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Miniaturisation reduces contrast sensitivity and spatial resolving power in ants

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Summary statement

Using pattern electroretinography, we show that miniaturisation in ants reduces both contrast sensitivity and spatial resolving power

Abstract

Vision is crucial for animals to find prey, locate conspecifics, and to 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 varied 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 per degree (cpd) compared to the ant with smallest eyes *Rhytidoponera inornata* that had 0.48 cpd. Maximum contrast sensitivity (minimum contrast threshold) in *M. tarsata* (2627 facets) was 15.51 (6.4% contrast detection threshold) at 0.1 cpd, while the smallest ant *R. inornata* (227 facets) had a maximum contrast sensitivity of 1.34 (74.1% contrast detection threshold) at 0.05 cpd. This is the first study to physiologically investigate contrast sensitivity in the context of insect allometry. Miniaturisation thus dramatically decreases maximum contrast sensitivity and also reduces spatial resolution, which could have implications for visually guided behaviours.

Keywords: Pattern electroretinogram, contrast sensitivity, spatial resolution, compound eye, acuity, lamina

Introduction

Size has profound implications for the biology of organisms. It plays a crucial role in the morphological and physiological design, 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 includes the ability to avoid predators and occupy niches that are inaccessible to larger animals (e.g., Peters, 1986). Reduced body size has implications on the development, physiology and has 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 been also used to estimate spatial resolving power based on the interommatidial angles, $\Delta\phi$ (e.g., Land, 1997a; Makarova et al., 2019; Snyder, 1977; Taylor et al., 2019). The interommatidial angles were measured either by tracking the pseudopupil or estimating the number of facets (Currea et al., 2018; Land, 1997a; Narendra et al., 2013). Intracellular recordings of the response of photoreceptors to sinusoidal grating 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), moths (Fischer et al., 2014); behavioural estimates: bumblebees (Spaethe and Chittka, 2003), fruit flies (Currea 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 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 allows 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 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* (Smith, F., 1858); *Myrmecia nigrocincta* (Smith, F., 1858); *Polyrhachis nr aurea* (Mayr, 1876); and *Rhytidoponera inornata* (Crawley, 1922) (Fig. 1A). The ants were collected on or around Macquarie University campus, Sydney (33.7738°S, 151.1126° E) between December 2017 and January 2018. We carried out PERG experiments on 4 to 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 at the widest part of their heads using ImageJ (U. S. National Institutes of Health, Bethesda, MD, US). 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 (U. S. National Institutes of Health, Bethesda, MD, US). The variation of medio-frontal facet diameters between species was greater than between individuals (Nested ANOVA: species accounted for 86.3% and individuals accounted for 0.3% variation in medio-frontal facet diameters). 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) to map the distribution of different sized lenses.

Pattern electroretinogram

In the same individuals for which we carried out morphometrics measurements we carried out electrophysiological experiments during the day between 09:00 to 16:00hrs. 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 five minutes, 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. We immobilised the ant by using beeswax on the mandibles, the constriction between 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 conducting gel (Livingstone International Pty Ltd, Australia). We used an active electrode with a diameter of 0.25 mm for *Myrmecia* and *Polyrhachis* species and 0.127 mm for *Rhytidoponera inornata*. As an indifferent electrode, we inserted a silver/silver-chloride electrode of 0.25 mm diameter into the mesosoma of the *Myrmecia* and a platinum electrode of 0.127 mm diameter for *Polyrhachis* and *Rhytidoponera* ants. ERGs were amplified using an alternating-current (AC)–coupled differential amplifier (DAM50, World Precision Instruments Inc., 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 analogue-to-digital converter (USB-6353 X-series, National Instruments; Austin, Texas, 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 frequencies (cycles per degree, cpd) and Michelson's contrast ($C= (I_{max}- I_{min})/(I_{max}+ I_{min})$, where I is intensity, (Michelson, 1927)). The stimuli were generated using Psychtoolbox 3 (Pelli, 1997) and MATLAB (R2015b, Mathworks, Natic, MA, US) controlled via custom Visual Basic software (NSH) written in Visual Studio (2013, Microsoft Corporation, Redmond, WA, US). The gratings had a mean irradiance of $1.75x10^{-4}$ Wcm^{-2}, measured using a radiometer (ILT1700, International Light Technologies, Peabody, MA, US), 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 minutes. 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 cpd) 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 cpd. 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 of the spatial frequency and contrast combination, fifteen repetitions of the response for five seconds each were averaged in the time domain and analysed to get 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 ten 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 ten 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 ERG response to sinusoidal grating stimulus to isolate post-receptoral response components. To identify whether ants provided robust and reliable neural signal in our PERG experiments, we measured neural response directly by electroretinogram, by measuring transients at light ON and OFF. For this, we measured neural responses in our largest study species *M.tarsata* and the smallest study species *R. inornata* using ON and OFF electroretinograms. Here, electrical response from the whole eye in response to changes in illumination (light ON and OFF) was measured. The resulting response waveform consists of summed changes in extracellular potentials produced by photoreceptors and second order neurons in the lamina. To measure ERGs, we prepared each ant (n=4 for each species) and electrodes attached to their left eye as described for PERG above. A cool white LED light source (5mm in diameter with an irradiance of 5.81×10^{-5} W cm^{-2}, C503C-WAS-CBADA151, Cree Inc, Durham, NC, USA) placed at 15 cm distance from the animal's eye was used as a stimulus. The ants were dark adapted for 5 minutes prior to stimulation. The stimulus of light ON and OFF for a duration of 5s each was presented using a custom MATLAB software (courtesy of Jan Hemmi). 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 alternating-current (AC)–coupled differential amplifier (DAM50, World Precision Instruments Inc., FL, USA) with a gain of 100 and bandpass filtered between 1 Hz and 1 kHz. The experimental setup was housed inside a Faraday cage at 22°C room temperature.

