

## RESEARCH ARTICLE

# Vision does not impact walking performance in Argentine ants

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## ABSTRACT

Many walking insects use vision for long-distance navigation, but the influence of vision on rapid walking performance that requires close-range obstacle detection and directing the limbs towards stable footholds remains largely untested. We compared Argentine ant (*Linepithema humile*) workers in light versus darkness while traversing flat and uneven terrain. In darkness, ants reduced flat-ground walking speeds by only 5%. Similarly, the approach speed and time to cross a step obstacle were not significantly affected by lack of lighting. To determine whether tactile sensing might compensate for vision loss, we tracked antennal motion and observed shifts in spatiotemporal activity as a result of terrain structure but not illumination. Together, these findings suggest that vision does not impact walking performance in Argentine ant workers. Our results help contextualize eye variation across ants, including subterranean, nocturnal and eyeless species that walk in complete darkness. More broadly, our findings highlight the importance of integrating vision, proprioception and tactile sensing for robust locomotion in unstructured environments.

**KEY WORDS:** Kinematics, Eye anatomy, Antennae, Speed, Obstacle, Uneven terrain

## INTRODUCTION

Walking involves long-distance navigation, avoidance of intermediate-range obstacles and coordination of the body to ensure stability on uneven terrain (Logan et al., 2010) (Fig. 1). In many insects, such as ants, navigation relies on visually sensing distant environmental features (Cheng et al., 2009; Graham and Cheng, 2009; Graham and Philippides, 2017; Narendra et al., 2013b; Wehner and Muller, 2006). At close range, vision informs slow reaching of the limb, such as during gap crossing (Niven et al., 2010; Pick and Strauss, 2005). However, when walking insects approach obstacles rapidly, visual sensory delays may preclude vision-based responses to obstacles and terrain features (Haselsteiner et al., 2014; Hölftje and Hustert, 2003). To test how vision impacts walking performance, we tracked ant walking speeds and step obstacle crossing in light and dark conditions.

The ability for vision to inform locomotion planning requires both visual detection of environmental features and rapid processing of this information. Visual detection of looming obstacles depends on their size and distance, plus the resolution and field of view of the animal. Numerous ant species react to large environmental landmarks for navigation. However, smaller objects at closer range may be undetectable or, if detectable, may limit the available reaction time.

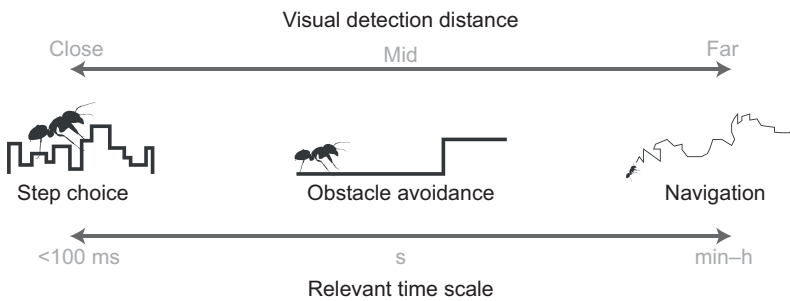
Ants dynamically adjust foraging trails to avoid new mid-range obstacles, but this behavior depends on the ant's eye resolution (Palavalli-Nettimi and Narendra, 2018). For near-field objects, insects often incorporate visual information during slow movements of the limb, such as limb targeting and gap crossing (Blaesing, 2004; Collett, 2002; Dürr, 2001; Niven et al., 2010, 2012; Pick and Strauss, 2005). This closed-loop control substantially guides walking in large animals (Wilkinson and Sherk, 2005) and humans (Patla and Greig, 2006; Reynolds and Day, 2005a,b; Smid and den Otter, 2013). For insect walking, visual guidance of the limbs and reaction to near-field obstacles may apply to slow limb-by-limb 'metachronal' movements in ambling walkers such as stick insects (Graham, 1972). However, it is less certain how vision influences fast-walking hexapedal insects, like cockroaches and ants, that typically use an alternating tripod gait (Full and Tu, 1990; Zollikofer, 1994). Stepping with three limbs at a time allows the insect's center of mass to stay within a triangular support area, providing passive stability to withstand perturbations without sensory feedback (Couzin-Fuchs et al., 2015; Jindrich and Full, 1999, 2002). Although the stability of this gait can limit the impact of vision, recent studies identify considerable gait variation during unconstrained walking on flat ground (Bender et al., 2011; Szczecinski et al., 2018). Uneven ground likely induces even greater deviations from the alternating tripod, increasing the potential importance of visual feedback.

Compared with large animals, small animals like ants experience greater substrate unevenness (Kaspari and Weiser, 1999) and walk with higher step frequencies (Heglund et al., 1974; Lee et al., 2016), furthering the need for secure foot placement but limiting the within-stride reflex time for sensory signals. Responding to vision requires transduction within the photoreceptors, processing and neural conduction to the responsive systems. Transduction and processing times for photoreceptors range between 30 and 150 ms in cockroaches (Heimonen et al., 2012; Ignatova et al., 2020), 35–60 ms in hawkmoths (Krishnan and Sane, 2014) and 30–250 ms in blow flies (Land and Collett, 1974; Laughlin and Weckstrom, 1993; Warzecha and Egelhaaf, 2000). Comparatively, neural conduction speeds are relatively fast at 0.5–3.7 m s<sup>-1</sup> in cockroaches (Pearson et al., 1970), with shorter transmission distances but likely narrower axons, and therefore slower conduction speeds, in smaller animals. While some ant species reach stride frequencies up to 40 Hz (Reinhardt and Blickhan, 2014; Pfeffer et al., 2019), Argentine ants walk at an average of 10–12 strides s<sup>-1</sup> (Clifton et al., 2020), limiting intrastride response times to within 100 ms. This cutoff lies well within the temporal acuity limit for honeybees (up to 100 Hz, corresponding to 10 ms) (Srinivasan and Lehrer, 1984; Srinivasan and Lehrer, 1985). To our knowledge, temporal acuity has not been measured in ants; however, given that photoreceptor properties vary with flight propensity (Frolov et al., 2017), the temporal acuity of walking ant workers is likely slower than that of honeybees. Therefore, fast movements of the limbs during walking may preclude visual feedback (Full and Koditschek, 1999). Nocturnal and subterranean species particularly confront feedback constraints because transduction speeds depend on both light levels and

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**Fig. 1. Impact of vision on *Linepithema humile* (Argentine ant) walking across spatiotemporal scales.** (A) Ants navigate by sensing long-distance visual features; however, visual perception of mid- or close-range objects can influence walking performance. Closer-range visual perception restricts effective feedback timing, with fast-step frequencies potentially preventing ants from using visual perception at these scales.

temperature (Frolov and Ignatova, 2020; Heimonen et al., 2012; Warrant, 2017; Warzecha and Egelhaaf, 2000). These data suggest that vision might not influence intrastride walking coordination but may inform obstacle avoidance at intermediate distances. However, few studies have directly tested the impact of vision on step-to-step walking performance with ‘effective blindness’ presented as a novelty (Gilbert, 1997; Jayaram et al., 2018), whereas it may be widespread among small, fast insects.

In addition to sensing their surroundings using vision, many walking insects rely on chemosensory and tactile information. Insect antennae span diverse morphologies, sensing chemicals, heat, humidity and mechanical feedback (Krishnan and Sane, 2015). During walking, both cockroaches and stick insects sweep their antennae to identify nearby obstacles (Baba et al., 2010; Dürr et al., 2001; Harley et al., 2009; Okada and Toh, 2004, 2006). Ants possess jointed antennae that they actuate to identify odors (Draft et al., 2018) and tactile features (Klotz and Reid, 1992) along foraging trails. Ant antennae also buttress against the walls of vertical tunnels, preventing workers from falling (Gravish et al., 2013), actively adjust and manipulate soil carried in their mandibles (Monaenkova et al., 2015), and allow conspecific discrimination (Ozaki et al., 2005; van Zweden and d’Ettorre, 2010). However, to the best of our knowledge, antennal activity has not been directly associated with ant walking performance or compared across lighting conditions. Mechanosensory feedback is faster than visual feedback (Sherman and Dickinson, 2004; Yarger and Fox, 2016); however, mechanical feedback stems from both antennae and the limbs (Bingman et al., 2017; Hebets, 2002; Isakov et al., 2016), which may differentially contribute under varying environmental conditions.

The Argentine ant (*Linepithema humile*) forages both diurnally and nocturnally as conditions permit (Abril et al., 2007; Human and Gordon, 1996), with workers regularly walking up to 60 m away from their nests (Hogg et al., 2018; Vega and Rust, 2003). Pheromone-based recruitment trails often cross a variety of substrates, including uneven terrain. Workers possess relatively large eyes compared with closely related species (Wild, 2004), but, unlike most other species (Knaden and Graham, 2016), visual cues do not impact navigation (Aron et al., 1993). The Argentine ant’s ability to travel relatively large distances in both light and dark conditions makes this species an appealing model for studying how vision impacts walking performance.

Effective walking is integral to the survival of Argentine ant workers and, by extension, colony-level performance. Thus, one would expect selective pressures to optimize sensory feedback towards maintaining foraging performance under varying environmental conditions. However, vision does not contribute to long-distance navigation in this species (Aron et al., 1993), and the latency of visual reaction times in invertebrates may prevent intrastride feedback, which is particularly relevant for identifying appropriate footholds on uneven terrain. Instead, we expect vision to impact the identification of and pre-

planning for close-range obstacles. We test the influence of vision by tracking full-body walking speeds on flat versus uneven terrain and in bright versus dark conditions. Because antennal activity could compensate for lost visual feedback in darkness, we also quantify and compare antennal activity. We show that darkness causes a relatively minor decrease in walking speed compared with terrain unevenness, with no measurable difference in step obstacle crossing or antennal activity. Vision, overall, plays a minor role in Argentine ant walking.

## MATERIALS AND METHODS

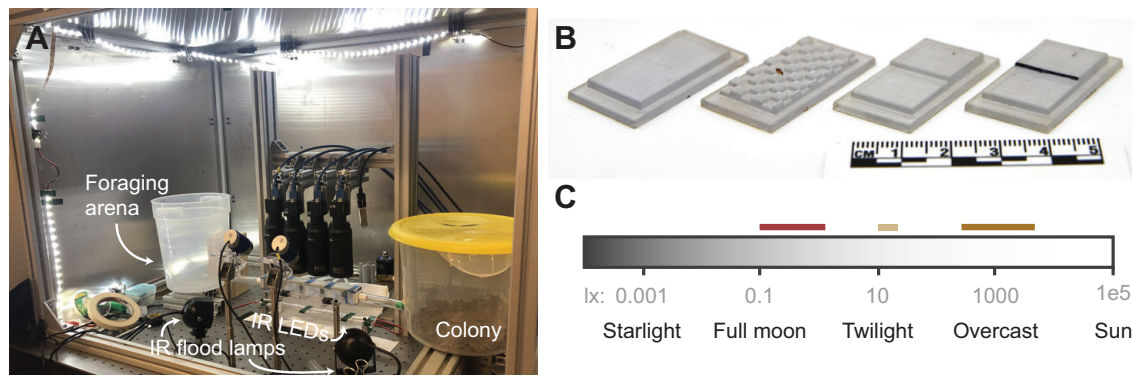
### Study animals

Argentine ants [*Linepithema humile* (Mayr 1868)] were collected from six locations in San Diego County, CA, USA, during February and March 2020. Argentine ants form supercolonies in southern California (Tsutsui et al., 2003; Thomas et al., 2006), so our reference to ‘colony fragments’ refers to groups of workers collected from different locations and on different dates. Once a large aggregation of ants was found, 100–500 workers and the surrounding soil were transferred to a plastic container and stored in a custom recording arena in the laboratory. Ants were housed overnight until recording began the following morning. Ants were housed in the laboratory for ~30 h in total. Data from one collection day were removed from analysis because many ants congregated within the tunnel.

### Details of experiment and recording

Ant colony fragments were collected between 07:00 h and 10:00 h then allowed to acclimate for at least 7 h in the laboratory before opening a pathway through a 3D-printed tunnel (Connex3 Objet 350, VeroClear material, Stratasys Inc., USA) to a foraging arena (Fig. 2A). The foraging arena contained food made from sugar, water and polymer crystals (to delay evaporation). After opening the foraging arena, the set-up was not disturbed for the remainder of the experiment. Overnight, ants from each colony fragment explored the foraging arena and developed a recruitment trail through the tunnel. Each colony fragment was then filmed during two recording sessions, from 07:00 h to 14:00 h on subsequent days. Temperature measurements were collected every 30 s while recording (Jepeak BE300263), ranging between 25.69°C and 25.94°C. After the recording session, the colony fragment was released back to its collection location, and the tunnel and substrates were cleaned in warm, soapy water. The equipment dried overnight, dissipating any collected pheromone trails before the first recording session for the next collected colony fragment.

