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RESEARCH ARTICLE

Ocellar structure is driven by the mode of locomotion and activity time in *Myrmecia* ants

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ABSTRACT

Insects have exquisitely adapted their compound eyes to suit the ambient light intensity in the different temporal niches they occupy. In addition to the compound eye, most flying insects have simple eyes known as ocelli, which assist in flight stabilisation, horizon detection and orientation. Among ants, typically the flying alates have ocelli while the pedestrian workers lack this structure. The Australian ant genus *Myrmecia* is one of the few ant genera in which both workers and alates have three ocellar lenses. Here, we studied the variation in the ocellar structure in four sympatric species of *Myrmecia* that are active at different times of the day. In addition, we took advantage of the walking and flying modes of locomotion in workers and males, respectively, to ask whether the type of movement influences the ocellar structure. We found that ants active in dim light had larger ocellar lenses and wider rhabdoms compared with those in bright-light conditions. In the ocellar rhabdoms of workers active in dim-light habitats, typically each retinula cell contributed microvilli in more than one direction, probably destroying polarisation sensitivity. The organisation of the ocellar retina in the day-active workers and the males suggests that in these animals some cells are sensitive to the pattern of polarised skylight. We found that the night-flying males had a tapetum that reflects light back to the rhabdom, increasing their optical sensitivity. We discuss the possible functions of ocelli to suit the different modes of locomotion and the discrete temporal niches that animals occupy.

KEY WORDS: Ocelli, Diurnal ants, Nocturnal ants, Winged male, Pedestrian ants, Tapetum, Alates

INTRODUCTION

To avoid predators or competitors and to maximise their chance of finding prey, animals are active at discrete times of the day (Kronfeld-Schor and Dayan, 2003). One of the most prominent abiotic factors that change between temporal niches is the ambient light intensity. On a cloud-free moonless night, light intensity is nearly 100 million times dimmer than during the day (O'Carroll and Warrant, 2017; Warrant, 2017). Hence, insects that operate in discrete temporal niches fine-tune their visual system to adapt to their respective light environments. Several studies on insects have focused on the compound eye specialisation that allows them to be active in dim light (Greiner, 2006a; Narendra et al., 2011; Warrant and Dacke, 2010, 2011). In this context, hymenopteran insects are interesting, as the night-active animals have an apposition eye design that is best suited for high light intensities. These nocturnal hymenopterans are nevertheless remarkably competent at night and use visual information to navigate in their dimly lit world (Freas et al., 2017; Narendra and Ramirez-Esquivel, 2017; Reid et al., 2011; Warrant and Dacke, 2016). Nocturnal insects improve the optical sensitivity of their compound eyes by developing larger lenses and wider rhabdoms (Greiner, 2006b; Greiner et al., 2007). They control for light flux through a light-sensitive pupillary mechanism (Menzi, 1987; Narendra et al., 2016a; Stavenga and Kuiper, 1977). They further improve the visual signal-to-noise ratio by integrating spatial and temporal information (Greiner et al., 2004; Stöckl et al., 2016a,b).

In addition to the compound eyes, several insects have simple eyes, known as ocelli. The ocelli typically comprise three simple eyes placed in a triangular formation on the dorsal surface of the head. Each ocellus consists of a lens, an iris, a corneagenous cell layer, a dorsal retina, a ventral retina and a neuropil (Narendra et al., 2016b; Ribi et al., 2011; Ribi and Zeil, 2017; Zeil et al., 2014). In some insects, the image resolved by the ocelli is under-focused (Berry et al., 2007a; Schuppe and Hengstenberg, 1993), while in some bees and wasps, the focal plane is near the retina (Hung and Ibbotson, 2014; Kelber et al., 2011; Ribi et al., 2011; Warrant et al., 2006). In cross-sections, the ocellar rhabdoms appear as elongate sheet-like structures (Kral, 1978; Ribi et al., 2011; Taylor et al., 2016) that are highly sensitive to polarised light in day-active bees (Geiser and Labhart, 1982; Ogawa et al., 2017) and ants (Fent and Wehner, 1985; Mote and Wehner, 1980), but not in night-active bees (Berry et al., 2011). The extent to which individual rhabdoms are sensitive to the polarisation pattern is dependent on the microvilli orientation and rhabdom straightness (Zeil et al., 2014). Typically, in flying insects, two paired retinular cells contribute microvilli that are oriented perpendicular to the long axis of the rhabdom with the microvilli aligned in a single direction. The ocellar lens is generally larger in nocturnal insects (Kerfoot, 1967; Narendra et al., 2011; Somanathan et al., 2009; Warrant et al., 2006), suggesting that the ocelli could be involved in capturing more light in dim-light conditions. Indeed, the ocelli allow bumblebees to extend their foraging duration into the dim-light periods (Wellington, 1974). The function of the ocelli has been most well studied in dragonflies and locusts, where they assist the animals in head stabilisation and horizon detection (Berry et al., 2007a; Berry et al., 2006, 2007b,c; Stange, 1981; Stange et al., 2002). But given the incredible structural diversity of ocelli (Zeil et al., 2014), they are likely to have different functions in different animals (Mizunami, 1995) or even multiple functions in the same animal (Ogawa et al., 2017; Taylor et al., 2016).

