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Visual odometry in the wolf spider *Lycosa tarantula* (Araneae: Lycosidae)

J. Ortega-Escobar[1],* and M. A. Ruiz[1]

ABSTRACT

The wolf spider *Lycosa tarantula* homes using path integration. The angular component of the displacement is measured using a polarized-light compass associated with the functioning of the anterior median eyes. However, how *L. tarantula* estimates the linear component of the displacement was not known prior to this investigation. The ability of *L. tarantula* to gauge the distance walked after being displaced from its burrow was investigated using experimental channels placed in an indoor setup. Firstly, we manipulated the perception of visual stimuli by covering all the spider's eyes. Secondly, we changed the optic flow supplied by a black-and-white grating ($\lambda=2$ cm) perceived either in the lateral or in the ventral field of view. Finally, the period of the lateral or ventral grating was changed from $\lambda=2$ cm to $\lambda=1$ cm. Our results indicate that visual information contributes to distance estimation because when the spider's eyes were covered, the spiders tended to search for the burrow at very variable distances. This visual information is created by the motion of the image as the spider walks, the motion in the lateral field of view being the most important. The preference of a lateral optic flow over the ventral flow can be explained by the difference in the resolution capacity of the posterior lateral eyes and the anterior lateral eyes.

KEY WORDS: Orientation, Spiders, Optic flow

INTRODUCTION

The wolf spider *Lycosa tarantula* (Linnaeus 1758) (Araneae, Lycosidae) homes by means of path integration. This mechanism implies the measurement of angular and linear displacement. Under natural conditions, the angular component of this displacement is measured using a polarized-light compass, which is associated with the functioning of the anterior median eyes (Ortega-Escobar and Muñoz-Cuevas, 1999). Under indoor conditions, we (Ortega-Escobar, 2002b; Ortega-Escobar, 2006) have shown that this spider needs some visual input for the integration of the angular component and that this input is only obtained through its anterior lateral eyes (ALEs). When the visual structure of the substratum (black-and-white grating) was rotated by 90 deg, there was a significant dispersion of the directional bearings (Ortega-Escobar, 2011) that was prevented when the ALEs were masked, although masking the other eyes had no effect. Therefore, it would appear that the angular component of the displacement seems to be processed through only one of the four pairs of eyes, i.e. the ALEs.

The use of linear displacement in homing (odometry) has been analyzed in several flying or walking insects, using channels of different lengths and visual textures. In these investigations, the

[1]Faculty of Psychology, University Autónoma of Madrid, 28049-Madrid, Spain.

*Author for correspondence (joaquin.ortega@uam.es)

insects were trained in one channel and tested in a different one in which the relevant stimuli had been changed, or some insect body parts modified. Several hymenopteran species have been studied using this method not only in relation to odometry but also with regard to the visual control of flight speed. Among the species studied are honeybees (*Apis mellifera*) either walking (Schöne, 1996) or flying (Baird et al., 2005; Dacke and Srinivasan, 2007; Esch et al., 2001; Si et al., 2003; Srinivasan et al., 1997; Srinivasan and Zhang, 2004), bumblebees (*Bombus terrestris*) (Baird et al., 2010), stingless bees (*Melipona seminigra*) (Hrncir et al., 2003; Eckles et al., 2012) and desert ants [*Cataglyphis fortis* (Ronacher and Wehner, 1995; Ronacher et al., 2000; Sommer and Wehner, 2004; Steck et al., 2009; Wittlinger et al., 2007; Wohlgemuth et al., 2002) and *Melophorus bagoti* (Narendra, 2007; Narendra et al., 2007; Narendra et al., 2008; Schwarz and Cheng, 2011; Schwarz et al., 2012)].

There have only been a few studies carried out on spiders using channels to investigate aspects of their orientation. Seyfarth and colleagues (Seyfarth et al., 1982) investigated how the wandering spider *Cupiennius salei* estimated the distance to a goal, in this case some prey, when the spiders were moved away either rectilinearly or through a semicircular corridor. All the spiders were blinded and divided into two groups: one group in which the lyriform organs (cuticular proprioceptors) on all leg femora had been surgically removed and one group of intact spiders. The intact spiders did not retrace their steps but walked back following a multiple-step trajectory that took them near to the point where they had abandoned the prey, and when they were near to their goal they made a Turner's loop as has been described in desert ants (Turner, 1907; Wehner and Srinivasan, 1981). The operated spiders had similar multiple-step trajectories but most of them did not return to their starting point. Regarding odometry, the distance estimated by the experimental animals (all of which were blinded) remained quite accurate, suggesting that no visual input was necessary to gauge the distance walked. Reyes-Alcubilla and colleagues (Reyes-Alcubilla et al., 2009) in their study of the wolf spider *L. tarantula*, used longitudinal channels to investigate the influence of active versus passive displacement, and the presence or absence of a visual landmark near the burrow, on the distance walked. After active displacement, and without a visual landmark, the spiders walked a mean distance of 27.6±9.3 cm (the burrow was 30 cm from the point of release). The authors deduced that *L. tarantula* could gauge the distance from the burrow by idiothetic information and that changing a visual landmark placed near the burrow had no effect on the search density distributions. However, they did not study the effect of blinding the spiders on their estimate of distance, or what would happen if the self-induced optic flow perceived by the spiders was changed.

