

RESEARCH ARTICLE

Miniaturisation reduces contrast sensitivity and spatial resolving power in ants

Ravindra Palavalli-Nettimi, Yuri Ogawa, Laura A. Ryan, Nathan S. Hart and Ajay Narendra*

ABSTRACT

Vision is crucial for animals to find prey, locate conspecifics and navigate within cluttered landscapes. Animals need to discriminate objects against a visually noisy background. However, the ability to detect spatial information is limited by eye size. In insects, as individuals become smaller, the space available for the eyes reduces, which affects the number of ommatidia, the size of the lens and the downstream information-processing capabilities. The evolution of small body size in a lineage, known as miniaturisation, is common in insects. Here, using pattern electroretinography with vertical sinusoidal gratings as stimuli, we studied how miniaturisation affects spatial resolving power and contrast sensitivity in four diurnal ants that live in a similar environment but vary in their body and eye size. We found that ants with fewer and smaller ommatidial facets had lower spatial resolving power and contrast sensitivity. The spatial resolving power was maximum in the largest ant *Myrmecia tarsata* at 0.60 cycles deg^{-1} compared with that of the ant with smallest eyes *Rhytidoponera inornata* at 0.48 cycles deg^{-1} . Maximum contrast sensitivity (minimum contrast threshold) in *M. tarsata* (2627 facets) was 15.51 (6.4% contrast detection threshold) at 0.1 cycles deg^{-1} , while the smallest ant *R. inornata* (227 facets) had a maximum contrast sensitivity of 1.34 (74.1% contrast detection threshold) at 0.05 cycles deg^{-1} . Miniaturisation thus dramatically decreases maximum contrast sensitivity and also reduces spatial resolution, which could have implications for visually guided behaviours. This is the first study to physiologically investigate contrast sensitivity in the context of insect allometry.

KEY WORDS: Pattern electroretinogram, Vision, Spatial resolution, Compound eye, Acuity, Lamina

INTRODUCTION

Size has profound implications for the biology of organisms. It plays a crucial role in morphological and physiological design, and dictates the performance of sensory systems and through this the lifestyle and the information-processing capacities of animals (Bonner, 2011; Calder, 1984; Schmidt-Nielsen, 1984). The evolution of extremely small body size within a lineage, reduction beyond which is not possible without functional consequences owing to anatomical and physiological constraints, is a phenomenon known as miniaturisation (Hanken and Wake, 1993). Miniaturisation is a widespread phenomenon across the animal kingdom (Hanken

and Wake, 1993). As many insects are polymorphic, they provide an opportunity to characterise the ecologically relevant benefits and costs associated with miniaturisation (e.g. Peeters and Ito, 2015). The benefits of being small include the ability to avoid predators and occupy niches that are inaccessible to larger animals (e.g. Peters, 1986). Reduced body size has implications for development and physiology, and places constraints on energetics and metabolic rates (e.g. Niven and Farris, 2012; Niven and Laughlin, 2008; Polilov, 2015; Ramirez-Esquivel, 2017). Vision is one of the sensory modalities where behavioural and neuronal responses can be recorded and quantified with exceptional accuracy (Jayatilaka et al., 2018; Nordström, 2012). Vision is indeed crucial for most insects for navigation, sexual selection, conspecific recognition, foraging and communication (Avarguès-Weber et al., 2011; Cronin et al., 2014; Stürzl et al., 2016; Tibbetts, 2002). Two visual capabilities that are fundamental to insects, and also to other animals, are spatial resolving power and contrast sensitivity. High spatial resolving power allows animals to discriminate between small objects and resolve fine detail whereas high contrast sensitivity (low contrast threshold) allows animals to discriminate objects as their achromatic contrast decreases.

The spatial resolving power and contrast sensitivity of insect compound eyes have been studied using several different techniques. Behavioural methods include optomotor experiments that rely on innate or reflex movements (Nityananda et al., 2015; Pick and Buchner, 1979) or Y-maze experiments where insects were trained to discriminate between horizontal and vertical gratings of differing spatial frequencies (Chakravarthi et al., 2016; Macuda et al., 2001; Srinivasan and Lehrer, 1988). Anatomical methods have also been used to estimate spatial resolving power based on interommatidial angle (e.g. Land, 1997a; Makarova et al., 2019; Snyder, 1977; Taylor et al., 2019). Interommatidial angle was measured either by tracking the pseudopupil or by estimating the number of facets (Curra et al., 2018; Land, 1997a; Narendra et al., 2013). Intracellular recordings of the response of photoreceptors to sinusoidal gratings of varying contrast and spatial frequency have also been investigated in several species (Catton, 1999; Rigosi et al., 2017). From studies that have used these different methods, we know that as eye size decreases, spatial resolving power reduces (anatomical estimates: *Cataglyphis* ants: Zollikofer et al., 1995; butterflies: Rutowski et al., 2009; bees: Jander and Jander, 2002; aphids: Doring and Spaethe, 2012; and moths: Fischer et al., 2014; behavioural estimates: bumblebees: Spaethe and Chittka, 2003; fruit flies: Curra et al., 2018; and psyllids: Farnier et al., 2015). However, the effect of miniaturisation on contrast sensitivity has not been studied.

Physiologically, spatial vision has primarily been estimated using optical or photoreceptor properties. However, discrimination of patterns occurs in the lamina, which is the first optic neuropil. The lamina is made up of retinotopically organised columnar units where each ommatidium maps to one laminar column. Laminar cells

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

*Author for correspondence (ajay.narendra@mq.edu.au)

 A.N., 0000-0002-1286-5373

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enhance visual signal contrast by filtering information both temporally and spatially (Mauss and Borst, 2017). We used a technique known as pattern electroretinography (PERG) that allowed us to measure both the spatial resolving power and contrast sensitivity simultaneously from the lamina. The PERG technique relies on the fact that the recorded signal is dominated by higher order neurons that individually respond to changing patterns of illumination, whereas the summed responses of all photoreceptors should show little modulation because the mean intensity of the stimulus is constant (Porciatti et al., 1993). The PERG technique has been used in ants to compare spatial vision in nocturnal and diurnal species (Ogawa et al., 2019), and also in mammals (e.g. Porciatti, 2007), birds (Ghim and Hodos, 2006) and sharks (Ryan et al., 2017). Here, we used the PERG technique to identify the effect of miniaturisation on spatial resolving power and contrast sensitivity in ants.

