SHORT COMMUNICATION

Does size affect orientation using celestial cues?

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Abstract

Insects are well known to orient using celestial cues. The pattern of polarised skylight is the dominant celestial compass information that insects use, which they detect using a specialised set of ommatidia. The number of ommatidia decreases with body size, and it is unknown how this reduction in the number of ommatidia affects the precision of orienting using celestial cues. We investigated this in eight different ant species that had varying numbers of ommatidia. We captured ants returning home, displaced them to an unfamiliar location and measured their precision in determining heading direction using celestial cues. The heading direction of the ants measured at a fixed distance from the release and also at a distance scaled to their body size was not correlated with the number of ommatidia. However, both the path straightness and walking speed were lower in smaller ants indicating the ability to orient at a finer scale was affected by miniaturisation.

Keywords Miniaturisation · Polarisation vision · Dorsal rim area · Compass cues

Introduction

Efficient navigation is crucial for most daily tasks including foraging, finding mates, establishing territories and parental care. Most animals rely predominantly on visual cues (e.g. landmark panorama, celestial compass) to gather information on routes and places (Åkesson et al. 2014). Even a deceptively simple task of walking in a straight line cannot be achieved without using an external compass (Cheung et al. 2008). A highly reliable compass cue that insects use is the pattern of polarised skylight (Wehner and Strasser 1985; Zeil et al. 2014). Insects detect this by a specialised set of photoreceptors present in the ommatidia located in the dorsal region of the compound eye (Labhart and Meyer 1999). The number of specialised ommatidia varies from 120 (2.5% of all ommatidia) in *Megalopta genalis* (Greiner et al. 2007), 150 (3.75%) in *Apis mellifera* (Wehner and Strasser 1985), 62 (1.72%) in *Myrmecia pyriformis* (Reid 2010) and 100 (7.69%) in *Cataglyphis bicolor* (Labhart 1986). While fewer

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ommatidia and smaller lenses affect spatial resolution (Land 1997), it is unknown how fewer specialised ommatidia affect an animal's ability to access polarised skylight.

The most convincing way of determining whether an animal can use a celestial compass is by capturing animals returning home and passively displacing them to a distant location where familiar visual landmark information is absent (e.g. Collett and Collett 2000; Zeil et al. 2014). If animals ignore the passive displacement and travel in the direction where the nest should have been, we can conclude that animals use a celestial compass. Here we investigated the accuracy of orientation, ability to maintain a straight path, and change in walking speed in eight ant species with varying body size and numbers of ommatidia.

