing. Eggs may also serve as a motivator to return, which could explain lower return rates when they were removed.

Table 2.

Nominal logistic regression of predictors of female harvestmen *Heteromitobates discolor* returning to their clutch site 3 h after removal.

Variable	df	χ^2	<i>p</i>
Proprioception	1,5	0.0	0.9458
Vision	1,5	18.7	<0.0001***
Dorsum paint	1,5	11.7	0.0006***
Vision × Proprio	1,5	12.1	0.0005***
Time	1,5	23.7	<0.0001***

We deprived animals of proprioception by not allowing them to walk and of vision by painting the eyes (“dorsum paint” is a control of painting). “Time” considers the hour of the day when females were back to the clutch. Only the first experimental period was considered here, see text ($N = 121$ navigation trials). Significant results are highlighted with asterisks.

Females that could walk as they moved away from the eggs were more successful in homing than those that could not. In arachnids, proprioception is used in homing by spiders, but such ability is hampered when slit sense organs on the legs are destroyed, suggesting that these organs play an important role (Seyfarth et al., 1982). It is unknown if arachnids outside spiders (Seyfarth et al., 1982) and harvestmen (this paper) also use slit sense organs for navigation, but we suspect it may be widespread. However, proprioception aided homing only when individuals were blinded, suggesting its role as a backup cue when visual cues are not available. Animals in general possibly rely on proprioceptive cues in dark or novel environments (Stackman

Table 3.

Nominal logistic regression of predictors of female harvestmen *Heteromitobates discolor* returning to their clutch site 3 h after removal.

Variable	df	χ^2	<i>p</i>
Vision	1,5	0.4	0.5521
Dorsum Paint	1,5	0.1	0.6994
Chemicals	1,5	0.0	0.9459
Vision × chemicals	1,5	1.3	0.2458
Time	1,5	29.4	<0.0001***

We deprived animals of vision by painting the eyes (“dorsum paint” is a control of painting). We removed the eggs and their chemicals and chemicals of the egg guarding females by washing the site with 90% ethanol. “Time” considers the hour of the day when females were back to the clutch. Only the second experimental period was considered here, see text ($N = 118$ navigation trials). Significant results are highlighted with asterisks.

& Herbert, 2002). More specifically, proprioceptive cues have been shown to be important when visual and mechanical cues are not available: fishes may use their fins as proprioceptive organs when deprived of the use of eyes and lateral line (Flammang & Lauder, 2013; Williams et al., 2013); hamsters use proprioceptive cues when in total darkness (references in Etienne et al., 1990); and step counting, among other mechanisms, help ants to navigate in the dark (Cheng et al., 2009). Arthropods known for using both proprioceptive and visual cues have a high success in homing (Steck et al., 2009; Müller & Wehner, 2010; Wolf, 2011). Our results suggest that this redundancy minimizes the chances of getting lost when one of the cues is unavailable (Collett & Collett, 2000; Wehner & Srinivasan, 2003; Cheng et al., 2012).

Paint on the dorsum negatively affected homing, and this was independent from the effects of paint on the eyes. While paint does not seem to have any long-lasting negative effects on females — we have recaptured previously painted females a year after experimentation, and paint has been widely used by researchers in previous studies (Gnaspini, 1996; Willemart & Gnaspini, 2004a, b; Buzzato et al., 2007; Chelini & Machado, 2012) — it does seem to interfere with successful homing, though identifying the mechanism by which it does requires further studies. We also found that females were more likely to return to their batches when they were displaced later in the day. This could be because they are nocturnal animals that move in and out of their shelter mainly later in the afternoon when it gets darker (Silva & Willemart, 2015). Unguarded eggs are also probably more vulnerable at night, as previously described in another Neotropical species (Chelini & Machado, 2012), and individuals displaced later in the day could have higher motivation to return (Goniosomatinae females do not leave their eggs/first instar immature unguarded to forage; Gnaspini, 1995).

Overall, our results suggest the use of multiple cues in homing, with vision being important, and some redundancy in cue use (i.e., proprioception in the absence of vision) that could increase overall homing abilities. We also provide the first demonstration that arachnids outside spiders use proprioception for navigation (Seyfarth et al., 1982). It is also one of the rare studies, with those by Meyer-Rochow & Liddle (1988, 2001), that suggest harvestmen use vision beyond mere distinction of bright/dark (Willemart et al., 2009). The importance of vision may be widely overlooked in the order Opiliones and future studies should consider it as a potentially important sensory modality in other contexts.