MATERIALS AND METHODS

Study animals

We studied four species of diurnal ants with a varying number of facets in their compound eye: *Myrmecia tarsata* F. Smith 1858; *Myrmecia nigrocincta* F. Smith 1858; *Polyrhachis nr. aurea* Mayr 1876; and *Rhytidoponera inornata* Crawley 1922 (Fig. 1A). The ants were collected on or around Macquarie University campus, Sydney, NSW, Australia (33.7738°S, 151.1126°E) between December 2017 and January 2018. We carried out PERG experiments on 4–6 individuals for each species. We used data for *Myrmecia tarsata* from Ogawa et al. (2019).

Morphometrics

To measure the head widths of the ants, we took photographs using a digital camera (Sony FDR AX100) and measured the widest part of their heads using ImageJ (US National Institutes of Health, Bethesda,

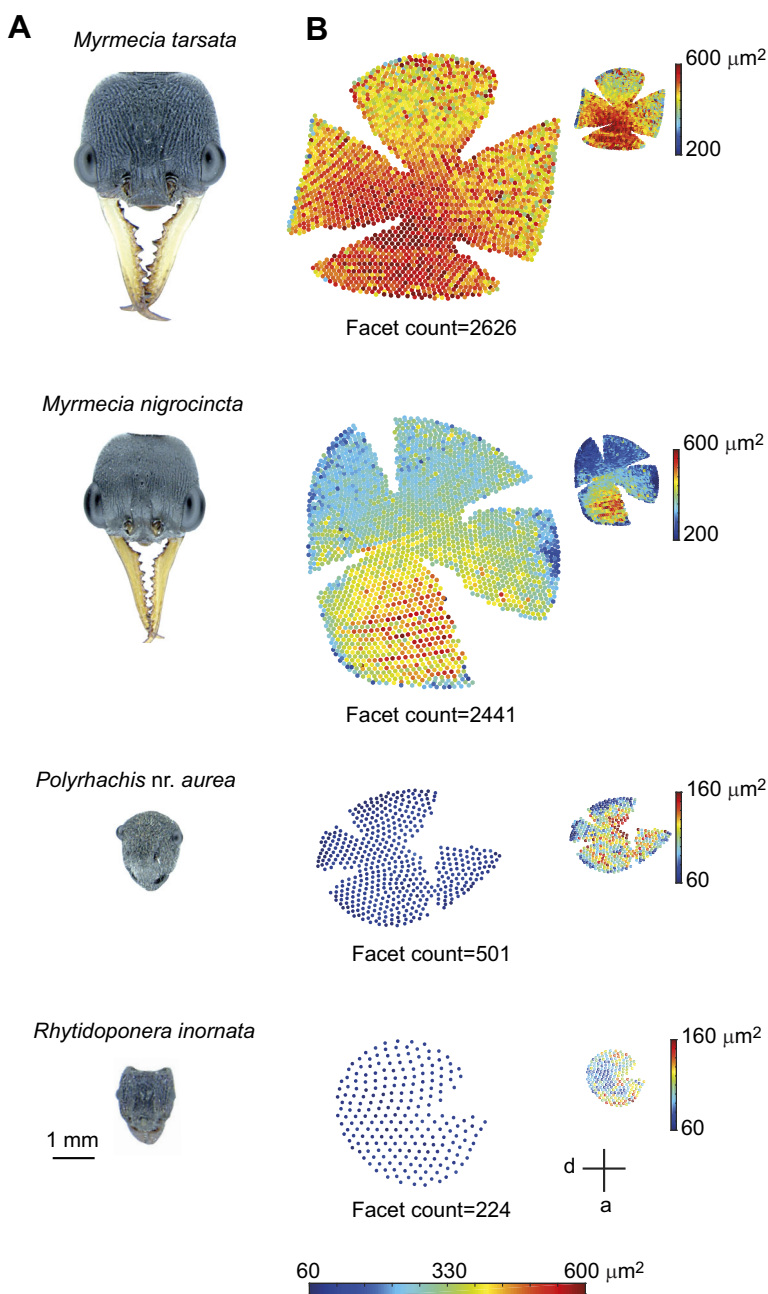


Fig. 1. Study species and corresponding eye map showing the variation in facet size. (A) Dorsal view of the head of the four study species. (B) Eye maps depict the facet size variation across species; the inset shows variation within each eye. The horizontal colour map scale indicates facet area for across-species comparison and the vertical colour map scale indicates facet area for comparison within each eye. The anterior (a) and dorsal (d) region of the eye are indicated bottom right.

MD, USA). For each eye on which we carried out PERG recordings, described below, we prepared eye replicas with transparent nail polish using well-established techniques (e.g. Ramirez-Esquivel et al., 2017). The eye replicas were photographed under a light microscope (Leica DM5000B, Leica Microsystems GmbH, Wetzlar, Germany). For each individual, we counted all the facets and measured facet diameter of an arbitrary 30 facets in the medio-frontal area of the eye using ImageJ. The variation of medio-frontal facet diameter between species was greater than that between individuals (nested ANOVA: species accounted for 86.3% and individuals accounted for 0.3% variation in medio-frontal facet diameter). Hence, we calculated the mean facet size of each species by taking an average of all facets in each individual and reporting the average of all individuals. In one randomly chosen individual for each species, we created an eye map using a custom-written program in MATLAB (courtesy of Richard Peters, La Trobe University, Australia) to map the distribution of different-sized lenses.

Pattern electroretinogram

In the same individuals for which we obtained morphometrics measurements, we performed electrophysiological experiments during the day between 09:00 h and 16:00 h. These experiments were carried out within a Faraday cage which was kept in a dark room at room temperature (21–24°C). Ants were first anaesthetised by cooling them on ice for 5 min, and their legs and antennae were removed. *Myrmecia* ants have a potent sting, hence we also removed their gaster. Each ant was further immobilised by mounting them on a plastic stage with their dorsal side up, and beeswax was then

applied to the mandibles, the constriction between the head and pronotum, and the petiole.