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, df = 17, $p << 0.01$), 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, R studio-team, 2016, Version 1.1.383). 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 were examined using *t*-test 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 study species

Among the four species, *M. tarsata* was the largest with twice the head width and 11 times more facets compared to *R. inornata* that 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, $p << 0.01$) and average medio-frontal facet diameter (Pearson correlation, r = 0.93, t_{17} = 10.37, $p << 0.01$). 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 to 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 response to different spatial frequencies of the stimulus over the recording session. We found that *M. tarsata* had the highest spatial resolving power of 0.60±0.004 cpd (mean±s.e.m), compared to 0.48±0.01 cpd 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 to the two bigger *Myrmecia* species (Fig. 5B) indicating that size explains the variation in contrast threshold. The variation in contrast sensitivity (1/contrast threshold) at the species level was best explained by medio-frontal facet diameter, spatial frequency of the stimuli, and an interaction between them (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 needed a higher and roughly similar contrast to detect the pattern. *M. nigrocincta* had a higher average contrast sensitivity (lower contrast threshold) for most of the spatial frequencies compared to *M. tarsata,* even though *M. nigrocincta* had smaller facets (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 photoreceptors and lamina in the final ERG waveform. But we were able to clearly see the OFF transient (Fig. 6 inset). In the final summed ERG 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 ERG waveform amplitudes are larger for *M. tarsata* with bigger eyes than *R. inornata* (Fig. 6), especially the OFF transient amplitudes for *M. tarsata* was higher (Fig. 6 inset). Because the OFF transient amplitude was lower for *R. inornata,* it leads 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 ±2.63 ms; mean±s.e.m) is comparable to that of the OFF transient peak for *M. tarsata* (t_1 = 17.75 ±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-receptoral 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 are inverted ERG waveforms of intracellular recordings which are typically measured in some studies (e.g. Alawi and Pak, 1971; Järvilehto and Zettler, 1973).

Discussion

Using PERG, we tested whether miniaturisation affects two key visual capabilities in ants. 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 cpd, while the smallest was 0.48 cpd. The maximum contrast sensitivity (minimum contrast threshold) of the largest species was 15.51 (6.4 % contrast detection threshold) at 0.1 cpd, while the smallest species was 1.34 (74.1 % contrast detection threshold) at 0.05 cpd. We discuss these results in light of the implications of miniaturisation in ants.

Our results show prominent differences in the visual capabilities of the ants that varied in head width. Measurements from the eye replicas across our four study species revealed that the number of ommatidia reduced and facet 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 larger ants (two *Myrmecia* species) had predominantly larger facets in comparison to 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 *M. tarsata,* but had higher average contrast sensitivity (lower contrast threshold) for most of the spatial frequencies (Fig. 5B, highest slope). Its spatial resolving power does not seem to be different compared to the smaller ants that had 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 likely 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 can shed more light into how its fast movement affects spatial resolving power and sensitivity.