Ocelli are found in almost all flying insects and in very few walking insects. The extent to which such different modes of locomotion influence the structure and design of the ocelli is relatively unexplored. Ants are ideal for such an investigation, as

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they are one of the few insects where the mode of locomotion differs between castes: the exclusively pedestrian workers that carry out the majority of tasks in the colony including nest maintenance, foraging and brood care, and the exclusively flying males that leave the nest to track and locate females and fight off competitors. Thus, any anatomical difference that may arise in the ocellar structure is not a species-specific trait but is driven by the mode of locomotion or caste-specific tasks. Ocelli are found in very few species of worker ants and, when present, their number varies between species from one to three (Zeil et al., 2014). In almost all ant species, the flying males have the largest ocelli (Moser et al., 2004; Narendra et al., 2011, 2016b). We here investigated the ocellar structure in the workers and males of an Australian ant genus, *Myrmecia* (aka bull ants, inch ants, jack jumpers). *Myrmecia* is one of the few ant genera in which workers have three ocelli on the dorsal surface of their head (Narendra et al., 2011). We focused our study on four species of *Myrmecia* that are sympatric and where worker activity ranges from strictly diurnal, to diurnal–crepuscular, crepuscular–nocturnal and truly nocturnal. Owing to difficulties in finding males of all four species, we focused on two: a day-flying male and night-flying male. We aimed to analyse the variation in the ocellar structure in both walking and flying ants that are active at different times of the day. We know from earlier work that the size of the ocelli in these ants does not increase linearly with body size, but the night-active animals tend to have larger ocelli compared with their diurnal counterparts (Narendra et al., 2011). An unusual aspect of this system is that in one of the species, *M. pyriformis*, workers are nocturnal whereas the males are day-active, giving us an opportunity to identify the effects of both activity time and mode of locomotion within a single species.

MATERIALS AND METHODS

Study species

We collected worker ants from three nests for each of the four sympatric *Myrmecia* species, *M. croslandi* Taylor 1991, *M. tarsata* Smith 1858, *M. nigriceps* Mayr 1862 and *M. pyriformis* Smith 1858 in Canberra, ACT, Australia. Specimens were collected in small jars and brought back live to the laboratory. Workers of the last three species exhibit distinct size polymorphism (Narendra et al., 2011) and we used the largest workers for morphometrics and histology (n=5 for each species). We collected males as they were leaving the nest. Males proved difficult to find, as they fly out of the nest only once a year and were produced only from a few of the nests that we observed. We here report on the males of *M. pyriformis*, which are day fliers, and males of *M. nigriceps*, which are night fliers. Working with these ants requires no ethics approval in Australia.

