

RESEARCH ARTICLE

Miniaturisation decreases visual navigational competence in ants

Ravindra Palavalli-Nettimi* and Ajay Narendra

ABSTRACT

Evolution of a smaller body size in a given lineage, called miniaturisation, is commonly observed in many animals including ants. It affects various morphological features and is hypothesised to result in inferior behavioural capabilities, possibly owing to smaller sensory organs. To test this hypothesis, we studied whether reduced spatial resolution of compound eyes influences obstacle detection or obstacle avoidance in five different species of ants. We trained all ant species to travel to a sugar feeder. During their return journeys, we placed an obstacle close to the nest entrance. We found that ants with higher spatial resolution exited the corridor – the area between the two ends of the obstacle – on average 10 cm earlier, suggesting they detected the obstacle earlier in their path. Ants with the lowest spatial resolution changed their viewing directions only when they were close to the obstacle. We discuss the effects of miniaturisation on visual navigational competence in ants.

KEY WORDS: Obstacle avoidance, Detour, Compound eye, Vision, Scaling

INTRODUCTION

Size has profound implications for the biology of most organisms (Bonner, 1965; Calder, 1984). The evolution of extremely small body size within a lineage, reduction beyond which is not possible owing to design limitations, is called miniaturisation (Hanken and Wake, 1993). It occurs in a wide range of animals, including mammals, birds, reptiles, amphibians, fishes, foraminiferans, molluscs, annelids, arachnids, insects, crustaceans and echinoderms (reviewed in Hanken and Wake, 1993). Miniaturisation has dramatic effects on anatomy and physiology, and influences the ecology, reproduction, evolutionary progress, behaviour and life history of animals (Bonner, 1965). In arthropods, miniaturisation affects the size of locomotory structures (Hanken and Wake, 1993; Polilov, 2015), absolute brain size (e.g. Gossen, 1949; Niven and Farris, 2012) and sensory structures (e.g. Makarova et al., 2015; Polilov, 2016; Ramirez-Esquivel et al., 2014; Spaethe and Chittka, 2003). While such effects of miniaturisation have been well documented, the behavioural consequences of size reduction have been less understood (Chittka and Niven, 2009; Niven and Farris, 2012). Miniaturisation is thought to lead to behavioural simplification owing to smaller sensory and motor organs, and also to overcome the high metabolic costs of maintaining a larger central nervous system (Eberhard and Wcislo, 2011). While behavioural complexity, which is largely an anthropocentric view, has been

used to compare animals of different sizes (Cole, 1985), the behavioural precision in small animals was first investigated in spiders (Eberhard, 2007). In web-building spiders, which varied by 5 orders of magnitude in weight, Eberhard showed that behavioural precision in web architecture did not noticeably decline in the smallest spiders (Eberhard, 2007, 2011).

However, a behaviour to document behavioural precision that is non-taxa specific has been lacking. The only study we are aware of that addresses this is in bumblebees, where larger workers were shown to have better visual target-detection abilities (Spaethe and Chittka, 2003). Clearly, visually guided behaviour in the context of navigation is a suitable non-taxa-specific behaviour to test the behavioural simplification hypothesis, as it is used by a variety of animals to traverse from a few centimetres (e.g. fiddler crabs; Zeil and Hemmi, 2006), to a few metres (e.g. ants; Narendra et al., 2013) or hundreds of kilometres (e.g. birds; Biro et al., 2007). Ants are a tractable system to investigate the role of miniaturisation in visual guidance, as (a) they exhibit tremendous size variation between species (Pie and Tschá, 2013), (b) they use visual cues to navigate (e.g. Graham and Cheng, 2009; McLeman et al., 2002; Reid et al., 2011) and (c) the visual system of a number of ant species has been well characterised (e.g. Gronenberg, 2008; Narendra et al., 2017). Ants navigate using familiar views en route or rely on the entire panorama (Bühlmann et al., 2011; Fukushima, 2001; Graham and Cheng, 2009; Kohler and Wehner, 2005; Mangan and Webb, 2012; Narendra, 2007). As most ants navigate in complex environments, familiar views often get occluded by obstacles in their path. Ants, and wasps walking with heavy prey, rely on their visual system to detect and detour around obstacles (Santschi, 1913; Schmidt et al., 1992; Thorpe, 1949). The main visual system in most ants is their compound eye, which is of an apposition design consisting of several ommatidia, with each ommatidium having its own lens, a crystalline cone and a photosensitive structure called the rhabdom. As each ommatidium resolves a point in space, the inter-ommatidial angle affects spatial resolution. Given a fixed area available for the eye, a greater number of ommatidia means a smaller inter-ommatidial angle and hence a higher spatial resolution (Land, 1997; Rutowski et al., 2009). Both solitary foraging ants and visual predators tend to have more facets, which invariably results in higher spatial resolution (Gronenberg, 2008; Land, 1997). The diameter of the facets varies across the eye, with the largest lenses typically found in the frontal region of the eye (Narendra et al., 2011; Perl and Niven, 2016; Schwarz et al., 2011). It should come as no surprise that an attempt has been made to address the behavioural effect of miniaturisation in ants (Bernstein and Bernstein, 1969). Among wood ants, individual workers were trained within a maze to determine their ability to compute the shortest possible path home. The length of the path that the wood ants chose was correlated with their head width and eye length, with bigger ants taking shorter paths. Here, we estimated the spatial resolution of different ant species and investigated whether reduced spatial resolution influences obstacle detection or obstacle avoidance.

Department of Biological Sciences, Macquarie University, Sydney, NSW 2109, Australia.

*Author for correspondence (rvndrpn@gmail.com)

 R.P., 0000-0002-4523-8245; A.N., 0000-0002-1286-5373

Received 9 January 2018; Accepted 15 February 2018

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 summers of 2016 and 2017. The surroundings comprised *Eucalyptus* trees, buildings, grass and small garden shrubs. We chose five different species of ants that varied in head width: *Camponotus aeneopilosus* Mayr 1862, *Camponotus piliventris* (Smith 1858), *Melophorus nemophilus* Heterick, Castalanelli & Shattuck 2017, *Rhytidoponera metallica* (Smith 1858) and *Notoncus ectatommoides* (Forel 1892) (Fig. 1). We located one nest for each of the five species on flat ground away from shrubs. All the species were day active, while *N. ectatommoides* was also active at night. Experiments were conducted during the day between 3 and 11 h after sunrise.

Variation in compound eye vision and spatial resolution

We counted the number of ommatidia and measured the diameter of individual lenses by preparing eye replicas of five individuals for each species. For this, we spread a thin layer of transparent nail polish over the cornea and, once dry, we carefully peeled it off (Narendra et al., 2011; Ramirez-Esquivel et al., 2017; Ribi et al., 1989). We then made an incision to flatten the replica, placed a coverslip over it, and photographed it using a bright-field microscope (Olympus BX50). From the images, we measured the diameter of all the facets in one eye of four individuals from each species. Facet diameter was measured across the longest distance for oval-shaped facets or the shortest distance between the parallel lines in hexagonal/pentagonal facets.

We estimated the spatial resolution as described by Narendra et al. (2016). Briefly, inter-ommatidial angle $\Delta\theta$ was calculated by assuming the eye has a hemispherical visual field: $\Delta\theta = \sqrt{[(Z/2)/N]}$, where Z is a sphere of 41,252.96 square degrees and N is facet count.