Though the smallest ant in our study, *R. inornata* had 8% of facets of the largest ant, *M. tarsata*, they 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 both 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 the photon capture, the signal to noise ratio and contrast discrimination (Warrant, 1999). To determine if this was the case in our ants, from our ERG recordings of the largest and the smallest ant we measured the duration of 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 to *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 fast moving whereas the smaller ants *P. nr aurea* and *R. inornata* are relatively slow moving and opportunists (Brown Jr., 2000). Hence the smaller, slow moving ants may have lower contrast sensitivity. Though high-resolution information in the visual panorama is not required for navigating (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 to 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 either 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 steering ability of tethered *Drosophila melanogaster* that was stimulated by moving sinusoidal gratings of different contrasts, 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 that have a peak value of 25 to 40 (Dvorak et al., 1980) and hoverflies that have a peak value of 40 to 100 (O'Carroll et al., 1996; O'Carroll et al., 2014; Straw et al., 2006). At present, it is difficult to compare our contrast sensitivity findings to previous work primarily due to differences in methods. Using PERG to measure contrast sensitivity makes our study quite unique, especially since 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 in the future.

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Conflict of interests

We have no conflicting interests

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Data availability

Data is available here:
https://ecologicalneuroscience.files.wordpress.com/2019/05/nettimi_etal_perg_mini_suppl.xlsx

Author contributions

R.P-N., Y.O., and A.N. contributed to conception and designed the study; R.P-N. and Y.O. collected the data; N.S.H. and L.A.R. wrote the data acquisition and analysis software and built the PERG equipment; R.P-N. performed the statistical analyses; R.P-N. wrote the first draft of the manuscript; Y.O and A.N. supervised the project.

