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Spatial Resolving Power and Contrast Sensitivity Are Adapted for Ambient Light Conditions in Australian *Myrmecia* Ants

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The eyes of most animals exhibit a trade-off between spatial resolving power and absolute sensitivity, which likely reflects functional adaptations for the animals' visual ecology. When animals operate in dim light conditions, the sensitivity of an eye needs to be increased because the signal-noise ratio of visual information is typically low, even though this potentially compromises spatial resolving power. Here, we investigated the spatial resolving power and contrast sensitivity in two congeneric ant species: the diurnal-crepuscular *Myrmecia tarsata* and the nocturnal *Myrmecia midas* using pattern electroretinography (PERG). Both ant species have a specialised zone in the medio-frontal region of the eye that has enlarged facets compared to the rest of the eye. Using the PERG technique, we found that spatial resolving power was 0.60 cycles per degree (cpd) in *M. tarsata*, while it was 0.57 cpd in *M. midas*. This variation in spatial resolving power is explained by differences in ommatidial facet diameters, which were significantly larger in the nocturnal *M. midas*. The contrast sensitivity reached a maximum of 15.5 at 0.1 cpd in *M. tarsata* and 21.2 at 0.05 cpd in *M. midas*. The contrast sensitivity functions did not differ significantly between the two species. In the diurnal-crepuscular *M. tarsata*, the specialised eye region with the largest facets provides both high spatial resolving power and contrast sensitivity making it an "acute zone". In contrast, in the nocturnal *M. midas* the specialised eye region with the largest facets improves the eye's sensitivity, making it a "bright zone". The increased sensitivity would be important under low luminance conditions and/or for discriminating objects of low contrast. We conclude that even closely related species active at different ambient light intensities have evolved different strategies to optimise their visual system to match their respective visual ecologies.

Keywords: vision, PERG, bright zone, acute zone, contrast sensitivity

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INTRODUCTION

An animal's behaviour is constrained by the anatomy and physiology of its sensory systems, which have evolved to extract ecologically relevant information from their habitat. The visual capabilities of an animal are typically characterised by their spatial resolving power and contrast sensitivity (Land, 1997). In order to discriminate small objects or fine details in a scene, animals require high spatial resolving power. Contrast sensitivity is a measure of the ability to discriminate visual stimuli

as their brightness contrast decreases. This is determined by the amount of light absorbed by each photoreceptor. Both spatial resolving power and contrast sensitivity in an eye are not uniform across the visual field and there is distinct regional specialisation (Walls, 1942). A classic example of regional specialisation is seen in the fiddler crab *Uca* spp, where their spatial resolving power varies across the eye, which matches to the information content and behavioural relevance of the corresponding parts of their visual field (Layne et al., 1997; Smolka and Hemmi, 2009). In insects, certain regions of the compound eye are associated with higher spatial resolving power (acute zones) that, for example, allow males to spot females against the sky background (Collett and Land, 1975; Beersma et al., 1977; Horridge, 1978; Zeil, 1979, 1983; Gonzalez-Bellido et al., 2011; Warrant, 2016). In contrast, in some insects, certain regions of the compound eye are most suited for increased light capture and improved absolute sensitivity (bright zone; van Hateren et al., 1989; Straw et al., 2006).

In addition, an eye also needs to be sensitive to a range of light intensities that the animal experiences (Snyder et al., 1977). This is because light intensity drops over 100 million times at night compared with a bright sunny day, which makes detecting reliable visual navigational information a challenge (Land and Nilsson, 2012). Indeed, a variety of nocturnal insects have evolved optical strategies to improve visual sensitivity to suit their nocturnal lifestyle (Land et al., 1999; Greiner et al., 2005; Warrant and Dacke, 2011; Stöckl et al., 2016a; Narendra et al., 2017). We have identified such optical adaptations in the Australian *Myrmecia* ants where congeneric and sympatric species range from being strictly diurnal, diurnal-crepuscular, and exclusively nocturnal. *Myrmecia,* similar to other ants and Hymenopterans, possess an apposition compound eye, which is an eye design well-suited for bright light conditions. The size of the lens and width of the rhabdoms gradually increase as species become nocturnal, resulting in a 27-fold increase in the sensitivity of the eye of the nocturnal ants (Greiner et al., 2007). The optical characteristics of their eyes are clearly related to periods of foraging activity that occur in different ambient light conditions as they become nocturnal (Narendra et al., 2011, 2016). *Myrmecia* ants, both diurnal and nocturnal, are well-known for their ability to forage individually and capture large prey (Narendra et al., 2013; Reid et al., 2013). However, their spatial resolving power and contrast sensitivity —especially in the context of the different light levels at which they operate—have not been measured.