Experimental procedure

Histological preparations of the ant ocelli

We fixed the ocelli under the light conditions at which animals were typically active. Ants were immobilised on ice, and their mandibles and sting were removed. Optimal fixation was achieved by cutting most of the compound eye and exposing the anterior, posterior and ventral head capsule. Specimens were fixed for 4 h at room temperature in a mixture of 2.5% glutaraldehyde and 4% paraformaldehyde in phosphate buffer (pH 7.2–7.5). This was followed by a series of buffer washes and post-fixation in 2% OsO_4 in distilled water for 1 h at room temperature. Samples were then dehydrated in an ethanol series, transferred to propylene oxide (or acetone) and embedded in Epoxy resin (Fluka, Sigma-Aldrich, St Louis, MO, USA). Embedded samples were split into three (one for the median ocelli, and two for the lateral ocelli) and re-embedded. This allowed us to ensure the orientation and the correct plane of sectioning could be chosen for both the median and lateral ocelli. Cross-sections of 1 µm thickness were cut on an ultra-microtome (Leica UC7, Wetzlar, Germany) using diamond knives (Diatome, Bienne, Switzerland). Sections for light microscopy were stained

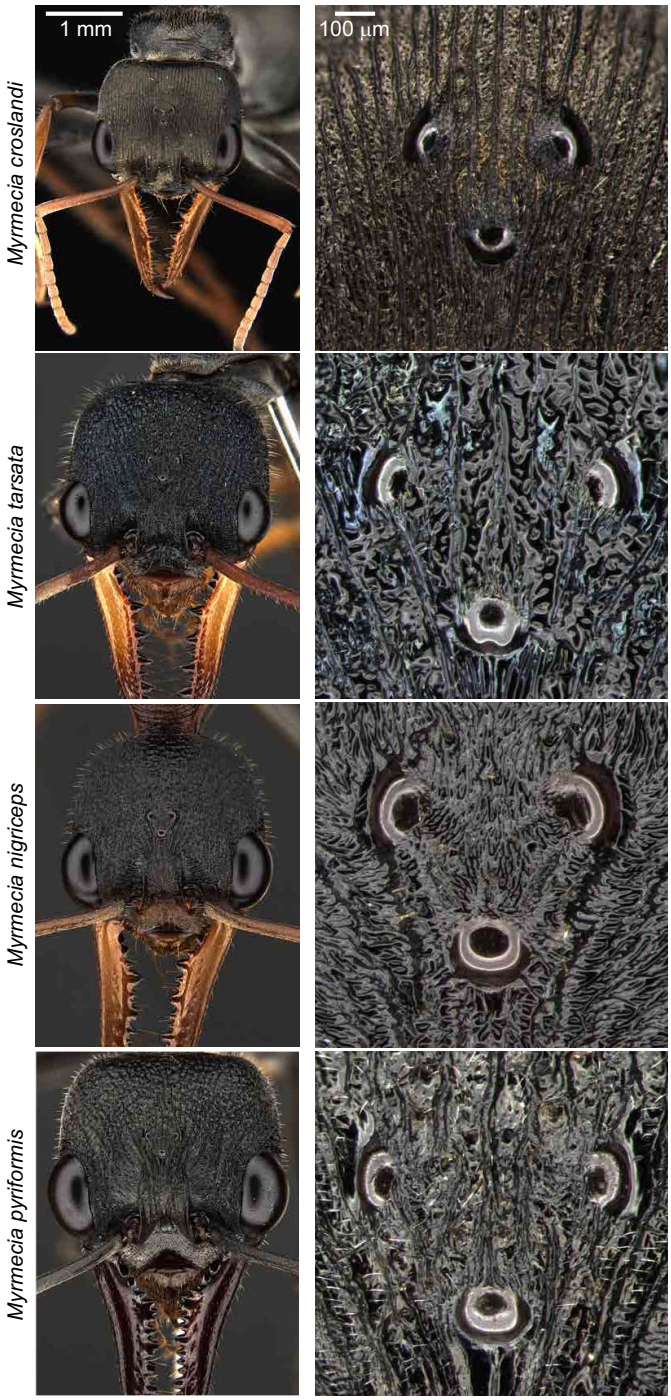

Fig. 1. Dorsal view of the workers of four *Myrmecia* ant species. Dorsal surface of the head of *Myrmecia* species ranging from diurnal *M. croslandi* to diurnal–crepuscular *M. tarsata*, crepuscular–nocturnal *M. nigriceps* and nocturnal *M. pyriformis*. Left column: overview images showing the position of the ocelli on the head and relative to each other. Right column: magnified view of the ocelli for each species. Scale bar for each panel is shown at the top of the column.