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Figures

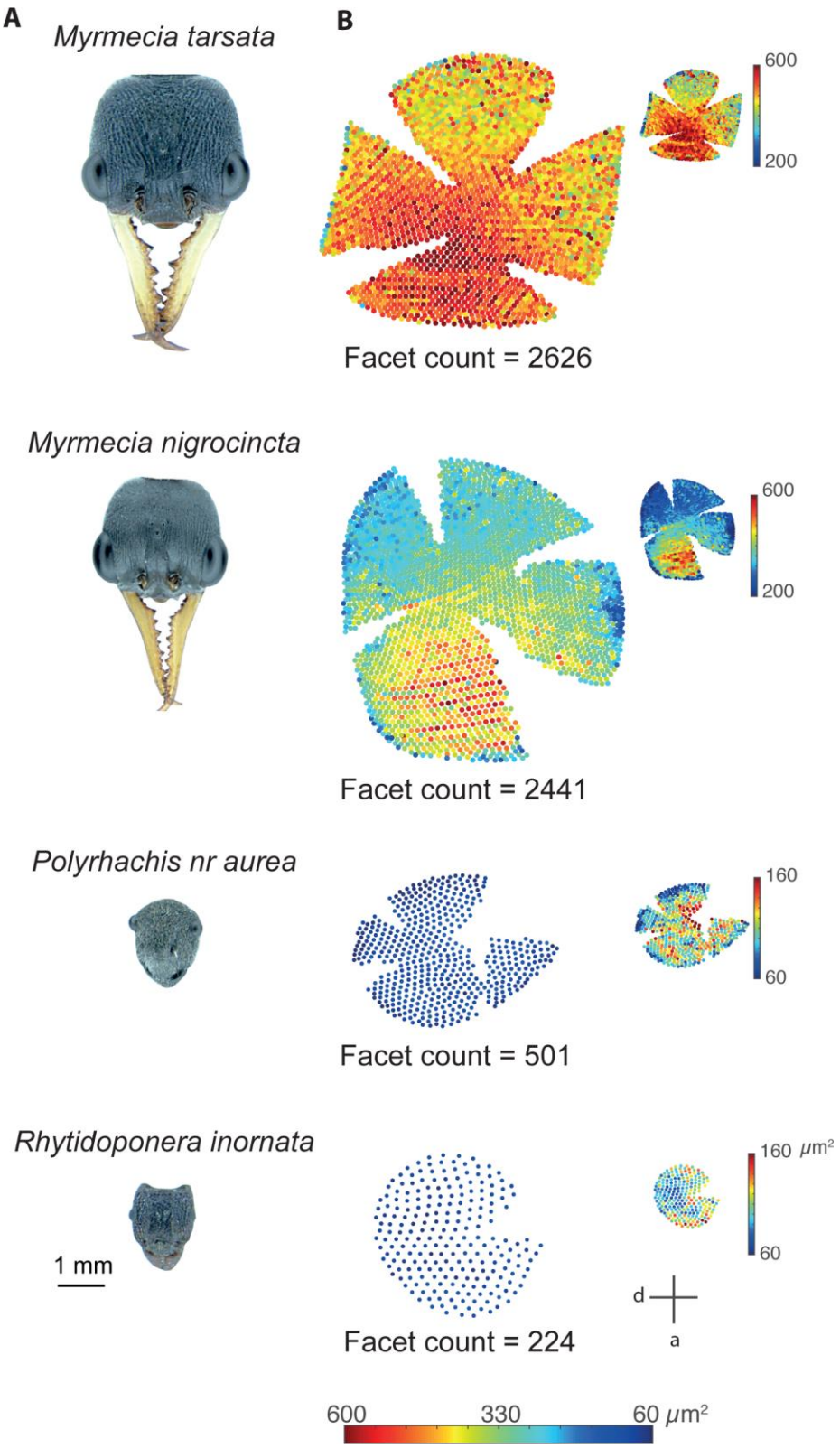


Figure 1. Study species and an eye map for each species showing the variation in facet size. (A) Dorsal view of study species. (B) Eye maps depict the facet size variation across species and the inset shows variation within each eye. Horizontal colour map scale indicates facet area for across species comparison; and the vertical colour map scale for within each eye. Anterior (a) and dorsal (d) region of the eye are indicated at bottom right.

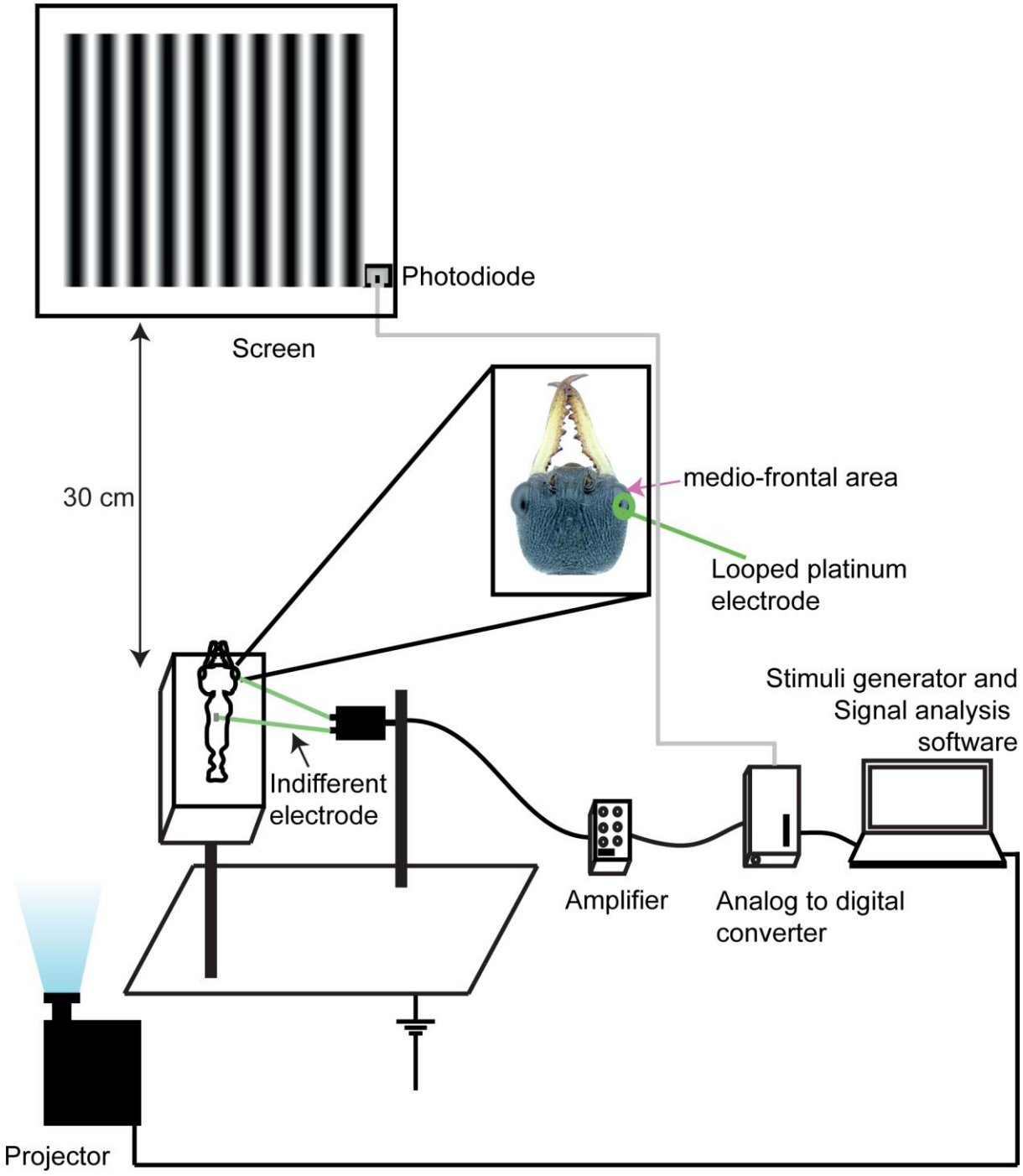


Figure 2. A schematic of the pattern electroretinography set-up. An ant was mounted on a plastic stage at 30 cm from a screen on to which the stimuli were projected. Electrical activity was recorded using a loop platinum electrode placed on the right eye ensuring the medio-frontal area of the eye (inset)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