In this study, we analyzed the effect of temporarily blinding all the eyes of the spider *L. tarantula* on the distance walked. This was carried out in order to ascertain whether the information was gauged
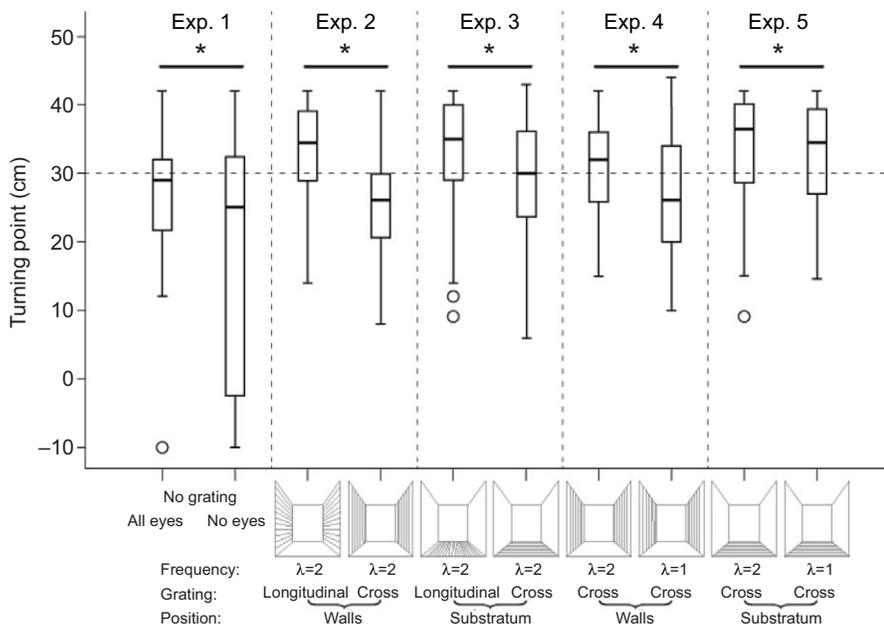
Fig. 1. Turning points of inbound searches by spiders. Data are shown for *Lycosa tarantula* in the control ('all eyes') and 'no eyes' conditions, and in the different channels with gratings in the walls or substratum. Boxes show medians and interquartiles, whiskers correspond to the extreme values and circles represent outliers. *Significant difference ($P<0.05$). The dashed line at 30 cm indicates the position of the virtual burrow; vertical dashed lines separate different experiments. Each experiment is summarized by two boxes, the one on the left corresponding to the control condition and the one on the right corresponding to the experimental condition.

solely by idiothetic means. We also modified the self-induced optic flow perceived either laterally or ventrally, to investigate the effect of this modification on the odometry of the spider and to ascertain whether this modification was important in both fields of view as perceived by different eyes.

RESULTS

Figs 1 and 2 represent, for all experiments and experimental conditions, the central tendency (median) and dispersion (interquartile range) of the distance walked and latency period, respectively.

Experiment 1: does the spider need visual information to gauge the distance walked?

This experiment was used as a control because there was no information available as to the kind of behavior *L. tarantula* would exhibit in the absence of visual information to estimate the distance walked. Initially, odometry was studied using spiders whose eyes were uncovered and consequently had complete visual information. Subsequently, this experiment was repeated using the same spiders but with their eyes masked. The animals were submitted to 10 trials with all their eyes uncovered; they were moved a distance of 30 cm away from their burrow, placed in a clear glass cup and transferred to the test channel. The walls of both the 'spider channel' and the 'test channel' were plain white. If the spider continued walking in the same direction to the end of the test channel, the distance walked was scored as negative. However, if the spider in the test channel made a 180 deg turn and walked towards the virtual position of the burrow, the distance walked was scored as positive.

After 10 control trials in the test channel with their eyes uncovered, all their eyes were covered following procedures described previously (Ortega-Escobar, 2006) and the spiders were submitted to 10 test trials. After the completion of the experiment, the eye coverings were removed and their integrity checked using a dissecting microscope.

In general, the spiders walked in contact with one of the walls of the channel and this pattern was intermixed with displacements from one wall to the other (Fig. 3, 'all eyes' condition). Displacements in

which the spiders walked through the central part of the channel were not observed. Spiders made their inbound path in a multiple-step trajectory, i.e. they walked, stopping several times until performing either the return loop or the search patterns. The locomotive behavior of the spiders with covered eyes was similar to that of the same animals when their eyes were uncovered; the homeward path was a multiple-step trajectory in contact with a wall that included crossings from one wall to the other (Fig. 3, 'no eyes' condition). The spiders did not make exploratory leg movements during displacement.

