RESEARCH ARTICLE

Vision does not impact walking performance in Argentine ants Glenna T. Clifton^{1,3,*}, David Holway² and Nicholas Gravish^{1,*}

ABSTRACT

Many walking insects use vision for long-distance navigation, but the influence of vision on rapid walking performance that requires closerange 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 intermediaterange 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; Höltje 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.

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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 withinstride 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⁻¹ 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⁻¹ (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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Relevant time scale

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 preFig. 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.

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 10⁸-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, 1000×500 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):

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

with Θ =41,253 deg² 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 200×200 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 (~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 offscreen 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 (~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 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 Bovet and Benhamou (1988). Briefly, each trackway section was split into $\sim 2 \text{ mm}$ steps (based on an average 2 mm step length observed in Clifton et al., 2020). The turning angle traveled over each step (ϕ) was used to determine the mean cosine (*c*) and mean sine (*s*) of the changes in direction. From there, sinuosity (*S*) was calculated as:

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

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 ~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 recalculated 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_total/2 by d_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~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(cross_time)~substrate+light+(1|colony); cross_angle~ substrate+light+(1|colony); speed_far~substrate+light+(1|colony); and log(speed_close)~substrate+light+(1|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 mixedeffect model was log(antennae_speed)~substrate+light+(1|colony). The model for step substrates included close versus far distance as an additional variable: log(antennae_speed)~substrate+light+ distance+(1|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 mm² with an interommatidial angle of 15.3 deg. Ommatidia facets were approximately hexagonally spaced with areas ranging from 184 μ m² to 302 μ m² and diameters ranging up to 20–26 μ 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 mm s⁻¹, with 5% of the distance traveled occurring at speeds above 28.4 mm s⁻¹ (Fig. 4A, Fig. S2). In darkness, preferred speed reduced by 4.7%, with a median speed of 18.3 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 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 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 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 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 $\pm 1 \text{ s.d.}$ 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. Boxes represent the median and interquartile range. Dashed lines show the median antennal speed values for 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, shortrange 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 eve. 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 \sim 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⁻¹ 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⁻¹, 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 'trailfollowing' 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 (Perna 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 shortlength 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 (~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 (~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 (~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.

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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.

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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.

Supplementary information

Supplementary information available online at

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Figure S1. Argentine ant ommatidia facet information. (A-B) Facet outlines from two Argentine ant workers. Facets were manually outlined with colors representing the facet area. The total number of facets for each eye is listed in the panel, however not all facets were able to be outlined. The arrows show the rostral and medial orientations of the head. (C) Boxplots showing the distribution of the longest dimensions across all outlined facets for each ant. Numbers represent the number of facets measured / number of facets total in the eye for each ant. (D) Boxplots showing the facet areas for each ant. Number of facets measured is the same as shown in panel C.

Fig. S2



Figure S2. Histograms of preferred forward walking speeds for each colony. The y-axis represents the percent of the total distance traveled by speed (x-axis). A kernel density estimate was applied to the underlying data. Yellow curves represent light conditions, red curves represent dark conditions. Dashed lines represent uneven substrates. The vertical lines display the median "preferred" walking speeds and the peak walking speeds (5% of total distance above that value). Preferred walking speeds were higher in darkness in only one colony (03/07/2020). Data from the 03/05/2020 colony was removed from analysis since the collection included a very large number of ants which congregated within the tunnel.





Figure S3. Additional analyses of step crossing performance and antennae correlation. (A) The angular excursion of ants while crossing the step does not differ based on light and dark conditions (p = 0.226). Slightly more angular crossings on the contrasted step substrate ($p < 10^{-1}$ 0.001) likely result from pheromone trail stochasticity. Boxes show median and guartile values. (B) Median forward walking speeds when the ant thorax was between 3 and 1 mm from the step did not depended on either substrate (chi-squared LRT test, p = 0.43) or lighting (chi-squared LRT test, p = 0.75) conditions. (C) The lateral position of the left (purple) and right (turquoise) antennae with respect to the body (left) and head (right) varies over time in example trials. (D) Cross-correlation of the right and left antennae under light (yellow) and dark (red) conditions. The noted sample sizes apply to both body and head panels. With respect to both the body and head, correlation values are significantly higher on flat terrain in light (Mann Whitney U, p < 10.001) but not in darkness (Mann Whitney U, p = 0.25). However, lighting only influenced correlation on flat (p < 0.001 for both body and head) and not uneven (p = 0.16 for body and head) substrates. Higher correlation values with respect to the body likely result from head motion. (E) Example auto-correlation left (purple) and right (turquoise) traces for the trials depicted in panel A. The lag associated with a correlation value drop to 1/e is defined as tau.