Experimental procedure

An area of 1 m² from the nest entrance was cleared of grass, small stones and debris, as much as possible. Ants were then trained to locate a feeder with sugar solution. The feeder was initially kept close to the nest entrance and then gradually moved in small steps in a straight line to a position 1 m from the nest. This training usually took 60–90 min. Following training, ants that left the feeder were tested randomly in the experimental or control condition. In the experimental condition, a black obstacle (rectangular block, 10 cm length×7 cm height) was placed 30 cm from the nest, such that its mid-point was in line with the nest and the feeder. The obstacle was put in place when the ant was at the feeder, ensuring that the ant was not disturbed. The path taken by the ant from the feeder to the obstacle was filmed with a video camera (Sony 4 K Handycam FDR AX100) at 25 frames s⁻¹, with a field of view covering the area between the feeder and the obstacle (Movie 1). When they were out of the field of view of the camera, the ants were monitored to make sure they reached their nest entrance. In the control condition, ants had to travel from the feeder to the nest without any obstacle. The data for both the control and experimental conditions were collected over a few days. We marked the obstacle location with two nails hammered fully into the ground, which ensured the obstacle was placed at the same location on successive days. Foragers of the five species did not appear to follow each other. Ants that exhibited any interaction with other ants while returning were excluded from the analysis. To ensure ants from the experimental condition were tested only once, homing ants were captured close to the nest entrance and were either marked using model paint (Citadel, Games Workshop, Lenton, UK) or detained and released at the nest entrance at the end of the experiment.

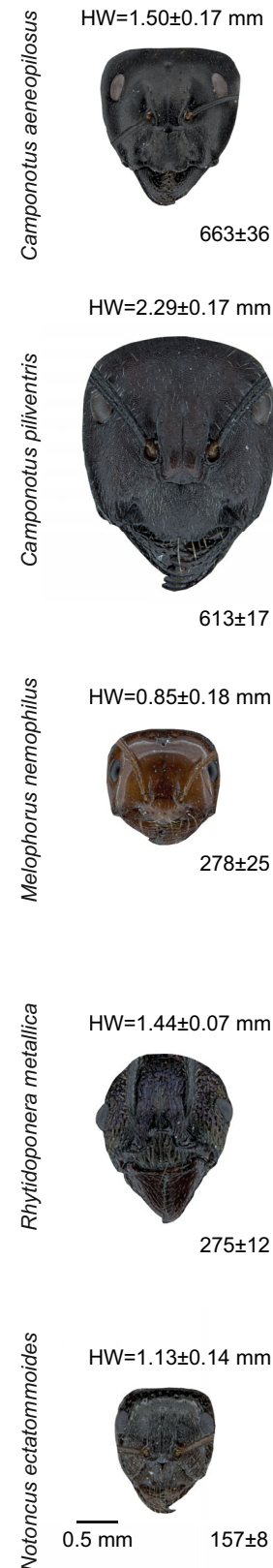


Fig. 1. Five study species with varying head width and facet count.

(A) *Camponotus aeneopilosus*, (B) *Camponotus piliventris*, (C) *Melophorus nemophilus*, (D) *Rhytidoponera metallica* and (E) *Notoncus ectatommoides*. Scale bar, 0.5 mm. Mean±s.d. facet count ($n=5$ for each species) is shown on the bottom right and head width (HW) is shown on the top left ($n=20$ for each species) of each image.

Data extraction from the videos

Video footage was converted to image sequences using Final Cut Pro version 10.2.2 (Apple Inc.). Head and gaster position were digitised using a custom-written Matlab program (MathWorks Inc., Natick, MA, USA), Digilite (courtesy of Jan Hemmi and Robert Parker). For the fast-moving *M. nemophilus*, we digitised every frame (40 ms inter-frame interval), whereas for the other ants we digitised every second frame (80 ms inter-frame interval). We used

the head position of the ant to re-create its path. As some ants did not take the shortest path home, we had to set a criterion to identify the paths that could be used for our analysis. For this, we defined a 'corridor' (grey box in Fig. 2), which is the area between two parallel lines extending from either side of the obstacle towards the feeder. We included paths that were entirely within the corridor until they reached the obstacle or paths that were 5 cm outside the corridor, provided the ants re-entered the corridor before

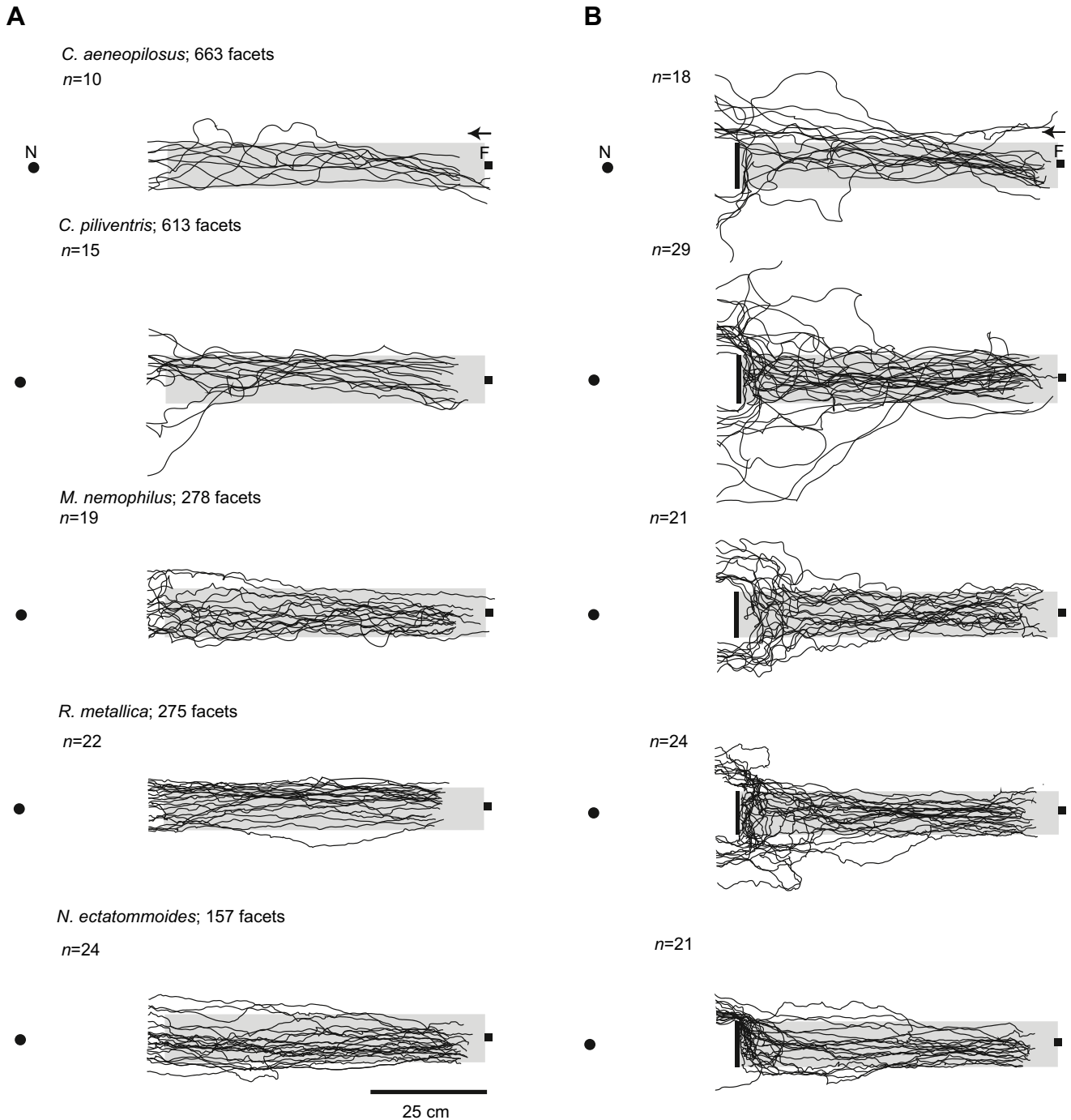


Fig. 2. Paths of homing ants with and without the obstacle. Paths of ants walking to their nest from the feeder (A) in the control condition and (B) in the presence of the obstacle. N, nest; F, feeder; *n*, sample size. Arrow indicates the walking direction of the ants (feeder to nest). The grey rectangle indicates the 'corridor' – the area between the two sides of the obstacle (black bar) extending to the feeder. For the control condition, the corridor starts at the fictive obstacle position. Control and obstacle paths for each species are in the same row.