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Table 1. Optical parameters of the median ocellus of *Myrmecia* ants

Parameter	*M. croslandi* worker (D)	*M. tarsata* worker (D/C)	*M. nigriceps* worker (C/N)	*M. pyriformis* worker (N)	*M. nigriceps* male (N)	*M. pyriformis* male (D)
Lens diameter (µm)	76.4±1.5	129.2±3.7	201.2±3.0	157.2±3.6	2184.0±35.8	2641±37.3
Lens size relative to head width	0.033±0.001	0.033±0.001	0.055±0.001	0.038±0.001	0.17±0.01	0.13±0.003
No. of rhabdoms	45.3±8.2	46.5±7.0	33.0±2.9	60.4±8.8	171.2±13.8	173.3±16.9
Rhabdom diameter (µm)	1.4±0.06	2.0±0.06	2.2±0.06	3.2±0.08	1.51±0.05	1.42±0.02
Rhabdom length (µm)*	d: 15.0±1.6	d: 11.5±0.9	d: 13.1±1.0	d: 16.7±0.9	d: 27.0±2.1	d: 23.4±1.6
	v: 6.4±0.5	v: 5.7±0.5	v: 8.4±0.4	v: 8.4±0.6	v: 8.7±1.5	v: 7.2±1.7
Retinula cell number	1167±26.9	475.3±8.5	334.6±12.7	176.0±10.4	291.3±3.7	872±21.9
Tapetum	Absent	Absent	Absent	Absent	Present	Absent

D, diurnal; D/C, diurnal–crepuscular; C/N, crepuscular–nocturnal; N, nocturnal.
*The length of the rhabdom in the dorsal (d) and ventral (v) retina is presented.

with Toluidine Blue and digitally photographed in a Zeiss photomicroscope (Oberkochen, Germany), while ultra-thin sections for transmission electron microscopy were stained with 6% saturated uranyl acetate (25 min) and lead citrate (5 min) before viewing with a Hitachi transmission electron microscope (Tokyo, Japan).

Analysis

For five individuals in each species, we determined the number of retinula cells and rhabdom diameter from cross-sections taken at the distal one-third of the retina. The ocellar rhabdoms varied dramatically in shape, which made measuring rhabdom diameter