Both spatial resolving power and contrast sensitivity of an eye can be estimated by anatomical measurements of optical properties. From their anatomy, it appears that the nocturnal *Myrmecia pyriformis* has lower spatial resolving power (interommatidial angle of 2.1°; Reid, 2010) compared to the diurnal *Myrmecia gulosa* (interommatidial angle of 1.7°; Via, 1977). Intracellular recordings also provide the information about the visual field of photoreceptors (Rigosi et al., 2017). One technique that can provide information of both the spatial resolving power and contrast sensitivity simultaneously is pattern electroretinography [PERG; (Porciatti, 2007)]. This technique allows us to measure responses of neurons in the lamina (the first optic ganglion), where sensitivity is improved by pooling the signals from photoreceptors in nocturnal insects (Kirschfeld, 1967; Warrant, 1993; Nilsson and Ro, 1994). The dendrites of the laminar monopolar cells (LMCs) extend into several neighbouring cartridges to connect the projections of the retinal axons from a single ommatidium, while in the diurnal insects they exhibit less branching (Greiner et al., 2005; Stöckl et al., 2016a,b). This strategy, known as spatial summation, increases photon capture without compromising spatial resolving power because it does not rely on changes in the optical components of the eye. Using PERG we can determine the spatial resolving power of an eye as the ability to resolve fine detail at different spatial frequencies of repeated dark and light stripes of gratings at high contrast. The PERG can also measure the contrast sensitivity, which is the ability to discriminate between adjacent stimuli on the basis of their differences in relative luminosity (contrast) rather than their absolute luminances (Ghim and Hodos, 2006). A contrast sensitivity function is obtained by measuring the contrast sensitivity over the range of the spatial frequencies of gratings and has become a common indicator of the ability of the visual system to process spatial frequency (Uhlrich et al., 1981; Ghim and Hodos, 2006; Porciatti, 2007; Ryan et al., 2017). The PERG technique has been used in humans (Bach et al., 2000), birds (Ghim and Hodos, 2006), turtles (Armington and Adolph, 1990), and sharks (Ryan et al., 2017). It may provide a more reliable estimate of spatial resolving power than anatomical methods especially for species in which behavioural estimates are difficult or time consuming to obtain (Ryan et al., 2017). Here we investigated the spatial resolving power and contrast sensitivities of two congeneric *Myrmecia* ants that are active at different times of the day using PERG.

MATERIALS AND METHODS

Animals

We studied workers of two congeneric *Myrmecia* species, which are active at different times of the day. *Myrmecia tarsata* is a diurnal-crepuscular species, with majority of its activity restricted to the bright periods of the day (Greiner et al., 2007; Narendra et al., 2011). *Myrmecia midas* is a nocturnal ant that restricts its foraging activity to the low light periods of twilight and night (Freas et al., 2017). Both species were caught from nests on the Macquarie University campus, Sydney NSW (33.7738°S, 151.1126°E). Both species exhibited distinct size polymorphism and we used medium sized workers in our study. In five individuals of each species (head widths: 3.21 ± 0.38 mm in *M. tarsata*; 3.91 ± 0.11 mm in *M. midas*, **Table 1**) we determined their spatial resolving power and contrast sensitivity. Research on ants does not require ethics approval in Australia. Nevertheless, we treated our animals with care.

Electrophysiology

Animals were kept on ice for 5 min before removing their legs and gaster. Each ant was fixed with its dorsal side facing up on a plastic stage with bees' wax and then mounted in a Faraday cage. We recorded the pattern electroretinogram (PERG) to determine the spatial resolving power and contrast sensitivity of the whole eye. The active electrode was a platinum wire

TABLE 1 | Summary of the spatial resolving power and contrast sensitivity.

	Diurnal-crepuscular *Myrmecia tarsata*	Nocturnal *Myrmecia midas*
Head width (mm) (means ± SE, $n = 5$)	3.21 ± 0.38	3.91 ± 0.11
Facet numbers (means ± SE, $n = 5$)	2,627 ± 120	3,590 ± 88
Facet diameter (μm) (means ± SE, $n = 5$)	22.40 ± 0.40	31.62 ± 0.47
Spatial resolving power (cpd) (means ± SE, $n = 5$)	0.60 ± 0.01	0.57 ± 0.01
Maximum contrast sensitivity ($n = 5$)	15.5 (6.4%)	21.2 (4.7%)
Spatial resolving power (cpd) with medio-frontal region only exposed (means ± SE, $n = 5$)	–	0.53 ± 0.02
Maximum contrast sensitivity with medio-frontal region only exposed ($n = 5$)	–	21.4 (4.7%)
Estimated interommatidial angle (deg)	0.83	0.88

of 0.25 mm diameter attached to the surface of the lateral side of the compound eye with conductive gel (Livingstone International Pty Ltd., New South Wales, Australia). A silver/silver-chloride wire of 0.1 mm diameter was inserted into the mesosoma and served as the indifferent electrode. ERGs were recorded using a differential amplifier (DAM50, World Precision Instruments Inc., FL, USA) connected to a computer via a 16-bit analogue-to-digital converter device (USB-6353, National Instruments, Austin, TX, USA). All experiments were carried out in a darkroom at room temperature (21–25°C). To exclude any effects of circadian rhythms on eye physiology, the experiments were carried out during each species' typical activity time, i.e., from 2 to 8 h after sunrise for *M. tarsata* and 1–6 h after sunset for *M. midas*. Animals were kept in dark for 1–3 h before recording.