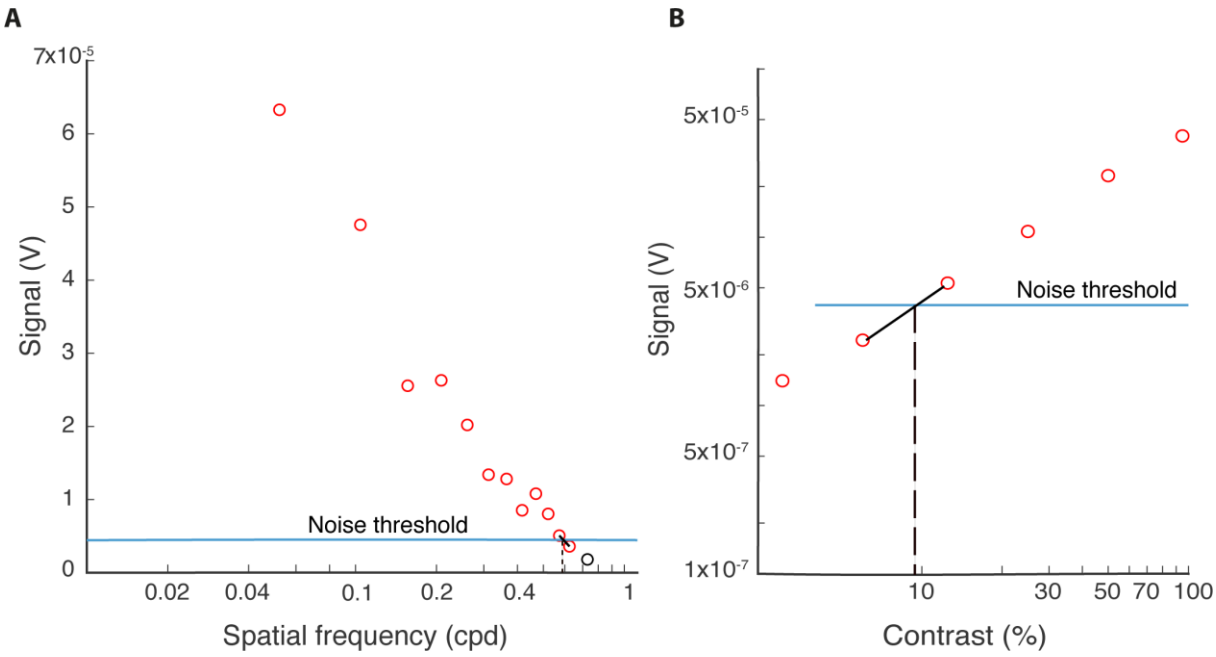


Figure 3. An example from one animal to demonstrate the estimation of spatial resolving power and contrast threshold from voltage signals obtained from an ant's eye. (A) Spatial resolving power, the highest spatial frequency to which the ant could respond (at 95% contrast). (B) Contrast threshold, the lowest contrast to which the ant could respond, is 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 voltage signal at 4 Hz; black data point (in A) means the peak value was not significantly different from the neighbouring 10 values of the Fast Fourier Transformed voltage signal (see methods for details). Spatial resolving power and contrast threshold are the x-axis values at the intersection of dotted lines. Contrast sensitivity was obtained by taking the inverse of contrast threshold.