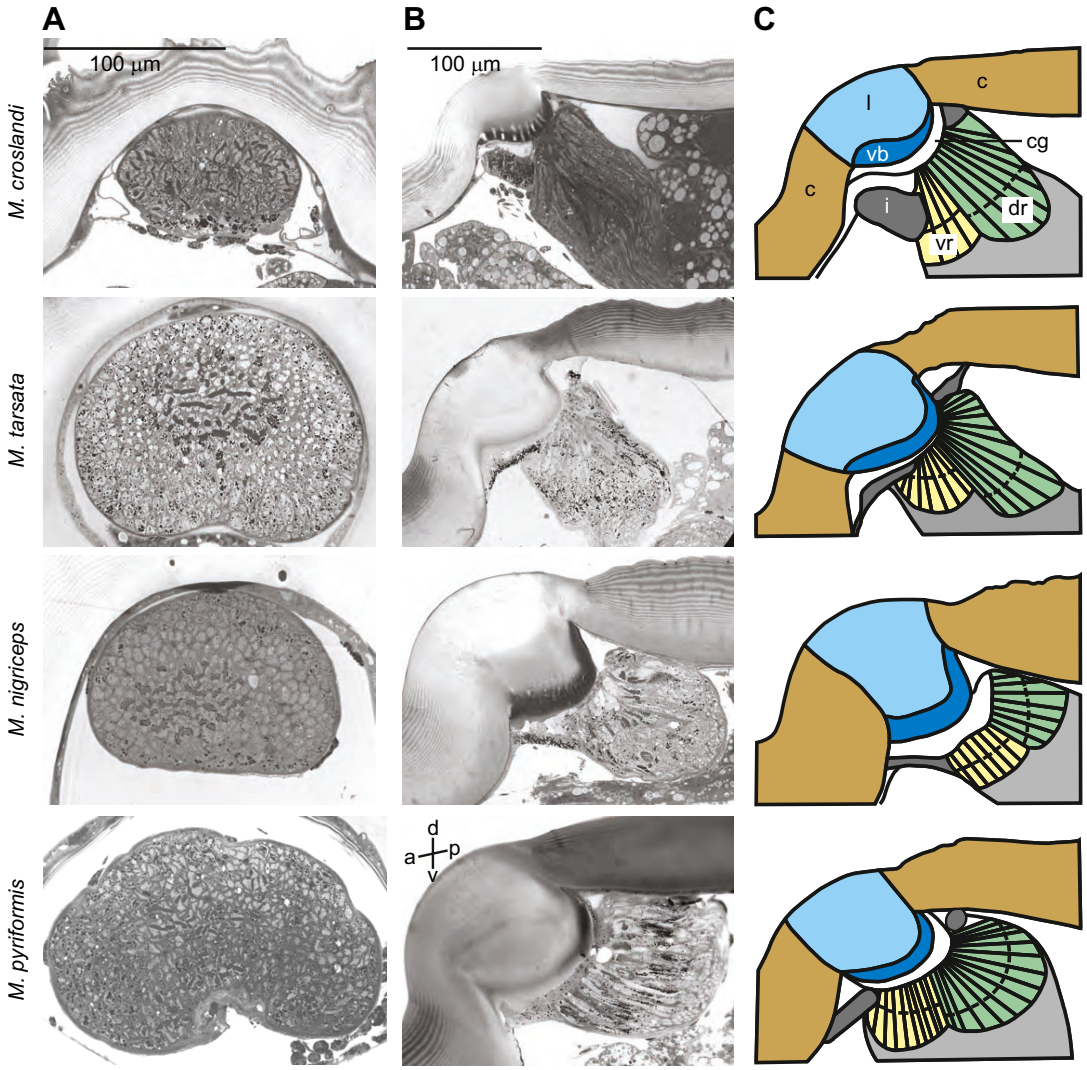

Fig. 2. Structure of the median ocellus in workers of *Myrmecia* ants. (A) Cross-section of the median ocellus where rhabdoms are dark grey in colour. (B) Longitudinal section of the median ocellus. (C) Schematic diagram of the longitudinal section of the median ocellus showing the different optical components: c, cuticle; l, lens; cg, corneagenous layer; vb, vitreous body; i, iris; vr, ventral retina looking at the sky; dr, dorsal retina looking at the horizon. Orientation of the sections and illustrations is shown: a, anterior; p, posterior; d, dorsal; v, ventral. Scale bar for each panel in A and B is shown at the top of the column.