Electroretinograms were measured to determine the spatial resolving power and contrast sensitivity (1/contrast threshold) of the whole eye (Fig. 2). As an active electrode, a looped platinum wire was carefully placed on the cornea of the ant's right eye with a conductive gel (Livingstone International Pty Ltd, Mascot, NSW, Australia). We used an active electrode with a diameter of 0.25 mm for *Myrmecia* species and *P. nr. aurea* and 0.127 mm for *R. inornata*. As an indifferent electrode, we inserted a silver/silver-chloride electrode of 0.25 mm diameter into the mesosoma of the *Myrmecia* species and a platinum electrode of 0.127 mm diameter for *P. nr. aurea* and *R. inornata*. Electroretinograms were amplified using an alternating current (AC)-coupled differential amplifier (DAM50, World Precision Instruments Inc., Sarasota, FL, USA) with a gain of 1000 and bandpass filtered between 0.1 Hz and 100 Hz. Amplified voltage signals were sent to a computer via a 16-bit analog-to-digital converter (USB-6353 X-series, National Instruments, Austin, TX, USA).

The PERG visual stimuli were projected by a digital light-processing projector (W1210ST, BenQ Corporation, Taipei, Taiwan) onto a white melamine screen (W51×H81 cm) placed at 30 cm from the ant's eye. For an ant facing the screen, such a preparation has been shown to stimulate the medio-frontal region of the eye in *Myrmecia* ants (Ogawa et al., 2019). The stimuli were vertical contrast-reversing sinusoidal gratings of different angular spatial frequency (cycles deg⁻¹) and Michelson's contrast [$C=(I_{\max}-I_{\min})/(I_{\max}+I_{\min})$], where I is intensity; Michelson,

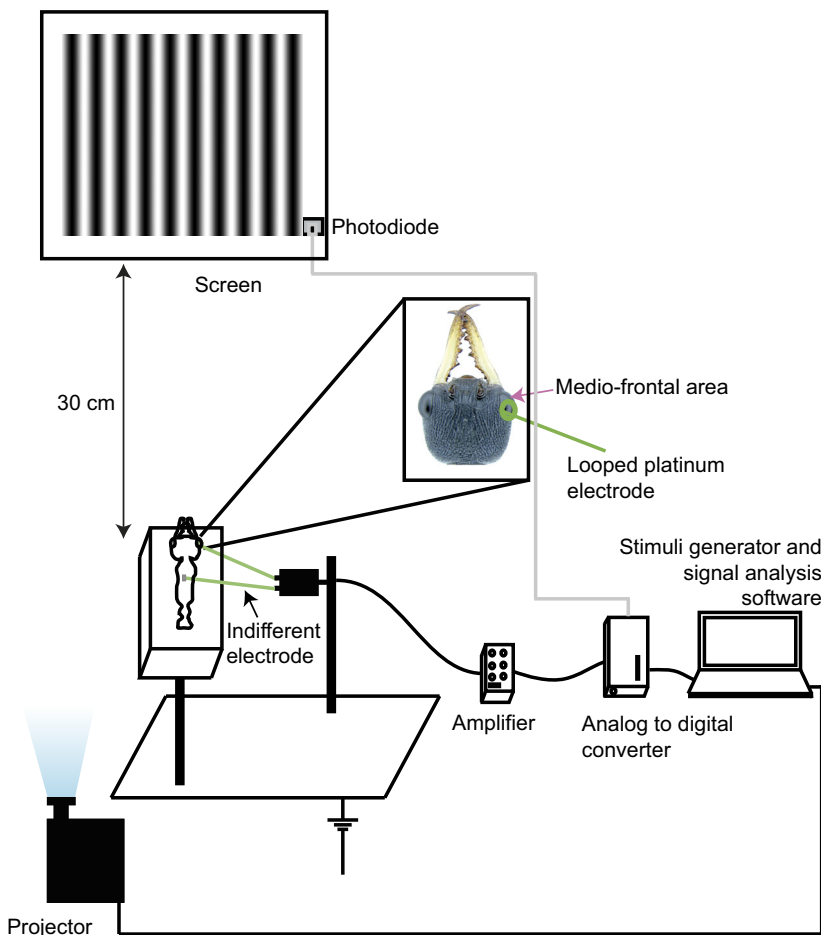


Fig. 2. Schematic diagram of the pattern electroretinography set-up. An ant was mounted on a plastic stage 30 cm from a screen on to which the stimuli were projected. Electrical activity was recorded using a loop platinum electrode placed on the right eye, ensuring the medio-frontal area of the eye (inset) was exposed to the stimuli. The photodiode on the screen was used to sync the stimulus to the response. See Materials and Methods for details of the set-up. Figure not to scale.

1927]. The stimuli were generated using Psychtoolbox 3 (Pelli, 1997) and MATLAB (R2015b, Mathworks, Natick, MA, USA) controlled via custom Visual Basic software (Nathan S. Hart, Macquarie University) written in Visual Studio (2013, Microsoft Corporation, Redmond, WA, USA). The gratings had a mean irradiance of 1.75×10^{-4} W cm $^{-2}$, measured using a radiometer (ILT1700, International Light Technologies, Peabody, MA, USA), and was kept constant for all the stimuli. The stimuli were reversed with a temporal frequency of 2 Hz.

Prior to the first recording, the ant was adapted to a uniform grey stimulus with same mean irradiance as the grating stimuli for 20 min. To measure the contrast sensitivity (1/contrast threshold) of the eye, the ant was presented with 11 spatial frequencies (0.6, 0.5, 0.45, 0.4, 0.35, 0.3, 0.25, 0.2, 0.15, 0.1 and 0.05 cycles deg $^{-1}$) and up to eight contrasts (95%, 85%, 75%, 50%, 25%, 12.5%, 6% and 3%) for each spatial frequency. In order to ensure the lowest spatial frequency that we tested was well below a noise threshold (described next), we presented the smallest two ants with an additional spatial frequency of 0.025 cycles deg $^{-1}$. But these values were not used in the final analyses as they were redundant.