The spiders with all their eyes uncovered walked a mean distance before turning or searching of 26.5 ± 11 cm ($t_{79}=-2.87$, $P<0.05$) but when their eyes were covered the mean distance was 18.4 ± 18.8 cm ($t_{79}=-5.55$, $P<0.001$). As can be seen in Fig. 1, there was more variation in the distance to the searching movements or turning point when the spiders had all their eyes covered ('no eyes' condition). Sometimes they did not turn and therefore walked away from the virtual burrow, which suggests that the proprioceptive information was insufficient for them to be able to return to the virtual burrow. Spiders with uncovered eyes were also observed to continue walking in the same direction they were walking in when taken from the spider channel, although this was a very small proportion when compared with the 'no eyes' condition (0.06% and 25%, respectively).

Regarding the turning points for inbound searches, the spiders with all their eyes uncovered searched for the fictive burrow (placed at 30 cm) in a place nearer to the fictive burrow than when all their eyes were covered (mixed effects model analysis with 'eye condition' as a fixed effect, $F_{1,101}=18.019$, $P<0.001$).

In the control condition, there were three animals that searched for the burrow or turned after having walked a mean distance of almost 30 cm, and five animals that searched or turned after having walked a mean distance slightly less than 30 cm. With the exception of two animals, the search for the burrow was mainly confined to the place where the virtual burrow was. In the 'no eyes' condition, five animals had a very large distribution of burrow-searching behavior while in the other three this behavior was similar to that observed in the control condition.
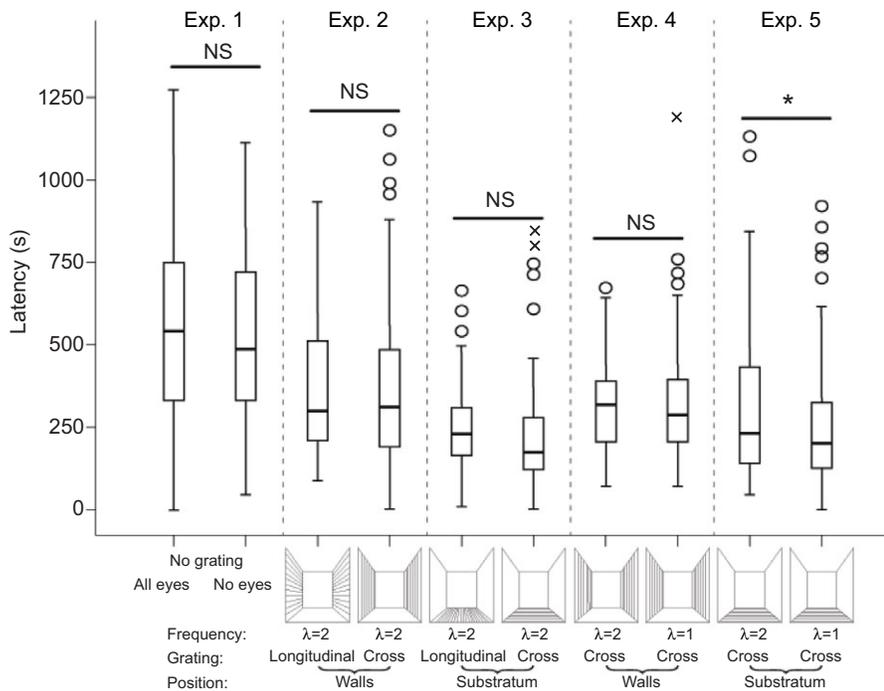
Fig. 2. Latency period to walk by all the groups under different conditions. The boxes show medians and interquartiles, whiskers correspond to the extreme values, circles represent outliers and crosses represent outliers at more than 1.5 interquartile ranges from the box edge. *Significant difference (P<0.05). Vertical dashed lines separate the different experiments. Each experiment is summarized by two boxes, the one on the left corresponding to the control condition and the one on the right corresponding to the experimental condition.

The mean latency periods to walk were 556.6±283.6 s when the spider's eyes were uncovered and 538.9±272.3 s when the spider's eyes were covered (Fig. 2). The difference between the latency period to walk in the two conditions was not significant (mixed effects model analysis, $F_{1,139}$=0.170, P=0.681). This suggests that covering the spider's eyes did not alter the general motivation of the spider to search for the burrow.

Experiment 2: does *L. tarantula* use lateral optic flow to estimate the distance walked?

As experiment 1 demonstrated, covering all of the eyes had a marked effect on the distance estimated by the spiders. This result implies that the spiders are using some other kind of sensory information to gauge the distance walked. In this and the following experiments we modified the optic flow perceived by the spider due to its self-displacement.