For each colony fragment, four 3D-printed substrates (Connex3 Objet 350, VeroClear material, Stratasys Inc.) were randomly positioned in the tunnel leading from the colony container to the foraging arena (Fig. 2B). In addition to a flat substrate, we included two substrates with a single 1 mm step (one with the edge of the step colored black using a Sharpie marker 12 h before testing so it could



**Fig. 2. Experimental set-up for quantifying *L. humile* walking performance with varying lighting.** (A) A fully enclosed arena housed a colony fragment of Argentine ants. Workers formed a pheromone trail through a 3D-printed tunnel connected to a foraging arena. Four high-speed machine vision cameras recorded ant motion in the tunnel. White LEDs, infrared (IR) flood lights and IR strip LEDs beneath the tunnel illuminated the enclosure. (B) Four 3D-printed substrates were inserted in random order into the tunnel floor, including a flat control, a 3 mm checkerboard uneven substrate and two step obstacles. (C) Light levels vary by over  $10^8$ -fold throughout the day. Argentine ants walk in complete darkness while in tunnels and forage throughout the day and night. We measured ant walking performance under dark (red), bright (yellowish-brown) and dusk (beige; results in Fig. S4) conditions. Lux values obtained from Cronin et al. (2014).

dry) and an uneven checkerboard with a box width of 3 mm and step height of 1 mm. A step height of 1 mm approximates the coxa ‘hip’ height of Argentine ant workers (estimated from publicly available photos of live ants), therefore challenging the ant without requiring vertical climbing.

During a recording session, two web cameras (YoLuke A860-Blue, Jide Technology, China) focused on the tunnel automatically detected an incoming ant and triggered one of four machine vision cameras (Blackfly S 13Y3M, Point Grey Research Inc., Canada). The cameras were each attached to a varifocal lens (20–100 mm; 13VM20100AS, Tamron, Japan). After being triggered by a web camera, each machine vision camera recorded for 3 s at 240 frames  $s^{-1}$  (720 frames total,  $1000 \times 500$  pixels) then was paused for 80 s to reduce the probability of re-recording the same ant. A still frame from the triggering web camera was saved in association with every video. In total, we recorded and analyzed over 4200 videos.

The experimental set-up was illuminated by white light-emitting diodes (LEDs; Lighting Ever, Daylight White, 6000 K) on a 12 h:12 h light:dark cycle. The tunnel was backlit using infrared (IR) LEDs (SMD3528-300-IR, 850 nm). During the recording session, the white LEDs were turned off every hour for 30 min using a wall outlet timer (15119, General Electric). During these dark periods, two IR flood lights (850 nm; Tendelux, Shenzhen, China) turned on to illuminate the tunnel from the side, enabling ant detection by the web cameras. Ants possess two or three spectrally distinct photoreceptor types (Ogawa et al., 2015). Although the spectral sensitivities of Argentine ants has not been measured, no ant species has demonstrated a sensitivity to light above 650 nm (Aksoy and Camlitepe, 2018; Briscoe and Chittka, 2001; Ogawa et al., 2015). Therefore, the use of 850 nm lighting most likely acts as complete darkness for Argentine ants. The illumination of the two lighting conditions was  $<1.8$  versus 200–2500 lx (Extech LT40, NH, USA) (Fig. 2C). The measured light lux levels ranged according to the orientation of the luxmeter, which was held at the tunnel location and rotated, replicating the possible directions the ants were facing while walking. Our measured lux values align with light levels measured for bull ants foraging on a dark evening and in bright light (Narendra et al., 2013b). A separate dataset of nine colony fragments was collected in August 2019, comparing dark and dusk light levels (60 lx), with summary figures in Fig. S4. Findings from the dusk light dataset aligned with those presented here, with the exception that ants walked, on average, at faster

speeds. Speed variation might result from collecting ants during the summer, owing to the dramatic seasonal cycles within Argentine ant colonies (Markin, 1970).

Determining the visual acuity of a compound eye requires measuring both external (e.g. facet) and internal (e.g. rhabdom) dimensions. While external features alone cannot specify visual capacity, facet dimensions and distributions enable cross-species comparisons and a first-order estimation of visual resolution. To quantify the facet dimensions of Argentine ant workers, we painted the heads of 20 individuals with clear nail polish (as in Narendra et al., 2016). We removed the nail polish molds and visualized each eye using a microscope (Olympus BX51,  $40\times$ ). The eye molds were not flat and unable to be flattened with release cuts because of their small size. Instead, we took photos at multiple focal planes ( $>10$  per eye) and focus stacked the images (Python; <https://github.com/cmcguinness/>). This method is most likely to underestimate the area of facets towards the edge of the eye, but will not overestimate facet measurements. We selected the five clearest eyes for analysis. The facets in each eye were manually outlined and measured in ImageJ (Schneider et al., 2012), using a calibration from photos of a resolution test target (NBS 1952, Thorlabs Inc., NJ, USA). We calculated interommatidial angle by assuming the eye has a hemispherical field of view and dividing by the number of facets (Narendra et al., 2016; Palavalli-Nettimi and Narendra, 2018):

$$\Delta\theta = \sqrt{\frac{\Theta/2}{N}}, \quad (1)$$

with  $\Theta=41,253 \text{ deg}^2$  in a circle and  $N$  facets.

### Overview of tracking ants in high-speed videos

Each video was analyzed to track the body and antennae of all ants in view. This approach consisted of three steps. First, ant-centered videos were generated by coarse estimation of ant locations in each frame then associating identified ants across frames, resulting in ‘trackways’. Second, trackways were used to generate ant-centered videos. Third, the ant-centered videos were processed using a deep-learning-based tracking software (Pereira et al., 2019), outputting the locations of four landmarks along the body and three points along the antennae. These data were then processed to remove any likely poor tracking by the program. Each step is detailed below.

### Full-body tracking

Tracking ant locations in each frame was achieved by identifying all ants in each frame and associating individuals across frames. Briefly, to identify ants in each frame, we normalized the video using background division, used image processing to isolate the body of each ant, and fitted contours to each ant body to estimate the location and orientation. Ants identified in each frame were associated across frames using a Kalman filter generating trackways (Straw et al., 2011). For details, see the supplementary material in Clifton et al. (2020).

### Generation of ant-centered videos

The facing of the ant in each Kalman-associated trackway was estimated by attempting to find the asymmetries in the isolated contour due to the antennae. Then ant orientation was processed to remove any 90 or 180 deg jumps caused by errors. Once the orientation was consistent throughout a trackway, it was filtered using a moving average (window size=11 frames, ignoring any windows with fewer than two non-nan values). The  $x$ - and  $y$ -coordinates of the trackways were also filtered using a low-pass Butterworth filter (Scipy,  $N=2$ ,  $\omega_n=0.2$ ).

Each trackway was used to generate ant-centered images with a dark background. Each background-divided frame was rotated so that the direction of the ant facing aligned with the  $+x$  direction (`imutils.rotate_bound`, Python) and cropped to a shape of  $200 \times 200$  pixels around the center of the ant. Each cropped, background-divided image was then inverted and adjusted to increase the contrast between ant and background. To remove any other ants in the cropped frame, we identified any non-central, large foreground objects and removed those that intersected with the edge of the cropped frame. Further details can be found in Clifton et al. (2020). This process resulted in a video with an isolated, light-colored ant centered in the frame that was walking towards the right against a dark background.

Although our method of finding the head orientation of the ant in each frame was mostly accurate, occasionally we generated a video with the ant facing towards the left. To identify and flip these videos, we used a support vector machine (SVM). A training group of 1345 ant-centered pictures was used to train the SVM to classify three ant-facing categories: right (ant faces in  $+x$  direction), left (ant faces in  $-x$  direction) or blank (the ant-centered picture is completely black, as would be generated if the ant-facing angle=nan). The first 100 frames of each trackway-cropped video were classified as left, right or blank. If the number of left-facing images outnumbered the right-facing images, the ant-facing angles and ant-centered images for the trackway were rotated by 180 deg.

### LEAP: deep-learning tracking of body and antennae

We used a recent deep-learning approach, implemented in MATLAB and Python (Pereira et al., 2019), to track landmarks on the ant in each video. We specified a skeleton of connected points to track, including six along the body (gaster tip, waist, neck, mandible, eyes) and three along each antennae. We manually identified these points in a training set of 140 frames. The LEAP tracker then predicted the landmark locations in all videos, requiring between 3 s and 30 s, depending on the number of frames. The LEAP network was trained using the following settings: scale=1, kernel for confidence maps=5, mirrored images enabled, leap\_cnn network architecture, 64 base filters, 5 deg rotation angle, 25 epochs, 50 batches per epoch, 50 samples per batch, validation fraction=0.1, AMSGRAD enabled, learning rate reduction factor=0.1 after three epochs, with the learning rate changing by less than  $1e-5$ .

The general kinematic variability of ant walking, especially on uneven terrain, resulted in some inaccurate tracking predictions. Identifying and removing these points was a multistep process, with full details in the supplementary methods of Clifton et al. (2020). Briefly, predicted landmarks with a low confidence value (output from the LEAP tracker) were removed, along with points that jumped by more than 10 pixels ( $\sim 0.33$  mm) within one frame. We also removed any data point that deviated from the average position of that landmark (calculated using the middle 50% of points) by more than two-thirds of the middle 50% range. The  $x$ - and  $y$ -coordinates of each landmark were then low-pass filtered (Scipy, Butterworth,  $N=2$ ,  $\omega_n=0.3$ ), while removing sections of the tracking with fewer than nine consecutive non-nan values. If, after this processing, fewer than 50 finite (non-nan) data points remained in the full landmark trace, that trace was removed from further analysis. Past comparisons of this approach with manually tracked landmarks demonstrated a high accuracy of the resulting post-processed data points.

### General analysis

The lighting condition for each video was determined by evaluating the associated web camera still frames. The lighting timer was not accurate in switching the lighting exactly every 30 min, so instead the timing of the lighting switches was determined by comparing the average illumination in each photo. Any videos within 5 s of a lighting switch were removed from further analysis.

Ants close to the edge of the video frame may be partially off-screen and therefore were not always reliably tracked. Similarly, ants near the edge of a substrate could behave unusually, owing to the transition from the flat tunnel floor. To identify these instances, image processing was used to locate the dark edges of the substrate and the step in each video. Any data points where the center of the ant was within 2 mm ( $\sim 64$  pixels) of a substrate edge were removed. The information on the step location was later used for analyzing ant walking while approaching and climbing the step.

While ants primarily walked through the tunnel to and from the foraging arena, they displayed some behavioral variation, including antennal cleaning and conspecific interactions. To identify and be able to remove these stationary behaviors from future analysis (e.g. walking speed comparisons), we low-pass filtered the instantaneous speed (Scipy, Butterworth,  $N=2$ ,  $\omega_n=0.6$ ) and removed any sections where this average walking speed dipped below  $3 \text{ mm s}^{-1}$ .

### Body speed and trajectory analysis, flat and uneven substrates

The low-pass filtered  $x$ - and  $y$ -coordinates of the waist throughout each trackway (excluding slow behaviors as described above) were used to calculate instantaneous velocity, which was then low-pass filtered again to remove the large accelerations present in ant walking. The resulting velocity was decomposed relative to the orientation of the ant, generating a forward speed. The distance traveled at each speed was used to create a 'histogram' of walking speeds (see Fig. 4A). The preferred, 'average' speed was calculated from the median of the distribution, while the top speed was determined from the cut-off for the fastest 5% of the total distance traveled under each condition (e.g. light on flat substrate). To test whether the distributions differed from each other, we used a Mann–Whitney  $U$ -test, with 2 mm of distance traveled representing one data point.

To test whether the trajectories of the ants differed on flat versus uneven substrates or under light versus dark conditions, we calculated the straightness, sinuosity and fractal dimension for each trackway. Straightness was defined as the total distance traveled divided by the

net distance traveled. Sinuosity was calculated based on Bovee and Benhamou (1988). Briefly, each trackway section was split into  $\sim 2$  mm steps (based on an average 2 mm step length observed in Clifton et al., 2020). The turning angle traveled over each step ( $\phi$ ) was used to determine the mean cosine ( $c$ ) and mean sine ( $s$ ) of the changes in direction. From there, sinuosity ( $S$ ) was calculated as:

$$S = 2 \times \left( p \times \left( \frac{1 + c^2 - s^2}{(1 - c)^2 + s^2} \right) + b^2 \right)^{-0.5}, \quad (2)$$

where  $p$  is the average step length (2 mm) and  $b$  is the standard deviation of step length errors compared with the average step length. Finding  $b$  resulted from how we identified the ant location at each step. The pathway was divided into  $\sim 2$  mm steps by identifying when the actual distance traveled approached a multiple of 2 mm. The errors of the actual step lengths from an ideal 2 mm were compiled, and the standard deviation of this distribution represented  $b$ . We also re-calculated sinuosity using  $b=0.5$  mm and saw no change in the results.

