Innate visual attraction in wood ants is a hardwired behaviour seen across different motivational and ecological contexts

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ABSTRACT

Ants are expert navigators combing innate and learnt navigational strategies. Whereas we know that the ants' feeding state segregates visual navigational memories in ants navigating along a learnt route, it is an open question if the motivational state also affects the ants' innate visual preferences. Wood ant foragers show an innate attraction to conspicuous visual cues. These foragers inhabit cluttered woodland habitat and feed on honeydew from aphids on trees, hence, the attraction to 'tree-like' objects might be an ecologically relevant behaviour that is tailored to the wood ants' foraging ecology. Foragers from other ant species with different foraging ecologies show very different innate attractions. We investigated here the innate visual response of wood ant foragers with different motivational states, i.e. unfed or fed, as well as males that have a short life span and show no foraging activity. Our results show that ants from all three groups orient towards a prominent visual cue, i.e. the wood ants' innate visual attraction is not context dependent, but a hardwired behaviour seen across different motivational and ecological contexts.

KEY WORDS

Visual orientation, innate behaviour, navigation, foraging ecology, wood ants, *Formica rufa*

INTRODUCTION

Ants cleverly combine innate and learnt navigational strategies to travel between their nest and feeding sites [1, 2]. Innate strategies such as path integration (e.g. [3, 4]), pheromone trails (e.g. [5]), attractive food odours [6], or innately attractive visual cues (e.g. [7, 8]) are key when unfamiliar with an environment. These innate responses can structure the ants' paths, hence, act as a scaffold and facilitate the learning of information relevant for navigation. Wood ant foragers show an innate attraction to large and conspicuous objects [7, 9-11]. These foragers feed on honeydew from aphids on trees [12], hence, the attraction to 'tree-like' objects might be an ecologically relevant behaviour that is tailored to the wood ants' foraging ecology [13]. Foragers from other ant species with different foraging ecologies show innate attractions different to wood ants (e.g. [8, 14]). Furthermore, behavioural experiments in *Drosophila* have shown that both flying and walking fruit flies show innate visual responses tuned to the flies' behaviour requirements [15, 16], with small objects being aversive and large thin objects being attractive. Aversive behaviour towards small objects potentially helps flies to avoid collision with other flying insects or predators, whereas bar-like objects may represent attractive feeding sites. Olfactory and visual information is commonly used to localize a food source, hence, the question arises if olfactory and visual responses vary with feeding state. Indeed, behavioural experiments in fruit flies and parasitotic wasps have revealed that the animals' feeding state can modulate innate olfactory and/or visual responses [17, 18].

Experienced foragers can also learn visual information for navigation [19-21] and, we know that motivational state (i.e. fed vs unfed) plays an important role in organizing visual navigational memories, allowing for different behaviours in fed and unfed ant foragers [22, 23]. More specifically, visual memories are primed by the ants' feeding state and this controls the choice between foodward and homeward route memories [22].

We investigate here if the innate visual response seen in wood ant foragers is also a context dependent behaviour tuned to foraging ecology. To do so, we recorded the innate visual response of wood ant foragers with different motivational states, i.e. unfed or fed, as well as males that have a short life span and show no foraging activity [24]. We found that ants from all three groups orient towards the visual cue, i.e. the wood ants' innate visual attraction is not flexible, but a hardwired behaviour seen across different motivational and ecological contexts.

METHODS

Ants

Experiments were performed with laboratory kept wood ants *Formica rufa L.* collected from Ashdown forest, East Sussex, UK. Ants were kept in the laboratory under a 12 h light: 12 h darkness cycle at 25-27° C. Ants were fed *ad libitum* with sucrose, dead crickets and water. During the experiments, food was limited to a minimum to increase the ants' foraging motivation, but water was permanently available.

We recorded the innate visual response from naïve wood ants from three different groups: unfed female foragers, fed female foragers and unfed males. To get fed foragers, foragers were fed with sucrose before the behavioural recording. We only recorded males that walked on the platform. Males are winged, however, they are not good flyers, i.e. most of the released males walked.