The PERG visual stimuli were projected by a digital light processing (DLP) projector (W1210ST, BenQ corporation, Taipei, Taiwan) on a white screen (51 cm width × 81 cm height) at a distance of 30 cm from the ant's head. For detailed descriptions on methods see Ryan et al. (2017). The stimuli were vertical contrast-reversing sinusoidal gratings of different spatial frequencies (cycles per degree, cpd) and Michelson's contrasts (Michelson, 1927), generated using Psychtoolbox 3 (Pelli, 1997) and MATLAB (R2015b, Mathworks, Natick, MA, US) and controlled via custom Visual Basic software (NSH) written in Visual Studio (2013, Microsoft Corporation, Redmond, WA, US). The mean irradiance of the grating stimuli was 1.75×10^{-4} W/cm^2 measured using a calibrated radiometer (ILT1700, International Light Technologies, Peabody, MA, US). A temporal frequency of 2 Hz was used for all stimuli.

Prior to the first recording, the ant was adapted to a uniform grey stimulus with the same mean irradiance as the grating stimuli for 20 min. To measure the contrast sensitivity function of the ants, they were presented with 14 spatial frequencies (0.73, 0.67, 0.62, 0.57, 0.52, 0.47, 0.41, 0.36, 0.31, 0.26, 0.21, 0.16, 0.1, 0.05 cpd), and up to eight contrasts (95%, 85 75, 50, 25, 12.5, 6, 3) with the same mean irradiance for each spatial frequency. The spatial frequencies of the gratings were presented in the order of decreasing frequencies of every second spatial frequency. To evaluate any degradation of the response over time, the interleaved spatial frequencies were then presented in ascending order. At each spatial frequency, all eight different contrasts were tested in decreasing order. For each combination of the stimuli, 15 repetitions of the response for 5 s each were averaged in the time domain. The averaged responses were then analysed using a Fast Fourier Transform, FFT. The non-visual electric signal (i.e., background noise) was measured as a control at two spatial frequencies (0.1 and 0.05 cpd) at 95% contrast with a black board used to shield the ant from the visual stimuli before and after the experimental series. The maximum signal out of the four control runs was used as the noise threshold.

Estimation of Spatial Resolving Power and Contrast Threshold

An F-test was used to assess whether the response signal at the second harmonic (4 Hz) of the FFT response spectrum differed significantly from 10 neighbouring frequencies, five on either side, for each spatial frequency and contrast combination. Spatial resolving power and contrast threshold were obtained by interpolating from the last point above the noise threshold whose amplitude at 4 Hz was also significantly greater than the 10 surrounding frequencies, and the first point below the noise threshold. If the first point below the noise threshold was not significantly greater than the 10 surrounding frequencies, the last point above the threshold was considered as the spatial resolving power, without interpolating between two data points. Contrast sensitivity is defined as the inverse of contrast threshold.

Identifying the Stimulation Region

For the experiments described above, the entire surface of the compound eye was exposed to the stimuli. Thus, it was possible that regions other than the medio-frontal region were activated by the visual stimuli. To identify whether this was the case, in five other individuals of one species, *M. midas*, we blocked the entire compound eye except the medio-frontal region using black paint. We carried out the PERG experiments with these occluded animals as described previously. From cornea replicas (see below) we determined the number of the facets and measured the diameter of 30 facets that were selected within the non-occluded medio-frontal region.

Size, Number, and Distribution of Facets

To determine the number and size of the facets we prepared eye replicas of all tested individuals. We used a thin layer of colourless nail polish using a well-established technique described in Narendra et al. (2011) and Ramirez-Esquivel et al. (2017). The replicas were photographed under a light microscope (Leica DM5000B, Leica Microsystems GmbH, Wetzlar, Germany) and we counted all the facets in each eye in all five animals for each species. Using ImageJ (U. S. National Institutes of Health, Bethesda, MD, US) we measured the diameter of 30 facets

that were distributed across the medio-frontal region in each individual. Although the average facet size in the medio-frontal region varied significantly between individuals, variation of facet size was greater between species compared to between individuals (Nested ANOVA: species and individual accounted for 65.6 and 12.2% of the variance of facet diameter, respectively, see **Supplementary Material**). Hence to determine the mean facet diameter of each species, we calculated the average facet diameter of each individual and reported the average of all individuals. For one individual of each species, we created an eye map using a custom-written program in MATLAB (courtesy Richard Peters, La Trobe University).