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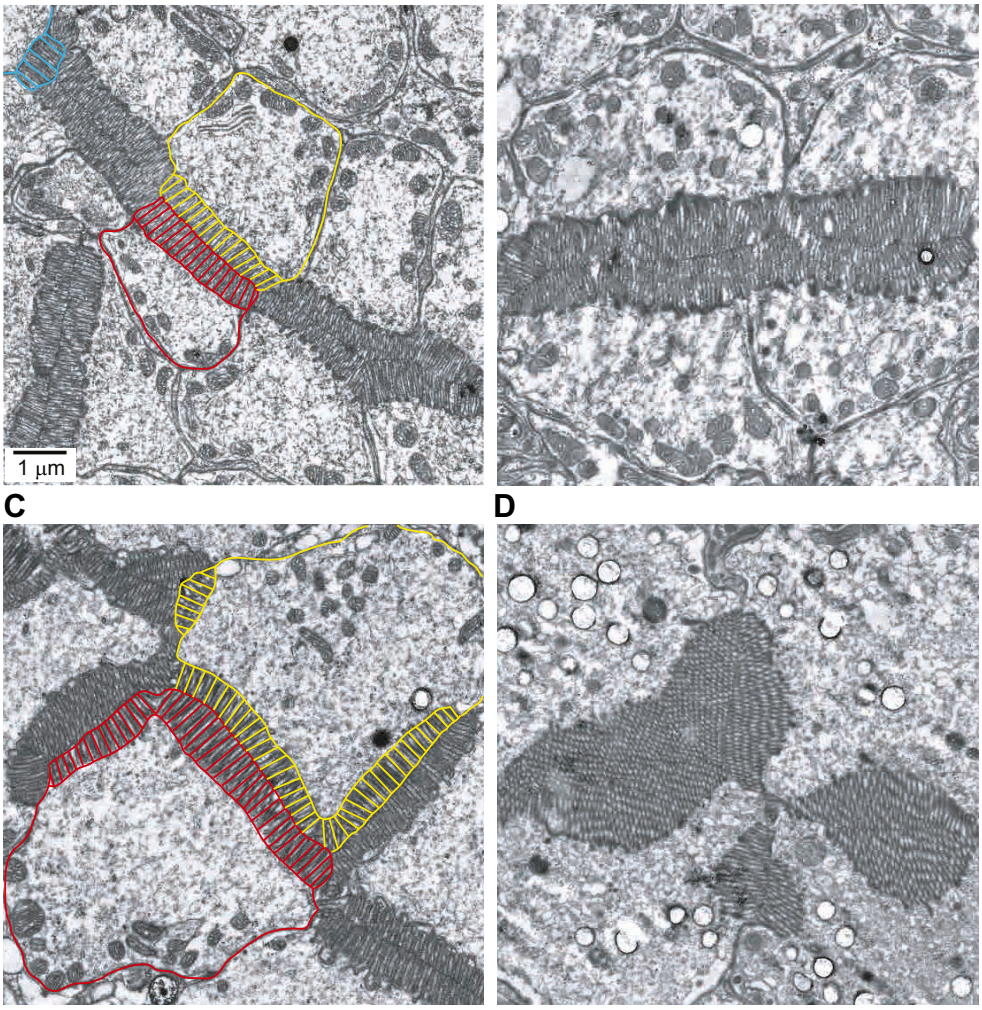

Fig. 3. Transmission electron micrographs of ocellar rhabdom cross-sections in workers of *Myrmecia* ants. Rhabdom cross-sections of (A) *M. croslandi* (diurnal), (B) *M. tarsata* (diurnal–crepuscular), (C) *M. nigriceps* (crepuscular–nocturnal) and (D) *M. pyriformis* (nocturnal). In A, a day-active ant, two retinula cells (in red and yellow) are shown that contribute microvilli in one direction towards a single rhabdom. In the top left of the panel lies a single retinula cell that contributes microvilli perpendicular (in blue) to the microvilli orientation contributed by the other retinula cells. In C, a crepuscular–nocturnal ant, two retinula cells (in red and yellow) are shown that each contribute microvilli in multiple orientations. Scale bar in A applies to all panels.

a challenge. We hence determined the cross-sectional area of each rhabdom irrespective of its geometry and calculated the diameter of the circle equivalent to this area. For five workers and two males of each species, we measured the distal to proximal rhabdom length from longitudinal sections along the anterior–posterior plane in the median ocellus.

The magnitude of polarisation sensitivity of individual receptors depends on rhabdom straightness in cross-sections (Zeil et al., 2014). Hence, we measured in one animal for each caste the straightness of all rhabdoms in cross-sections by digitising five equidistant positions along the long axis of each rhabdom using the custom-written software Digilite (©Jan Hemmi and Robert Parker) in Matlab (MathWorks, Natick, MA, USA). We then determined rhabdom straightness by determining the segment orientation (between points 1–2, 1–3, 1–4) and calculated the difference between the average segment orientation and the absolute orientation (between points 1–5), a method that has been used to analyse ocellar rhabdom straightness in honeybees (Ribi et al., 2011) and in ants (Narendra et al., 2016b). We also determined the global organisation of rhabdom orientation in both the lateral and median ocellus.

Measurement of the number of retinula cells and rhabdoms, and rhabdom width was carried out blind. Two independent observers measured these in a random sample with an accuracy of >90%.

RESULTS

External ocellar structure

In all species and castes, the external surface of the median and lateral ocelli was smooth and convex (Fig. 1). Among workers, the median ocellus was smallest in the day-active *M. croslandi* and was largest in the crepuscular–nocturnal *M. nigriceps* (Fig. 1, Table 1). The median ocellus was more than twice as large in the males compared with their respective workers. Although male ants had a smaller head compared with the workers, the relative size of the median ocellus was larger in males compared with the workers (Table 1). Among males, the absolute size of the median ocellus was larger in the day-flying *M. pyriformis*, but the relative size of the median ocellus was larger in the night-flying *M. nigriceps*.