Angular errors increase with distance travelled (Cheung et al. 2008) and hence we identified the initial heading direction of ants at a fixed distance from the release point. Bigger ants tend to have longer legs (Kaspari and Weiser 1999), which correlates with larger strides and faster walking speed (Zollikofer 1994), which can lead to straighter path and better orientation precision (Cheung et al. 2007). Hence, we also measured the initial heading direction at a distance scaled to the body size of the ant to account for differences in stride length. In addition, we measured both walking speed and path straightness in ants of different sizes to identify how miniaturisation affects orientation precision at a fine scale.
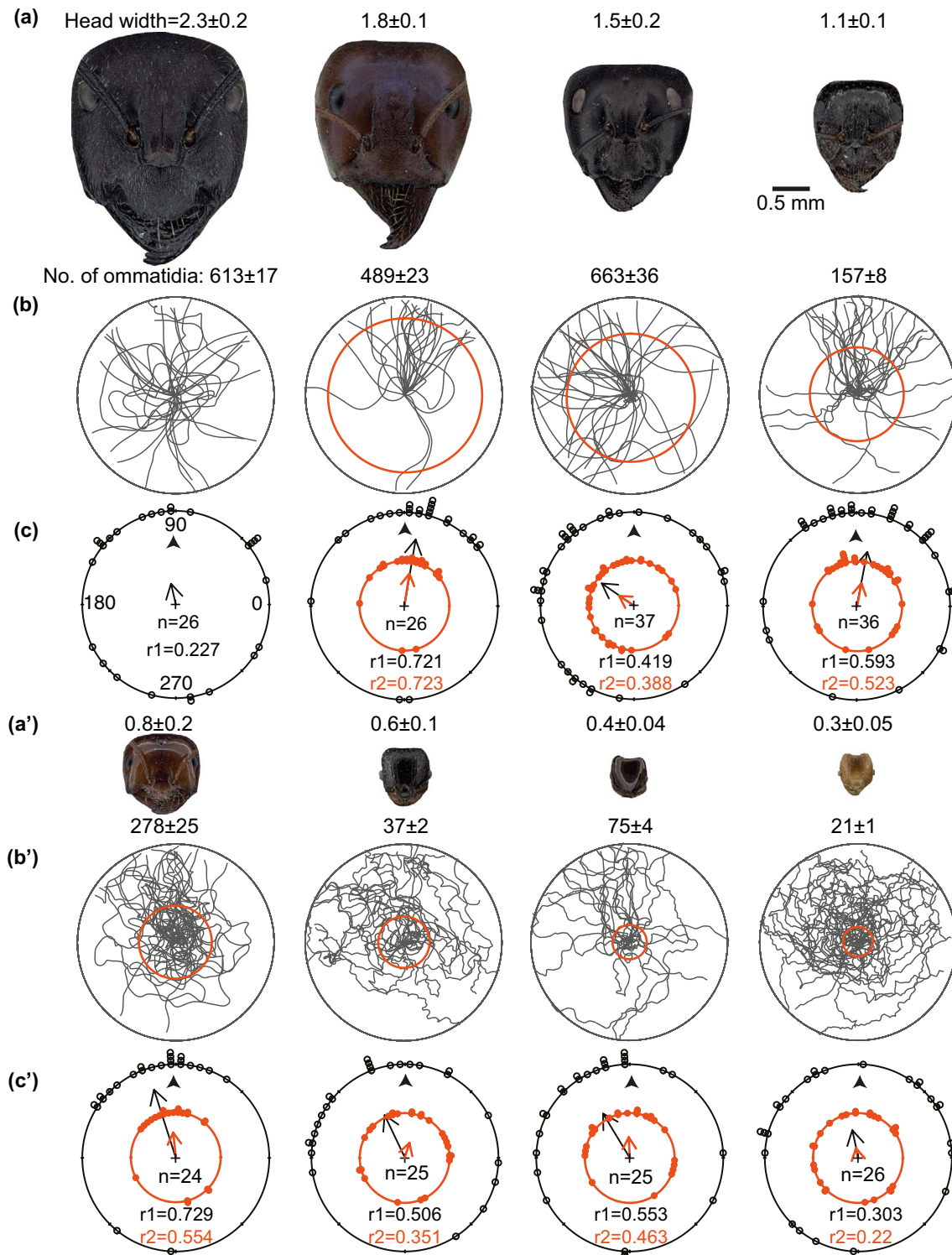
Fig. 1 Ant species of different sizes and their ability to follow a home vector. (**a, a′**) Dorsal view of (left–right) *C. piliventris, I. purpureus, C. aeneopilosus, N. ectatommoides, M. nemophilus, Pheidole* sp 1, *M. rothsteini* and *Pheidole* sp 2. Mean ± s.d. of head width (*n* = 20) and ommatidia number (*n* = 5) are shown. (**b, b′**) Paths of each species on the circular platform. Ants were released at the centre of the platform. (**c, c′**) Circular plots for each species show heading direc-tion for raw data (in black) and data from scaled recording area (in red). The red circle indicates the scaled recording area correspond-ing to ants' head width. Filled black arrow: fictive home direction. Length of the mean vector (r1 for raw data, r2 data from scaled recording area), mean direction (black arrow: raw data, red arrow: data from scaled recording area) and sample size (*n*) are shown

Materials and methods

Study site and species

We carried out experiments at the Australian National University campus (35°16′50.14″S, 149°06′42.13″E) in the Austral summer of 2016. We studied eight day-active ant species: *Camponotus piliventris* Smith 1858, *Iridomyrmex purpureus* Smith 1858, *Camponotus aeneopilosus* Mayr 1862, *Notoncus ectatommoides* Forel 1892, *Melophorus nemophilus* Heterick, Castalanelli and Shattuck 2017, *Pheidole* sp 1, *Monomorium rothsteini* Forel 1902, and *Pheidole* sp 2 (Fig. 1a, a′). For each species, we measured the head width ($n = 20$) and determined the number of ommatidia in five animals for each species by preparing eye replicas (Narendra et al. 2011). Experiments were conducted between 4–6 h or 9–11 h after sunrise, and only during clear sunny days.