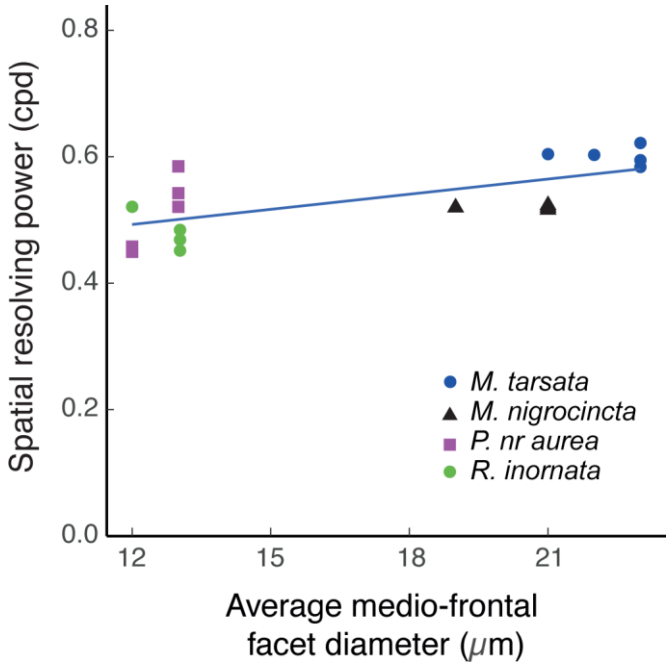
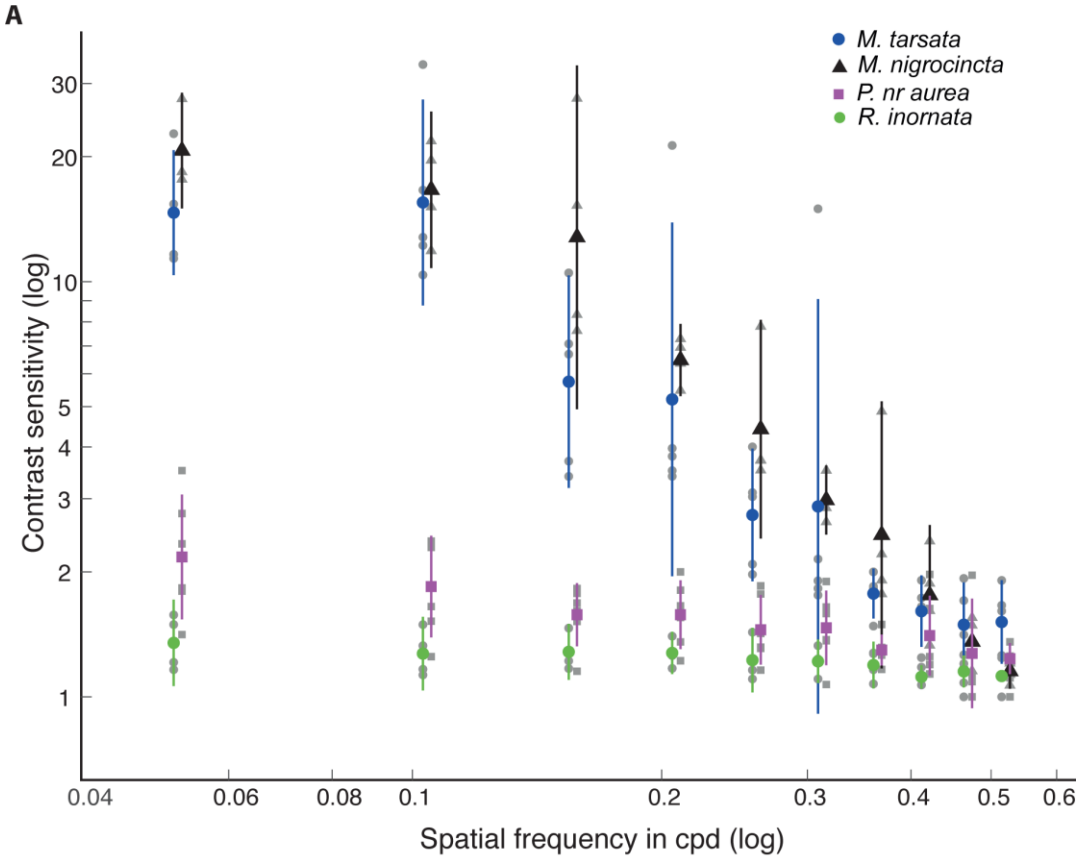


Figure 4. Relationship between spatial resolving power and average medio-frontal facet diameter in four ant species. Data from each species are shown in a different colour and symbol. This nomenclature is similar to that used in Figures 5 and 6. Regression line is based on the estimates of linear model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