Ocellar anatomy

The retina in both the median (Fig. 2) and lateral ocelli (Fig. S1) in all castes and species was bipartite, with a dorsal retina which appears to face the horizon and a ventral retina which appears to face the sky. Except for the viewing angles, we found little structural difference between the median and lateral ocelli and hence we will describe here the structure of the median ocellus. Ant ocellar rhabdoms, in cross-sections, varied dramatically in shape, ranging from linear to circular or having odd geometries. Worker ants had fewer rhabdoms compared with the males (Table 1). Among

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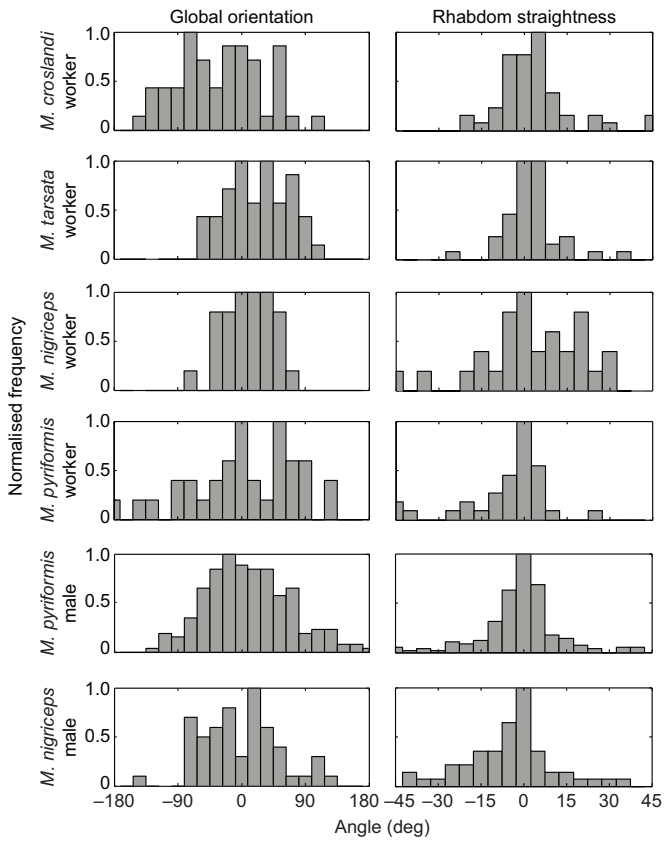

Fig. 4. Histograms showing frequency distribution of global orientation and rhabdom straightness in the median ocellus of *Myrmecia* ants. For rhabdom straightness, 0 deg indicates least deviation from a straight line. See Materials and methods for details.

workers, the nocturnal ants had the greatest number of ocellar rhabdoms, while in males, the number of ocellar rhabdoms was remarkably similar between the day- and night-flying animals. In cross-sections, the rhabdoms were most narrow in the diurnal ants and their size increased gradually as animals became nocturnal (Fig. 2, Table 1). In males too, the rhabdoms were wider in the nocturnal species compared with the day fliers (Table 1). In all species and castes, the distal–proximal length of the rhabdoms in the dorsal retina was nearly twice that of the rhabdoms in the ventral retina, which was clear from longitudinal sections (Fig. 2B, Table 1).