The spatial frequencies of the gratings were presented in the order of decreasing frequency of every second spatial frequency. Then, the interleaved spatial frequencies were presented in an ascending order to assess any degradation of the response over time. At each spatial frequency, different contrasts were tested in decreasing order. For each spatial frequency and contrast combination, 15 repetitions of the response for 5 s each were averaged in the time domain and analysed to obtain the mean response in the frequency domain using a fast Fourier transform (FFT). Consequently, the amplitude of the second harmonic (4 Hz) of the FFT response spectrum was recorded for each stimulus (see detailed methods in Ryan et al., 2017). To measure any non-visual responses (i.e. background noise) for each ant, we ran a control protocol at two of the lowest spatial frequencies tested for each species at 95% contrast with a black board to shield the ant from the visual stimuli before and after the experimental

series. The maximum recorded voltage signal at the second harmonic of the FFT out of the four control runs was used as the noise threshold.

Estimation of spatial resolving power and contrast sensitivity

To assess whether the response signal at the second harmonic (4 Hz) of the FFT response spectrum differed from background neural noise, for each spatial frequency and contrast combination we checked whether the response peak value differed significantly from 10 neighbouring frequencies, five on either side, using an *F*-test. Spatial resolving power (at 95% contrast) and contrast threshold values were obtained by interpolating from the last point above the noise threshold (blue lines in Fig. 3) and the first point below the noise threshold. The intersection point of the interpolated line and the noise threshold was considered the spatial resolving power or contrast threshold (Fig. 3). If the first point below the noise threshold was not significantly greater than the 10 surrounding frequencies, the spatial frequency of the last point above the threshold was considered as the spatial resolving power. Contrast sensitivity was obtained by calculating the inverse of the contrast threshold.

Electroretinogram

PERG uses higher harmonics of the electroretinogram response to a sinusoidal grating stimulus to isolate post-receptor response components. To identify whether ants provided a robust and reliable neural signal in our PERG experiments, we measured the neural response directly by electroretinogram (ERG), by measuring transients at light ON and OFF. For this, we measured neural responses in our largest study species *M. tarsata* and smallest study species *R. inornata* using ON and OFF electroretinograms. Here, the electrical response from the whole eye to changes in illumination (light ON and OFF) was measured. The resulting response waveform consists of summed changes in extracellular potentials

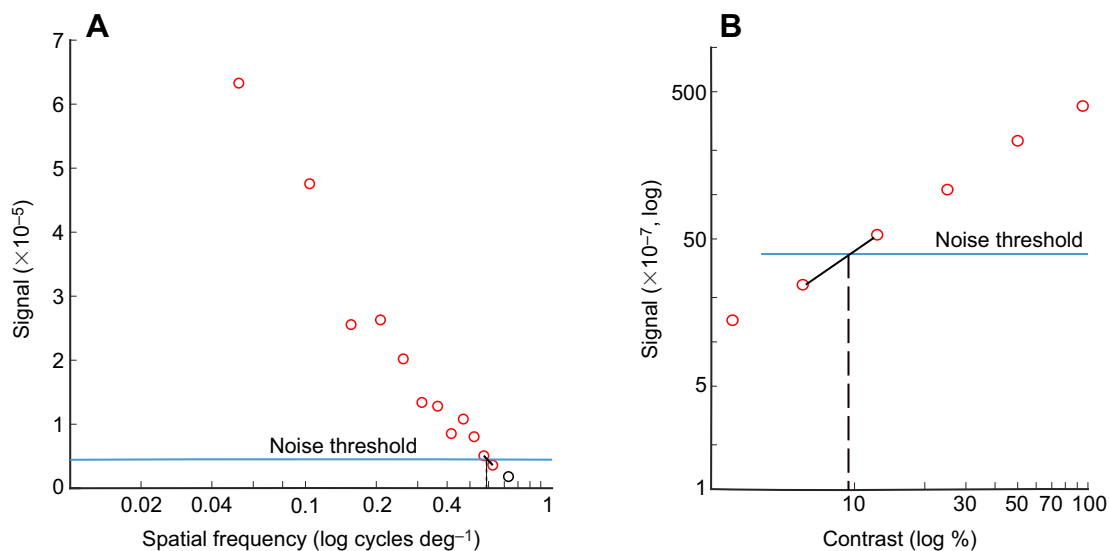


Fig. 3. Estimation of spatial resolving power and contrast threshold from voltage signals obtained from an ant's eye. (A) Spatial resolving power, the highest spatial frequency to which the ant could respond (at 95% contrast). (B) Contrast threshold, the lowest contrast to which the ant could respond, shown for one spatial frequency of the stimuli. Blue lines represent maximum signal from control treatments where the ant was shielded from the stimuli. Red data points indicate significant peaks in the voltage signal at 4 Hz; the black data point in A indicates that the peak value was not significantly different from the neighbouring 10 values of the fast Fourier transformed voltage signal (see Materials and Methods for details). Spatial resolving power and contrast threshold are the x-axis values at the intersection of the dashed lines in A and B, respectively. Contrast sensitivity was obtained by taking the inverse of contrast threshold. Example data shown were obtained from one animal.