Experimental procedure

We trained ants to a sugar feeder placed 0.3–3.0 m from the nest entrance. Ants returning from the feeder were individually captured in foam-stoppered vials with a black sleeve. Foragers returning home from naturally occurring food locations were also caught opportunistically between 2.4–11.0 m from the nest. For each captured individual, we noted the direction and distance from either the feeder or the capture location to the nest (home vector*)*. The captured ants were placed in a dark box and released at a single distant location (500–1000 m away from the nest). The displacement site was in the middle of an oval, which was unfamiliar to the ants. Successful orientation would thus require the use of a celestial compass. Ants were released in the middle of a circular wooden platform (40 cm diameter, elevated 15 cm off the ground). The platform had a circular hole in the centre into which the foam-stoppered vial with the black sleeve fitted tightly. To release the ant, the foam stopper was replaced by a flat piece of cardboard with a central hole of 5 mm diameter. Ants had to climb up the vial to reach the surface of the platform before they could access visual information. We filmed the ants with a camera (Sony FDR AX100) until they left the platform or for 15 min, whichever occurred first, along with a compass and a scale.

The footage was converted to image sequences using Final Cut Pro (v10.2.2, Apple Inc). Head position was digitised every third frame (*Pheidole* sp 2), or every second frame (*Pheidole* sp 1) or every frame (the remainder species), using a custom-written Matlab program (courtesy of Jan Hemmi and Robert Parker). To identify the heading direction of each individual, we calculated the angle between the exit point (*x/y* coordinates of the last point on the platform or at 15 min) and the origin. We compared the heading direction of each individual to its home vector, to identify whether ants followed the home vector when displaced to the unfamiliar location. Compared to smaller ants, bigger ants have long legs and would require fewer strides to cover the same distance (Zollikofer 1994). Hence, we measured the heading direction, walking speed, path straightness (ratio of straight line distance and the sum of all the segments of the path) by scaling the recording area to the head width, proxy for body size and leg length (Kaspari and Weiser 1999*)* of each species ('scaled recording area' from here in).

Statistical analyses

We compared heading directions to the expected home vector direction (90°; see Fig. 1c, c′) using a *V* test (Batschelet 1981). We calculated the length of the mean vector (*r*) which ranges from 0 to 1, with values closer to '0' indicating a scattered distribution. We determined whether the difference between heading directions and the predicted home vector was correlated to the distance at which animals were captured. Average speed and path straightness data were not normally distributed. Hence, for between-species comparison of average speed and path straightness, we used the Kruskal–Wallis test and the Dunn's post hoc test with Bonferroni correction ($\alpha = 0.05$) where applicable. Statistical analyses were carried out in RStudio (Version 1.1.383; RStudio-team 2016) and Oriana 4 (Kovach Computing services).

Results

Variation in the study species

There was a sevenfold difference in head size between the largest species, *C. piliventris*, to the smallest species, *Pheidole* sp 2, that were studied (Table 1). The number of ommatidia increased with head width (Fig. 1a, a′) (Pearson's correlation, $r = 0.89$ [95% CI 0.51, 0.98], $t_6 = 4.87$, $p < 0.01$) and varied dramatically from 663 ± 36 to 21 ± 1 (mean ± s.d.) (Fig. 1).

Heading direction

We found that the distribution of heading direction of ants of all sizes was significantly oriented towards the home direction (*V* test; Table 1; Fig. 1). A similar pattern was found when their heading direction was analysed after scaling the recording area to head width (red circle in Fig. 1; Table 1). Within each species, the heading direction did not differ between the data from scaled recording area and

Table 1 Circular statistics for heading directions of ants on the platform

Sl. no	Species	Head width, mean ± s.d (mm), $n = 20$	Length of mean vector (r)		Mean vector (ϕ)		V test (expected mean: 90°)	
			Raw data	Data from scaled recording area	Raw data	Data from scaled recording area	Raw data	Data from scaled recording area
1	*C. piliventris*	2.3 ± 0.2	0.227	0.227	103.1	103.1	$V = 0.222, p = 0.05$	$V = 0.222, p = 0.05$
2	*I. purpureus*	1.8 ± 0.1	0.721	0.723	80.4	81.2	$V = 0.711, p \ll 0.01$	$V = 0.715, p \ll 0.01$
3	*C. aeneopilosus*	1.5 ± 0.2	0.419	0.388	145.5	147	$V = 0.238, p = 0.02$	$V = 0.211, p = 0.03$
4	*N. ectatommoides*	1.1 ± 0.1	0.593	0.523	78.9	79.6	$V = 0.582, p \ll 0.01$	$V = 0.514, p \ll 0.01$
5	*M. nemophilus*	0.8 ± 0.2	0.729	0.554	107.7	96.1	$V = 0.729, p \ll 0.01$	$V = 0.551, p \ll 0.01$
6	*Pheidole* sp 1	0.6 ± 0.1	0.506	0.351	115.1	72.1	$V = 0.457, p \ll 0.01$	$V = 0.334, p < 0.01$
7	*M. rothsteini*	0.4 ± 0.04	0.553	0.463	121.2	92.6	$V = 0.473, p \ll 0.01$	$V = 0.462, p \ll 0.01$
8	*Pheidole* sp 2	0.3 ± 0.05	0.303	0.22	102.4	101.2	$V = 0.296, p = 0.01$	$V = 0.216, p = 0.06$