Statistical Analysis

We tested whether facet diameters differ between species by performing analysis of variance (ANOVA) in a linear mixed-effects model in RStudio (Version 1.1.419, RStudio, Inc. Boston, MA, US). Species was used as a random effect. We used a linear model to assess the relationship between spatial resolving power and facet diameter. To determine the relationship between contrast sensitivity and facet diameters in *M. tarsata* and *M. midas*, we used a linear mixed-effects model using a restricted maximum likelihood (REML) estimation method. We carried this out in the *lme4* package of R (https://cran.r-project.org/web/packages/lme4/index.html) to examine the relationship between contrast sensitivity, facet diameter and spatial frequency of stimuli. Prior to data analysis, we log-transformed contrast sensitivity and spatial frequency data. Facet diameter and spatial frequency were used as fixed effects. Animal identity nested within species was used as a random effect. The significances of the fixed effect terms were examined using a *t*-test with Satterthwaite approximation for degree of freedom (lmerTest package). Final residuals were checked graphically for compliance with model assumptions.

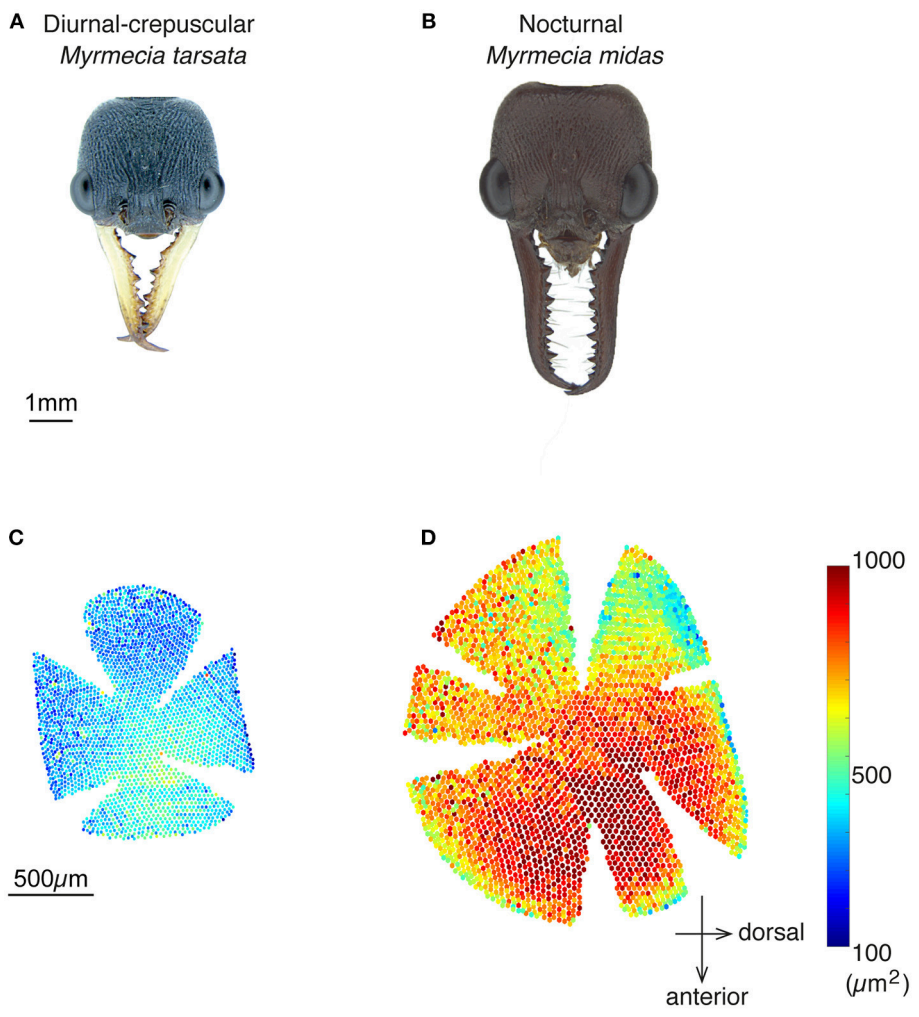

FIGURE 1 | Spatial variability in facet diameters in the compound eyes of worker ants of the diurnal-crepuscular *Myrmecia tarsata* and nocturnal *Myrmecia midas*. **(A,B)** Dorsal view of the two ants. **(C,D)** Eye maps depicting the number of facets and the size of the facets in one individual. Eye maps of the two species are shown in the same scale. Each dot represents a facet, with its centre indicating the position of the facet. The size of the lens is indicated by the vertical colour map and not by the size of individual dots. Anterior and dorsal side of the eye are indicated.

In the nocturnal *M. midas*, we determined whether spatial resolving power differed between animals that had their entire eye exposed (intact) or had only the medio-frontal region of their eye exposed by using a linear model in RStudio. To assess the relationship between contrast sensitivity, facet diameter and spatial frequency in two conditions of *M. midas* (entire eye exposed (intact) or eyes with medio-frontal region only exposed), we again used the mixed-effects model, which was used to assess the contrast sensitivity functions in *M. tarsata* and *M. midas* as described previously.

RESULTS

Facet Numbers and Diameters

The nocturnal ant *M. midas* had more facets compared to the diurnal-crepuscular *M. tarsata* (**Table 1**). From the eye maps of both species, it is clear that larger facets were localised in the medio-frontal region of the eye (**Figure 1**). The nocturnal ants had significantly larger facets (31 μm) in the medio-frontal region compared to its diurnal relative (22 μm) (**Table 1**; **Supplementary Table 1** and **Supplementary Figures 1A,B**).