Table 1. Variation in facet count, head width, inter-ommatidial angle (proxy for spatial resolution) and facet diameter of the study species

Species	Head width (mm) (<i>n</i> =20)	Facet count per eye (<i>n</i> =5)	Inter-ommatidial angle (deg) (<i>n</i> =5)	Facet diameter range (µm) (<i>n</i> =4)
<i>C. aeneopilosus</i>	1.50±0.17	663.0±35.7	5.58	18.8±0.8 to 6.9±0.6
<i>C. piliventris</i>	2.29±0.17	612.8±16.6	5.81	20.4±0.5 to 7.5±1.1
<i>M. nemophilus</i>	0.85±0.18	278.0±25.5	8.61	15.3±0.8 to 5.7±0.4
<i>R. metallica</i>	1.44±0.07	275.4±11.8	8.66	18.5±1.2 to 6.5±0.9
<i>N. ectatommoides</i>	1.13±0.14	156.8±8.1	11.46	21.9±2.1 to 9.0±1.6

Means±s.d. are listed for head width and facet count per eye. Facet diameter range is represented as the maximum to the minimum diameter (mean±s.d.). *n*, sample size.

encountering the obstacle and then detouring. Often, ants groomed while departing from the feeder, during which they initially walked slightly away from the corridor. Hence, we excluded the initial paths from the feeder and only analysed paths starting 10–15 cm from the feeder.

Measurements used for obstacle detection/avoidance

We used four measurements to determine whether and how miniaturisation influences visual navigation: exit distance, gaze direction, path straightness and change in velocity (see below).

Exit distance

We assumed that when ants first detect the obstacle, they make a decision to either detour or search for familiar views that were occluded by the obstacle. For this, the ants most likely have to exit the corridor. Hence, we identified the point at which ants first exit the corridor (grey box in Fig. 2) and walk for at least 3 cm distance outside the corridor. Alternatively, if an ant made a search loop (Fig. S1) before exiting the corridor, the point where the loop started was considered as the point of obstacle avoidance. At most, four ants in each species showed searching behaviour. A straight-line distance from the obstacle to this point gave the ‘exit distance’. If ants did not exit the corridor in the control condition, their exit distance was considered to be zero.

Gaze direction relative to nest

Obstacle detection may also drive animals to look away from their nest direction to find familiar views. In all the five species we studied, the head moves independently of the rest of the body but to a significantly lesser extent than for *Myrmecia* ants (Zeil et al., 2014). We therefore used the head and gaster position to determine the gaze direction of the animal relative to the nest at different distances from the obstacle: 0–5 cm, 5–10 cm, 10–15 cm, 15–30 cm, 30–45 cm, 45–60 cm. For each distance interval and for each species, we developed histograms of gaze direction that were normalised to the maximum. These histograms show the frequency of gaze direction relative to the nest at different distances from the obstacle.

Path straightness

We measured path straightness of each species both with and without the obstacle. We determined this for the path before the first exit and also for the entire path (from the obstacle to a distance of 60 cm). We measured straightness as the ratio of the straight-line distance and the sum of all the segments of the path.

Change in velocity

We identified the distance from the obstacle at which ants walked slowest and decelerated the most. We measured this along the path up to the first exit and also for the entire path from the obstacle to a distance of 60 cm. We also calculated the average speed of ants at six different distances from the obstacle in both control and

experimental conditions: 0–5 cm, 5–10 cm, 10–15 cm, 15–30 cm, 30–45 cm, 45–60 cm.

Statistical analysis

We compared each of the different measurements between the experimental (with the obstacle) and control (without the obstacle) conditions using the Wilcoxon rank-sum test. For between-species comparison within each condition, we used the Kruskal–Wallis test, and the Dunn *post hoc* test with Bonferroni correction ($\alpha=0.05$) when applicable. We calculated the circular standard deviations for gaze direction relative to the nest, and also compared their mean vectors using the Watson–Williams *F*-test using Oriana 4 (Kovach Computing Services: <http://www.kovcomp.co.uk/>). All other statistical analyses were carried out in RStudio (version 1.1.383; www.rstudio.com).

RESULTS

Variation in compound eye vision and spatial resolution

The five ant species we studied varied in head width (0.85–2.29 mm) and facet count (157–663 facets; Table 1, Fig. 1). Their inter-ommatidial angle ranged from 11.46 deg in the smallest ant (*N. ectatommoides*) to 5.58 deg in the biggest ant (*C. aeneopilosus*) (Table 1). *Notoncus ectatommoides* had the largest facets while *M. nemophilus* had the smallest facets (Table 1).

Paths taken by different ants

Paths of ants heading to the nest were distinctly different with and without the obstacle (Fig. 2). In the absence of the obstacle, ants with more facets (*C. aeneopilosus*: 663 and *C. piliventris*: 613) had more spread in their paths compared with ants with fewer facets (Fig. 2A). In the presence of the obstacle, all the species initially walked towards the obstacle, then detoured around it to reach the nest (Fig. 2B). We found that only *N. ectatommoides* (157 facets) workers typically detoured to the right of the obstacle, which may be due to the presence of a tree on the right (Table 2; exact binomial test, 1/21 left detours, sample estimate=0.047, $P<0.01$). Almost all the ants headed towards the nest after walking past the edge of the obstacle (data not shown).

Table 2. Detour direction in different ants

Species	No. of ants detouring		<i>P</i> -value
	Left	Right	
<i>C. aeneopilosus</i>	5	13	0.09
<i>C. piliventris</i>	12	17	0.46
<i>M. nemophilus</i>	11	10	1.00
<i>R. metallica</i>	11	13	0.84
<i>N. ectatommoides</i>	1	20	<0.01

P-values were calculated using the binomial test.

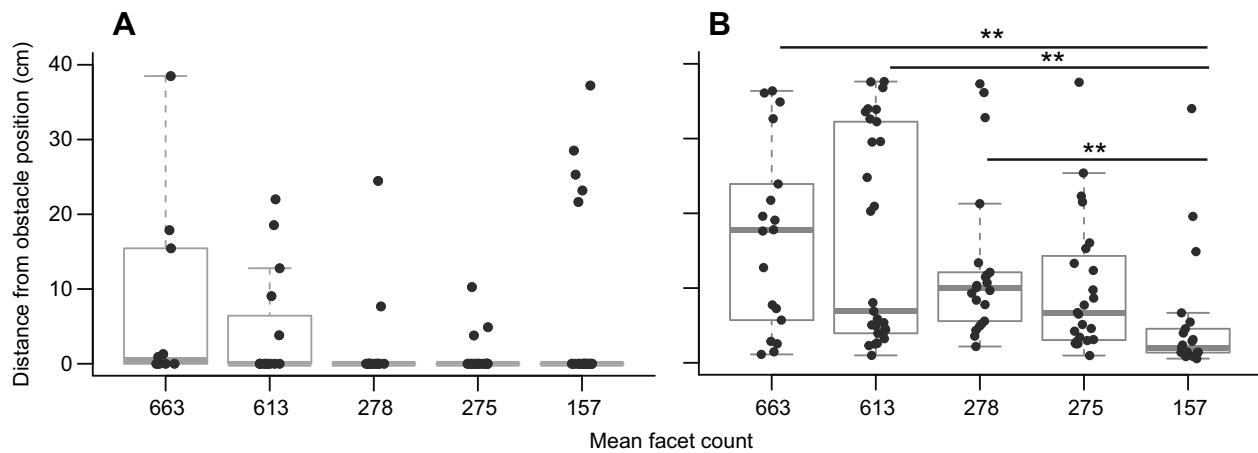


Fig. 3. Exit distance of ants with different numbers of facets. The distance at which ants exit the foraging corridor relative to the obstacle (at 0 cm) is shown for ants (A) without an obstacle in their path (control) and (B) with an obstacle. Significant differences for between-species comparisons using Dunn's *post hoc* test with Bonferroni correction (after Kruskal–Wallis test) are represented using line and asterisks notation: ** $P \leq 0.01$. Sample sizes are given in Fig. 2. Thick lines are the median; boxes indicate the 25th to 75th quartiles and whiskers indicate the 10th and 90th percentile.