Table 1. Variation in size and spatial vision of the study species

	<i>M. tarsata</i> (n=5)	<i>M. nigrocincta</i> (n=4)	<i>P. nr. aurea</i> (n=6)	<i>R. inornata</i> (n=4)
Head width (mm)	3.21±0.17	2.15±0.04	1.23±0.03	1.31±0.02
Facet number per eye	2627±53	2483±42	522±11	227±7
Medio-frontal facet diameter (µm)	22.4±0.36	20.5±0.5	12.5±0.2	12.75±0.25
Spatial resolving power (cycles deg ⁻¹)	0.60±0.004	0.52±0.0005	0.51±0.02	0.48±0.01
Maximum contrast sensitivity	15.51±0.7 at 0.1 cycles deg ⁻¹	20.68±0.6 at 0.05 cycles deg ⁻¹	2.17±0.5 at 0.05 cycles deg ⁻¹	1.34±0.5 at 0.05 cycles deg ⁻¹

Means±s.e.m. are listed for head width, facet number, medio-frontal facet diameter, spatial resolving power and contrast sensitivity. Sample sizes (*n*) are indicated below the species name.

produced by photoreceptors and second-order neurons in the lamina. To measure ERGs, we prepared each ant (*n*=4 for each species) with electrodes attached to their left eye as described for PERG above. A cool white LED light source (5 mm in diameter with an irradiance of 5.81×10^{-5} W cm⁻², C503C-WAS-CBADA151, Cree Inc., Durham, NC, USA) placed at a distance of 15 cm from the animal's eye was used as a stimulus. The ants were dark adapted for 5 min prior to stimulation. The stimulus of light ON and OFF for a duration of 5 s each was presented using custom MATLAB software (courtesy of Jan Hemmi, University of Western Australia). Ten such consecutive repetitions of ON and OFF responses were averaged to obtain an overall ERG waveform for each ant. ERGs were amplified using an AC-coupled differential amplifier (DAM50, World Precision Instruments Inc.) with a gain of 100, bandpass filtered between 1 Hz and 1 kHz. The experimental set-up was housed inside a Faraday cage at room temperature (22°C).

Data analyses

The number of facets and the size of each facet are known to affect contrast sensitivity and resolving power (Land and Nilsson, 2002). We found that facet count and medio-frontal facet diameter were co-linear in the four studied species (Pearson's correlation: $r=0.97$, $t=19.1$, d.f.=17, $P \ll 0.01$), so we used only medio-frontal facet diameter for subsequent analyses. To assess the relationship between contrast sensitivity, spatial frequency and medio-frontal facet diameter, we used a linear mixed-effects model by restricted maximum likelihood ('lme4' package, v1.1.383; <https://cran.r-project.org/web/packages/lme4/index.html>). We used inverse transformation of contrast sensitivity, i.e. contrast threshold, in the model to meet the assumption of homogeneity of variance (Zar, 2010). Medio-frontal facet diameter and spatial frequency were used as fixed effects, and animal ID nested within species was used as a random effect. The significance of the fixed effect terms was examined using *t*-tests with Satterthwaite approximation for degrees of freedom ('lmerTest' package). Residuals of the model were inspected visually to check for the assumptions of normality and homogeneity of variance. We used a linear model to determine the relationship between spatial resolving power and medio-frontal facet diameter.

RESULTS

Size variation in the study species

Among the four species, *M. tarsata* was the largest with twice the head width and 11 times more facets compared with *R. inornata*, which had the smallest eyes (Table 1, Fig. 1). Head width was positively correlated with facet count (Pearson correlation, $r=0.89$, $t_{17}=8.18$, $P \ll 0.01$) and average medio-frontal facet diameter (Pearson correlation, $r=0.93$, $t_{17}=10.37$, $P \ll 0.01$). While facets in

the medio-frontal area of the eye were largest within each species (an exception being the smallest ant), both the *Myrmecia* species had larger facets compared with those of the smaller species, *P. nr. aurea* and *R. inornata* (Fig. 1B).

Spatial resolving power

From the PERG recordings, we did not find any degradation of the response to different spatial frequencies of the stimulus over the recording session. We found that *M. tarsata* had the highest spatial resolving power at 0.60 ± 0.004 cycles deg⁻¹ (mean±s.e.m), compared with 0.48 ± 0.01 cycles deg⁻¹ in the smallest study species, *R. inornata* (Table 1). We found that the medio-frontal facet diameter explained the variation in spatial resolving power (Fig. 4, Table 2). Species with smaller facets had lower resolving power, although *M. nigrocincta* had less resolving power than expected (Fig. 4). This species also had the least variation between individuals, while *P. nr. aurea* had the most variation (Fig. 4).

Contrast sensitivity

Maximum contrast sensitivity decreased (minimum contrast threshold increased) with decreasing number and size of facets (Table 1, Fig. 5A). The two smaller species, *P. nr. aurea* and *R. inornata*, had different slopes and intercepts in the regression model when compared with the two bigger *Myrmecia* species

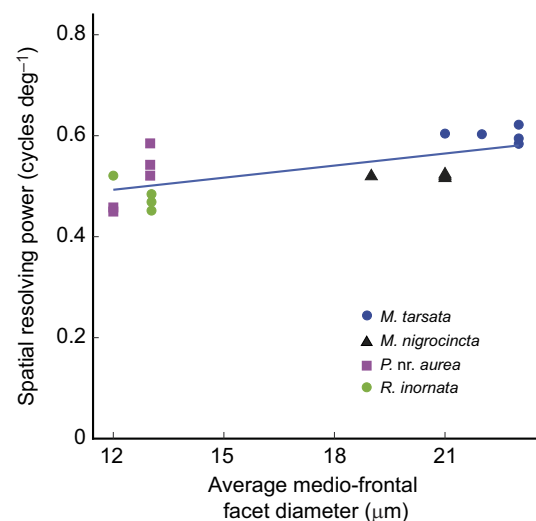


Fig. 4. Relationship between spatial resolving power and average medio-frontal facet diameter in four ant species. Data from each species are shown in a different colour and symbol. This nomenclature is similar to that used in Figs 5 and 6. The regression line is based on the estimates of a linear model fit as shown in Table 2.