When stimuli are presented in the lateral field, they are perceived mainly by the posterior median eyes (PMEs) and posterior lateral eyes (PLEs) (Land, 1985; Kovoor et al., 1992). The visual fields of *L. tarantula* are different for each eye (Land, 1985; Kovoor et al., 1992). The anterior median eyes (AMEs) are directed towards the zenith and do not have a role in detecting the visual characteristics of the stimuli used in this study. The ALEs are oriented towards the substratum and when the spider walks they maintain a constant distance from it. The PMEs look forward and the PLEs look towards the lateral field of view. In our experiment using lateral grating, this could be perceived both by the PMEs for stripes placed frontally at a certain distance, given that PME visual fields extend from 0 deg frontal to 60–80 deg lateral (Land, 1985), and by the PLEs, which would perceive the stripes moving immediately lateral to the spider. In this experiment we did not discriminate between these two possibilities because we consider that an eye that looks frontally, like the PME, cannot be used reliably to obtain optic flow.

The spiders were displaced in the same channels as described in Materials and methods (see 'General procedure'). In this experiment the walls of the spider channel were always lined with a grating of

black-and-white stripes (width of the stripe=1 cm and λ=2 cm) orientated in the longitudinal direction of the channel, a condition that supplies very little optic flow. For training, the walls of both the spider and test channels were lined with the same longitudinal-stripe grating. For the test, the walls of the test channel were lined with the same grating but orientated perpendicular to the long axis of the channel. Ten trials were carried out in each condition for each spider.

As in experiment 1, the spiders walked in contact with one of the walls of the channel and this pattern was intermixed with displacements from one wall to the other (Fig. 3).

The mean distance the spiders walked in the longitudinally striped test channel (training) was 34±6.6 cm. The spiders walked and searched for the burrow further than the point where they needed to (t_{79}=4.98, P<0.001). In the cross-striped test channel (test) the mean distance walked was 24.9±8.1 cm (t_{79}=−5.35, P<0.001). The fixed effect 'stripe orientation' had a significant effect over the distance walked (mixed effects model analysis, $F_{1,124}$=72.567, P<0.001).

Mean latency periods to walk were 380.9±217.2 and 387.9±259.5 s for training and test conditions, respectively. There was no significant difference in the latency to walk due to the stripe orientation (mixed effects model analysis, $F_{1,108}$=0.416, P=0.520).

Experiment 3: does *L. tarantula* use ventral optic flow to estimate the distance walked?

Stimuli in the ventral visual field are perceived by ALEs, whose visual fields are directed towards the substratum (Land, 1985; Kovoor et al., 1992).

In this experiment, the walls of the spider channel and test channel were plain white. The substratum of the spider channel always consisted of a black-and-white grating (λ=2 cm) orientated parallel to the longitudinal axis of the channel. For training, the substrates of both channels were longitudinally striped. For the test, the stripe orientation was perpendicular to the longitudinal axis of the channel (cross-striped grating). Ten trials were carried out in each condition for each one of the eight female spiders.
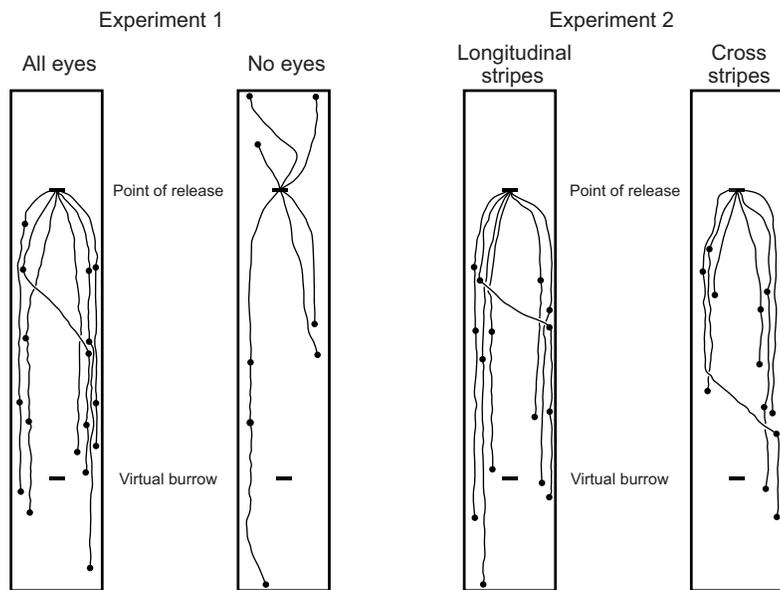
Fig. 3. Examples of real trajectories of experiments 1 and 2. The trajectories of experiments 3–5 are similar to those of experiment 2. For each experiment, six trajectories for each condition are shown. Trajectories begin at the top part of the rectangle (which represents a top view of the test channel) at the point of release. The black dots represent where the spider stops. In the 'no eyes' condition there are three trajectories in which the spiders walked in the direction of the outbound path. In both experiments there are trajectories in which the animal crosses from one wall to the other.