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Appendix

Table A1.

Nominal logistic regression of predictors of female harvestmen *Heteromitobates discolor* returning to their clutch site 20–24 h after removal.

Variable	df	χ^2	<i>p</i>
Proprioception	1,9	1.2	0.2694
Vision	1,9	1.6	0.2128
Dorsum paint	1,9	4.9	0.0263*
Eggs	1,9	0.1	0.7951
Chemicals	1,9	3.6	0.0583
Proprio × Vision	1,9	5.4	0.0204*
Chemicals × Vision	1,9	0.1	0.7889
Eggs × Vision	1,9	1.2	0.2640
Time released	1,9	20.1	<0.0001***

We deprived animals of proprioception by not allowing them to walk and of vision by painting the eyes (“dorsum paint” is a control of painting). We removed the eggs and their chemicals and chemicals of the egg guarding females by washing the site with 90% ethanol. “Time” considers the hour of the day when females were back to the clutch Both experimental periods were considered here, see text ($N = 239$ navigation trials). Significant results are highlighted with asterisks.

Table A2.

Nominal logistic regression of predictors of female harvestmen *Heteromitobates discolor* returning to their clutch site 24 h after removal.

Variable	df	χ^2	<i>p</i>
Proprioception	1,5	1.1	0.2846
Vision	1,5	5.1	0.0241*
Dorsum paint	1,5	6.1	0.0134*
Vision × Proprio	1,5	5.5	0.0186*
Time	1,5	10.4	0.0013**

We deprived animals of proprioception by not allowing them to walk and of vision by painting the eyes (“dorsum paint” is a control of painting). “Time” considers the hour of the day when females were back to the clutch. Only the first experimental period was considered here, see text ($N = 121$ navigation trials). Significant results are highlighted with asterisks.

Table A3.

Nominal logistic regression of predictors of female harvestmen *Heteromitobates discolor* returning to their clutch site 24 h after removal.

Variable	df	χ^2	<i>p</i>
Vision	1,5	3.7	0.0547
Dorsum paint	1,5	0.7	0.3911
Chemicals	1,5	3.3	0.0681
Vision \times Chemicals	1,5	0.1	0.7028
Time	1,5	10.7	0.0011**

We deprived animals of vision by painting the eyes (“dorsum paint” is a control of painting). We removed the eggs and their chemicals and chemicals of the egg guarding females by washing the site with 90% ethanol. “Time” considers the hour of the day when females were back to the clutch. Only the second experimental period was considered here, see text ($N = 118$ navigation trials). Significant results are highlighted with asterisks.

Table A4.

Time to return (up to 3 h, first set of trials).

Variable	df	χ^2	<i>p</i>
Repeat of Table 1			
Proprioception	1,9	1.3	0.2509
Vision	1,9	1.5	0.2135
Dorsum paint	1,9	8.6	0.0034**
Eggs	1,9	1.0	0.3200
Chemicals	1,9	0.2	0.6652
Proprio × Vision	1,9	6.5	0.0109*
Chemicals × Vision	1,9	0.0	0.8031
Eggs × Vision	1,9	1.6	0.2115
Time released	1,9	25.4	<0.0001***
Repeat of Table 2			
Proprioception	1,5	1.1	0.2941
Vision	1,5	6.8	0.0090**
Dorsum paint	1,5	7.9	0.0050**
Vision × Proprio	1,5	6.3	0.0121*
Time	1,5	11.7	0.0006***
Repeat of Table 3			
Vision	1,5	0.3	0.5679
Dorsum paint	1,5	0.0	0.9138
Chemicals	1,5	0.1	0.8129
Vision × Chemicals	1,5	0.2	0.6395
Time	1,5	28.5	<0.0001***

Parametric survival, Weibull distribution. Censored at 3 h. These are a repeat of the above, but with time to return in 30 min blocks: the first corresponds to Table 1, the second to Table 2, the third to Table 3. Significant results are highlighted with asterisks.