Table 2. Summary of the linear model fit for testing the relationship between spatial resolving power and average medio-frontal facet diameter

Parameter	Estimate	s.e.m.	t-value	P-value
Intercept	0.39	0.03	11.19	<<0.01
Average medio-frontal facet diameter	7.98	2.03	3.92	<0.01

(Fig. 5B), indicating that size explains the variation in contrast threshold. The variation in contrast sensitivity ($1/\text{contrast threshold}$) at the species level was best explained by medio-frontal facet diameter, spatial frequency of the stimuli and an interaction between the two (Table 3). The bigger ants could perceive a low angular frequency pattern at a much lower contrast than the smaller ants, but for a higher angular frequency, both the smaller and bigger ants

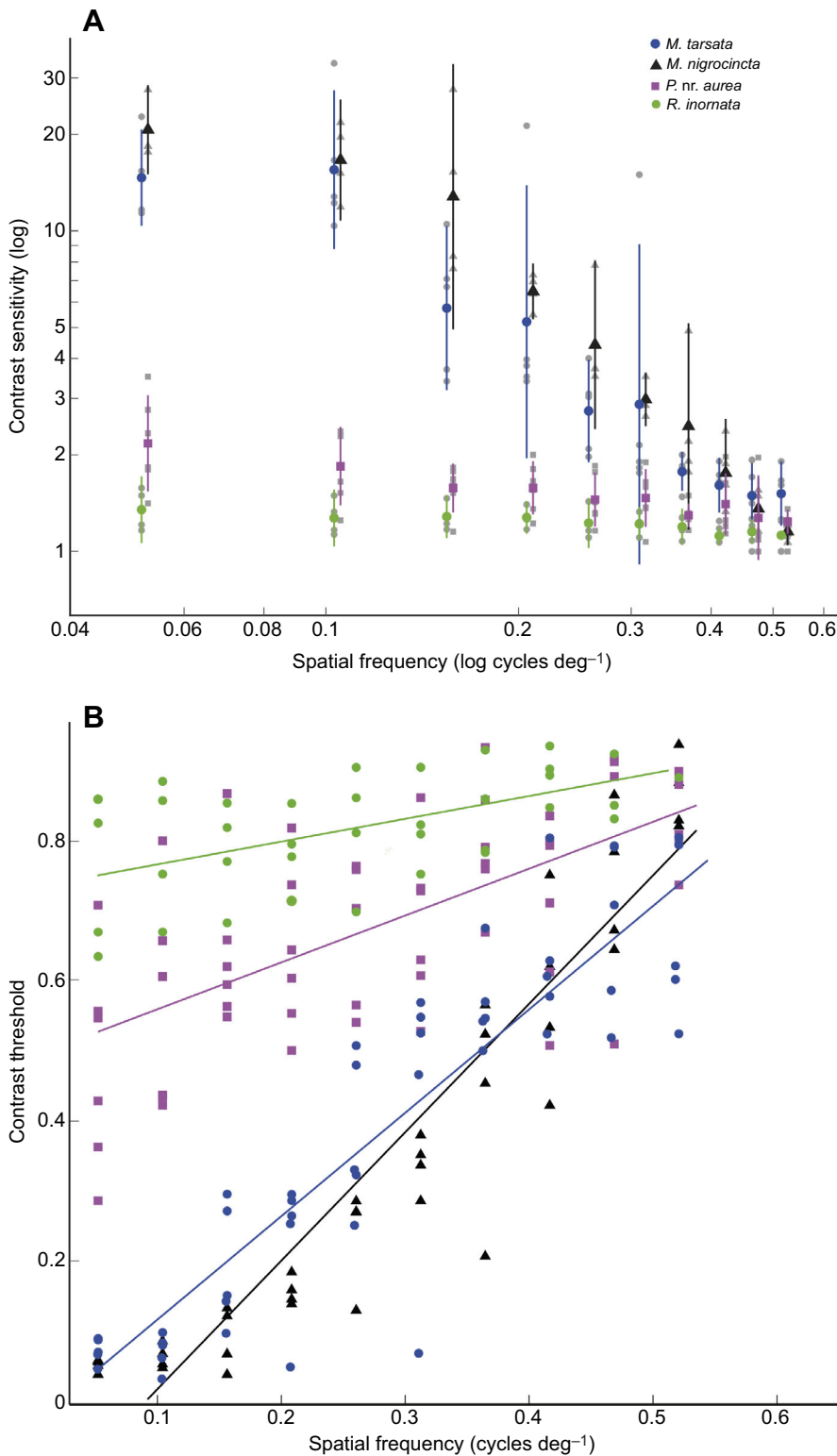


Fig. 5. Relationship between contrast sensitivity, contrast threshold and spatial frequency of the stimuli. (A) Contrast sensitivity data are shown as means \pm 95% confidence interval for each species. A lower contrast sensitivity value indicates a higher contrast threshold; ants could detect gratings of that particular spatial frequency only when sinusoidal gratings (stimuli) had a higher contrast. Note that a log scale is used on both the axes, and the data points for each species are slightly shifted for clearer visualisation. (B) Relationship between contrast threshold and spatial frequency. The raw data used for linear mixed model analyses are plotted with a unique colour for each study species. Each data point within each species corresponds to the contrast threshold for a given spatial frequency for an individual ant. Linear mixed model fit lines are shown for each species, indicating that smaller species have shallower slopes.

Table 3. Summary of linear mixed model fit by restricted maximum likelihood for testing the relationship between contrast threshold, average medio-frontal facet diameter and spatial frequency

Parameter	Estimate	s.e.m.	d.f.	t-value	P-value
Intercept	1.43	0.18	3.01	7.74	<0.01
Average medio-frontal facet diameter	-0.07	0.01	3.14	-6.58	<0.01
Spatial frequency	-0.74	0.18	169.06	-4.10	<<0.01
Average medio-frontal facet diameter: spatial frequency	0.11	0.01	168.79	10.78	<<0.01

Mixed model parameters: fixed effects are average medio-frontal facet diameter and spatial frequency, and random effects are ant ID nested within species. The *t*-tests for fixed effects used Satterthwaite approximations to degrees of freedom (d.f.). The variance in each of the random effects is less than 1%.

needed a higher and roughly similar contrast to detect the pattern. *Myrmecia nigrocincta* was an exception where despite having small facets they had a higher average contrast sensitivity (lower contrast threshold) compared with *M. tarsata* (Fig. 5A).