The fractal dimension of each trackway section was determined by segmenting the trackway by multiple lengths and comparing the net distances traveled for those segments to the segment lengths. For example, a 7 mm-long trackway was segmented into 0.15 mm travel distances, and the net distances traveled for all sections were summed. This approach was repeated for sections of 0.63, 1.10, 1.58, 2.05, 2.52, 2.99 and 3.47 mm. A linear regression (Scipy, linregress) of the summed net distances versus section length resulted in a slope of 2.07. The fractal dimension ( $D$ ) was defined as  $D=1$ –slope. The segment lengths chosen for each trackway were chosen as ranging inclusively from 0.15 mm to half of the total distance traveled, with a step value of the total distance traveled/15 [i.e. (0.15 to  $d_{\text{total}}/2$  by  $d_{\text{total}}/15$ )].

These values were calculated for all sections of each ant trackway and then averaged. Any sections with fewer than 20 data points ( $<83.3$  ms) or where the ant traveled  $<3$  mm were excluded. For sinuosity and fractal dimension calculations, any time we did not observe a location of the ant within 2 pixels (0.06 mm) of the segmented distance (e.g. every 2 mm for sinuosity), the trackway section was excluded.

To determine whether there was a significant influence of lighting (light versus dark) on trackway tortuosity, we used non-parametric rank testing. The distributions of straightness, sinuosity and fractal dimensions were highly skewed and unable to be normalized for parametric testing. Instead, the data for each colony fragment and substrate (flat versus array) were analyzed using a Mann–Whitney  $U$ -test, testing the factor of light [wilcox.test( $Y \sim \text{light}$ ), R]. The resulting  $P$ -values are listed in Table S1.

### Step-crossing analysis

To determine whether step-crossing performance alters under light and dark conditions, we calculated four measures: (1) step-crossing time, (2) step-crossing angle, and forward speed (3) far from the step and (4) close to the step. We only included trials in which the ant stepped upwards. For step-crossing time, crossing was defined as starting with the waist at a distance of 4 mm from the step (before any observed deceleration) and ending when the waist passes 1 mm away from the step. For step-crossing angle, we measured the net angular displacement of the ant from crossing starting to stopping. If an ant were to detect the step and veer to walk along the edge, this would be represented as a large crossing angle. For forward speed far from the step, the instantaneous forward speed of each ant was low-pass filtered (Scipy, Butterworth,  $N=2$ ,  $\omega_n=0.6$ ). We calculated the median low-pass filtered speed of each ant when the waist was

between 6 mm and 4 mm before the step. Any trials with fewer than 10 data points within this window were removed. For forward speed close to the step, we calculated the median low-pass filtered speed of each ant when the waist was between 3 mm and 1 mm before the step. Any trials with fewer than 10 data points within this window were removed.

To statistically test for differences in these parameters caused by substrate type (contrasted versus uncontrasted steps) and lighting (light versus dark), we used linear mixed-effect modeling (lme4::lmer function in R; Bates et al., 2015). Crossing time and speed close to the step were normalized using a logarithmic function. The goodness of fit of these models was confirmed by examining quantile–quantile and residual plots. We used the following full models:  $\log(\text{cross\_time}) \sim \text{substrate} + \text{light} + (1|\text{colony})$ ;  $\text{cross\_angle} \sim \text{substrate} + \text{light} + (1|\text{colony})$ ;  $\text{speed\_far} \sim \text{substrate} + \text{light} + (1|\text{colony})$ ; and  $\log(\text{speed\_close}) \sim \text{substrate} + \text{light} + (1|\text{colony})$ . These models were compared with reduced models, removing either the substrate or light factors, and analyzed with a chi-squared likelihood ratio test (LRT; anova function in R).

### Antennal activity analysis

Average antennal speeds were calculated on flat and uneven substrates, and with ants far and close to step obstacles. For flat and uneven terrain, ants were analyzed as they walked through the center of the substrate, with the waist between 11 mm and 19 mm from the left edge of the 30 mm-long substrate. The analysis windows on step substrates coincided with those used for average body speed far and close to the step, from 6 mm to 4 mm and 3 mm to 1 mm before the step, respectively. The tracking of each antenna was referenced to the body of the ant using the vector from the waist to the neck. Instantaneous speed with respect to the body was used to find the median speed estimates for each antenna, which were then averaged.

Estimates of average antennal speed on flat and uneven terrain were analyzed for an influence of light and substrate in a similar manner to the step-crossing metrics above. The exact linear mixed-effect model was  $\log(\text{antennae\_speed}) \sim \text{substrate} + \text{light} + (1|\text{colony})$ . The model for step substrates included close versus far distance as an additional variable:  $\log(\text{antennae\_speed}) \sim \text{substrate} + \text{light} + \text{distance} + (1|\text{colony})$ . As above, chi-squared LRTs (anova function in R) tested the influence of each fixed variable.

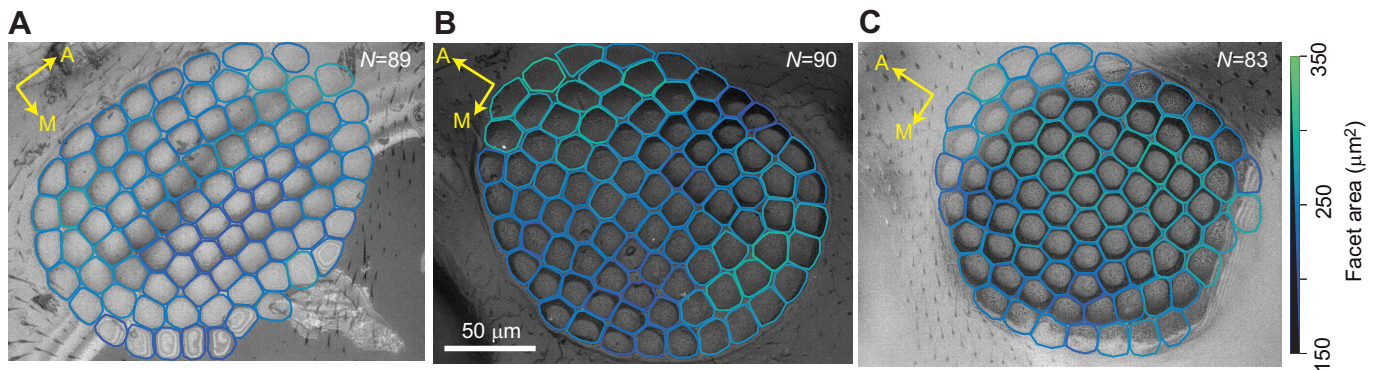
## RESULTS

### Argentine ant eye anatomy

The compound apposition eyes of insects are comprised of ommatidia, which each include a lens and multiple receptors. Quantifying the visual acuity of compound eyes requires measurements of external features (e.g. ommatidia number, facet dimensions) and internal features (e.g. rhabdom dimensions) (Land, 1997a). Argentine ant workers possess between 80 and 100 ommatidia per eye (Wild, 2004). We measured eye area to be  $0.026 \text{ mm}^2$  with an interommatidial angle of  $15.3$  deg. Ommatidia facets were approximately hexagonally spaced with areas ranging from  $184 \mu\text{m}^2$  to  $302 \mu\text{m}^2$  and diameters ranging up to  $20$ – $26 \mu\text{m}$  (Fig. 3, Fig. S1). Medial and lateral facets were relatively smaller, with the largest facets along the anterior and posterior margins of the eye (Fig. 3).

### Ant walking speeds on flat and uneven terrain

On flat ground under visual spectrum illumination, ants preferred to walk at a forward speed of  $19.2 \text{ mm s}^{-1}$ , with 5% of the distance traveled occurring at speeds above  $28.4 \text{ mm s}^{-1}$  (Fig. 4A, Fig. S2). In darkness, preferred speed reduced by 4.7%, with a median speed of  $18.3 \text{ mm s}^{-1}$  (Mann–Whitney  $U$ -test,  $P < 0.001$ ). This pattern was



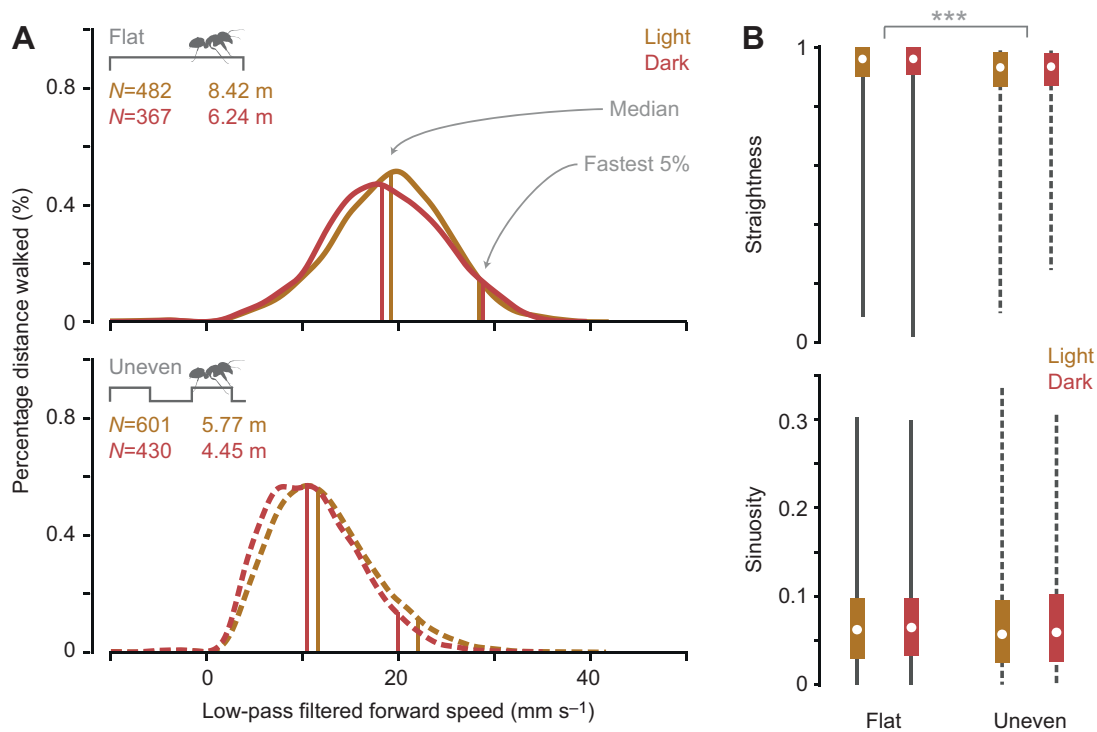
**Fig. 3. Facet outlines of the eyes of three *L. humile* workers.** (A–C) Facets were manually outlined with colors to represent facet area. The total number of facets for each eye is listed in the panels; however not all facets were able to be outlined. The arrows show the anterior (A) and medial (M) orientations of the head. The scale bar shown in B applies across the figure.

consistent across all colony fragments except one, which exhibited increased speed in the darkness (Fig. S2). Ants slowed down considerably on the checkerboard substrate under light conditions, with a median speed of  $11.6 \text{ mm s}^{-1}$ , 39.6% of the preferred speed under flat/light conditions. Compared with this large decrease, preferred speed on uneven substrates declined only by  $1.1 \text{ mm s}^{-1}$  in darkness (9.5%; Mann–Whitney  $U$ -test,  $P < 0.001$ ). Because these speed distributions represent the forward speed aligned with the ant's orientation, shifts in speed preference could derive from changes in turning or trailway shape. We compared the tortuosity of paths under all conditions using measures of straightness, sinuosity and fractal dimension (Fig. 4B) (Almeida et al., 2010; Benhamou,