Exit distance

We found that the ants with the lowest number of facets (*N. ectatommoides*: 157) exited the corridor significantly closer to the obstacle compared with the three species with more facets (*C. aeneopilosus*: 663, *C. piliventris*: 613, *M. nemophilus*: 278) (Fig. 3B; Kruskal–Wallis $\chi^2 = 21.126$, d.f.=4, $P < 0.01$; Table S2A). Ants with the highest number of facets exited the corridor 10 cm earlier than ants with the lowest number of facets (Table S3). The exit distance of different ant species did not differ within the control group (without obstacle; Fig. 3A; Kruskal–Wallis $\chi^2 = 7.3429$, d.f.=4, $P = 0.118$). The exit distance of all ant species differed

between the control and experimental group, with ants exiting the corridor earlier in the presence of the obstacle (Fig. 3; Table S1A).

Gaze direction relative to nest

In the control condition, all ants were well oriented and looked towards the nest even when they were farther from it (Fig. 4; control, last five rows). When ants were close to the nest position (0–5 cm from the fictive obstacle), their viewing directions changed and they looked on either side of the nest position (Fig. 4; control, top row). In the presence of the obstacle, initially (15–60 cm from the obstacle; Fig. 4, obstacle, bottom three rows) all the ants were well oriented

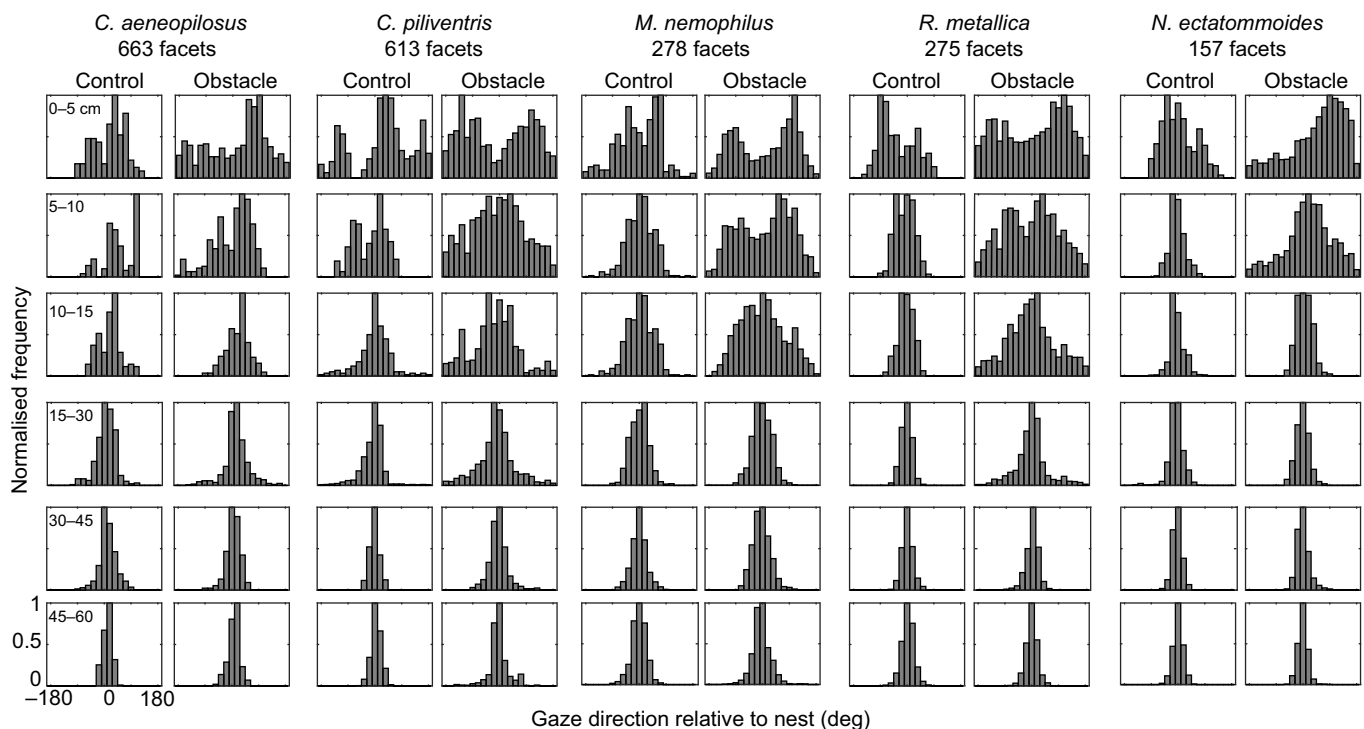


Fig. 4. Normalised gaze direction (relative to nest) for ants at different distances from the obstacle. Histograms for the control and obstacle condition for each species are shown as well as the mean facet count. The nest is at 0 deg. The numbers within the plot in the first column indicate the distance from the obstacle. Corresponding circular statistics are given in Table 3 and Table S4.

Table 3. Descriptive statistics for gaze direction relative to nest of ants as they walked towards the obstacle

Distance from obstacle (cm)	Species	Mean vector		Circular s.d. (deg)
		Angle (deg)	Length	
0–5	<i>C. aeneopilosus</i>	82.106	0.224	99.182
	<i>C. piliventris</i>	176.232	0.129	115.95
	<i>M. nemophilus</i>	93.229	0.107	121.196
	<i>R. metallica</i>	119.248	0.128	116.283
	<i>N. ectatommoides</i>	91.51	0.371	80.685
5–10	<i>C. aeneopilosus</i>	15.629	0.557	61.947
	<i>C. piliventris</i>	6.181	0.244	99.068
	<i>M. nemophilus</i>	27.624	0.194	103.841
	<i>R. metallica</i>	0.819	0.197	103.209
	<i>N. ectatommoides</i>	28.859	0.406	76.884
10–15	<i>C. aeneopilosus</i>	21.447	0.804	37.859
	<i>C. piliventris</i>	17.832	0.433	74.108
	<i>M. nemophilus</i>	7.448	0.386	79.03
	<i>R. metallica</i>	6.67	0.371	80.68
	<i>N. ectatommoides</i>	4.577	0.901	26.156
15–30	<i>C. aeneopilosus</i>	16.254	0.791	39.188
	<i>C. piliventris</i>	10.698	0.603	57.635
	<i>M. nemophilus</i>	1.611	0.856	31.928
	<i>R. metallica</i>	3.106	0.686	49.715
	<i>N. ectatommoides</i>	1.755	0.929	21.971
30–45	<i>C. aeneopilosus</i>	5.755	0.918	23.649
	<i>C. piliventris</i>	4.294	0.866	30.684
	<i>M. nemophilus</i>	3.719	0.876	29.526
	<i>R. metallica</i>	2.743	0.935	21.04
	<i>N. ectatommoides</i>	0.012	0.925	22.565
45–60	<i>C. aeneopilosus</i>	7.621	0.945	19.331
	<i>C. piliventris</i>	2.37	0.857	31.883
	<i>M. nemophilus</i>	4.436	0.877	29.399
	<i>R. metallica</i>	0.1	0.945	19.246
	<i>N. ectatommoides</i>	1.127	0.961	16.128

The nest is at 0 deg. The closer the mean vector length is to one, the more directed the distribution.

and looked towards the nest. Ants with the lowest number of facets (*N. ectatommoides*) continued to be well oriented towards their nest even at 10–15 cm from the obstacle (Fig. 4), as evidenced from the smaller circular standard deviation in their viewing directions (Fig. 4, Table 3). Viewing directions of the ant with the lowest number of facets varied when the ants were 5–10 cm from the obstacle.