Raw data: heading direction at the last point on the platform; data from scaled recording area: heading direction at the distance on the platform scaled to ants' headwidth

the raw data (Watson–Wheeler test; Table S1). Overall, the length of the mean vector was not correlated to head width (Pearson's correlation, raw data: $r = -0.18$ [95% CI $-0.78, 0.60$], $t_6 = -0.442$, $p = 0.67$; scaled recording area: $r = 0.11$ [95% CI $-0.64, 0.75$], $t_6 = 0.25$, $p = 0.8$) or to the number of ommatidia (raw data: $r = -0.14$ [95% CI $-0.77, 0.62$], $t_6 = -0.36$, $p = 0.73$; scaled recording area: $r = 0.1$ [95% CI $-0.65, 0.75$], $t_6 = 0.25$, $p = 0.81$). We found that the difference between the heading direction (for both data from scaled recording area and raw data) and the expected home vector direction for each species was not correlated to the distance at which animals were captured from the nest (Table S2).

Average walking speed and path straightness

The size of the animal was positively correlated with walking speed (Pearson's correlation, raw data: $r = 0.78$ [95% CI 0.17, 0.96], $t_6 = 3.06$, $p = 0.02$; scaled recording area: $r = 0.81$ [95% CI 0.26, 0.96], $t_6 = 3.44$, $p = 0.01$), and with path straightness (raw data: $r = 0.86$ [95% CI 0.41, 0.97], $t_6 = 4.25$, $p < 0.01$; scaled recording area: $r = 0.86$ [95% CI 0.41, 0.97], $t_6 = 4.24$, $p < 0.01$). Larger ants were significantly faster than the smaller ants (Fig. 2a; Table S3a, b). Ants with head width less than 0.8 mm had less straight paths than the bigger ants (Fig. 2b; Table S3c, d).

Discussion

We studied whether miniaturisation affects ant's ability to orient using celestial compass. The smallest ant previously known to use a celestial compass was *Formica rufa* with 400 ommatidia (Jander 1957). We found that ants with as few as

21 ommatidia could orient using a celestial compass, suggesting that the ability to derive compass information from celestial cues is not dependent on size or phylogeny. Foragers of *Apis mellifera* could orient accurately using polarised skylight even with less than 50% of their specialised ommatidia (Wehner and Strasser 1985).