Compared with experiments 1 and 2, there was no difference in the way the spiders walked over the artificial substratum used in this experiment (paper over a plastic sheet in both channels). After making a 180 deg turn, the spiders also walked keeping in contact with the walls and alternating with movements from one wall to the other.

The mean distance walked by the spiders in the training trials was 33.1±8.2 cm (t_{79}=3.37, P<0.001) and in the test trials it was 29.4±9.1 cm (t_{79}=−0.54, P=0.590). The fixed effect 'stripe orientation' had a significant effect over the distance walked in the channel (mixed effects model analysis, $F_{1,127}$=11.258, P<0.001).

Mean latency periods to walk were 256.2±136.5 and 219.0±159.5 s for training and test conditions, respectively. The mean difference between the two latencies to walk was not significant (mixed effects model analysis, $F_{1,109}$=2.236, P=0.138).

Experiment 4: can *L. tarantula* distinguish the grating period of the cross-stripes lining the walls?

In some experiments carried out using insects (e.g. honeybees) in cross-striped channels, the stripe width was changed to induce a smaller (wider channel) or larger (narrower channel) image motion for the visual system. In this experiment, instead of changing the width of the channel, we altered the period of the cross-stripes from λ=2 cm (stripe width=1 cm) in the spider channel to λ=1 cm (stripe width=0.5 cm) in the test channel.

The spiders were displaced in the same channels as described in Materials and methods (see 'General procedure'). In this experiment the walls of the spider channel were always lined with cross-stripes (λ=2 cm). For training, the walls of the test channel were lined with cross-stripes of period λ=2 cm. For the test, the walls of the test channel were lined with cross-stripes of period λ=1 cm. As in previous experiments, 10 trials were carried out in each condition for each spider.

When the stripe period of the lateral grating was changed from λ=2 cm to λ=1 cm in the test channel, the mean distance walked by the spiders changed from 30.8±7.1 cm (t_{79}=1.06, P=0.292) to 27.0±9.1 cm (t_{79}=−2.94, P=0.004). The fixed effect 'stripe period' had a significant effect on the distance walked (mixed effects model analysis, $F_{1,123}$=10.576, P<0.001). This shows that with a half-period cross-stripe grating the spiders looked for the burrow at a shorter distance. Bearing in mind the large reduction in the distance walked observed in experiment 2 (see above), in this experiment an increase in image motion on the eye due to the half-period of the grating further decreased the distance the spiders walked.

The mean latency period to walk for spiders under control and test conditions was 325.9±145.5 and 329.0±186.7 s, respectively. The fixed effect 'stripe period' had no significant effect on the latency to walk (mixed effects model analysis, $F_{1,83}$=0.303, P=0.584).

Experiment 5: can *L. tarantula* distinguish the grating period of the cross-stripes lining the substratum?

In this experiment we changed the period of the cross-stripes in the substratum from λ=2 cm to λ=1 cm.

The walls of the spider channel and test channel were plain white; the substratum always consisted of a cross-striped grating. For training, the stripe width in the substrates of both channels was 1 cm (λ=2 cm). For the test, the stripe width in the test channel was 0.5 cm (λ=1 cm). As in previous experiments, 10 trials were carried out in each condition for each spider.

For training, the mean distance walked was 33.6±8.3 cm (t_{79}=3.84, P<0.001) while for test the mean distance walked was 32.1±8.6 cm (t_{79}=2.20, P=0.030); the difference in the distance walked was small but significant (mixed effects model analysis, $F_{1,120}$=4.797, P=0.030).

The mean latency period to walk for the spiders under training and test conditions was 307.5±230 and 258.9±196.6 s, respectively. The fixed effect 'stripe period' had a small, significant effect on the latency to walk (mixed effects model analysis, $F_{1,99}$=4.022, P=0.048).

DISCUSSION

These studies are different to those that have been carried out using insects (honeybees, desert ants), where there was a reward in the form of food. In our study the reward for *L. tarantula* was to find and enter the burrow, a place where it would feel secure from predators, would be able to stalk its prey and could avoid high temperatures.

Our study shows that *L. tarantula* females need visual information to estimate the distance walked as they need to estimate the angles turned during the outward journey (Ortega-Escobar, 2006). The

spiders that searched for the burrow with visual information through all their eyes began to search for it at a point a little before the fictive burrow position. When all their eyes were masked, the searching behavior of the spiders became more variable in a high percentage of displacements (25%). The spiders walked in the same direction they had walked previously, instead of making the 180 deg turn and looking for the burrow in the correct direction. Furthermore, when all their eyes were masked, the distance walked was very variable. However, in this condition no difference in the pattern of leg movements was observed. This is contrary to the findings of Schmid (Schmid, 1997) for *Cupiennius salei* where, under infrared illumination not perceived by the spider, the forelegs were constantly moving up and down.