Spatial Resolving Power

The amplitude of the PERG response at the second harmonic of the stimulus modulation frequency decreased with increasing spatial frequency or decreasing contrast of the visual stimuli. The point at which the amplitude fell below the noise threshold was used to define the spatial resolving power at the highest contrast. Spatial resolving power was 0.60 ± 0.01 cpd and 0.57 ± 0.01 cpd (mean ± SE) in the diurnal-crepuscular and nocturnal species, respectively (**Table 1**). We found that facet diameter explained the variation in the spatial resolving power (**Figure 2**, **Table 2**).

Contrast Sensitivity

The contrast threshold was measured as the point at which the response amplitude fell below the noise threshold whilst decreasing the contrast of the visual stimuli. The contrast threshold was typically lower at lower spatial frequency (0.05 cpd) and increased at higher spatial frequencies. No contrast threshold was recorded for the highest spatial frequency (0.73 cpd) because responses for that frequency never reached the threshold. Contrast thresholds for all spatial frequencies were used to calculate the contrast sensitivities (1/contrast threshold) shown in **Figure 3**. In *M. tarsata*, the contrast sensitivity reached a maximum of 15.5 at 0.1 cpd. In *M. midas* the highest contrast sensitivity was 21.2 at 0.05 cpd and declined with increasing spatial frequency. We found that variation in contrast sensitivity was explained by the spatial frequency of gratings, but not by the facet diameter (**Table 3**).

Stimulating Only the Medio-Frontal Region of the Eye

To ascertain the region of the compound eye that was stimulated, we occluded the compound eye except the medio-frontal region and measured both spatial resolving power and contrast sensitivity. We determined this only in the nocturnal *M. midas*. In the occluded animals, the medio-frontal region of the eye consisted of 431.2±34.8 facets. Their average facet diameter was 29.1±0.83 μm, which was comparable to the facet sizes found in intact *M. midas* (31 μm). The spatial resolving power of the eye at the highest contrast when the medio-frontal region only was exposed was 0.53 ± 0.02 cpd ($N = 5$) and was comparable to the *M. midas* with intact eyes [0.57 ± 0.01 cpd; $R^2 = 0.23$, $F_{(1, 8)} = 2.36$, $p < 0.16$, **Tables 1**, **4**, **Figure 2**]. The variation in contrast sensitivity for intact eyes and eyes with medio-frontal region only exposed was explained by spatial frequency of stimuli, but not by the facet diameter (**Figure 4**; **Table 5**).

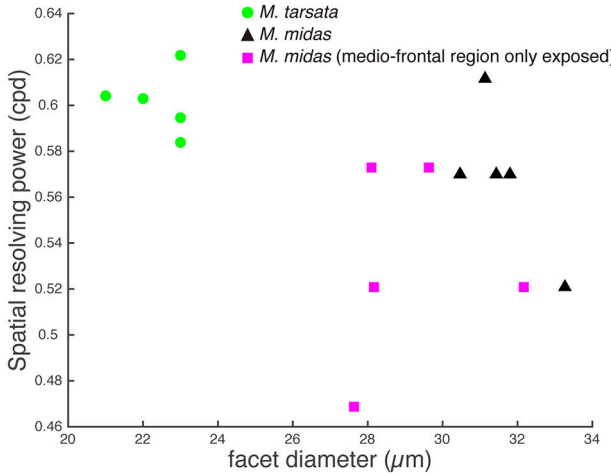

FIGURE 2 | Relationship between the spatial resolving power and facet diameter in the medio-frontal region of the eye in *Myrmecia* ants. Green circles: diurnal-crepuscular *M. tarsata*; black triangles: nocturnal *M. midas*; magenta squares: *M. midas* with medio-frontal region of the eye only exposed. Slightly smaller ants were used to measure the facet diameters of *M. midas* with medio-frontal region of the eye only exposed (magenta squares). Hence their facets were slightly smaller. However, their average facet sizes were comparable to the intact eyes and this variation did not cause a difference in the spatial resolving power.

TABLE 2 | Summary of linear model fit for testing the relationship between spatial resolving power and facet diameter in *Myrmecia* ants.

Parameter	Estimate	SE	*t*-value	*p*-value
Intercept	0.69	0.04	16.32	<0.0001
Facet diameter	−3.95	1.55	−2.55	0.03

Spatial resolving power = −3.95 facet diameter + 0.69.*

DISCUSSION

We measured the spatial resolving power and contrast sensitivity using a pattern electroretinogram technique in two congeneric *Myrmecia* ants that were active at different times of the day. We found that the spatial resolving power of *Myrmecia* ants was correlated with their facet diameter in the medio-frontal region of the compound eye. Spatial resolving power was higher in the diurnal-crepuscular *M. tarsata* that had smaller facets compared to the nocturnal *M. midas* that had larger facets. The contrast