So does having fewer ommatidia affect orientation precision? We found variation in the heading directions of ants both within and between species (Fig. 1). For instance, ants in our study that had the most number of ommatidia (*Camponotus* species) and the least number of ommatidia (*Pheidole* species) had the biggest scatter in their heading directions (low r values). We explored whether this variation could be due to differences in the distance at which animals were captured relative to the nest. The error in homing direction and capture distance was not correlated, but we did not have enough variation in the capture distance for smaller ants to be conclusive. Analysis of walking speed and path straightness at a finer scale provided the strongest evidence that fewer number of ommatidia indeed affected orientation precision (Fig. 2). Animals with fewer ommatidia had less straight paths and walked slower. Although smaller animals had fewer ommatidia, reduced body size, and in turn stride length, it did not explain the slow walking speed and reduced path straightness. We determined this by measuring walking speed and path straightness in an area that was scaled to the body size of each species. While the ability to orient using compass information appears to be common across ants of different sizes, it may not be the primary mode of navigation. For instance, some species of *Camponotus* orient primarily using terrestrial landmark information (Klotz and Reid 1993), whereas *Iridomyrmex purpureus* relies mainly on pheromone trails (Card et al. 2016). The latter species uses celestial compass information when they cannot access
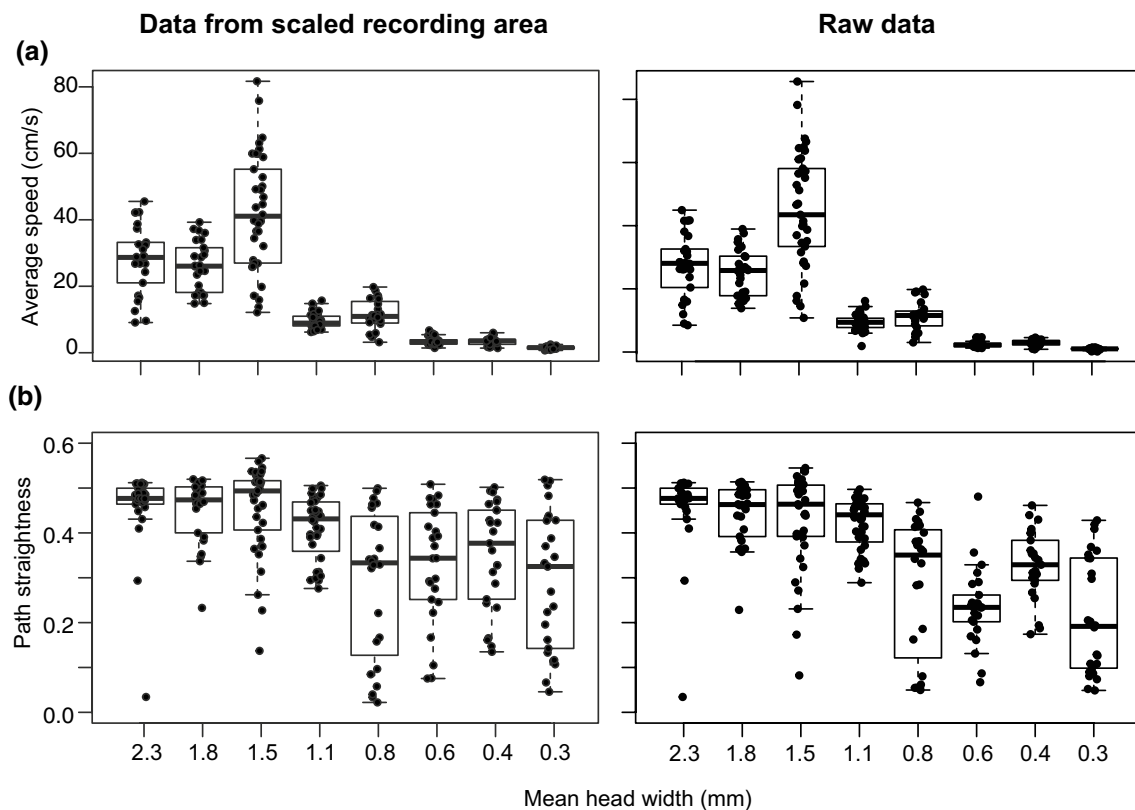
Fig. 2 Relationship between average walking speed and path straightness of ants with size. *x* axes: head width (mm) of (left–right) *C. piliventris, I. purpureus, C. aeneopilosus, N. ectatommoides, M. nemophilus, Pheidole* sp 1, *M. rothsteini* and *Pheidole* sp 2. **a** Average walking speed, **b** path straightness. Data from scaled recording area: left column, raw data: right column. Corresponding statistics for between-species comparisons are in electronic supplementary material, Table S3. The thick line indicates median, box indicates 25th to 75th quartile and Whiskers indicate 10th and 90th percentile

their primary navigational cue. It is possible that some of the variation seen in the heading direction, path straightness and walking speed is because of this reason. We have assumed the relationship between stride length and body size in our animals follow a similar linear pattern as described in 11 other ant species (Zollikofer 1994). However, stride length could vary between species due to biomechanical constraints and this needs to be characterised in future studies.

We show that reduced ability to access celestial compass information results in a less straight path and reduced walking speed. However, the ability to initially orient towards the nest using a celestial compass was common across ants of all sizes.

Compliance with ethical standards

Data accessibility The data are provided in the electronic supplementary material and https://ecologicalneuroscience.files.wordpress.com/2018/03/displ_data_codes.zip.

Conflict of interest We have no competing interests.

Ethical approval Research on ants does not require animal ethical approval in Australia.

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