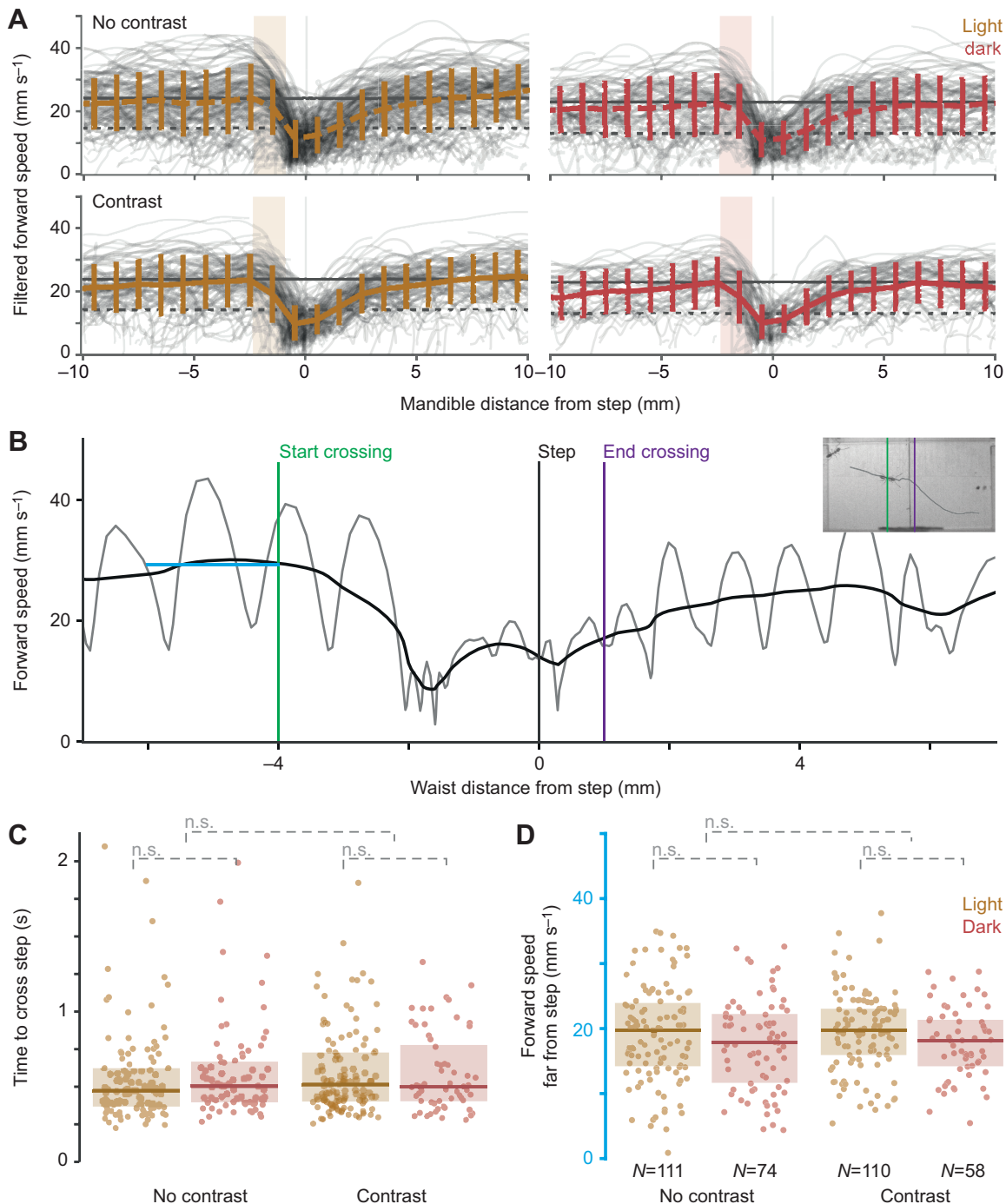
2004; Nams, 1996). Although paths on the uneven substrate were less straight than those on flat ground (Table S1), there was no influence of lighting ( $P = 0.33$ – $0.85$ ) (Table S2).

#### Step crossing in darkness and light

Argentine ant workers walked towards a step obstacle at an average speed similar to that found on flat substrates in both light and dark conditions (Fig. 5A, solid horizontal lines). Workers decelerated when the mandible reached  $\sim 2 \text{ mm}$  from the step (Fig. 5A). While crossing the step, forward velocity remained low and then returned to the average flat ground speed once the worker had moved beyond  $5 \text{ mm}$  beyond the step. The decrease in speed when crossing the



**Fig. 4. Preferred *L. humile* walking speed and tortuosity on flat and uneven substrates.** (A) Distributions showing the distance traveled across speeds for ants on flat (top) and uneven (bottom) substrates in light (yellowish-brown) and dark (red). Vertical lines represent the median speeds and the cutoff for the fastest 5% speed. Light versus dark distributions were significantly different on both flat and uneven substrates (Mann–Whitney,  $P < 0.001$ ) but with a relatively small effect size (4.7% and 9.5%, respectively). (B) Measures of straightness and sinuosity of trackways on flat (solid lines) and uneven (dashed lines) terrain in light (yellowish-brown) and dark (red) conditions. Boxes represent interquartile range with whiskers spanning all data points without excluding outliers. Substrate type significantly, but minorly, impacted straightness (Mann–Whitney,  $***P < 0.001$ ), but lighting had no influence on either metric (see Tables S1 and S2).



**Fig. 5. Performance of *L. humile* workers crossing a 1-mm step obstacle.** (A) Low-pass filtered forward speeds did not decelerate until the ant's antennae reached the step edge in both light (yellowish-brown) and dark (red) conditions. Vertical bars represent  $\pm 1$  s.d. of all speeds observed in 1-mm windows. Horizontal lines represent preferred, median speeds observed on flat (solid lines) and uneven (dashed lines) substrates. Transparent red and yellowish-brown boxes represent the normal location of the antennal tips in front of the mandible (1–2.3 mm). (B) Step crossing was defined as starting when the ant waist reached 4 mm from the step (green line) and continued until crossing 1 mm after the step (purple line). Low-pass filtering the speed removed the relatively large accelerations within each step. The median forward speed far from the step (blue line) was determined from when the ant waist was between 6 mm and 4 mm in front of the step. (C) The time to cross the step did not depend on substrate type (chi-squared LRT test,  $P=0.30$ ) or lighting (chi-squared LRT test,  $P=0.82$ ). Boxes show the median and interquartile range of the distributions. (D) The forward speed far from the step does not differ for substrate ( $P=0.73$ ) or lighting ( $P=0.07$ ) conditions. n.s., not significant.

single step was consistent with the preferred speed on the uneven checkerboard substrate of the same step height (Fig. 5A, dashed horizontal lines).

To reliably compare step-crossing performance in all conditions, we defined step crossing as starting at a distance of 4 mm from the

step (before any observed deceleration) and ending when the petiole of the worker passed 1 mm beyond the step (Fig. 5B). The time required for workers to cross the step did not differ between light and dark conditions (LME chi-squared test,  $P=0.82$ ) or between high-contrast and low-contrast steps (LME chi-squared test,

$P=0.30$ ) (Fig. 5C). Despite a 6.5% difference in walking speed far from the step (Fig. 5D; LME chi-squared test,  $P=0.07$ ), speeds close to the step did not differ (Fig. S3B; LME chi-squared test,  $P=0.75$ ).

### Antennal activity in light and darkness

We found that on the uneven substrate, antennal speed with respect to the body was 35.1% faster than that on flat ground (Fig. 6B; LME chi-squared test,  $P<0.001$ ); however, lighting did not have a significant effect (LME chi-squared test,  $P=0.17$ ). Antennal speed decreased in darkness, with a 0.2% and 1.0% decrease on flat and uneven terrain, respectively. We also found that antennal speeds increased for ants close to a step (LME chi-squared test,  $P<0.001$ ), but with no significant impact of step contrast ( $P=0.03$ ) or lighting ( $P=0.84$ ). Qualitatively, Argentine ant workers moved their antennae over a larger area while walking on uneven terrain or while close to the step (Fig. 6C); however, lighting conditions did not appear to influence exploration range.

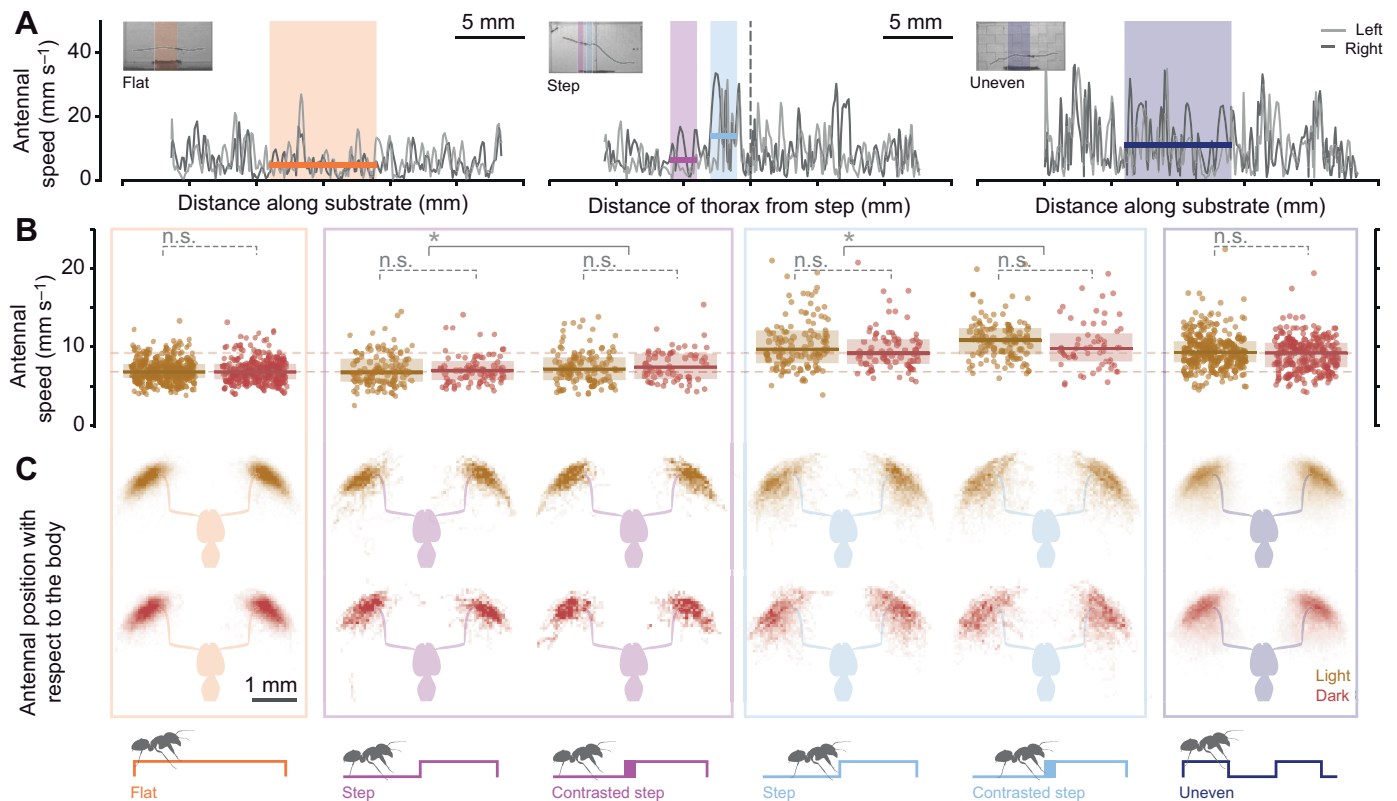
## DISCUSSION

### Argentine ant eye anatomy

The eyes of Argentine ant workers, with 80–100 ommatidia, have fewer facets compared with the eyes of many other species that have been studied (most with larger body sizes): *Melophorus bagoti* with 590 (Schwarz et al., 2011), *Polyrhachis sokolova* with 596 (Narendra et al., 2013a), *Camponotus pennsylvanicus* with 375–660 (Klotz et al., 1992), *Formica rufa* with 380–815 (Perl and

Niven, 2016), *Formica integroides* with 700 (Bernstein and Finn, 1971), *Camponotus consobrinus* with 800 (Narendra et al., 2016), *Cataglyphis bicolor* with 1200 (Menzi, 1987), four *Myrmecia* species with 2300–3600 (Greiner et al., 2007; Klotz et al., 1992) and *Gigantiops destructor* with 4100 (Gronenberg and Hölldobler, 1999). The relatively low number of facets in Argentine ants could be linked to the smaller body size of workers compared with other studied species (Perl and Niven, 2016). However, vision-based navigation does not require high resolution (Milford, 2013; Stürzl et al., 2015), and ants with as few as 72–80 ommatidia alter foraging patterns in response to landscape variation (Pratt et al., 2001). Therefore, Argentine ants with 80–100 ommatidia can likely sense long-range visual cues despite their observed disregard of these cues while foraging (Aron et al., 1993).

Three species – *Solenopsis invicta*, *Solenopsis richteri* and *Temnothorax rugatulus* (Baker and Ma, 2006; Ramirez-Esquivel et al., 2017) – have workers that overlap in size and ommatidia number with Argentine ant workers, but have eyes with smaller individual facets. The Argentine ant's relatively larger facets might increase the optical sensitivity of worker eyes. Optical sensitivity is a complex trait that depends on numerous anatomical factors, but larger facets increase light capture and are often associated with vision-demanding behaviors such as nocturnal foraging and flight (Greiner, 2006; Land, 1997a; Sheehan et al., 2019). With relatively large facets at the anterior and posterior margins of the eye, the Argentine ant could benefit from improved vision along the ground and skyline.