Electroretinogram

ERG waveforms consist of four major components: a cornea negative transient, a sustained slow decaying ON component which plateaus, a cornea negative OFF transient and a cornea positive sustained decaying OFF component (Fig. 6). Photoreceptor hyperpolarisation contributes to the cornea negative transient, and the sustained ON, while its depolarisation contributes to the cornea positive sustained OFF component (e.g. Popkiewicz and Prete, 2013). ERG responses originating from the second-order neurons in the lamina typically consist of ON and OFF transients (voltage change spikes) at the beginning and the end of the stimulus

(Coombe, 1986). In our case, an ON transient from the lamina was not evident from the summed voltage response from both the photoreceptors and lamina in the final electroretinogram waveform. But we were able to clearly see the OFF transient (Fig. 6, inset). In the final summed electroretinogram waveform, the presence of the cornea negative OFF transient from the lamina leads to a drop in the voltage (c in Fig. 6 inset) of the cornea positive OFF component originating from depolarising photoreceptors (d in Fig. 6). In addition, the electroretinogram waveform amplitudes were larger for *M. tarsata* (with bigger eyes) than for *R. inornata* (Fig. 6); in particular, the OFF transient amplitude for *M. tarsata* was higher (Fig. 6, inset). Because the OFF transient amplitude was lower for *R. inornata*, it led to a saturating peak, so for this species we measured the duration of the peak for which the amplitude did not change more than 0.1% (see Fig. 6, inset). The OFF transient saturation duration for *R. inornata* ($t_2=13.85\pm 2.63$ ms; mean \pm s.e.m.) is comparable to that of the OFF transient peak for *M. tarsata* ($t_1=17.75\pm 1.03$ ms; Fig. 6, inset), suggesting the presence of a transient in both ants. Thus, we were able to confirm the presence of the post-receptor neural signals from the lamina in the ants, which shows that PERG responses are indeed from the second-order neurons in the lamina. Note that all our recordings were extracellular and thus they were inverted electroretinogram waveforms of intracellular recordings which are typically measured (e.g. Alawi and Pak, 1971; Järvilehto and Zettler, 1973).

DISCUSSION

Using PERG, we tested whether miniaturisation affects two key visual capabilities in ants: contrast sensitivity and spatial resolving power. We found that, on average, smaller ants had dramatically reduced contrast sensitivity and lower spatial resolving power (Table 1). The largest of the four species we studied had a spatial resolving power of 0.60 cycles deg^{-1} , while for the smallest it was

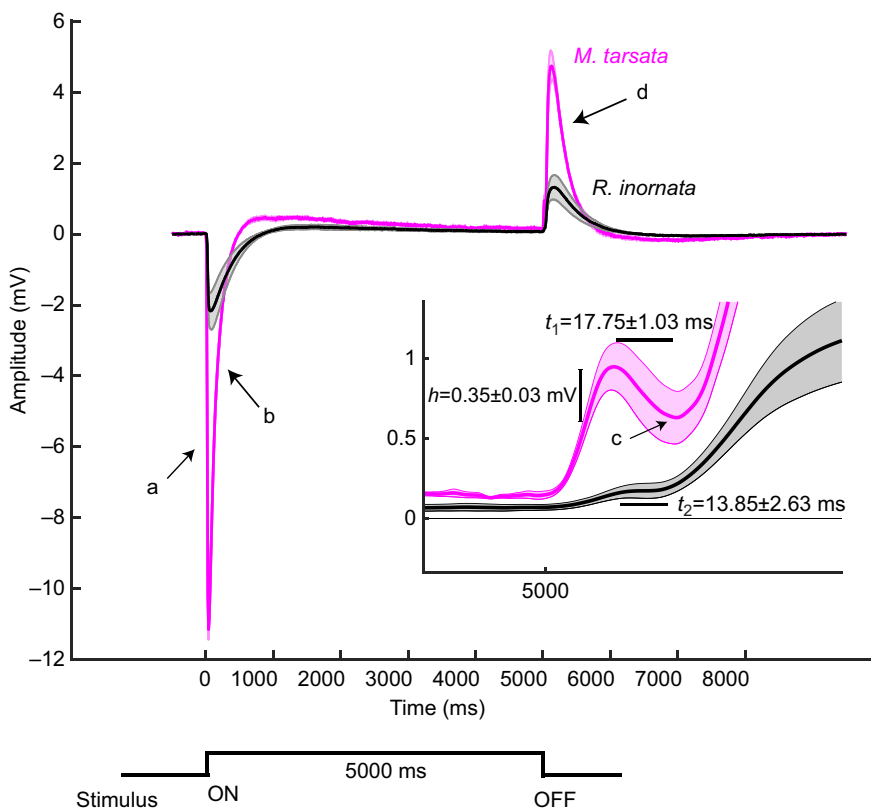


Fig. 6. Electroretinograms of *M. tarsata* and *R. inornata* species. The electroretinograms (means \pm s.e.m.; $n=4$ for each species) consist of four waveforms: a cornea negative transient, which is not evident here because the voltage response is the sum of both the photoreceptors and second-order neurons in the lamina (a), a sustained slow-decaying ON component which plateaus (b), a cornea negative OFF transient (c; inset), and a cornea positive sustained decaying OFF component (d). The OFF transient peak (c) contributed by the lamina can be seen in the inset. The amplitude of the OFF transient peak (\pm s.e.m.) for *M. tarsata* (h) and the duration of the OFF transient (\pm s.e.m.) for both ants (t_1 for *M. tarsata*, t_2 for *R. inornata*) are indicated.

0.48 cycles deg^{-1} . The maximum contrast sensitivity (minimum contrast threshold) of the largest species was 15.51 (6.4% contrast detection threshold) at 0.1 cycles deg^{-1} , while that of the smallest species was 1.34 (74.1% contrast detection threshold) at 0.05 cycles deg^{-1} . We discuss these results in the light of the implications of miniaturisation in ants.