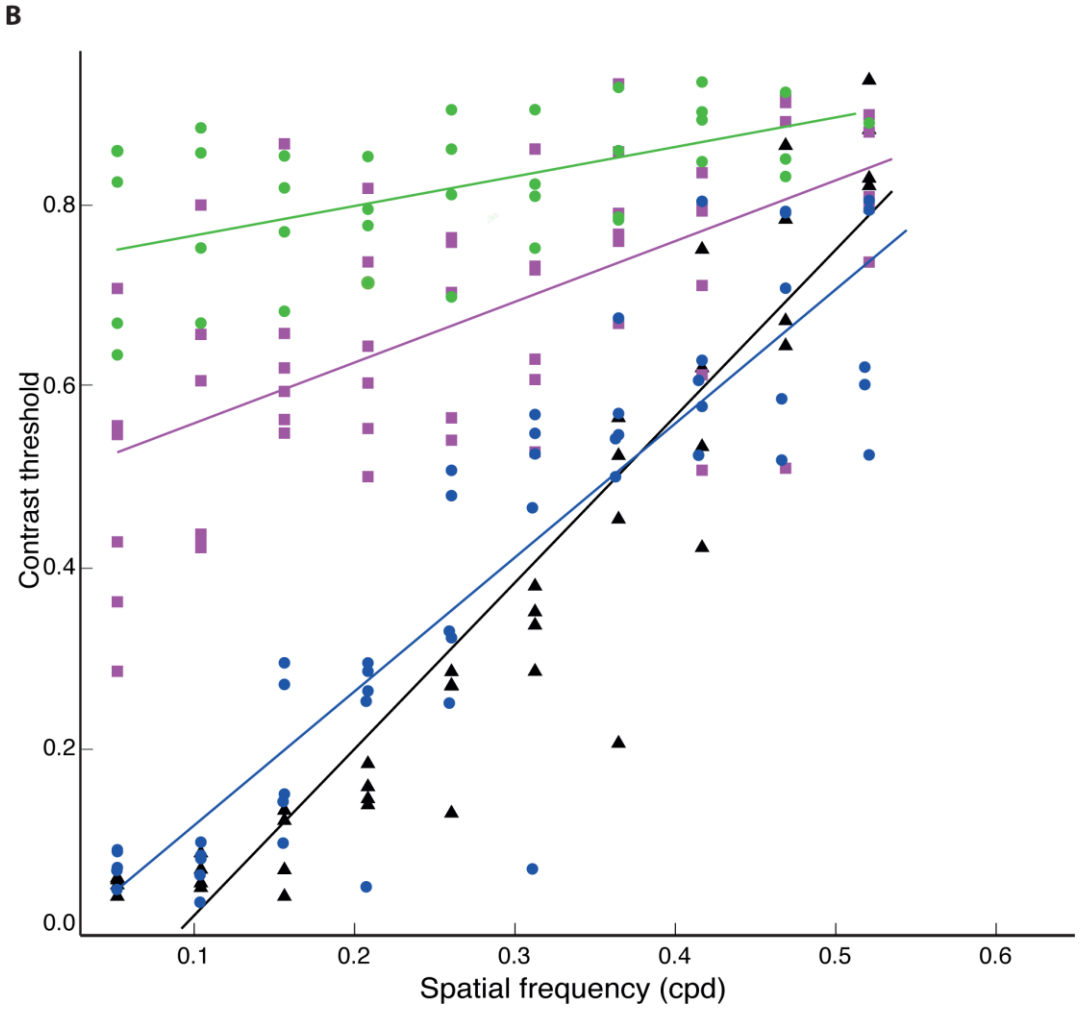


Figure 5. Relationship between contrast sensitivity, contrast threshold, and spatial frequencies of the stimuli. (A) Mean ± 95% confidence intervals of contrast sensitivity are indicated for each species. A lower contrast sensitivity value indicates a higher contrast threshold; ants could detect gratings of that particular spatial frequency only when sinusoidal gratings (stimuli) had a higher contrast. Note that a log scale is used on both the axis, and the data points for each species are slightly shifted for a clearer visualisation. (B) Relationship between contrast threshold and spatial frequencies. The raw data used for linear mixed model analyses are plotted. Data for each study species are shown with a unique colour. 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, and they indicate that smaller species have lower slopes.