Experimental setup

Ants from these three groups were released in the centre of a circular platform (120 cm in diameter) surrounded by a cylinder (diameter 3 m, height 1.8 m) with white walls (Figure 1A). A 20° wide black rectangle (height: 90 cm, width: 52 cm) was placed on the inner wall of the surrounding cylinder. As a control, additional ants from the three groups were recorded when the visual cue was absent. Ants were only recorded once. To remove possible olfactory traces, the surface of the platform was covered with white paper which was rotated between recordings. Further, the visual cue was rotated between recordings to avoid the use of cues other than the black rectangle. The centre of the platform consisted of a cylindrical holding chamber of 6.5 cm diameter, which was remotely lowered to release the ant onto the platform. The ants' position was recorded every 20 ms using a tracking video camera (Trackit, SciTrackS GmbH). Paths were analysed in Matlab with similar analyses as done previously [11].

RESULTS

Paths from unfed female foragers, fed female foragers and unfed males that show no foraging activity were recorded in the behavioural arena in the presence of a conspicuous visual object (Figure 1A). Ants from all three groups were directed (Rayleigh test; all $p \ll 0.001$) and the ants walked towards the visual cue (Figure 1B). The 95% CI for the ants' final headings overlaid with the visual cue and the groups did not differ from each other (Watson Williams tests; all $p > 0.05$). Whereas ants from all three groups approached the visual cue (Figure 1B), we observed differences in the ants' walking speed (Figure 2Ai) and path straightness (Figure 2Aii). Males walked significantly faster and straighter than fed and unfed foragers. Unfed and fed foragers differed in their walking speed but not in the path straightness (Figure 2).

In the absence of the visual cue (Figure 1C), unfed foragers were not directed (Rayleigh test; $p > 0.05$), whereas the other two groups were directed (Rayleigh test; males, $p < 0.01$; fed foragers, $p < 0.05$) but showed a very large spread in directions. Males walked significantly faster and straighter than fed and unfed foragers (Figure 2B). Unfed and fed foragers did not differ from each other.

DISCUSSION

Conspicuous objects initiate innate behaviour in many insects, including ants (fruit flies: [25-27], locusts: [28], ladybirds: [29], mantids: [30], leaf hoppers: [31], ants: [7-11, 14]), with many of these innate behaviours being ecologically tuned (e.g. prey detection, predator avoidance or landing site detection). Similarly, innate visual responses of different ant species are tailored to the ants' habitat. Whereas wood ant foragers are attracted to large conspicuous objects [7, 9-11], desert ants avoid them [8, 14]. Wood ants (*Formica rufa*) inhabit cluttered woodland habitat where they predominantly feed on honeydew from aphids on trees [12]. *Cataglyphis fortis* desert ants forage on dead arthropods that are unpredictably distributed on the ground in the food-scarce terrain of the Saharan salt pans [6]. *C. fortis* avoid large objects such as bushes, potentially to avoid predators. Hence, the differences in the innate bias of wood and desert ant foragers make sense from an ecological point of view and seems to be tuned to their foraging ecology. What we do not know, however, is whether these innate visual responses vary with an ants' motivational state or caste. To test this, we recorded the innate visual behaviour of unfed foragers, fed foragers and males that have a short life span and show no foraging activity. We show here that the innate visual attraction to conspicuous objects in wood ants is not a context dependent behaviour, but a hardwired sensori-motor behaviour seen across different motivational and ecological contexts. It is possible that males are attracted to conspicuous objects in order to gain elevation to assist in dispersal. However, any role in innate visual orientation that assists in foraging would expect to be modulated by the ants' motivational state.

There are several examples of flexible innate behaviours in insects. For example, it was shown in parasitoid wasps that the insects' individual feeding state controls their innate behaviour [18]. Unfed wasps are attracted by flower odours and yellow targets that indicate food while fed wasps are attracted to host odours and are not attracted by yellow colours [18]. Furthermore, experiments with hawkmoths have shown that the moths' innate colour preference depends on ambient light conditions [32]. These moths are crepuscular and their colour preferences are tuned to illuminance and background. This flexible behaviour allows them to successfully forage under different light conditions. These examples show that insects are equipped with innate visual preferences, but they maintain necessary behavioural flexibility and behaviour is tuned to foraging ecology.