Path straightness

We first analysed the paths of ants before they exited the corridor. In the control condition, we found that the path straightness differed between ants with facet numbers of 613 and 278 and between ants with facet numbers of 278 and 275 (Fig. 5B; Kruskal–Wallis $\chi^2=16.958$, d.f.=4, $P=0.0019$; Table S2B). In the presence of the obstacle, we found there was no difference in path straightness between the different ant species (Fig. 5B; Kruskal–Wallis $\chi^2=4.647$, d.f.=4, $P=0.325$). Comparing the path straightness of ants with and without the obstacle, we found that it decreased in the two species with the lowest number of facets (Fig. 5B, coloured boxes; Table S1B).

Analysing the entire path, we found that in the control condition, path straightness differed only between ants with facet numbers of 278 and 275 (Fig. 5C; Kruskal–Wallis $\chi^2=15.08$, d.f.=4, $P=0.0045$; Table S2C). In the presence of the obstacle, we found that the path straightness differed between ants with facet numbers of 663 and 278 and between ants with facet numbers of 278 and 157 (Fig. 5C; Kruskal–Wallis $\chi^2=19.582$, d.f.=4, $P<0.001$; Table S2D). Comparing the path straightness of ants with and without the obstacle, we found that it differed for all species, except those with

the maximum and minimum number of facets (Fig. 5C, coloured boxes; Table S1C).

Distance from the obstacle where ants attained the lowest speed or decelerated the most

We found no clear facet count-dependent pattern in the distance at which different species were slowest or decelerated the most for paths before they exited the corridor and for the entire path. For both variables, only two species (*M. nemophilus*: 278 facets and *N. ectatommoides*: 157 facets) were significantly affected by the presence of the obstacle when compared with control ants (Fig. 6, coloured boxes; Table S1D–G). Similarly, within the control and obstacle treatments, we did not find an effect of facet number (Fig. 6; distance at lowest speed: control, before exit: Kruskal–Wallis $\chi^2=15.529$, d.f.=4, $P=0.0037$, Table S2E; obstacle, before exit: Kruskal–Wallis $\chi^2=6.524$, d.f.=4, $P=0.163$; control, entire path: Kruskal–Wallis $\chi^2=16.401$, d.f.=4, $P=0.002$, Table S2F; obstacle, entire path: Kruskal–Wallis $\chi^2=4.031$, d.f.=4, $P=0.401$; distance at highest deceleration: control, before exit: Kruskal–Wallis $\chi^2=21.201$, d.f.=4, $P<0.001$, Table S2G; obstacle, before exit: Kruskal–Wallis $\chi^2=8.09$, d.f.=4, $P=0.08$; control, entire path: Kruskal–Wallis $\chi^2=8.760$, d.f.=4, $P=0.067$; obstacle, entire path: Kruskal–Wallis $\chi^2=2.032$, d.f.=4, $P=0.729$).

Average walking speed at different distances from the obstacle

We analysed the change in the ants' walking speed as they approached the obstacle. In the control condition, we did not find a significant change in walking speed (Fig. 7, left column; Dunn's *post hoc* test with Bonferroni correction). In the presence of the obstacle, only the ants with 278 facets (*M. nemophilus*) were significantly faster at 0–5 cm distance from the obstacle than when they were 45–60 cm from it (Fig. 7C; Dunn's *post hoc* test after Bonferroni correction, $Z=3.58$, $P=0.005$).

DISCUSSION

We studied whether reduced spatial resolution influences obstacle detection or obstacle avoidance in different species of ants. We found that ants with higher spatial resolution exited the familiar corridor earlier, suggesting they detected the obstacle earlier in their path. Ants that had the lowest spatial resolution changed their viewing direction only when closer to the obstacle. We did not find any clear patterns in path straightness and speed when ants were compared with and without the obstacle.

Obstacle avoidance behaviour has implications for navigation by both walking insects such as ants (Collett et al., 1992; McLeman et al., 2002; Schmidt et al., 1992; Thorpe, 1949) and flying insects such as bees (Dittmar et al., 2010), and even for collective food transport (McCreery et al., 2016) and animal cognition (Kabadayi et al., 2017). Miniaturisation should affect this behaviour as miniature ants tend to have reduced sensory capabilities. Indeed, our results suggest that the ants with higher spatial resolution respond earlier by exiting the familiar corridor (Fig. 3; Table S3). In addition, the species with the lowest facet count (*N. ectatommoides*) reached very close to the obstacle before detouring (Fig. 2B). In this species, the viewing direction changed greatly when ants were close to the obstacle (Fig. 4). This means the ants with fewer facets may not have detected the obstacle visually until they were close to it. Our results corroborate with previous studies on bumblebee target-detection abilities (Spaethe and Chittka, 2003) and with those on wood ants which showed bigger ants take shorter paths than smaller ants over consecutive trips to a food source (Bernstein and Bernstein, 1969).