**Fig. 6. Antennal function for *L. humile* walking on flat or uneven terrain and while crossing a 1-mm step obstacle.** (A) Antennal speeds with respect to the body of the ant were averaged across 8 mm on flat (orange) and uneven (navy) substrates and while ants were far from (4–6 mm, purple) and close to (1–3 mm, light blue) the step. (B) Distributions of observed antennal speeds in light (yellowish-brown) and dark (red) conditions on flat (lower) and uneven (upper) terrain. At distances far from the step, antennal speeds resemble those on flat ground. Close to the step, antennal speeds slightly exceed those observed on uneven ground (LMER anova,  $*P<0.05$ ; n.s., not significant). (C) Antennal spatial patterns appear similar under light and dark conditions, but cover a larger range when on uneven terrain or when close to the step. Opacity shows relative spatial density.



### Darkness minimally influences walking speeds on flat and uneven terrain

We found that Argentine ant walking speeds were minimally impacted by darkness, with a <5% overall reduction on flat ground for every replicate except one, which increased average walking speeds in darkness. This finding differs from walking observations in both cockroaches (Baba et al., 2010; Ye et al., 2003) and blow flies (Kress and Egelhaaf, 2012), which slow down by 35% and >50%, respectively, in darkness. Although fruit flies use optic flow to regulate walking speed (Creamer et al., 2018), they mostly maintain normal speeds in darkness (5–8% reduction) (Howard et al., 2019). To the best of our knowledge, walking speeds in ants have been associated with light levels only in the bull ant, *Myrmecia piriformis*, which showed a logistic increase in speed with luminance (Narendra et al., 2013b). Ant walking speed likely contributes to colony-level survival because it can increase rates of food acquisition (Fewell, 1988) and decrease risks associated with foraging, such as predation (Hurlbert et al., 2008; Jayatilaka et al., 2011) and desiccation (Schilman et al., 2005). Therefore, nocturnal foraging in Argentine ants likely does not incur these costs. Interestingly, we observed a greater influence of darkness on speed for walking on uneven versus flat terrain. This finding suggests that ants rely more on vision when negotiating challenging or uncertain terrain, highlighting the importance of studying locomotion and behavioral responses on non-flat terrain.

### Darkness does not impact crossing a step obstacle

The checkerboard substrate tested above introduced continuous, short-range unevenness. Although darkness does not appear to influence the ability of Argentine ant workers to cross this continuously varying terrain, vision may still help to identify obstacles at a mid-range distance and to induce pre-planning. Comparisons with other ant species suggest that Argentine ant workers, which possess only <100 ommatidia per compound eye, likely cannot identify mid-range obstacles (Palavalli-Nettimi and Narendra, 2018). To test whether workers respond to a looming obstacle, we compared their performance crossing a single 1-mm step in light and dark conditions. Step obstacles are common in perturbation studies (Birn-Jeffery et al., 2014; Gart and Li, 2018; Harley et al., 2009; Theunissen et al., 2015; Watson et al., 2002), and are often used to test neuromechanical control under new conditions. A step height of 1 mm approximates worker coxal height while standing, therefore inducing a step and not vertical climbing, which shifts walking kinematics (Weihmann and Blickhan, 2009). Given an interommatidial angle of ~15 deg, Argentine ants should be able to visually resolve the step at a distance of at least 3.49 mm (Land, 1997b). We tested step crossing with a low-contrast step and a high-contrast step, both in light and dark conditions, to determine whether worker ants adjust their walking kinematics prior to reaching the step. Workers only decelerated when their mandible (and therefore eyes) reached within 2 mm of the step, with no difference in the time needed to cross the step or the approach speed between light and dark conditions. Although speeds far from the step differed between light and dark conditions, these differences likely derive from slower preferred speeds in darkness and are not due to visual detection and planning for the step obstacle.

Our findings suggest that vision does not impact the ability of Argentine ant workers to identify and cross step obstacles. Ants often decelerated only when their antennae reached the step (Fig. 5A, Fig. S3A,B); this behavior likely represents a reliance on tactile sensation instead of vision. Antennal-based strategies have been observed in tiger beetles (Zurek and Gilbert, 2014), stick insects (Schütz and Dürr, 2011) and cockroaches (Baba et al., 2010; Gart and

Li, 2018; Harley et al., 2009), with all observed to adjust speed or body positioning before reaching obstacles. Our observations that Argentine ants decelerate to slower speeds, but do not stop as the mandible nears the step edge (Fig. 5A), differ from maximally sprinting cockroaches that collide with a tall vertical obstacle before climbing (Jayaram et al., 2018). Argentine ant workers appear to decelerate of their own control rather than through interactions with an obstacle. Additionally, because we observed similar walking speeds when near the obstacle and when walking on the uneven substrate, the checkerboard substrate we used could act as a series of step obstacles, each requiring an associated deceleration.

Worker ants demonstrated the ability to rapidly decelerate once in the vicinity of a step. To generate these body accelerations, ants likely generate large friction forces through friction-inducing hairs on their distal tarsi and an actively controlled adhesive organ next to their claws (Endlein and Federle, 2015). As a consequence, ants are likely capable of decelerating from their average speed (25.9 mm s<sup>-1</sup> on flat ground) to rest within a single step. The latency between an antennal contact and reactionary behavior has not been measured in ants; however, reaction times are ~40 ms in stick insects (Schütz and Dürr, 2011), 25 ms in cockroaches (Ye and Comer, 1996; Ye et al., 2003) and <10 ms in hawk moths (Krishnan and Sane, 2014). Argentine ants walk at 5–15 strides s<sup>-1</sup>, corresponding to a minimum stride duration of 66 ms (Clifton et al., 2020). Therefore, it is likely that tactile sensation from the antennae is sufficiently fast to enable decelerations within one stride of encountering an obstacle.

### Antennal activity does not differ in light and darkness

In the absence of light, ants may still gather sensory information about substrate structure through tactile contact of the antennae and limbs. If Argentine ant workers use vision for walking, we expect that their antennal activity would increase in darkness to compensate for lost visual information. Counter to our expectations, antennal speed did not increase in darkness, with a 0.2% and 1.0% decrease on flat and uneven terrain, respectively. Apart from this small decrease in antennal speed, lighting condition did not influence overall antennal activity. Ant antennae contain hundreds of sensory receptors (Nakanishi et al., 2009), gathering critical tactile and chemosensory information about the environment. Ant antennae function in determining pheromone trail location (Draft et al., 2018), trail polarity (Jackson et al., 2004), nest location (Steck et al., 2011) and nestmate recognition (Frasnelli et al., 2012). In other insects, antennae have also been shown to facilitate wall following (Mongeau et al., 2013) and obstacle identification (Zurek and Gilbert, 2014). For cockroaches and stick insects, vision directs antennal motion towards obstacles while standing (Comer and Baba, 2011), but has not been directly tested for an influence on the highly coordinated antennal motions observed during walking (Dürr et al., 2001; Krause et al., 2013; Okada and Toh, 2004). Both sighted and blinded stick insects re-target their feet after sensing an obstacle with their antennae, suggesting that antennal sensation might be sufficient for navigating uneven terrain (Schütz and Dürr, 2011). Our findings for Argentine ants similarly support a dominant, albeit context-specific, role of antennal activity during walking.

In Argentine ants, the spatial range of the antennae depended on substrate structure, but was not influenced by lighting. The spatial patterns observed on flat ground mimic carpenter ant ‘trail-following’ behavior, while the spatial patterns when walking near a step obstacle and on uneven terrain resembled ‘exploration’ (Draft et al., 2018). Our findings suggest that antennal function and ant behavior likely shift as a result of substrate unevenness, but are unaffected by vision in Argentine ants. Unlike observations in

carpenter ants (Frasnelli et al., 2012), we find that Argentine ants did not tightly correlate antennal motion relative to the head ( $P < 0.5$ ; Fig. S3C–F). Since antennae evolved from modified appendages (Kaufman et al., 1980; Krishnan and Sane, 2015), variation in antennal coordination on uneven terrain may reveal neural control mechanisms associated with derived functions.

### Darkness does not diminish walking performance in Argentine ants

The experiments presented here recorded walking speeds and step obstacle negotiation metrics for Argentine ants in visual spectrum and IR (undetectable) light conditions. Importantly, the ants were observed walking along pheromone trails to a steady food source. Many ant species deposit pheromones from exocrine glands on their abdomen or limbs to recruit other foragers to a found food source (Morgan, 2009). These pheromones primarily serve to direct walking, although some species also deposit specific chemicals to signal nest location (Steck et al., 2009). Argentine ant workers turn in response to chemical sensation asymmetry between the antennae, but do not alter walking speed based on pheromone concentration (Perma et al., 2012). The use of olfactory landmarks has not been observed in this species. Therefore, although the Argentine ants we studied likely relied on pheromone trails to guide their walking direction across the substrates, it is unlikely that these trails informed the identification and crossing of step obstacles or the ability to traverse uneven terrain. Our choice to record ant walking along pheromone trails removes the potential impact of visually guided long-distance navigation, and isolates the impact of vision on walking due to environmental variability at intermediate and short-length scales.

We found that several metrics of walking performance did not substantially differ in ant workers walking in the absence of vision. However, our results do not demonstrate that vision plays no role in walking under all conditions. First, darkness could have induced behavioral changes not captured by the metrics measured here. For example, ant workers could shift their preferred stride length (Clifton et al., 2020) or the sampling strategy of the antennae. Second, ants may incorporate visual cues when available but prioritize other senses in darkness. Many ant species integrate multiple sensory modalities during navigation (Evison et al., 2008), and can prioritize either vision or chemosensation based on the environmental information available (Jones et al., 2019). In one species, the reliance on visual cues for navigation depends on foraging experience, with experienced foragers favoring visual landmarks and novices favoring chemical trails (Card et al., 2016). Ants demonstrate flexibility in using redundant senses during navigation, which could also apply when avoiding obstacles or tackling uneven terrain. Given the observed minor influence of darkness on walking speeds and step crossing in Argentine ants, vision is not necessary for maintaining walking performance. However, vision may still provide sensory information under favorable conditions. Our finding that antennal activity does not increase in darkness suggests that vision contributes to a lesser extent, if at all, to obstacle avoidance and step choice.

Argentine ants demonstrate no difference in walking performance in different light conditions when walking along pheromone trails; however, most tested insects show some influence from vision. Grasshoppers slow down before even their antennae reach an obstacle (Pearson and Franklin, 1984) and miss targeted footholds with their forelimbs more frequently when blinded (Niven et al., 2010). In darkness, blow flies alter body posture and use their forelegs to probe surfaces, reducing walking speeds by 50% on rough terrain (Kress

and Egelhaaf, 2012). Cockroaches with occluded ocelli prefer to tunnel under versus climb over an obstacle (Harley et al., 2009), whereas cockroaches with occluded compound eyes collide more frequently with obstacles than when their vision is unimpeded (Baba et al., 2010). To our knowledge, only two studies show no influence of vision: cockroaches sprinting ( $\sim 25$  body lengths  $s^{-1}$ ) then transitioning to vertical climbing (Jayaram et al., 2018) and a diurnal species of tiger beetle effectively ‘blinded’ by motion blur resulting from fast running speeds (up to 120 body lengths  $s^{-1}$ ) (Zurek and Gilbert, 2014). Unlike these two examples, Argentine ant workers walk at moderate speeds ( $\sim 10$  body lengths  $s^{-1}$ ) yet also maintain similar walking behavior in light and dark conditions. This observation could be explained by low-resolution eyes in Argentine ants that preclude high-resolution vision even in light conditions. However, robotic tests show that even low-resolution vision can permit successful navigation (Milford, 2013). Alternatively, Argentine ant workers could derive all necessary sensory feedback for walking from chemosensory and proprioceptive cues using the antennae and limbs.

### Conclusions

Despite possessing large eyes compared with closely related species (Wild, 2004), Argentine ant workers do not appear to require vision while walking on flat and uneven terrain, or while crossing over step obstacles. In contrast to minor differences between light and dark conditions, terrain unevenness and obstacles prompted large changes in walking speed and antennal activity, highlighting the importance of considering nontraditional laboratory conditions when studying movement control and behavior.

Our findings combined with results from a previous study testing Argentine ant navigation (Aron et al., 1993) indicate that workers do not require vision for walking or navigation. Given that eyes are costly to maintain (Niven et al., 2007), and given numerous independent examples of ant species with either highly reduced eyes or no eyes at all (Deharveng and Bedos, 2018; Pape and University of Arizona, 2016; Tierney et al., 2018), the presence of eyes in Argentine ants seems at odds with our findings. Vision may complement chemosensory signals while walking even if not required to maintain obstacle avoidance and negotiation of uneven terrain. Additionally, vision could serve functions not associated with walking, such as short-range perception of conspecifics and predators, or perception of light patterns to entrain circadian rhythms. Alternatively, vision might play a crucial role for Argentine ant queens or males (which both have relatively large eyes) remaining in the worker caste because of developmental constraints. However, a harvester ant queen demonstrates plasticity in optic lobe brain volume across life stages, proposing possible workaround for constraints among castes (Julian and Gronenberg, 2002).