The behavior of *L. tarantula* with all its eyes masked was in contrast to that shown by *C. salei* (Seyfarth et al., 1982) studied in a similar way. *Cupiennius salei* was able to return to the point from where it was moved after capturing its prey. As all its eyes were masked, this indicates that all the information must have been obtained by its proprioceptors. Consequently, when Seyfarth and colleagues destroyed the lyriform organs on all the femora, the success rate was less than 50%. Seyfarth and colleagues associated this idiothetic orientation in *C. salei* with the nocturnal behavior of this species (Seyfarth et al., 1982). *Lycosa tarantula* females also show nocturnal behavior (Ortega-Escobar, 2002a; Ortega et al., 1992) under indoor conditions where there is reduced light intensity (40 lx) during the day. However, in indoor conditions with a higher light intensity, *L. tarantula* females have been observed in the upper part of the burrow or at a distance of 3–4 cm from it (J.O.-E., unpublished). In outdoor conditions, during the spring, summer or autumn seasons, the females can also be observed in the upper part of the burrow, and during hot temperatures they can be observed returning to the burrow from distances of ~30–40 cm (J.O.-E., unpublished). Therefore, the distance used in these experiments equates well to normal distances for outdoor conditions. Unlike the desert ant *C. fortis*, and similarly to the honeybee, *L. tarantula* does not forage in a featureless landscape but one in which there is an abundance of moving visual stimuli both in the lateral and ventral fields of view.

Although our study was carried out in indoor conditions in the absence of any compass input, for example a sun compass or a polarized light compass, the spiders were well motivated because all of them tried to find the burrow after a multiple-step displacement followed by a Turner's loop. Even those spiders with all their eyes masked looked for the burrow and were not less motivated because there was not a significant difference in the latency to walk period.

When lateral optic flow was induced by a grating perpendicular to the walking direction, the spiders searched for the fictive burrow 9.6 cm before the site of the burrow, approximately one-third of the distance they had to walk. This effect was seen in all the animals in this group. This means that lateral optical flow is very important for the spider to locate the burrow. When the optic flow was generated in the ventral field of view, the spiders also searched for the burrow at a distance (3.7 cm) before the actual site, but the effect was less marked.

Could this effect be related to the resolution capabilities of the different eyes? In order to resolve fine details, the eyes must have retinas with fine mosaics of photoreceptors. One measure of the resolution is the inter-receptor angle subtended by two receptors (Land, 1985; Land and Nilsson, 2002). This angle has been calculated for *L. tarantula* females and males (Kovoor and Muñoz-Cuevas, 1997): in females the angles are 1.70 deg for ALEs, 0.63 deg for PMEs and 0.78 deg for PLEs. Clearly, the ALEs are less

discriminative than the PMEs and PLEs, and this could explain why the ventral optic flow is less effective than the lateral optic flow.

From previous data (Kovoor and Muñoz-Cuevas, 1997) we can see that the PLE retina of *L. tarantula* is capable of resolving gratings finer than those used in this investigation. However, this is the first study carried out on this spider using this kind of stimulus and we did not know whether this would be an important factor in gauging the distance to the burrow. These experiments show that optic flow does have an influence on gauging the distance to the burrow. Therefore, we propose in future experiments to investigate the effect of gratings in the millimeter range or of random dot patterns.

In the absence of similar research conducted using spiders, we shall relate our results to those obtained for other walking arthropods. Similar studies with self-induced optic flow have been carried out using desert ants *C. fortis* (Ronacher and Wehner, 1995; Ronacher et al., 2000; Wittlinger and Wolf, 2013). Contrary to our results, the optic flow in the lateral visual field of the ant is 'neither sufficient nor necessary for correct distance estimation' (Ronacher et al., 2000). Ronacher and Wehner carried out an experiment similar to this study, placing visual patterns on the substratum (Ronacher and Wehner, 1995). They trained ants using a stationary grating of 10 mm black-and-white-stripes (λ=20 mm) and then tested the ants using stationary patterns in which the stripe width was 5 or 20 mm. There was no significant influence of the spatial frequency on the distance travelled. In addition, they covered the ventral half of the eyes of some of the ants and found that this condition did not affect the distance walked in a test channel. Similar results were obtained by Wittlinger and Wolf in the course of a study in which they analyzed the effect of amputating two of the walking legs of *C. fortis* (Wittlinger and Wolf, 2013). In this study they had one experimental group in which the ventral half of the compound eye was covered but they found that the distance walked was not statistically different from that of the group in which the ventral half was uncovered.