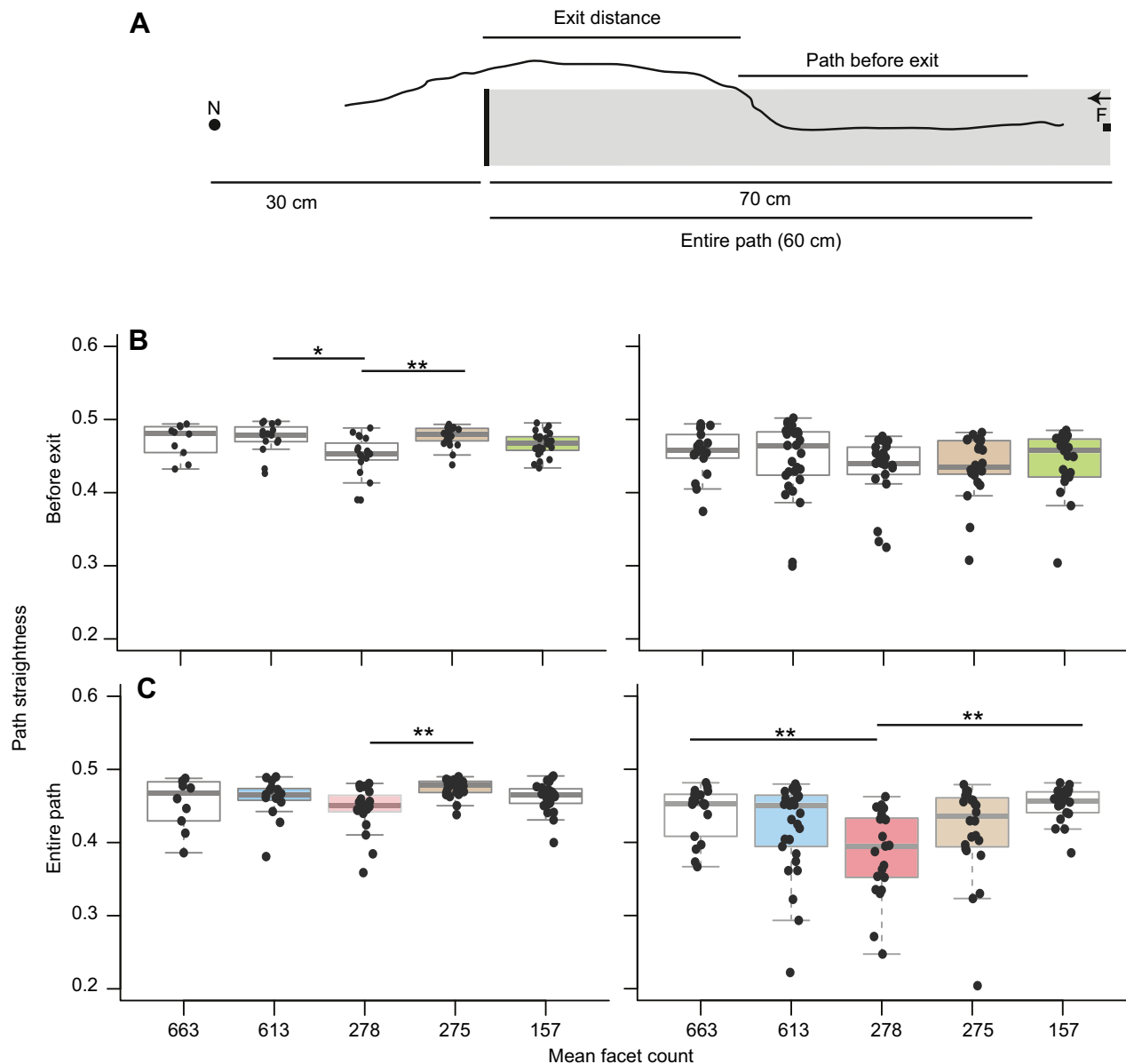


Fig. 5. Path straightness of ant species with different facet numbers. (A) Illustration of distances measured in this study. (B,C) Straightness of paths (B) before exit and (C) for the entire path. Left: control condition; right: obstacle condition. Coloured boxes indicate a significant difference between data for ants in corresponding control and obstacle treatments (Wilcoxon rank-sum test; Table S1). Significant differences for between-species comparisons using Dunn's *post hoc* test with Bonferroni correction (after Kruskal–Wallis test) are represented using line and asterisks notation: ** $P \leq 0.01$, * $P \leq 0.05$. Sample size and figure conventions are as for Figs 2 and 3.

But, in addition, we show that in obstacle-avoidance behaviour, the difference is probably due to sensory constraints of spatial resolution in the miniature ants.

Although we suggest a correlation between spatial resolution and obstacle detection, miniaturisation could have affected anatomical features involved in locomotor systems. For example, even though *M. nemophilus* and *R. metallica* have a similar number of facets, the former detoured well before the obstacle (Fig. 2). This may be because thermophilic ants such as *M. nemophilus* walk faster (Fig. 7), in a more meandering way (Fig. 5B,C), and turn quickly (Wahl et al., 2015; Zollikofer, 1994). Fast-moving ants have faster photoreceptors, and tend to have higher spatial resolution, allowing them to visualise a clearer (less blurry) image (de Souza and Ventura, 1989), and this could have allowed them to detect the

obstacle earlier. In addition, when the ants detect the obstacle that obstructed their learned panorama, we expected them to have the lowest speed, or to have decelerated or walked a less straight path. But we did not find any consistent difference between control and experimental ants. We did not control for age or prior experience of ants in our experiments, and both these factors may have driven some of the variation that we see.

Clearly, *N. ectatommoides* detoured mostly to the right side of the obstacle (Fig. 2B, last row; Table 2), while no such bias was seen in the control condition (Fig. 2A, last row). While it is tempting to discuss this in the context of laterality, for which there is evidence in ants and bees at both the population and individual level (Basari et al., 2014; Frasnelli et al., 2012; Hunt et al., 2014; Ong et al., 2017), we think that is not the case here. We think the bias may be

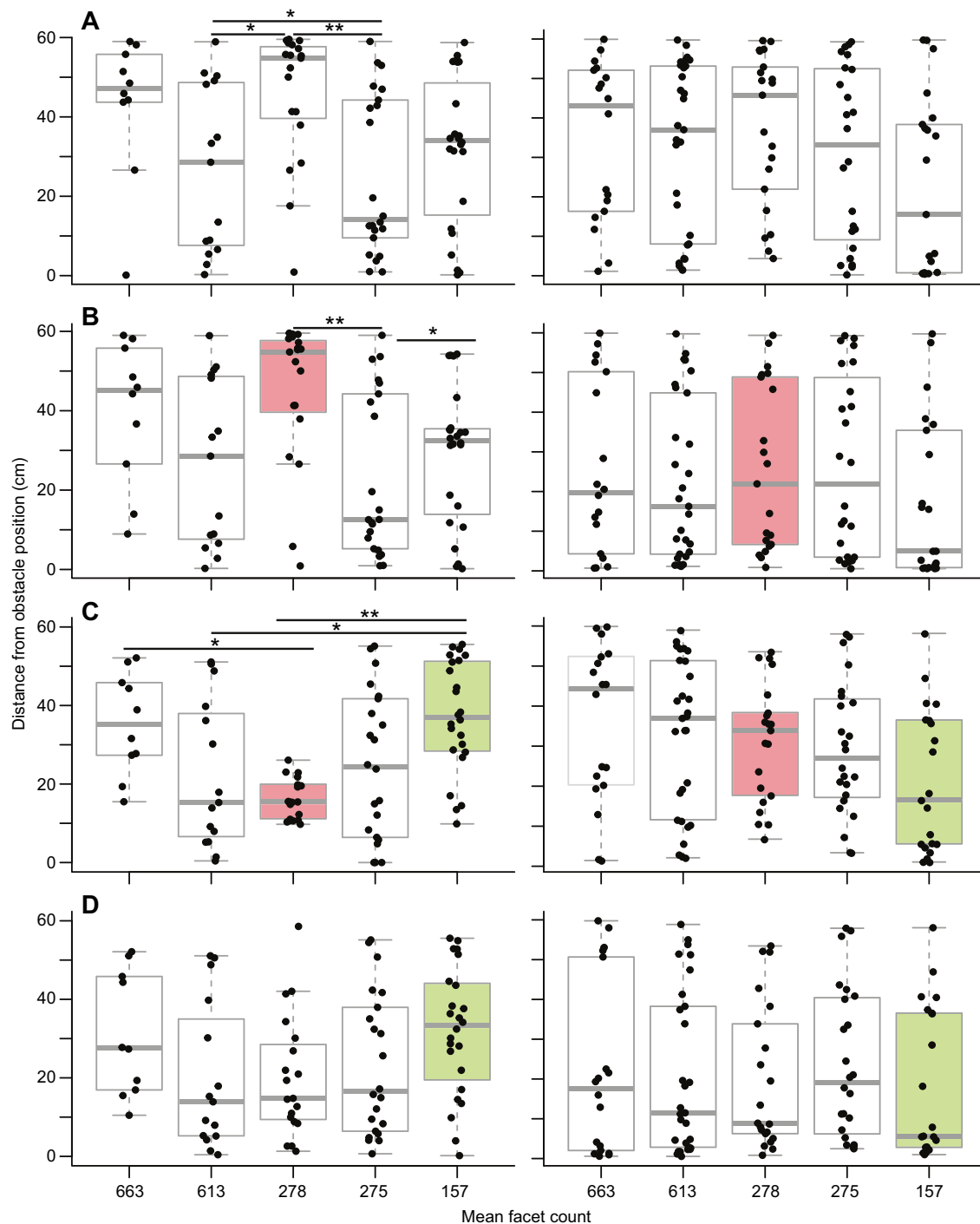


Fig. 6. Distance from the obstacle at which ants with different facet numbers were slowest or decelerated the most. (A,B) Distance from the obstacle position at which the ants attained the lowest speed (A) before the first exit and (B) for the entire path. (C,D) Distance from the obstacle position at which the ants decelerated the most (C) before the first exit and (D) for the entire path. Left: control condition; right: obstacle condition. Coloured boxes indicate a significant difference between data for ants in corresponding control and obstacle treatments (Wilcoxon rank-sum test; Table S1D–G). For between-species comparison, Dunn's *post hoc* test with Bonferroni correction (after Kruskal–Wallis test) was used: ** $P \leq 0.01$, * $P \leq 0.05$. Sample size and figure conventions are as for Figs 2 and 3.

due to the presence of a conspicuous tree on one side of their panorama, which may have been a familiar visual cue to these animals. Ants are known to use distinct landmarks as beacons to guide their routes (Collett et al., 2007; Graham et al., 2003; Narendra, 2007). However, *N. ectatommoides* walked much closer to the obstacle than other ants before detouring. A small proportion

of these ants (24%) came in contact with the obstacle before detouring. This could mean that they might need a mechanosensory input to sense obstacles, similar to beetles and cockroaches (Baba et al., 2010; Pelletier and McLeod, 1994).