Given that there are many examples of flexibility, there is the question why wood ants do not show any flexibility in their innate visual orientation. Perhaps, ant foragers rely on their multimodal navigation toolkit to overcome potential problems with an inflexible innate visual reflex. Fed foragers will have path integration information and odour cues and visual information [1] as they attempt to return to their nest and these sources of orientation information may be essential in overcoming innate visual attractions that may otherwise disrupt a homeward journey.

FUNDING

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DATA ACCESSIBILITY

The dataset generated during this study is available from the University of Sussex research repository (hosted by Figshare) [33]: https://doi.org/10.25377/sussex.14270330.

AUTHORS' CONTRIBUTIONS

CB and PG conceived the study. CB performed the experiments, analysed the data, and drafted the manuscript. PG revised the manuscript. Both authors have approved and agree to be accountable for all content of the manuscript.

FIGURES

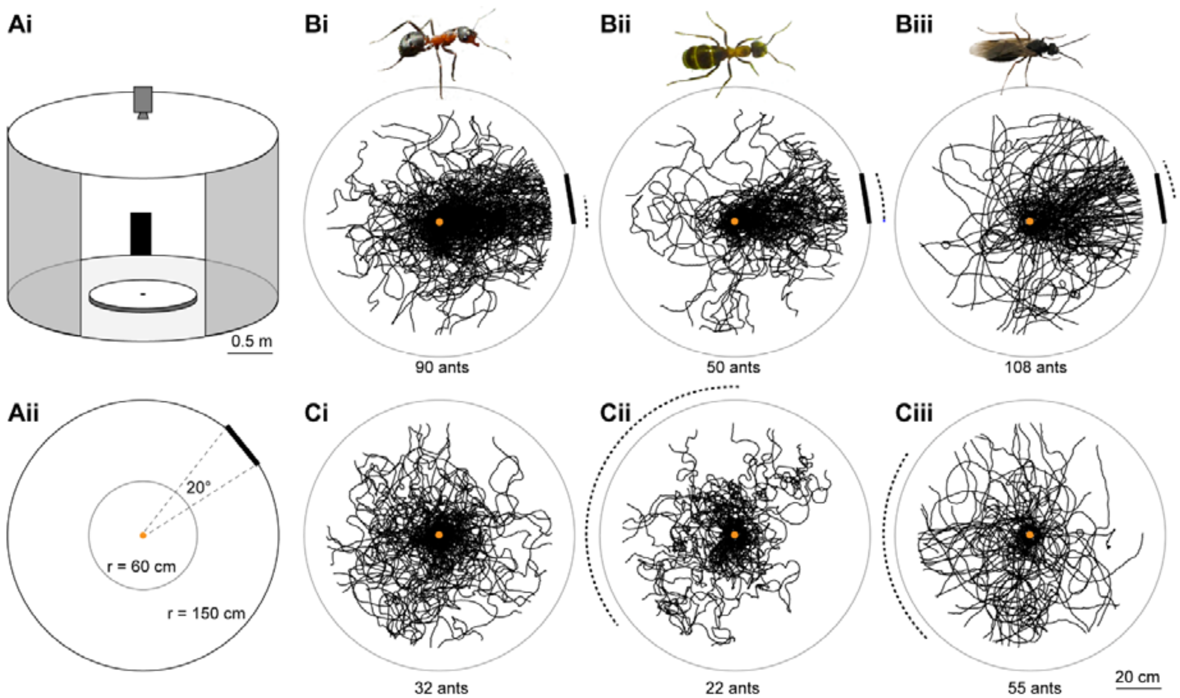

Figure 1: Innate visual attraction in wood ants is seen across different motivational and ecological contexts. (Ai) The experimental arena in which naïve ants were recorded. Circular white platform (radius: 60 cm) is located in the centre of a cylinder (radius: 1.5 m, height: 1.8 m). A 20° wide black rectangle (height: 90 cm, width: 52 cm) is mounted at the inner wall of the surrounding cylinder. A camera recorded the ants' paths from above. A small door permitted access to the arena shown here open and larger for clarity. **(Aii)** A top-down view of the arena shown in Ai. **(B)** Paths of ants released at the centre of the arena in the presence of the visual cue are shown as black lines. If the data is directed, dotted arcs show 95% confidence intervals (CIs) of the heading directions. The visual cue is shown at the platform edge instead of on the cylinder wall. Bi: unfed female foragers; Bii: fed female foragers; Biii: unfed males. **(C)** As in B but ants were recorded without the visual cue.