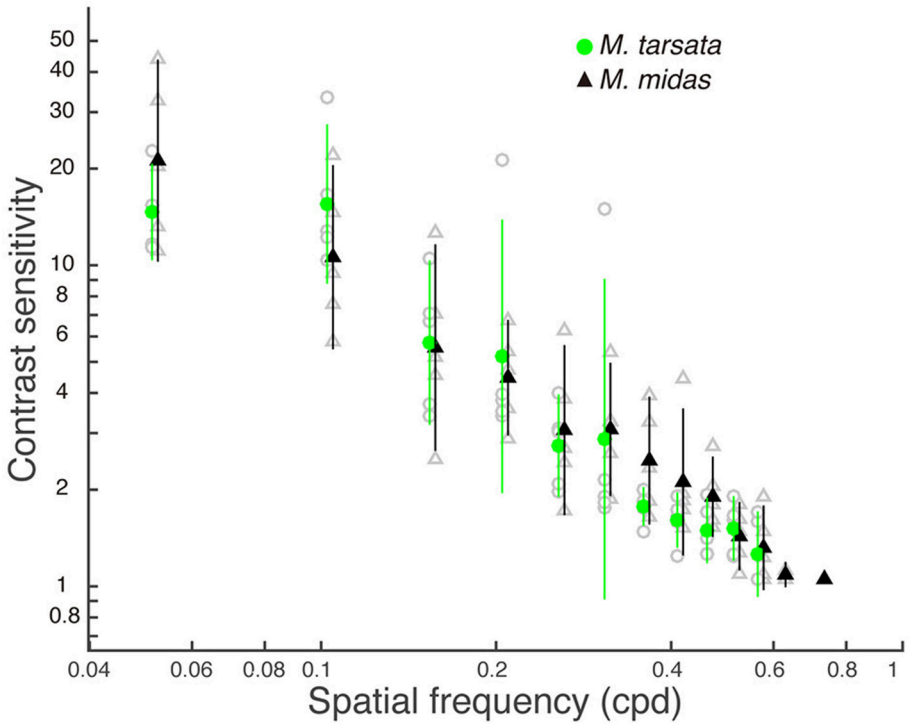

FIGURE 3 | Contrast sensitivity functions for *Myrmecia* ants obtained from pattern electroretinogram (PERG) measurements. Data are means ± 95% confidence intervals of contrast sensitivity (1/contrast threshold) measured from five individuals of *M. tarsata* (filled green circles) and *M. midas* (filled black triangles). Individual measurements are shown as open grey symbols for *M. tarsata* (circles) and *M. midas* (triangles). Data points for each species are slightly shifted to either left or right from tested spatial frequencies for clarity.

TABLE 3 | Summary of the linear mixed-effects model analysis by restricted maximum likelihood for testing the relationship between contrast sensitivity, spatial frequency, and facet diameter in *Myrmecia* ants.

Parameter	Estimate	SE	*df*	*t*-value	*p*-value
Intercept	−0.29	0.31	12.54	−0.95	0.36
Facet diameter	5.11	11.21	12.42	0.46	0.66
Spatial frequency	−1.21	0.24	101.01	−5.14	<0.0001
Facet diameter:spatial frequency	2.27	8.56	101.02	0.27	0.79

*Model: contrast sensitivity~facet diameter*spatial frequency + (1|species/animal ID). The t-tests for fixed effects use Satterthwaite approximations to degrees of freedom (df). The variance in each of the random effects is < 2%.*

TABLE 4 | Summary of linear model fit for testing whether the spatial resolving power differ between conditions (intact *M. midas* vs. *M. midas* with medio-frontal region of the eye only exposed).

Parameter	Estimate	SE	*t*-value	*p*-value
Intercept	0.61	0.04	15.82	<0.0001
Condition	−0.04	0.02	−1.54	0.16

*Spatial resolving power = −0.04*condition+ 0.61.*

sensitivity of *M. tarsata* and *M. midas* reached a the maximum of 15.5 (6.4% Michelson's contrast) and 21.2 (4.7% Michelson's contrast), respectively, at low spatial frequency. As the variation in contrast sensitivity was not explained by facet diameters, the results suggested that contrast sensitivity functions did not differ between species.

Spatial Resolving Power

The medio-frontal region of the compound eye in the diurnal-crepuscular *M. tarsata* was composed of considerably smaller facets (22.4 μm) compared to the nocturnal *M. midas* (31.62 μm). Both the spatial resolving power and contrast sensitivity function did not differ between fully exposed eyes and eyes with medio-frontal region only exposed in *M. midas*. This indicates that only the medio-frontal region of the compound eye was responsible for the majority of the recorded PERG signal when the entire eye was exposed. The larger lens diameter in nocturnal *M. midas* might improve photon capture, an adaptation for dim-light conditions (Narendra et al., 2011), but it may come at the expense of spatial resolving power (Warrant and McIntyre, 1993; Warrant, 1999). Our results from the PERG show that the spatial resolving power is lower in ants with larger lenses (*M. midas*) compared to ants with smaller lenses (*M. tarsata*) (**Figure 2**; **Table 2**). Larger lenses with lower spatial resolving power seem to be an adaptation for a nocturnal lifestyle in *M. midas*. However

TABLE 5 | Summary of the linear mixed-effects model analysis by restricted maximum likelihood for testing the relationship between contrast sensitivity, spatial frequency and facet diameter in intact *M. midas* and *M. midas* with medio-frontal region of the eye only exposed.