Our results show prominent differences in the visual capabilities of ants that varied in head width. Measurements from the eye replicas across our four study species revealed that the number of ommatidia was reduced and facets became smaller in size as head width decreased (Fig. 1) – a pattern that has been observed in other insects (e.g. Currea et al., 2018; Fischer et al., 2014; Rutowski et al., 2009). The medio-frontal region of the eye of the larger ants (two *Myrmecia* species) had predominantly larger facets in comparison to those of the smallest species (Fig. 1). This region in the larger ants can be either an ‘acute zone’ with decreased interommatidial angles and higher resolving power than the rest of the eye or a ‘bright zone’ with an increased photon catch and higher sensitivity (Land and Nilsson, 2002).

Whether the medio-frontal region of the eye is an acute zone or a bright zone has implications for the ecology and/or behaviour of the animal. Consider the jack jumper ant *M. nigrocincta*, which had smaller facets than those of *M. tarsata*, but higher average contrast sensitivity (lower contrast threshold) for most spatial frequencies (Fig. 5B, steepest slope). Its spatial resolving power does not seem to be different from that of the smaller ants with smaller facets (Fig. 4). This suggests that the medio-frontal region in *M. nigrocincta* might be a bright zone with higher sensitivity rather than an acute zone with higher spatial resolving power. Typically, fast-moving or flying insects have acute zones, but there are exceptions. For example, fast-moving male hoverflies have bright zones with increased contrast sensitivity rather than increased resolving power (Straw et al., 2006). The higher average contrast sensitivity in the jack jumper ant is probably an adaptation to the rapid visual pursuit of small flying targets, such as bees or flies, and jumping behaviour, which are typical of this species. Increased contrast sensitivity is particularly useful in visually tracking and catching prey mid-air against the background of the canopy and sky (Land, 1997b). Future studies on the temporal resolution of the jumping ant should shed more light into how its fast movement affects spatial resolving power and sensitivity.

Although the smallest ant in our study, *R. inornata*, had 8% of the facets of the largest ant, *M. tarsata*, it still had 80% of their spatial resolution but only 8% of their contrast sensitivity. This suggests that *R. inornata* requires spatial resolving power more than contrast sensitivity. This also indicates that spatial resolution and contrast sensitivity do not decrease similarly with size. The reduced contrast sensitivity can also be attributed to the decreased number of facets, with *M. nigrocincta* being an outlier given their unique foraging behaviour, as discussed earlier. Smaller individuals of *Drosophila melanogaster* have sacrificed contrast sensitivity to improve spatial resolution (Currea et al., 2018). But these flies rely on temporal summation to improve contrast sensitivity. Increased integration duration of photoreceptors enhances visual sensitivity by increasing photon capture, signal to noise ratio and contrast discrimination (Warrant, 1999). To determine whether this was the case in our ants, from our electroretinogram recordings of the largest and smallest ant, we measured the duration of the ON response (first peak in Fig. 6) as the full-width of the response at half the maximum amplitude. The duration of the ON response was short in *M. tarsata* (106.95 ± 3.84 ms; $n=4$) compared with that in *R. inornata* (261.35 ± 53.49 ms; $n=4$). This shows that *R. inornata* have longer integration times, which may allow them to improve their low contrast

sensitivity by temporal summation. How this potentially improved contrast sensitivity inferred from longer integration times, which is dissimilar to the low contrast sensitivity that we measured at the laminar second-order neurons, improves the animal’s response is unclear at this stage.

It is also possible that the smaller ants in our study may not require high contrast sensitivity to forage and navigate in their surroundings. Both *Myrmecia* ants that we studied are generalist predators and are fast moving whereas the smaller ants *P. nr. aurea* and *R. inornata* are relatively slow moving and opportunists (Brown, 2000). Hence the smaller, slow-moving ants may have lower contrast sensitivity. Though high-resolution information in the visual panorama is not required for navigation (Milford, 2013; Wystrach et al., 2016), it would be essential for avoiding or detouring obstacles. Indeed, smaller ants detect and detour around obstacles only when they are significantly closer to it, compared with the larger ants (Palavalli-Nettimi and Narendra, 2018). Miniaturisation decreases spatial resolving power and contrast sensitivity in ants, thus affecting certain visually guided behaviours.

Contrast sensitivity has been measured in a number of insects physiologically or estimated behaviourally. Behavioural experiments suggest that contrast sensitivity might be dependent on behavioural task, with bumblebees having a high contrast sensitivity of 33 during flight control (Chakravarthi et al., 2017), but exceptionally low contrast sensitivity of 1.57 during object discrimination tasks (Chakravarthi et al., 2016). Monitoring the steering ability of tethered *D. melanogaster* that was stimulated by moving sinusoidal gratings of different contrasts, and spatial and temporal frequencies showed that both smaller and larger flies had a contrast sensitivity (lowest discernible contrast) of 2.22 (Currea et al., 2018). Physiologically, contrast sensitivity has been measured from motion-detecting neurons in blowflies, which have a peak value of 25–40 (Dvorak et al., 1980), and hoverflies, which have a peak value of 40–100 (O’Carroll et al., 1996; O’Carroll and Wiederman, 2014; Straw et al., 2006). At present, it is difficult to compare the contrast sensitivity values from our work with previous work because of differences in methods. Using PERG to measure contrast sensitivity makes our study unique, especially as contrast sensitivity cannot be estimated anatomically. We hope our study will encourage the use of PERG in other insects, which will allow for a direct comparison between species in the future.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: Y.O., A.N.; Methodology: R.P.-N., Y.O., L.A.R., N.S.H., A.N.; Software: Y.O., L.A.R., N.S.H.; Validation: Y.O.; Formal analysis: R.P.-N., Y.O., N.S.H.; Investigation: R.P.-N., Y.O.; Resources: L.A.R., N.S.H., A.N.; Data curation: R.P.-N.; Writing - original draft: R.P.-N.; Writing - review & editing: Y.O., L.A.R., N.S.H., A.N.; Visualization: R.P.-N., Y.O., A.N.; Supervision: A.N.; Project administration: A.N.; Funding acquisition: A.N.

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Data availability

Raw data for contrast sensitivity, acuity and facet diameter are available in Excel format: https://ecologicalneuroscience.files.wordpress.com/2019/05/nettimi_et_al_perp_mini_suppl.xlsx

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