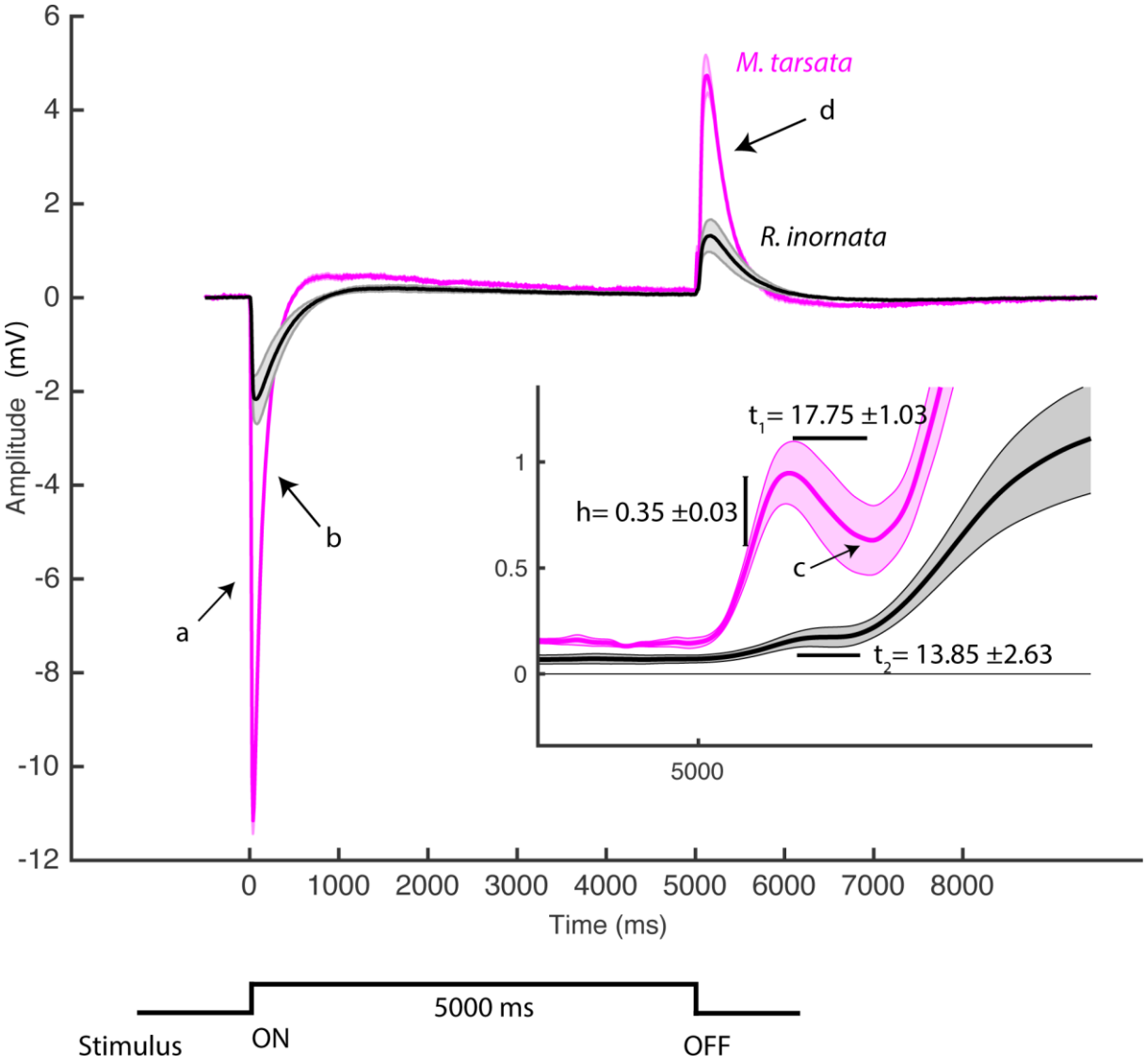


Figure 6. Electroretinograms (mean ± s.e.m; n=4 each) of *M. tarsata* and *R. inornata* species. The ERGs consist of four waveforms: a cornea negative transient, which is not evident here because the voltage response is the sum of both the photoreceptors and second order neurons in the lamina (a), a 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 ± s.e.m for *M. tarsata* (h) and the duration of the OFF transient ± s.e.m for both ants (t_1 for *M. tarsata,* t_2 for *R. inornata*) are indicated.

Tables

Table 1. Variation in size and spatial vision of the study species. Mean ± s.e.m are listed for head width, facet number, medio-frontal facet diameter, spatial resolving power and contrast sensitivity. Sample sizes are indicated below the species name.

	M. tarsata n=5	*M. nigrocincta* n=4	*P. nr aurea* n=6	*R. inornata* 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 using PERG, *cpd*	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 cpd	20.68±0.6 at 0.05 cpd	2.17±0.5 at 0.05 cpd	1.34±0.5 at 0.05 cpd

Table 2. Summary of linear model fit for testing the relation 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

Table 3. Summary of linear mixed model fit by restricted maximum likelihood for testing the relation between contrast threshold, average medio-frontal facet diameter, and spatial frequency. Mixed model parameters: fixed effects are average medio-frontal facet diameter and spatial frequency, and random effects are antID nested within species. The t-tests for fixed effects use Satterthwaite approximations to degrees of freedom (*df*). The variance in each of the random effects is less than 1%.

Parameter	**Estimate**	**s.e.m**	***df***	***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