Other studies have clearly shown that *C. fortis* uses proprioceptive information to measure the distance travelled (Wittlinger et al., 2007; Wolf, 2011). In these studies, leg and stride lengths were manipulated by elongating the legs (the so-called 'stilts ants') or by shortening them (the so-called 'stumps ants'). These modifications in leg length were carried out after the ants had arrived at a feeder where they had been trained. On their return journey both the stilts and stumps ants misjudged the distance to walk, with the stilts ants overestimating the homing distance while the stumps ants underestimated it (Wittlinger et al., 2007). The authors suggest the functioning of a stride integrator whose sensory input and localization in the central nervous system can only be speculated.

Using the Australian desert ant *Melophorus bagoti*, Schwarz and colleagues (Schwarz et al., 2012) have shown that the systematic drift towards the fictive nest observed in longitudinal channels by Narendra and colleagues (Narendra et al., 2008) is not a stereotypical part of the search behavior of these ants. Schwarz and colleagues found that the systematic drift, sometimes accompanied by overshooting the fictive nest, was due to the training conditions, which comprised a nest enclosed by a rectangular box (Schwarz et al., 2012). They called this fact a learned rule, and following this learned rule, the ants should run along the channel because their goal is at the end of it. Could a similar learned rule be functioning in our study with *L. tarantula*? It is true that in the training conditions of experiments 2, 3 and 5 the spiders overshot the location of the fictive burrow, but in the test conditions they did not, with the exception of experiment 5. This suggests that the learned rule of walking towards the end of the channel is not functioning in this case.

Schöne carried out experiments using walking honeybees (Schöne, 1996), another well-studied insect. In this study, the bees walked along a channel that led them to the hive entrance. There were two ways of inducing optic flow: (1) the transparent floor of the channel is moved over a stationary grating of black-and-white stripes (λ=1 cm or alternatively 0.5 or 2 cm); (2) the transparent floor is stationary and the grating is moved. In both cases the floor or the grating could be moved either in the bee's direction or against it. When there was a decrease in the optic flow, the bees increased their walking speed and over-estimated the distance. When there was an increase of the optic flow, the opposite situation was found. In both cases, there was an influence of the ventral optic flow on the estimation of distance.

However, the majority of the work on odometry in bees has been carried out on flying bees trained to fly in channels with different visual stimuli (Baird et al., 2005; Dacke and Srinivasan, 2007; Esch et al., 2001; Si et al., 2003; Srinivasan et al., 1997; Srinivasan and Zhang, 2004). Srinivasan and colleagues found that when honeybees were trained and tested in a tunnel with little optic flow (longitudinal grating) they looked for the trained reward throughout the entire tunnel (Srinivasan et al., 1997). They also found that the search distribution was not altered by changing the period of the grating that lined the walls of the tunnel. This would suggest that the honeybee odometer relies primarily on visual information and is not modified by the period of the grating. As in the honeybee, in *L. tarantula* the odometer is also mainly visual and sensed overall by the lateral regions of the visual field, probably through the PLEs, and to a lesser degree by the ventral region of the visual field through the ALEs.

It is necessary to carry out similar studies in the subjective night of the spiders by changing their photoperiod and observing them under infrared light to determine whether their nocturnal displacements are also controlled by visual information or only by proprioceptive methods.

MATERIALS AND METHODS

Adult virgin females of *L. tarantula* were used for all experiments. These animals had been captured from a wild population in Madrid (central Spain; 40°32′N, 3°42′W) and had been through the final two to three molts in the laboratory; the age of all the animals was very similar and all the trials were conducted after maturation. The spiders were maintained in individual containers measuring 17×13×8 cm with sufficient substratum (earth) to move around and dig burrows. They were fed blow flies (*Calliphora vomitoria*) and given water twice a week.

General procedure

The spiders were placed in a terrarium that was divided into three channels of the same length and width (Fig. 4). The channels were 52 cm long and 9.5 cm wide, with 10 cm high walls. The spiders were placed in the terrarium 3 days before the beginning of the study and they lived in these channels for the duration of the experiment. Light was provided by four, 36 W fluorescent bulbs positioned 160 cm above the terrarium. At 12 cm from one end of the lateral channels, an artificial burrow measuring 17 cm deep and 2.5 cm in diameter was built.

The spiders left silk threads all along the spider channels, 1 and 2, and around the burrow only during their nocturnal displacements. There was no burrow in the middle test channel (channel 3). However, in the place where the burrow should have been, at a distance of 30 cm from the point of release, there was a point known as the virtual burrow. To ensure that there were no silk threads, which could have acted as a cue, the substrate in the test channel was thoroughly cleaned with a brush after each test.