We find that darkness does not compromise Argentine ant walking performance. As such, workers foraging at night can benefit from reduced risks of thermal stress or predation (Greiner, 2006; Wcislo et al., 2004). Argentine ant workers likely rely on tactile or chemical cues to inform walking strategies and obstacle negotiation. Antennae and proprioceptive limbs likely provide sensory feedback to ensure secure foot placement and limb coordination while maintaining relatively fast walking speeds ( $\sim 10$  body lengths  $s^{-1}$ ) (Zill et al., 2010). These strategies could provide relevant inspiration for legged robotics and autonomous vehicles (Cowan et al., 2005; Rudy et al., 2013). Furthermore, our findings demonstrate that possessing eyes does not necessarily correspond to an active role of vision in every task. This dissociation highlights the importance of testing functional interpretations for anatomical structures. Overall, our findings

suggest that vision does not impact walking performance in Argentine ant workers, highlighting the importance of integrating vision, proprioception and tactile sensing for robust locomotion in unstructured environments.

#### Acknowledgements

We thank Prof. Michael Tolley for 3D printer access and Ben Shih and Chris Cassidy for printing help.

#### Competing interests

The authors declare no competing or financial interests.

#### Author contributions

Conceptualization: G.T.C., D.H., N.G.; Methodology: G.T.C.; Software: G.T.C., N.G.; Formal analysis: G.T.C.; Resources: N.G.; Writing - original draft: G.T.C.; Writing - review & editing: D.H., N.G.; Visualization: G.T.C.; Supervision: N.G.; Funding acquisition: N.G.

#### Funding

Funding support for this research was provided by the Army Research Office under grant W911NF-17-1-0145, the University of California, San Diego Chancellor's Research Excellence Scholarships, and support from the Department of Mechanical and Aerospace Engineering.

#### Data availability

The datasets generated during and analyzed during the current study are publicly available on the Harvard Dataverse: <https://doi.org/10.7910/DVN/G3X8TW>. The code used for analysis is available at GitHub: [https://github.com/gtclifton/Ant\\_LightDark](https://github.com/gtclifton/Ant_LightDark).

#### Supplementary information

Supplementary information available online at <https://jeb.biologists.org/lookup/doi/10.1242/jeb.228460.supplemental>

#### References

- Abril, S., Oliveras, J. and Gómez, C. (2007). Foraging activity and dietary spectrum of the Argentine ant (Hymenoptera: Formicidae) in invaded natural areas of the northeast Iberian Peninsula. *Environ. Entomol.* **36**, 1166-1173. doi:10.1603/0046-225X(2007)36[1166:FAADSO]2.0.CO;2
- Aksoy, V. and Camlitepe, Y. (2018). Spectral sensitivities of ants – a review. *Anim. Biol.* **68**, 55-73. doi:10.1163/15707563-17000119
- Almeida, P. J. A. L., Paulo, J. A., Vieira, M. V., Kajin, M., Forero-Medina, G. and Cerqueira, R. (2010). Indices of movement behaviour: conceptual background, effects of scale and location errors. *Zoologia (Curitiba)* **27**, 674-680. doi:10.1590/S1984-46702010000500002
- Aron, S., Beckers, R., Deneubourg, J. L. and Pasteels, J. M. (1993). Memory and chemical communication in the orientation of two mass-recruiting ant species. *Insectes Soc.* **40**, 369-380. doi:10.1007/BF01253900
- Baba, Y., Tsukada, A. and Comer, C. M. (2010). Collision avoidance by running insects: antennal guidance in cockroaches. *J. Exp. Biol.* **213**, 2294-2302. doi:10.1242/jeb.036996
- Baker, G. T. and Ma, P. W. K. (2006). Morphology and number of Ommatidia in the compound eyes of *Solenopsis invicta*, *Solenopsis richteri*, and their hybrid (Hymenoptera: Formicidae). *Zool. Anz. A J. Comp. Zool.* **245**, 121-125. doi:10.1016/j.jcz.2006.06.001
- Bates, D., Mächler, M., Bolker, B. and Walker, S. (2015). Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* **67**. doi:10.18637/jss.v067.i01
- Bender, J. A., Simpson, E. M., Tietz, B. R., Daltorio, K. A., Quinn, R. D. and Ritzmann, R. E. (2011). Kinematic and behavioral evidence for a distinction between trotting and ambling gaits in the cockroach *Blaberus discoidalis*. *J. Exp. Biol.* **214**, 2057-2064. doi:10.1242/jeb.056481
- Benhamou, S. (2004). How to reliably estimate the tortuosity of an animal's path: straightness, sinuosity, or fractal dimension? *J. Theor. Biol.* **229**, 209-220. doi:10.1016/j.jtbi.2004.03.016
- Bernstein, S. and Finn, C. (1971). Ant compound eye: Size-related ommatidium differences within a single wood ant nest. *Experientia* **27**, 708-710. doi:10.1007/BF02136977
- Bingman, V. P., Graving, J. M., Hebets, E. A. and Wiegmann, D. D. (2017). Importance of the antenniform legs, but not vision, for homing by the neotropical whip spider. *J. Exp. Biol.* **220**, 885-890. doi:10.1242/jeb.149823
- Birn-Jeffery, A. V., Hubicki, C. M., Blum, Y., Renjewski, D., Hurst, J. W. and Daley, M. A. (2014). Don't break a leg: running birds from quail to ostrich prioritise leg safety and economy on uneven terrain. *J. Exp. Biol.* **217**, 3786-3796. doi:10.1242/jeb.102640
- Blaesing, B. (2004). Stick insect locomotion in a complex environment: climbing over large gaps. *J. Exp. Biol.* **207**, 1273-1286. doi:10.1242/jeb.00888
- Bovet, P. and Benhamou, S. (1988). Spatial analysis of animals' movements using a correlated random walk model. *J. Theor. Biol.* **131**, 419-433. doi:10.1016/S0022-5193(88)80038-9
- Briscoe, A. D. and Chittka, L. (2001). The evolution of color vision in insects. *Annu. Rev. Entomol.* **46**, 471-510. doi:10.1146/annurev.ento.46.1.471
- Card, A., McDermott, C. and Narendra, A. (2016). Multiple orientation cues in an Australian trunk-trail-forming ant, *Iridomyrmex purpureus*. *Australian J. Zool.* **64**, 227-232.
- Cheng, K., Narendra, A., Sommer, S. and Wehner, W. (2009). Traveling in clutter: navigation in the Central Australian desert ant *Melophorus bagoti*. *Behav. Bproc* **80**, 261-268.
- Clifton, G. T., Holway, D. and Graving, N. (2020). Uneven substrates constrain walking speed in ants through modulation of stride frequency more than stride length. *R. Soc. Open Sci.* **7**, 192068. doi:10.1098/rsos.192068
- Collett, T. S. (2002). Insect vision: controlling actions through optic flow. *Curr. Biol.* **12**, R615-R617. doi:10.1016/S0960-9822(02)01132-6
- Comer, C. and Baba, Y. (2011). Active touch in orthopteroid insects: behaviours, multisensory substrates and evolution. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **366**, 3006-3015. doi:10.1098/rstb.2011.0149
- Couzin-Fuchs, E., Kiemel, T., Gal, O., Ayali, A. and Holmes, P. (2015). Intersegmental coupling and recovery from perturbations in freely running cockroaches. *J. Exp. Biol.* **218**, 285-297. doi:10.1242/jeb.112805
- Cowan, N. J., Ma, E. J., Cutkosky, M. and Full, R. J. (2005). A biologically inspired passive antenna for steering control of a running robot. *Springer Tracts Adv. Robot.* 541-550. doi:10.1007/11008941\_58
- Cremer, M. S., Mano, O. and Clark, D. A. (2018). Visual control of walking speed in *Drosophila*. *Neuron* **100**, 1460-1473.e6. doi:10.1016/j.neuron.2018.10.028
- Cronin, T. W., Johnsen, S., Justin Marshall, N. and Warrant, E. J. (2014). Visual Ecology. Princeton University Press.
- Deharveng, L. and Bedos, A. (2018). Diversity of terrestrial invertebrates in subterranean habitats. *Cave Ecol.* 107-172. doi:10.1007/978-3-319-98852-8\_7
- Draft, R. W., McGill, M. R., Kapoor, V. and Murthy, V. N. (2018). Carpenter ants use diverse antennae sampling strategies to track odor trails. *J. Exp. Biol.* **221**. doi:10.1242/jeb.185124
- Dürr, V. (2001). Stereotypic leg searching movements in the stick insect: kinematic analysis, behavioural context and simulation. *J. Exp. Biol.* **204**, 1589-1604.
- Dürr, V., König, Y. and Kittmann, R. (2001). The antennal motor system of the stick insect *Carausius morosus*: anatomy and antennal movement pattern during walking. *J. Comp. Physiol. A* **187**, 131-144. doi:10.1007/s003590100183
- Endlein, T. and Federle, W. (2015). On heels and toes: how ants climb with adhesive pads and tarsal friction hair arrays. *PLoS ONE* **10**, e0141269. doi:10.1371/journal.pone.0141269
- Evison, S. E. F., Petchey, O. L., Beckerman, A. P. and Ratrieks, F. L. W. (2008). Combined use of pheromone trails and visual landmarks by the common garden ant *Lasius niger*. *Behav. Ecol. Sociobiol.* **63**, 261-267. doi:10.1007/s00265-008-0657-6
- Fewell, J. H. (1988). Energetic and time costs of foraging in harvester ants, *Pogonomyrmex occidentalis*. *Behav. Ecol. Sociobiol.* **22**, 401-408. doi:10.1007/BF00294977
- Frasnelli, E., Iakovlev, I. and Reznikova, Z. (2012). Asymmetry in antennal contacts during trophallaxis in ants. *Behav. Brain Res.* **232**, 7-12. doi:10.1016/j.bbr.2012.03.014
- Frolov, R. V. and Ignatova, I. I. (2020). Electrophysiological adaptations of insect photoreceptors and their elementary responses to diurnal and nocturnal lifestyles. *J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol.* **206**, 55-69. doi:10.1007/s00359-019-01392-8
- Frolov, R. V., Matsushita, A. and Arikawa, K. (2017). Not flying blind: a comparative study of photoreceptor function in flying and non-flying cockroaches. *J. Exp. Biol.* **220**, 2335-2344. doi:10.1242/jeb.159103
- Full, R. J. and Koditschek, D. E. (1999). Templates and anchors: neuromechanical hypotheses of legged locomotion on land. *J. Exp. Biol.* **202**, 3325-3332.
- Full, R. J. and Tu, M. S. (1990). Mechanics of six-legged runners. *J. Exp. Biol.* **148**, 129-146.
- Gart, S. W. and Li, C. (2018). Body-terrain interaction affects large bump traversal of insects and legged robots. *Bioinspir. Biomim.* **13**, 026005. doi:10.1088/1748-3190/aaa2d0
- Gilbert, C. (1997). Visual control of cursorial prey pursuit by tiger beetles (Cicindelidae). *J. Comp. Physiol. A* **181**, 217-230. doi:10.1007/s003590050108
- Graham, D. (1972). A behavioural analysis of the temporal organisation of walking movements in the 1st instar and adult stick insect (*Carausius morosus*). *J. Comp. Physiol.* **81**, 23-52. doi:10.1007/BF00693548
- Graham, P. and Cheng, K. (2009). Which portion of the natural panorama is used for view-based navigation in the Australian desert ant? *J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol.* **195**, 681-689. doi:10.1007/s00359-009-0443-6
- Graham, P. and Philippides, A. (2017). Vision for navigation: what can we learn from ants? *Arthropod Struct. Dev.* **46**, 718-722. doi:10.1016/j.asd.2017.07.001
- Graving, N., Monaenkova, D., Goodisman, M. A. D. and Goldman, D. I. (2013). Climbing, falling, and jamming during ant locomotion in confined environments. *Proc. Natl. Acad. Sci. USA* **110**, 9746-9751. doi:10.1073/pnas.1302428110