Tau can be interpreted as the time-scale that positional information of an antennae informs future positions. (F) Tau distributions under dark and light conditions. Tau values were slightly, but significantly higher for flat versus uneven substrates under all conditions (p for light/dark for body: <0.001/0.04; for head: <0.001/0.03). Darkness was associated with slightly higher tau values on uneven ground (p < 0.001 for body and head), but not for flat terrain (p = 0.16 for body, 0.12 for head).



Figure S4. Preferred walking speeds, measures of trailway tortuosity, and step crossing performance for ants walking in darkness (red) and low, dusk light (yellow). (A) Histograms showing the distance traveled across speeds for ants on flat (above) and uneven (below) substrates in dusk light and darkness. Vertical lines represent the median speeds and the cutoff for the fastest 5% speed. In darkness, preferred speed reduced by 2.7%, with a median speed of 25.2 mm/s (Mann-Whitney U test, p < 0.001). Ants slowed down considerably on the checkerboard substrate under light conditions, with a median speed of 15.4 mm/s, 40.5% of the preferred speed under flat/light conditions. Similar to on flat substrates, preferred speed declined only by 1.9% in dark conditions on the uneven substrate (median speed 15.1 mm/s; Mann-Whitney U test, p = 0.03). (B) Measures of straightness and sinuosity of trackways on flat (solid lines) and uneven (dashed lines) terrain in dusk light (yellow) and dark (red) conditions. Boxes represent interguartile range with whiskers spanning all data points without excluding outliers. (C) Violin plots showing the fractal dimension of all trailways in dark and dusk light. (C) The time to cross the step differed for contrasted vs. un-contrasted substrates (chi-squared LRT test, p < 0.001), but did not depend on lighting (chi-squared LRT test, p = 0.04). Boxes show the median and interquartile range of the distributions. (D) The forward speed far from the step does not differ for substrate (p = 0.404) or lighting (p = 0.6053) conditions.

Table S1

Statistical analysis of *substrate*-induced differences in trackway straightness, sinuosity, and fractal dimension. Colony number refers to the day of recording in February or March 2020. "N" refers to the number of datapoints for each colony, lighting, and test variable. P-values were generated using a Mann-Whitney U test. Bolded p-values are < 0.01. The accuracy of these measures has been disputed in the literature, we therefore only report values for straightness and sinuosity within the body of the paper.

Colony	25	03	04	05	06	07	NO COLONY
Straightness							
N_Dark	173	191	123	0	75	118	680
N_Light	245	207	114	0	105	188	859
p_Dark	6.09E-11	1.75E-07	0.009		0.167	0.002	1.39E-17
p_Light	1.08E-08	0.004	0.138		0.066	0.003	2.93E-13
Sinuosity							
N_Dark	165	188	113	0	73	110	649
N_Light	236	204	112	0	95	173	820
p_Dark	0.131	0.102	0.094		0.004	0.142	0.058
p_Light	0.023	0.354	0.004		0.371	0.287	0.004
Fractal Dimension							
N_Dark	151	183	97	0	63	84	578
N_Light	215	192	100	0	66	130	703
p_Dark	1.53E-07	0.002	0.176		0.852	4.08E-04	4.92E-09
p_Light	7.50E-08	0.003	0.484		0.608	0.031	6.30E-09

Table S2

Statistical analysis of *light*-induced differences in trackway straightness, sinuosity, and fractal dimension. Colony number refers to the day of recording in August 2019. "N" refers to the number of datapoints for each colony, substrate, and test variable. P-values were generated using a Mann-Whitney U test. Bolded p-values are < 0.01. The accuracy of these measures has been disputed in the literature, we therefore only report values for straightness and sinuosity within the body of the paper.

Colony	25	03	04	05	06	07	NO COLONY
Straightness							
N_Dark	260	206	124	0	89	154	833
N_Light	234	194	113	0	91	152	784
p_Dark	0.211	0.610	0.930		0.069	0.675	0.806
p_Light	0.765	0.025	0.057		0.016	0.090	0.060
Sinuosity							
N_Dark	243	201	112	0	80	133	769
N_Light	234	193	113	0	88	150	778
p_Dark	0.890	0.568	0.063		0.419	0.272	0.302
p_Light	0.840	0.827	0.147		0.154	0.678	0.160
Fractal Dimension							
N_Dark	202	184	88	0	56	85	615
N_Light	232	193	109	0	73	129	736
p_Dark	0.811	0.442	0.655		0.536	0.293	0.830
p_Light	0.222	0.582	0.797		0.076	0.034	0.481