Parameter	Estimate	SE	*df*	*t*-value	*p*-value
Intercept	0.14	0.78	14.41	0.17	0.86
Facet diameter	−8.52	25.50	14.33	−0.33	0.74
Spatial frequency	−1.46	0.65	98.24	−2.25	0.03
Facet diameter:spatial frequency	9.66	21.24	98.23	0.46	0.65

*Model: contrast sensitivity~facet diameter * spatial frequency + (1|condition/animal ID). The t-tests for fixed effects use Satterthwaite approximations to degrees of freedom (df). The variance in each of the random effects is <2%.*

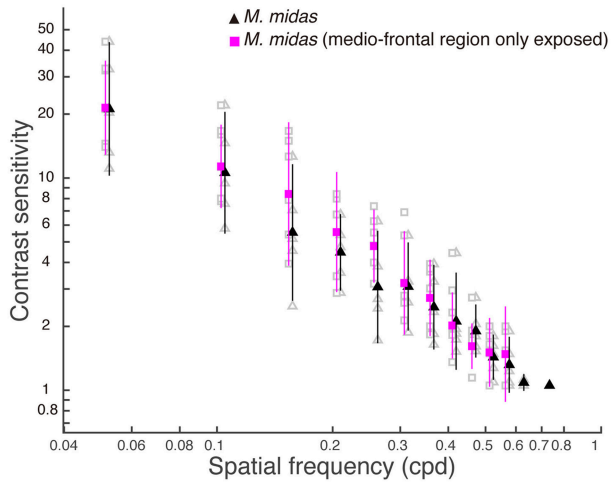

FIGURE 4 | Contrast sensitivity functions for intact eyes and eyes with medio-frontal region only exposed in *M. midas*. Data are means ± 95% confidence intervals of contrast sensitivity (1/contrast threshold) measured from five individuals of intact (filled black triangles) and *M. midas* with medio-frontal region of the eye only exposed (filled magenta squares). Individual measurements are shown as open grey symbols for intact (triangles) and *M. midas* with medio-frontal region of the eye only exposed (squares). Data points for each condition are slightly shifted to either left or right from tested spatial frequencies for clarity.

this may also be an effect of nocturnal animals typically being larger which enables them to accommodate larger lenses (e.g., Narendra et al., 2017).

In order to compare the spatial resolving power of the two *Myrmecia* ants that we studied with other species, we estimated their interommatidial angles (Δø) as 1/(2*spatial resolving power) (Land, 1997). This was 0.83° for the diurnal-crepuscular *M. tarsata* and 0.88° for the nocturnal *M. midas*. Interommatidial angles have been reported in a diurnal *Myrmecia gulosa* as 1.7° (Via, 1977) and in a nocturnal *Myrmecia pyriformis* as 2.1° (Reid, 2010). In *M. gulosa*, the interommatidal angle was determined by tracking the pseudopupil (Via, 1977), whereas in the nocturnal *M. pyriformis*, the interommatidial angle was calculated by dividing the average diameter of the facets by the curvatures of the eye in the medio-frontal region (Reid, 2010). Based on these interommatidial angles, the spatial resolving power can be predicted as 0.29 cpd for *M. gulosa* and as 0.24 cpd for *M. pyriformis*. Thus, the spatial resolving power of *M. gulosa* and *M. pyriformis* estimated by anatomical results is much lower than the values obtained for *M. tarsata* (0.57 cpd) and *M. midas* (0.6 cpd) by PERG. This difference occurs despite the time of activity and the visual tasks of these different species being comparable. This discrepancy in the spatial resolving power is likely due to the difference in methods, such as not taking into account the neural connectivity in the lamina for anatomical estimates which can only provide a theoretical upper limit of spatial resolving power (Caves et al., 2018).

This is not unusual and indeed different measures of spatial resolving power yield different estimates even in the same species (Horridge, 2005; Caves et al., 2016, 2018; Ryan et al., 2017). In honeybees, the optical data suggests that bees have interommatidial angles of 1–1.3°(Land 1997, references therein) or 1.7° (Horridge, 2005). These anatomical estimates differ markedly from those obtained using behavioural discrimination tests, which were 0.26 cpd, giving a Δø of 1.92° (Srinivasan and Lehrer, 1988), or Δø of 1.78 based on the spatial resolving power of 0.28 cpd (Horridge, 2003). A recent study using intracellular recording techniques showed that single photoreceptors in the light-adapted state are capable of responding to objects as small as 0.6 × 0.6° (Rigosi et al., 2017), which is at least 5 times smaller than the smallest features bees are known to behaviourally resolve (Lehrer and Bischof, 1995; Giurfa et al., 1996; Giurfa and Vorobyev, 1998). The differences in results may be due to different experimental conditions including light intensity or the eye's adaptation state, and the recording level in the visual processing pathway.