- Greiner, B.** (2006). Adaptations for nocturnal vision in insect apposition eyes. *Int. Rev. Cytol.* **250**, 1–46. doi:10.1016/S0074-7696(06)50001-4
- Greiner, B., Narendra, A., Reid, S. F., Dacke, M., Ribi, W. A. and Zeil, J.** (2007). Eye structure correlates with distinct foraging-bout timing in primitive ants. *Curr. Biol.* **17**, R879–R880. doi:10.1016/j.cub.2007.08.015
- Gronenberg, W. and Hölldobler, B.** (1999). Morphologic representation of visual and antennal information in the ant brain. *J. Comp. Neurol.* **412**, 229–240. doi:10.1002/(SICI)1096-9861(19990920)412:2<229::AID-CNE4>3.0.CO;2-E
- Harley, C. M., English, B. A. and Ritzmann, R. E.** (2009). Characterization of obstacle negotiation behaviors in the cockroach, *Blaberus discoidalis*. *J. Exp. Biol.* **212**, 1463–1476. doi:10.1242/jeb.028381
- Haselsteiner, A. F., Gilbert, C. and Wang, Z. J.** (2014). Tiger beetles pursue prey using a proportional control law with a delay of one half-stride. *J. R. Soc. Interface* **11**, 20140216. doi:10.1098/rsif.2014.0216
- Hebets, E. A.** (2002). Relating the unique sensory system of amblypygids to the ecology and behavior of *Phrynus parvulus* from Costa Rica (Arachnida, Amblypygi). *Can. J. Zool.* **80**, 286–295. doi:10.1139/z02-006
- Heglund, N. C., Taylor, C. R. and McMahon, T. A.** (1974). Scaling stride frequency and gait to animal size: mice to horses. *Science* **186**, 1112–1113. doi:10.1126/science.186.4169.1112
- Heimonen, K., Immonen, E.-V., Frolov, R. V., Salmela, I., Juusola, M., Vähäsyrinki, M. and Weckström, M.** (2012). Signal coding in cockroach photoreceptors is tuned to dim environments. *J. Neurophysiol.* **108**, 2641–2652. doi:10.1152/jn.00588.2012
- Hogg, B. N., Nelson, E. H., Hagler, J. R. and Daane, K. M.** (2018). Foraging distance of the argentine Ant in california vineyards. *J. Econ. Entomol.* **111**, 672–679. doi:10.1093/jeet/tox366
- Höltje, M. and Hustert, R.** (2003). Rapid mechano-sensory pathways code leg impact and elicit very rapid reflexes in insects. *J. Exp. Biol.* **206**, 2715–2724. doi:10.1242/jeb.00492
- Howard, C. E., Chen, C.-L., Tabachnik, T., Hormigo, R., Ramdya, P. and Mann, R. S.** (2019). Serotonergic modulation of walking in *Drosophila*. *Curr. Biol.* **29**, 4218–4230.e8. doi:10.1016/j.cub.2019.10.042
- Human, K. G. and Gordon, D. M.** (1996). Exploitation and interference competition between the invasive Argentine ant, *Linepithema humile*, and native ant species. *Oecologia* **105**, 405–412. doi:10.1007/BF00328744
- Hurlbert, A. H., Ballantyne, F. and Powell, S.** (2008). Shaking a leg and hot to trot: the effects of body size and temperature on running speed in ants. *Ecol. Entomol.* **33**, 144–154. doi:10.1111/j.1365-2311.2007.00962.x
- Ignatova, I. I., Saari, P. and Frolov, R. V.** (2020). Latency of phototransduction limits transfer of higher-frequency signals in cockroach photoreceptors. *J. Neurophysiol.* **123**, 120–133. doi:10.1152/jn.00365.2019
- Isakov, A., Buchanan, S. M., Sullivan, B., Ramachandran, A., Chapman, J. K. S., Lu, E. S., Mahadevan, L. and de Bivort, B.** (2016). Recovery of locomotion after injury in *Drosophila melanogaster* depends on proprioception. *J. Exp. Biol.* **219**, 1760–1771. doi:10.1242/jeb.133652
- Jackson, D. E., Holcombe, M. and Ratnieks, F. L. W.** (2004). Trail geometry gives polarity to ant foraging networks. *Nature* **432**, 907–909. doi:10.1038/nature03105
- Jayaram, K., Mongeau, J.-M., Mohapatra, A., Birkmeyer, P., Fearing, R. S. and Full, R. J.** (2018). Transition by head-on collision: mechanically mediated manoeuvres in cockroaches and small robots. *J. R. Soc. Interface* **15**, 20170664. doi:10.1098/rsif.2017.0664
- Jayatilaka, P., Narendra, A., Reid, S. F., Cooper, P. and Zeil, J.** (2011). Different effects of temperature on foraging activity schedules in sympatric *Myrmecia* ants. *J. Exp. Biol.* **214**, 2730–2738. doi:10.1242/jeb.053710
- Jindrich, D. L. and Full, R. J.** (1999). Many-legged maneuverability: dynamics of turning in hexapods. *J. Exp. Biol.* **202**, 1603–1623.
- Jindrich, D. L. and Full, R. J.** (2002). Dynamic stabilization of rapid hexapedal locomotion. *J. Exp. Biol.* **205**, 2803–2823.
- Jones, S., Czaczkes, T. J., Gallager, A. J., Oberhauser, F. B., Gourlay, E. and Bacon, J. P.** (2019). Copy when uncertain: lower light levels increase trail pheromone depositing and reliance on pheromone trails in ants. *Anim. Behav.* **156**, 87–95. doi:10.1016/j.anbehav.2019.08.007
- Julian, G. E. and Gronenberg, W.** (2002). Reduction of brain volume correlates with behavioral changes in queen ants. *Brain Behav. Evol.* **60**, 152–164. doi:10.1159/000065936
- Kaspari, M. and Weiser, M. D.** (1999). The size–grain hypothesis and interspecific scaling in ants. *Funct. Ecol.* **13**, 530–538. doi:10.1046/j.1365-2435.1999.00343.x
- Kaufman, T. C., Lewis, R. and Wakimoto, B.** (1980). Cytogenetic analysis of chromosome 3 in *DROSOPHILA MELANOGASTER*: the homoecotic gene complex in polytene chromosome interval 84a–B. *Genetics* **94**, 115–133.
- Klotz, J. H. and Reid, B. L.** (1992). The use of spatial cues for structural guideline orientation in *Tapinoma sessile* and *Camponotus pennsylvanicus* (Hymenoptera: Formicidae). *J. Insect Behav.* **5**, 71–82. doi:10.1007/BF01049159
- Klotz, J. H., Reid, B. L. and Gordon, W. C.** (1992). Variation of ommatidia number as a function of worker size in *Camponotus pennsylvanicus* (DeGeer) (Hymenoptera: Formicidae). *Insectes Soc.* **39**, 233–236. doi:10.1007/BF01249297
- Knaden, M. and Graham, P.** (2016). The sensory ecology of ant navigation: from natural environments to neural mechanisms. *Annu. Rev. Entomol.* **61**, 63–76. doi:10.1146/annurev-ento-010715-023703
- Krause, A. F., Winkler, A. and Dürr, V.** (2013). Central drive and proprioceptive control of antennal movements in the walking stick insect. *J. Physiol. Paris* **107**, 116–129. doi:10.1016/j.jphysparis.2012.06.001
- Kress, D. and Egelhaaf, M.** (2012). Head and body stabilization in blowflies walking on differently structured substrates. *J. Exp. Biol.* **215**, 1523–1532. doi:10.1242/jeb.066910
- Krishnan, A. and Sane, S. P.** (2014). Visual feedback influences antennal positioning in flying hawk moths. *J. Exp. Biol.* **217**, 908–917. doi:10.1242/jeb.094276
- Krishnan, A. and Sane, S. P.** (2015). Antennal mechanosensors and their evolutionary antecedents. *Adv. Insect Physiol.* **59**–99. doi:10.1016/bs.aip.2015.06.003
- Land, M. F.** (1997a). Visual acuity in insects. *Annu. Rev. Entomol.* **42**, 147–177. doi:10.1146/annurev.ento.42.1.147
- Land, M. F.** (1997b). The resolution of insect compound eyes. *Isr. J. Plant Sci.* **45**, 79–91. doi:10.1080/07929978.1997.10676675
- Land, M. F. and Collett, T. S.** (1974). Chasing behaviour of houseflies (*Fannia canicularis*). *J. Comp. Physiol.* **89**, 331–357. doi:10.1007/BF00695351
- Laughlin, S. B. and Weckstrom, M.** (1993). Fast and slow photoreceptors? A comparative study of the functional diversity of coding and conductances in the Diptera. *J. Comp. Physiol. A* **172**, 593–609. doi:10.1007/BF00213682
- Lee, T., Jang, S., Jeong, M. and Cho, D.-I. D.** (2016). Allometric scaling of insects and animals for biomimetic robot design considerations. 2016 16th International Conference on Control, Automation and Systems (ICCAS), Gyeongju. 1541–1546. doi:10.1109/ICCAS.2016.7832508
- Logan, D., Kiemel, T., Dominici, N., Cappellini, G., Ivanenko, Y., Lacquaniti, F. and Jeka, J. J.** (2010). The many roles of vision during walking. *Exp. Brain Res.* **206**, 337–350. doi:10.1007/s00221-010-2414-0
- Markin, G. P.** (1970). The seasonal life cycle of the argentine ant, *iridomyrmex humilis* (Hymenoptera: Formicidae), in Southern California. *Ann. Entomol. Soc. Am.* **63**, 1238–1242. doi:10.1093/aesa/63.5.1238
- Menzi, U.** (1987). Visual adaptation in nocturnal and diurnal ants. *J. Comp. Physiol. A* **160**, 11–21. doi:10.1007/BF00613437
- Milford, M.** (2013). Vision-based place recognition: how low can you go? *Int. J. Robot. Res.* **32**, 766–789. doi:10.1177/0278364913490323
- Monaenkova, D., Gravish, N., Rodriguez, G., Kutner, K., Goodisman, M. A. D. and Goldman, D. I.** (2015). Behavioral and mechanical determinants of collective subsurface nest excavation. *J. Exp. Biol.* **218**, 1295–1305. doi:10.1242/jeb.113795
- Mongeau, J.-M., Demir, A., Lee, J., Cowan, N. J. and Full, R. J.** (2013). Locomotion- and mechanics-mediated tactile sensing: antenna reconfiguration simplifies control during high-speed navigation in cockroaches. *J. Exp. Biol.* **216**, 4530–4541. doi:10.1242/jeb.083477
- Morgan, E. D.** (2009). Trail pheromones of ants. *Physiol. Entomol.* **34**, 1–17. doi:10.1111/j.1365-3032.2008.00658.x
- Nakanishi, A., Nishino, H., Watanabe, H., Yokohari, F. and Nishikawa, M.** (2009). Sex-specific antennal sensory system in the ant *Camponotus japonicus*: structure and distribution of sensilla on the flagellum. *Cell Tissue Res.* **338**, 79–97. doi:10.1007/s00441-009-0863-1
- Nams, V. O.** (1996). The VFracal: a new estimator for fractal dimension of animal movement paths. *Landsch. Ecol.* **11**, 289–297. doi:10.1007/BF02059856
- Narendra, A., Raderschall, C. A. and Robson, S. K. A.** (2013a). Homing abilities of the Australian intertidal ant *Polyrhachis sokolova*. *J. Exp. Biol.* **216**, 3674–3681. doi:10.1242/jeb.089649
- Narendra, A., Reid, S. F. and Raderschall, C. A.** (2013b). Navigational efficiency of nocturnal *Myrmecia* ants suffers at low light levels. *PLoS ONE* **8**, e58801. doi:10.1371/journal.pone.0058801
- 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. doi:10.1038/srep22331
- Niven, J. E., Anderson, J. C. and Laughlin, S. B.** (2007). Fly photoreceptors demonstrate energy-information trade-offs in neural coding. *PLoS Biol.* **5**, e116. doi:10.1371/journal.pbio.0050116
- Niven, J. E., Buckingham, C. J., Lumley, S., Cuttle, M. F. and Laughlin, S. B.** (2010). Visual Targeting of Forelimbs in Ladder-Walking Locusts. *Curr. Biol.* **20**, 86–91. doi:10.1016/j.cub.2009.10.079
- Niven, J. E., Ott, S. R. and Rogers, S. M.** (2012). Visually targeted reaching in horse-head grasshoppers. *Proc. Biol. Sci.* **279**, 3697–3705. doi:10.1098/rspb.2012.0918
- Ogawa, Y., Falkowski, M., Narendra, A., Zeil, J. and Hemmi, J. M.** (2015). Three spectrally distinct photoreceptors in diurnal and nocturnal Australian ants. *Proc. Biol. Sci.* **282**, 20150673. doi:10.1098/rspb.2015.0673
- Okada, J. and Toh, Y.** (2004). Spatio-temporal patterns of antennal movements in the searching cockroach. *J. Exp. Biol.* **207**, 3693–3706. doi:10.1242/jeb.01201
- Okada, J. and Toh, Y.** (2006). Active tactile sensing for localization of objects by the cockroach antenna. *J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol.* **192**, 715–726. doi:10.1007/s00359-006-0106-9
- Ozaki, M., Wada-Katsumata, A., Fujikawa, K., Iwasaki, M., Yokohari, F., Satoji, Y., Nisimura, T. and Yamaoka, R.** (2005). Ant nestmate and non-nestmate