This study did not include ants with the highest or lowest spatial resolution. Nevertheless, we were still able to see that reduced

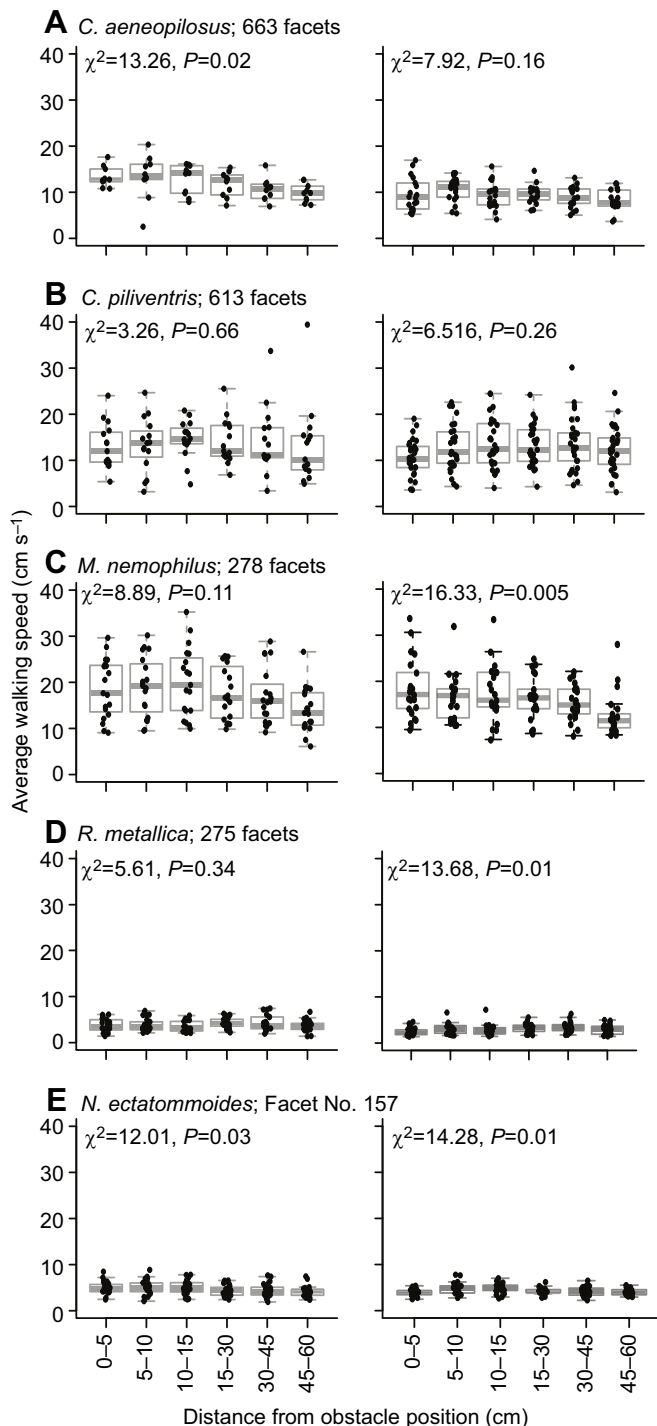


Fig. 7. Average walking speed for ants with different facet numbers heading towards the obstacle position. Left: box plots for the control condition; right: box plots for the obstacle condition. Mean facet count is indicated at the top of each plot. The average speeds across different intervals were compared using the Kruskal–Wallis test (χ^2 and P -values are shown in each plot). Sample size and figure conventions are as for Figs 2 and 3.

spatial resolution affects visual navigation, especially in the context of obstacle avoidance. Miniaturisation thus appears to decrease visual navigational competence in ants. While we have focused on visual guidance in this study, it remains to be seen whether the choice of compass cues (celestial/terrestrial) differs between the large and miniature animals.

Acknowledgements

We thank Jochen Zeil for hosting us at the ANU and for his comments on the manuscript. We are grateful to the members of the Macquarie University Neuroethology group for their comments and to Justin O'Donnell and Jack Westacott for their assistance in digitising some of the video footage.

Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: R.P.-N., A.N.; Methodology: R.P.-N., A.N.; Validation: R.P.-N., A.N.; Formal analysis: R.P.-N.; Investigation: R.P.-N.; Resources: A.N.; Data curation: R.P.-N.; Writing - original draft: R.P.-N.; Writing - review & editing: R.P.-N., A.N.; Visualization: R.P.-N.; Supervision: A.N.; Funding acquisition: A.N.

Funding

R.P.-N. is funded by International Macquarie University Research Excellence Scholarship (iMQRES 2015141). The research was supported by the Australian Research Council (FT140100221 and DP150101172).

Data availability

Raw data used for this study can be accessed at: https://ecologicalneuroscience.files.wordpress.com/2018/02/obstacle_data.zip

Supplementary information

Supplementary information available online at <http://jeb.biologists.org/lookup/doi/10.1242/jeb.177238.supplemental>

References

- Baba, Y., Tsukada, A. and Comer, C. M. (2010). Collision avoidance by running insects: antennal guidance in cockroaches. *J. Exp. Biol.* **213**, 2294–2302.
- Basari, N., Bruendl, A. C., Hemingway, C. E., Roberts, N. W., Sendova-Franks, A. B. and Franks, N. R. (2014). Landmarks and ant search strategies after interrupted tandem runs. *J. Exp. Biol.* **217**, 944–954.
- Bernstein, S. and Bernstein, R. A. (1969). Relationships between foraging efficiency and the size of the head and component brain and sensory structures in the red wood ant. *Brain Res.* **16**, 85–104.
- Biro, D., Freeman, R., Meade, J., Roberts, S. and Guilford, T. (2007). Pigeons combine compass and landmark guidance in familiar route navigation. *Proc. Natl. Acad. Sci. USA* **104**, 7471–7476.
- Bonner, J. T. (1965). *Size and Cycle: An Essay on the Structure of Biology*. Princeton, USA: Princeton University Press.
- Bühlmann, C., Cheng, K. and Wehner, R. (2011). Vector-based and landmark-guided navigation in desert ants inhabiting landmark-free and landmark-rich environments. *J. Exp. Biol.* **214**, 2845–2853.
- Calder, W. A. (1984). *Size, Function, and Life History*. Cambridge, Massachusetts: Harvard University Press.
- Chittka, L. and Niven, J. (2009). Are bigger brains better? *Curr. Biol.* **19**, R995–R1008.
- Cole, B. J. (1985). Size and behavior in ants: constraints on complexity. *Proc. Natl. Acad. Sci. USA* **82**, 8548–8551.
- Collett, T. S., Dillmann, E., Giger, A. and Wehner, R. (1992). Visual landmarks and route following in desert ants. *J. Comp. Physiol. A* **170**, 435–442.
- Collett, T. S., Graham, P. and Harris, R. A. (2007). Novel landmark-guided routes in ants. *J. Exp. Biol.* **210**, 2025–2032.
- de Souza, J. M. and Ventura, D. F. (1989). Comparative study of temporal summation and response form in hymenopteran photoreceptors. *J. Comp. Physiol. A* **165**, 237–245.
- Dittmar, L., Stürzl, W., Baird, E., Boeddeker, N. and Egelhaaf, M. (2010). Goal seeking in honeybees: matching of optic flow snapshots? *J. Exp. Biol.* **213**, 2913–2923.
- Eberhard, W. G. (2007). Miniaturized orb-weaving spiders: behavioural precision is not limited by small size. *Proc. R. Soc. B* **274**, 2203–2209.
- Eberhard, W. G. (2011). Are smaller animals behaviourally limited? Lack of clear constraints in miniature spiders. *Anim. Behav.* **81**, 813–823.
- Eberhard, W. G. and Wcislo, W. T. (2011). Grade changes in brain-body allometry, morphological and behavioural correlates of brain size in miniature spiders, insects and other invertebrates. In *Advances in Insect Physiology* (ed. J. Casas), pp. 155–214. Burlington: Academic press: Elsevier Ltd.
- Frasnelli, E., Iakovlev, I. and Reznikova, Z. (2012). Asymmetry in antennal contacts during trophallaxis in ants. *Behav. Brain Res.* **232**, 7–12.
- Fukushi, T. (2001). Homing in wood ants, *Formica japonica*: use of the skyline panorama. *J. Exp. Biol.* **204**, 2063–2072.
- Gossen, M. (1949). Untersuchungen an Gehirnen verschieden grosser, jeweils verwandter Coleopteren- und Hymenopterenarten. *Zool. Jb. Abt. Allgem. Zool. Physiol.* **62**, 1–64.