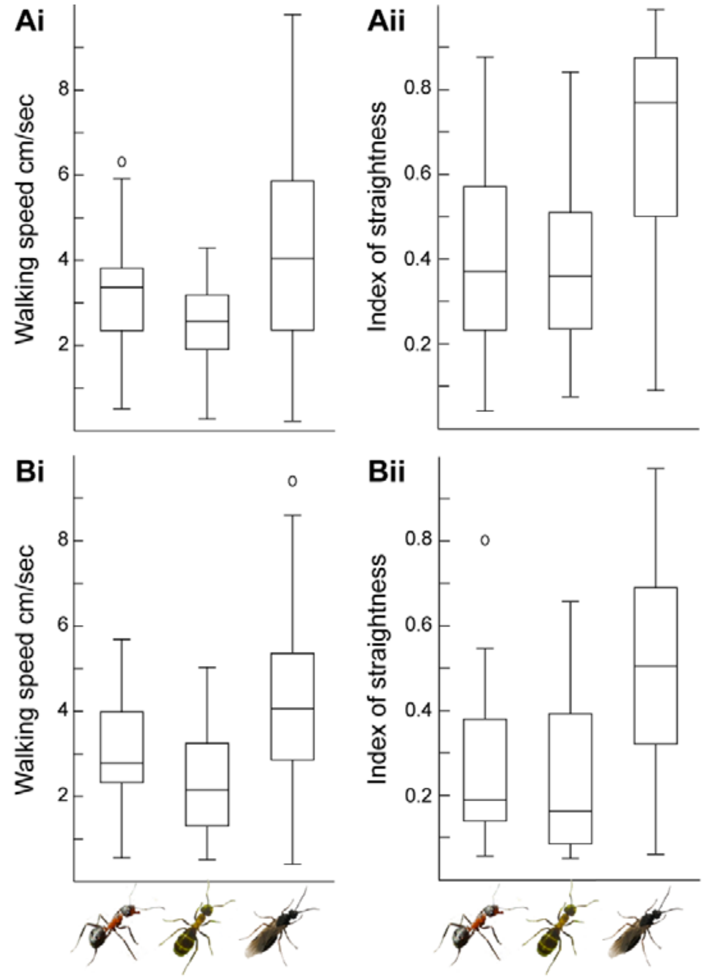

Figure 2: Walking speed and path straightness of ants with different motivational and ecological contexts. (A) Path characteristics in the presence of the visual cue. Unfed, n = 90 ants; fed, n = 50 ants; males, n = 108 ants. **(Ai)** Walking speed of ants differed significantly between the three groups (left: unfed foragers; middle: fed foragers; right: males). Kruskal Wallis with Mann Whitney test and Bonferroni correction; unfed vs fed, $p < 0.001$; unfed vs males, $p < 0.05$; fed vs males, $p < 0.001$. **(Aii)** Path straightness of males was significantly higher than of fed and unfed foragers (Kruskal Wallis with Mann Whitney test and Bonferroni correction; unfed vs males, $p < 0.001$; fed vs males, $p < 0.001$). There was no difference between the paths of fed and unfed foragers (Kruskal Wallis with Mann Whitney test and Bonferroni correction; unfed vs fed, $p > 0.05$). **(B)** Path characteristics in the absence of the visual cue. Unfed, n = 32 ants; fed, n = 22 ants; males, n = 55 ants. **(Bi)** Walking speed of males was significantly higher than observed in unfed and fed foragers but the latter two groups did not differ from each other. Kruskal Wallis with Mann Whitney test and Bonferroni correction; unfed vs fed, $p > 0.05$; unfed vs males, $p < 0.01$; fed vs males, $p < 0.001$. **(Bii)** Path straightness of males was significantly higher than of fed and unfed foragers (Kruskal Wallis with Mann Whitney test and Bonferroni correction; unfed vs males, $p < 0.001$; fed vs males, $p < 0.001$). There was no difference between the paths of fed and unfed foragers (Kruskal Wallis with

Mann Whitney test and Bonferroni correction; unfed vs fed, $p > 0.05$). Boxplots: median, 25th and 75th percentiles (edges of the boxes) and whiskers for extreme values not considered as outliers (circles).

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