Clearly, spatial resolving power measured by PERG is higher than the theoretical maximum based on anatomical estimates. We suggest two reasons for this. One, PERG measures all signals that improve signal-to-noise ratio (SNR) in the lamina, which includes temporal and spatial summation strategies. Spatial summation may reduce spatial resolving power due to the large visual overlap. However, increasing the photon capture, which improves the SNR should reduce any potential decrease in spatial resolving power (Land, 1997; Theobald et al., 2006). Temporal summation could also decrease spatial resolution, but mostly at high frequencies, i.e., at the limit of the temporal resolving power (Warrant, 1999). Since the temporal frequency of our stimuli was low (2 Hz), spatial resolving power may actually improve, which is what we found. Secondly, interommatidial angle estimated from anatomy assumes that the closest neighbouring ommatidia are the horizontally or vertically adjacent ommatidia. PERG estimates do not make this assumption. This is crucial because, if there is a slight distortion in the ommatidial array, the neural wiring of neighbouring laminar cartridges could potentially reduce the distance between physiological neighbouring ommatidia compared to anatomical neighbours, which would also improve spatial resolving power. Thus, the PERG potentially provides a more reliable estimate of spatial resolving power than anatomical methods. Further PERG

studies in other animals whose spatial resolving power are known from anatomical and behavioural techniques, such as honeybees, are required to evaluate this hypothesis.

Contrast Sensitivity

Contrast sensitivity is the ability to discriminate patterns as their brightness contrast decreases (O'Carroll and Wiederman, 2014). In this study, the PERG measurements of contrast sensitivity revealed a maximum of 15.5 (6.4% contrast) at 0.1 cpd in *M. tarsata* and 21.2 (4.7% contrast) at 0.05 cpd and in *M. midas*. In most previous studies, the minimum amount of contrast (contrast threshold) of a grating to evoke a response has been measured from motion detecting neurons. In insects, contrast sensitivity (1/contrast threshold) estimates have ranged from 25 to 40 in blowfly (Dvorak et al., 1980), in both diurnal and nocturnal hawkmoths (O'Carroll et al., 1996, 1997), in honeybees (Bidwell and Goodman, 1993), in bumblebees (O'Carroll et al., 1996) and in hoverfly (Straw et al., 2006). These suggest that the maximum contrast sensitivity in the diurnal-crepuscular *Myrmecia* ant is slightly lower than that found in the motion detection neurons of other insects. Contrast sensitivity changes depending on the behavioural task. This was shown in bumblebees *Bombus terrestris* where behavioural estimates had a peak contrast sensitivity of at least 33 (3% contrast) in the motion detection system (Chakravarthi et al., 2017), which is much lower than the value of 1.57 (63.6% contrast) for an object discrimination test (Chakravarthi et al., 2016).

The ability of the eye to capture light, which likely limits contrast sensitivity, in *M. tarsata* must be the same as nocturnal *M. midas*, because their contrast sensitivity functions are not significantly different. However, the optical sensitivity in *M. tarsata* was lower than nocturnal ants based on their facet and rhabdom diameters (Greiner et al., 2007). In addition to the size of the photosensitive structure, the sensitivity of a compound eye depends on the overall number of facets and on the size of the individual facets (Horridge, 1977). The diurnal-crepuscular *M. tarsata* had fewer and smaller facets compared to the nocturnal *M. midas*, and correspondingly they had a lower maximum contrast sensitivity. In addition, the critical flicker fusion frequency, that is the fastest flickering light an animal can still perceive as flickering, is lower in *M. midas* (84.6 $\pm$ 3.2 Hz) compared to *M. tarsata* (154.0 $\pm$ 8.5 Hz) (unpublished data). This indicates that nocturnal ants employ a slower temporal resolution to improve the sensitivity of eyes, unlike the diurnal-crepuscular ants. Nevertheless, *M. tarsata* maintained a reasonable contrast sensitivity despite having smaller facets that led to higher spatial resolving power than *M. midas*. The diurnal *Myrmecia* ants, including *M. tarsata*, are known to have larger optic lobes compared to their nocturnal relatives (Sheehan et al., 2019). The large optic lobes in diurnal ants may increase the absolute sensitivity compared to their nocturnal counterparts. This raises the possibility that *M. tarsata* with their small lenses might maintain a slightly higher contrast sensitivity than expected by neural summation mechanisms, which improves the sensitivity without compromising spatial resolving power.

DATA AVAILABILITY STATEMENT

The datasets for this study are available in the **Supplementary Material**.

AUTHOR CONTRIBUTIONS

YO, LR, and AN designed the study. YO and RP-N data collection. YO, LR, and RP-N data analysis. YO and OS eye maps. NH, LR, YO, and OS built the equipment and wrote the software. YO first draft of the manuscript. YO and AN wrote sections of the manuscript. All authors revised manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2019.00018/full#supplementary-material

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