- Graham, P. and Cheng, K. (2009). Ants use the panoramic skyline as a visual cue during navigation. *Curr. Biol.* **19**, R935-R937.
- Graham, P., Fauria, K. and Collett, T. S. (2003). The influence of beacon-aiming on the routes of wood ants. *J. Exp. Biol.* **206**, 535-541.
- Gronenberg, W. (2008). Structure and function of ant (Hymenoptera: Formicidae) brains: strength in numbers. *Myrmecological News* **11**, 25-36.
- Hanken, J. and Wake, D. B. (1993). Miniaturization of body size: organismal consequences and evolutionary significance. *Annu. Rev. Ecol. Syst.* **24**, 501-519.
- Hunt, E. R., O'Shea-Wheller, T., Albery, G. F., Bridger, T. H., Gumn, M. and Franks, N. R. (2014). Ants show a leftward turning bias when exploring unknown nest sites. *Biol. Lett.* **10**, 20140945.
- Kabadayi, C., Bobrowicz, K. and Osvath, M. (2017). The detour paradigm in animal cognition. *Anim. Cogn.* **21**, 21-35.
- Kohler, M. and Wehner, R. (2005). Idiosyncratic route-based memories in desert ants, *Melophorus bagoti*: how do they interact with path-integration vectors? *Neurobiol. Learn. Mem.* **83**, 1-12.
- Land, M. F. (1997). The resolution of insect compound eyes. *Isreal J. plant Sci.* **45**, 79-91.
- Makarova, A., Polilov, A. and Fischer, S. (2015). Comparative morphological analysis of compound eye miniaturization in minute hymenoptera. *Arthropod Struct. Dev.* **44**, 21-32.
- Mangan, M. and Webb, B. (2012). Spontaneous formation of multiple routes in individual desert ants (*Cataglyphis velox*). *Behav. Ecol.* **23**, 944-954.
- McCreery, H. F., Dix, Z. A., Breed, M. D. and Nagpal, R. (2016). Collective strategy for obstacle navigation during cooperative transport by ants. *J. Exp. Biol.* **219**, 3366-3375.
- McLeman, M. A., Pratt, S. C. and Franks, N. R. (2002). Navigation using visual landmarks by the ant *Leptothorax albigipennis*. *Insectes Soc.* **49**, 203-208.
- Narendra, A. (2007). Homing strategies of the Australian desert ant *Melophorus bagoti*. II. Interaction of the path integrator with visual cue information. *J. Exp. Biol.* **210**, 1804-1812.
- Narendra, A., Reid, S. F., Greiner, B., Peters, R. A., Hemmi, J. M., Ribi, W. A. and Zeil, J. (2011). Caste-specific visual adaptations to distinct daily activity schedules in Australian *Myrmecia* ants. *Proc. R. Soc. B* **278**, 1141-1149.
- Narendra, A., Gourmaud, S. and Zeil, J. (2013). Mapping the navigational knowledge of individually foraging ants, *Myrmecia croslandi*. *Proc. R. Soc. B* **280**, 20130683.
- Narendra, A., Ramirez-Esquivel, F. and Ribi, W. A. (2016). Compound eye and ocellar structure for walking and flying modes of locomotion in the Australian ant, *Camponotus consobrinus*. *Sci. Rep.* **6**, 22331.
- Narendra, A., Kamhi, J. F. and Ogawa, Y. (2017). Moving in dim light: Behavioral and visual adaptations in nocturnal ants. *Integr. Comp. Biol.* **57**, 1104-1116.
- Niven, J. E. and Farris, S. M. (2012). Miniaturization of nervous systems and neurons. *Curr. Biol.* **22**, R323-R329.
- Ong, M., Bulmer, M., Groening, J. and Srinivasan, M. V. (2017). Obstacle traversal and route choice in flying honeybees: evidence for individual handedness. *PLoS ONE* **12**, e0184343.
- Pelletier, Y. and McLeaod, C. D. (1994). Obstacle perception by insect antennae during terrestrial locomotion. *Physiol. Entomol.* **19**, 360-362.
- Perl, C. D. and Niven, J. E. (2016). Differential scaling within an insect compound eye. *Biol. Lett.* **12**, 20160042.
- Pie, M. R. and Tschä, M. K. (2013). Size and shape in the evolution of ant worker morphology. *PeerJ* **1**, e205.
- Polilov, A. A. (2015). Small is beautiful: Features of the smallest insects and limits to miniaturization. *Annu. Rev. Entomol.* **60**, 103-121.
- Polilov, A. A. (2016). *At the Size Limit-Effects of Miniaturization in Insects*, 1st edn. Cham, Switzerland: Springer International Publishing.
- Ramirez-Esquivel, F., Zeil, J. and Narendra, A. (2014). The antennal sensory array of the nocturnal bull ant *Myrmecia pyriformis*. *Arthropod Struct. Dev.* **43**, 543-558.
- Ramirez-Esquivel, F., Ribi, W. A. and Narendra, A. (2017). Techniques to investigate the anatomy of the ant visual system. *JOVE* **129**, e56339.
- 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.
- Ribi, W. A., Engels, E. and Engels, W. (1989). Sex and caste specific eye structures in stingless bees and honey bees (Hymenoptera: Trigonidae, Apidae). *Entomol. Gen.* **14**, 233-242.
- Rutowski, R. L., Gislén, L. and Warrant, E. J. (2009). Visual acuity and sensitivity increase allometrically with body size in butterflies. *Arthropod Struct. Dev.* **38**, 91-100.
- Santschi, F. (1913). Comment s'orientent les fourmis. *Rev. Suisse Zool.* **21**, 347-426.
- Schmidt, I., Collett, T. S., Dillier, F.-X. and Wehner, R. (1992). How desert ants cope with enforced detours on their way home. *J. Comp. Physiol. A* **171**, 285-288.
- Schwarz, S., Narendra, A. and Zeil, J. (2011). The properties of the visual system in the Australian desert ant *Melophorus bagoti*. *Arthropod Struct. Dev.* **40**, 128-134.
- Spaethe, J. and Chittka, L. (2003). Interindividual variation of eye optics and single object resolution in bumblebees. *J. Exp. Biol.* **206**, 3447-3453.
- Thorpe, W. H. (1949). A note on detour experiments with *Ammophila pubescens*. *Behav. II* **31**, 257-263.
- Wahl, V., Pfeffer, S. E. and Wittlinger, M. (2015). Walking and running in the desert ant *Cataglyphis fortis*. *J. Comp. Physiol. A* **201**, 645-656.
- Zeil, J. and Hemmi, J. M. (2006). The visual ecology of fiddler crabs. *J. Comp. Physiol. A* **192**, 1-25.
- Zeil, J., Narendra, A. and Stürzl, W. (2014). Looking and homing: how displaced ants decide where to go. *Philos. Trans. R. Soc. B* **369**, 20130034.
- Zollikofer, C. P. E. (1994). Stepping patterns in ants. II. Influence of body morphology. *J. Exp. Biol.* **192**, 107-118.