- discrimination by a chemosensory sensillum. *Science* **309**, 311-314. doi:10.1126/science.1105244
- Palavalli-Nettimi, R. and Narendra, A.** (2018). Miniaturisation decreases visual navigational competence in ants. *J. Exp. Biol.* **221**, jeb177238. doi:10.1242/jeb.177238
- Pape, R. and University of Arizona** (2016). The importance of ants in cave ecology, with new records and behavioral observations of ants in Arizona caves. *Int. J. Speleol.* **45**, 185-205. doi:10.5038/1827-806X.45.3.1936
- Patla, A. E. and Greig, M.** (2006). Any way you look at it, successful obstacle negotiation needs visually guided on-line foot placement regulation during the approach phase. *Neurosci. Lett.* **397**, 110-114. doi:10.1016/j.neulet.2005.12.016
- Pearson, K. G. and Franklin, R.** (1984). Characteristics of leg movements and patterns of coordination in locusts walking on rough terrain. *Int. J. Robot. Res.* **3**, 101-112. doi:10.1177/027836498400300209
- Pearson, K. G., Stein, R. B. and Malhotra, S. K.** (1970). Properties of action potentials from insect motor nerve fibres. *J. Exp. Biol.* **53**, 299-316.
- Pereira, T. D., Aldarondo, D. E., Willmore, L., Kislin, M., Wang, S. S.-H., Murthy, M. and Shaevitz, J. W.** (2019). Fast animal pose estimation using deep neural networks. *Nat. Methods* **16**, 117-125. doi:10.1038/s41592-018-0234-5
- Perl, C. D. and Niven, J. E.** (2016). Differential scaling within an insect compound eye. *Biol. Lett.* **12**, 20160042. doi:10.1098/rsbl.2016.0042
- Perna, A., Granovskiy, B., Garnier, S., Nicolis, S. C., Labédan, M., Theraulaz, G., Fourcassié, V. and Sumpter, D. J. T.** (2012). Individual rules for trail pattern formation in Argentine ants (*Linepithema humile*). *PLoS Comput. Biol.* **8**, e1002592. doi:10.1371/journal.pcbi.1002592
- Pfeffer, S. E., Wahl, V. L., Wittlinger, M. and Wolf, H.** (2019). High-speed locomotion in the Saharan silver ant, *Cataglyphis bombycina*. *J. Exp. Biol.* **222**: jeb198705 doi:10.1242/jeb.198705
- Pick, S. and Strauss, R.** (2005). Goal-driven behavioral adaptations in gap-climbing *Drosophila*. *Curr. Biol.* **15**, 1473-1478. doi:10.1016/j.cub.2005.07.022
- Pratt, S. C., Brooks, S. E. and Franks, N. R.** (2001). The use of edges in visual navigation by the ant *Leptothorax alpeipennis*. *Ethology* **107**, 1125-1136. doi:10.1046/j.1439-0310.2001.00749.x
- Ramirez-Esquivel, F., Leitner, N. E., Zeil, J. and Narendra, A.** (2017). The sensory arrays of the ant, *Temnothorax rugatulus*. *Arthropod Struct. Dev.* **46**, 552-563. doi:10.1016/j.asd.2017.03.005
- Reinhardt, L. and Blickhan, R.** (2014). Level locomotion in wood ants: evidence for grounded running. *J. Exp. Biol.* **217**, 2358-2370. doi:10.1242/jeb.098426
- Reynolds, R. F. and Day, B. L.** (2005a). Visual guidance of the human foot during a step. *J. Physiol.* **569**, 677-684. doi:10.1113/jphysiol.2005.095869
- Reynolds, R. F. and Day, B. L.** (2005b). Rapid visuo-motor processes drive the leg regardless of balance constraints. *Curr. Biol.* **15**, R48-R49. doi:10.1016/j.cub.2004.12.051
- Rudy, R., Cohen, A. J., Pulskamp, J. S., Polcawich, R. G. and Oldham, K. R.** (2013). Antenna-like tactile sensor for thin-film piezoelectric micro-robots. Proceedings of the ASME Design Engineering Technical Conference. 1. doi:10.1115/DETC2013-13500
- Schilman, P. E., Lighton, J. R. B. and Holway, D. A.** (2005). Respiratory and cuticular water loss in insects with continuous gas exchange: comparison across five ant species. *J. Insect Physiol.* **51**, 1295-1305. doi:10.1016/j.jinsphys.2005.07.008
- Schneider, C. A., Rasband, W. S. and Eliceiri, K. W.** (2012). NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* **9**, 671-675. doi:10.1038/nmeth.2089
- Schütz, C. and Dürr, V.** (2011). Active tactile exploration for adaptive locomotion in the stick insect. *Philos. Trans. R. Soc. B Biol. Sci.* **366**, 2996-3005. doi:10.1098/rstb.2011.0126
- 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. doi:10.1016/j.asd.2010.10.003
- Sheehan, Z. B. V., Kamhi, J. F., Seid, M. A. and Narendra, A.** (2019). Differential investment in brain regions for a diurnal and nocturnal lifestyle in Australian *Myrmecia* ants. *J. Comp. Neurol.* **527**, 1261-1277. doi:10.1002/cne.24617
- Sherman, A. and Dickinson, M. H.** (2004). Summation of visual and mechanosensory feedback in *Drosophila* flight control. *J. Exp. Biol.* **207**, 133-142. doi:10.1242/jeb.00731
- Smid, K. A. and den Otter, A. R.** (2013). Why you need to look where you step for precise foot placement: the effects of gaze eccentricity on stepping errors. *Gait Posture* **38**, 242-246. doi:10.1016/j.gaitpost.2012.11.019
- Srinivasan, M. V. and Lehrer, M.** (1984). Temporal acuity of honeybee vision: behavioural studies using flickering stimuli. *Physiol. Entomol.* **9**, 447-457. doi:10.1111/j.1365-3032.1984.tb00787.x
- Srinivasan, M. and Lehrer, M.** (1985). Temporal resolution of colour vision in the honeybee. *J. Comp. Physiol. A* **157**, 579-586. doi:10.1007/BF01351352
- Steck, K., Hansson, B. S. and Knaden, M.** (2009). Smells like home: desert ants, *Cataglyphis fortis*, use olfactory landmarks to pinpoint the nest. *Front. Zool.* **6**, 5. doi:10.1186/1742-9994-6-5
- Steck, K., Hansson, B. S. and Knaden, M.** (2011). Desert ants benefit from combining visual and olfactory landmarks. *J. Exp. Biol.* **214**, 1307-1312. doi:10.1242/jeb.053579
- Straw, A. D., Branson, K., Neumann, T. R. and Dickinson, M. H.** (2011). Multi-camera real-time three-dimensional tracking of multiple flying animals. *J. R. Soc. Interface* **8**, 395-409. doi:10.1098/rsif.2010.0230
- Stürzl, W., Grixa, I., Mair, E., Narendra, A. and Zeil, J.** (2015). Three-dimensional models of natural environments and the mapping of navigational information. *J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol.* **201**, 563-584. doi:10.1007/s00359-015-1002-y
- Szczecinski, N. S., Büschges, A. and Bockemühl, T.** (2018). Direction-specific footpaths can be predicted by the motion of a single point on the body of the fruit fly *Drosophila melanogaster*. In *Biomimetic and Biohybrid Systems. Living Machines. Lecture Notes in Computer Science*, vol. 10928 (ed. V. Vouloutsi). Springer. doi:10.1007/978-3-319-95972-6\_51
- Theunissen, L. M., Bekemeier, H. H. and Dürr, V.** (2015). Comparative whole-body kinematics of closely related insect species with different body morphology. *J. Exp. Biol.* **218**, 340-352. doi:10.1242/jeb.114173
- Thomas, M. L., Payne-Makrisá, C. M., Suarez, A. V., Tsutsui, N. D. and Holway, D. A.** (2006). When supercolonies collide: territorial aggression in an invasive and unicolonial social insect. *Molecular Ecology* **15**, 4303-4315. doi:10.1111/j.1365-294X.2006.03038.x
- Tierney, S. M., Langille, B., Humphreys, W. F., Austin, A. D. and Cooper, S. J. B.** (2018). Massive parallel regression: a précis of genetic mechanisms for vision loss in diving beetles. *Integr. Comp. Biol.* **58**, 465-479. doi:10.1093/icb/icy035
- Tsutsui, N. D., Suarez, A. V. and Grosberg, R. K.** (2003). Genetic diversity, asymmetrical aggression, and recognition in a widespread invasive species. *Proc. Natl Acad. Sci. USA* **100**, 1078-1083. doi:10.1073/pnas.0234412100
- Van Zweden, J. and D'Ettorre, P.** (2010). Nestmate recognition in social insects and the role of hydrocarbons. In *Insect Hydrocarbons: Biology, Biochemistry, and Chemical Ecology* (ed. G. Blomquist and A. Bagnères), pp. 222-243. Cambridge: Cambridge University Press. doi:10.1017/CBO9780511711909.012
- Vega, S. Y. and Rust, M. K.** (2003). Determining the foraging range and origin of resurgence after treatment of Argentine Ant (Hymenoptera: Formicidae) in urban areas. *J. Econ. Entomol.* **96**, 844-849. doi:10.1093/jee/96.3.844
- Warrant, E. J.** (2017). The remarkable visual capacities of nocturnal insects: vision at the limits with small eyes and tiny brains. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **372**, 20160063. doi:10.1098/rstb.2016.0063
- Warzecha, A. and Egelhaaf, M.** (2000). Response latency of a motion-sensitive neuron in the fly visual system: dependence on stimulus parameters and physiological conditions. *Vision Res.* **40**, 2973-2983. doi:10.1016/S0042-6989(00)00147-4
- Watson, J., Ritzmann, R., Zill, S. and Pollack, A.** (2002). Control of obstacle climbing in the cockroach, *Blaberus discoidalis*. I. Kinematics. *J. Comp. Physiol. A Sens. Neural Behav. Physiol.* **188**, 39-53. doi:10.1007/s00359-002-0277-y
- Wcislo, W. T., Arneson, L., Roesch, K., Gonzalez, V., Smith, A. and Fernández, H.** (2004). The evolution of nocturnal behaviour in sweat bees, *Megalopta genalis* and *M. ecuadoria* (Hymenoptera: Halictidae): an escape from competitors and enemies? *Biol. J. Linn. Soc.* **83**, 377-387. doi:10.1111/j.1095-8312.2004.00399.x
- Wehner, R. and Müller, M.** (2006). The significance of direct sunlight and polarized skylight in the ant's celestial system of navigation. *Proc. Natl. Acad. Sci. USA* **103**, 12575-12579. doi:10.1073/pnas.0604430103
- Weihmann, T. and Blickhan, R.** (2009). Comparing inclined locomotion in a ground-living and a climbing ant species: sagittal plane kinematics. *J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol.* **195**, 1011-1020. doi:10.1007/s00359-009-0475-y
- Wild, A. L.** (2004). Taxonomy and distribution of the Argentine Ant, *Linepithema humile* (Hymenoptera: Formicidae). *Ann. Entomol. Soc. Am.* **97**, 1204-1215. doi:10.1603/0013-8746(2004)097[1204:TADOTA]2.0.CO;2
- Wilkinson, E. J. and Sherck, H. A.** (2005). The use of visual information for planning accurate steps in a cluttered environment. *Behav. Brain Res.* **164**, 270-274. doi:10.1016/j.bbr.2005.06.023
- Yarger, A. M. and Fox, J. L.** (2016). Dipteran halteres: perspectives on function and integration for a unique sensory organ. *Integr. Comp. Biol.* **56**, 865-876. doi:10.1093/icb/icw086
- Ye, S. and Comer, C. M.** (1996). Correspondence of escape-turning behavior with activity of descending mechanosensory interneurons in the cockroach, *Periplaneta americana*. *J. Neurosci.* **16**, 5844-5853. doi:10.1523/JNEUROSCI.16-18-05844.1996
- Ye, S., Leung, V., Khan, A., Baba, Y. and Comer, C. M.** (2003). The antennal system and cockroach evasive behavior. I. Roles for visual and mechanosensory cues in the response. *J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol.* **189**, 89-96. doi:10.1007/s00359-002-0383-x
- Zill, S. N., Keller, B. R., Chaudhry, S., Duke, E. R., Neff, D., Quinn, R. and Flannigan, C.** (2010). Detecting substrate engagement: responses of tarsal campaniform sensilla in cockroaches. *J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol.* **196**, 407-420. doi:10.1007/s00359-010-0526-4
- Zollikofer, C.** (1994). Stepping patterns in ants - influence of speed and curvature. *J. Exp. Biol.* **192**, 95-106.
- Zurek, D. B. and Gilbert, C.** (2014). Static antennae act as locomotory guides that compensate for visual motion blur in a diurnal, keen-eyed predator. *Proc. Biol. Sci.* **281**, 20133072. doi:10.1098/rspb.2013.3072