In all the experiments, the spiders were gently pushed 30 cm away from the burrow in their channel (channels 1 or 2), placed in a transparent glass cup and transferred to the same point in the test channel (channel 3), 30 cm
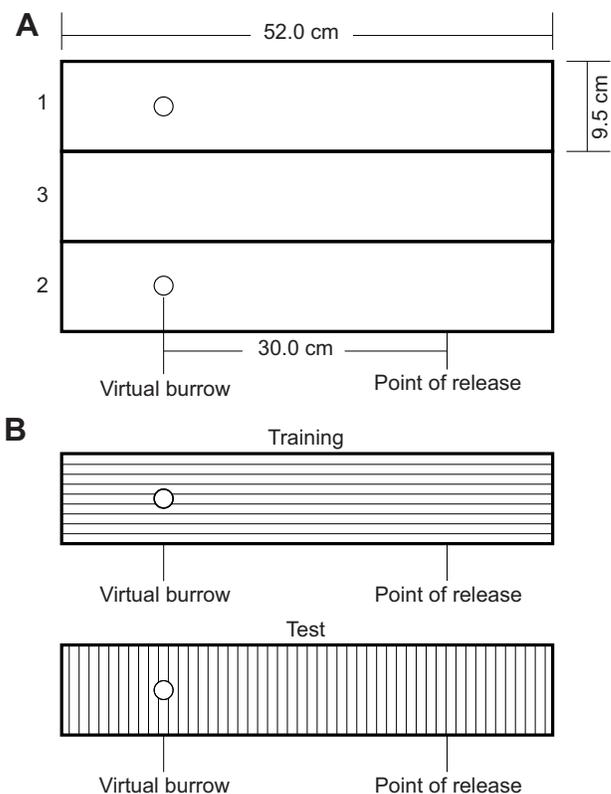
Fig. 4. Set-up used to study odometry by *L. tarantula*. (A) Top view of the terrarium divided into three channels (1–3). Channels 1 and 2 were the 'spider channels' each containing one spider. Channel 3 was the 'test channel'. Spiders walked along their channel and were transferred to the point of release of the test channel (channel 3) positioned 30 cm from the burrow. (B) Example of a top view of channel 3 for an experiment in which the grating was placed on the substratum. For training, the grating was longitudinally striped but it was cross-striped for testing.

from the virtual burrow. During transfer to the test channel, the spiders could see clearly through the glass cup. If the spider did not move after 20 min, it was taken via the glass cup and put back near to its burrow. The displacement of the spiders was filmed using a Panasonic SDR-H80 video camera positioned above the terrarium. As the spiders moved in contact with one wall of the channel (thigmotaxis), a paper ruler was placed in the center of the substratum to measure the distance walked. As a result of thigmotaxis, the visual information gathered through each PLE was different, being finest in the eye furthest from the wall (a 1 cm stripe equivalent to 8 deg) and coarser in the eye nearest to the wall (a 1 cm stripe equivalent to 29 deg). In any case, each stripe could be perceived from the nearer one by each of the PLEs (inter-receptor angle subtended by two receptors is 0.78 deg) (Kovoor and Muñoz-Cuevas, 1997). Spiders could walk between −10 cm (if they did not make a 180 deg turn in the direction of the virtual burrow and proceeded to the end of the channel furthest from the virtual burrow) and 42 cm (if they did make a 180 deg turn and walked towards the virtual burrow or even overshot it and proceeded towards the end nearest to the virtual burrow). It was considered that a spider had walked the correct distance to its burrow if it made search movements using leg pairs I and II, typical of burrow searching, or if it made a complete (180 deg) change in direction (Turner's loop). The time lapse between the moment the spider was placed on the test channel substratum and the moment it began to walk (latency) was also measured. This latency period is considered to be the measure of the motivation to search for the burrow.

In each experiment, eight fresh female spiders were used. Each spider was submitted to 10 training trials and 10 experimental trials. In each experiment, 80 training trials and 80 experimental trials were carried out. For each trial, the distance walked by the spider before searching as well its

latency period were measured. The eye-covering procedure is described in a previous paper (Ortega-Escobar, 2006).

Data analysis and statistics

All data were analyzed using the statistical software IBM SPSS 19.0. All levels of significance were set to $\alpha<0.05$. The distances walked and the latency period are reported as means ± s.d. and they are also illustrated using box-and-whisker plots, where the center is the median, the spread is the interquartile range (25% and 75% percentiles) and the whiskers are the 10th and 90th percentiles, depicting the dispersion of the data. One-sample t-tests were performed to compare the inbound distance against the burrow distance from the point of release (established as a fixed distance of 30 cm for all animals).

In all experiments a mixed effects longitudinal linear model (similar to a repeated measures ANOVA) was carried out to study the effect of changing any visual condition in the test channel. In the proposed model the two experimental conditions (training versus test) are treated as repeated measures. Within each experimental condition 10 trials were measured for each animal, giving a total of 20 measurements. Hence, repeated measurements for each animal should be nested within the experimental condition factor. In the model, the experimental condition factor was considered as a fixed effect, repeated measurements within condition were considered a random effect, and the different animals measured were also considered a random effect. Only differences between the two experimental conditions were interpreted and no *post hoc* tests were necessary because this effect has only two levels (training–test).

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