In bees and wasps, individual ocellar rhabdoms were composed of two paired retinula cells that contributed microvilli perpendicularly to the long axis of the rhabdom (Ribi et al., 2011; Zeil et al., 2014). In comparison, the structure of the ant ocellar rhabdom was surprisingly variable. Individual ocellar rhabdoms were composed of (mean±s.e.m.; n=5 ants) 25.9±1.2 retinula cells in *M. croslandi*, 10.3±0.8 in *M. tarsata*, 10.1±1.5 in *M. nigriceps* and 2.9±0.7 in *M. pyriformis*. In males, individual ocellar rhabdoms were composed of 5.04±0.4 retinula cells in day-flying *M. pyriformis* compared with 1.7±0.2 in night-flying *M. nigriceps*. Among workers active in bright light (*M. croslandi* and *M. tarsata*) and in males (*M. nigriceps*, *M. pyriformis*), we found a few rhabdoms that had, in addition, one retinula cell that contributed microvilli perpendicular to the microvilli orientation contributed by the other retinula cells (shown in blue Fig. 3A), which is characteristic of polarisation detectors. Among workers active in dim light (*M. nigriceps* and *M. pyriformis*), in the majority of the ocellar rhabdoms, a single retinula cell often contributed microvilli in more than one orientation, probably destroying the ability to compare e-vector intensities (Fig. 3). This was rarely seen in the ocelli of workers active in bright light and in the flying males.

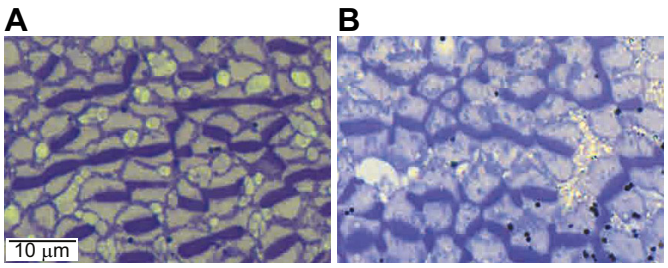

Fig. 5. Light-micrographs of ocellar retina cross-sections of male *Myrmecia* ants. Cross-section of the ocellar retina of a day-flying male *M. pyriformis* (left) and a night-flying male *M. nigriceps* (right). Scale bar for both panels is shown in A.

In several flying hymenopterans, the global orientation of ocellar rhabdoms is known to be consistent (Zeil et al., 2014). However, in the median ocellus of worker ants, in both the strictly day-active and strictly night-active ants (*M. croslandi* and *M. pyriformis*), the rhabdom orientation had a wide distribution (Fig. 4). In both *M. tarsata* and *M. nigriceps*, the global orientation was within ±90 deg relative to the horizontal. In the lateral ocellus, the orientation of the rhabdoms in all four species was consistent and was restricted to ±90 deg relative to the horizontal (Fig. S2). In both median and lateral ocelli, the majority of the rhabdoms were relatively straight, but there were more curved rhabdoms in workers active in dim light (Fig. 4; Fig. S2). Among males, the orientation of rhabdoms in both day and night fliers was largely within ±90 deg relative to horizontal (Fig. 4). The majority of the rhabdoms in male ants too were relatively straight (Figs 4 and 5).

In the nocturnal male *M. nigriceps* (only), we found a reflective crystal tapetal structure at the proximal part of the rhabdom (Fig. 6). The tapetal structures were visible under transmitted light as whitish-blue structures (Fig. 6B′), but were most distinct when viewed under dark field where they appeared as speckled structures with a silvery sheen (Fig. 6C′). Longitudinal sections further confirmed that the tapetum was present behind the rhabdom (Fig. 6D′). Such a tapetal structure was absent in the day-active males (Fig. 6, left) and in the workers of the four species.

DISCUSSION

Almost all known flying male ants have large ocelli. But because either the winged forms are hard to find or only a few species of worker ants have ocelli, this visual structure has not received much attention in ants. Here, we studied the workers and males of closely related species of *Myrmecia* ants and found that the ocellar structure is influenced by both the time at which animals are active and their mode of locomotion. Among pedestrian workers, the crepuscular–nocturnal species had the largest ocellar lens and the nocturnal species had the widest and greatest number of rhabdoms. Among males, the day fliers had the largest ocellar lens and the night fliers had the widest rhabdom. The night-flying males were the only animals with a reflective tapetum at the proximal region of the rhabdom, which most likely increases their optical sensitivity, allowing them to operate in dim-light conditions.

In the compound eye of nocturnal ants and other hymenopterans, the size of the lens and the width of the rhabdom tends to be larger than in their diurnal relatives, resulting in a 27- to 30-fold increase in optical sensitivity (Greiner, 2006b; Greiner et al., 2007; Narendra

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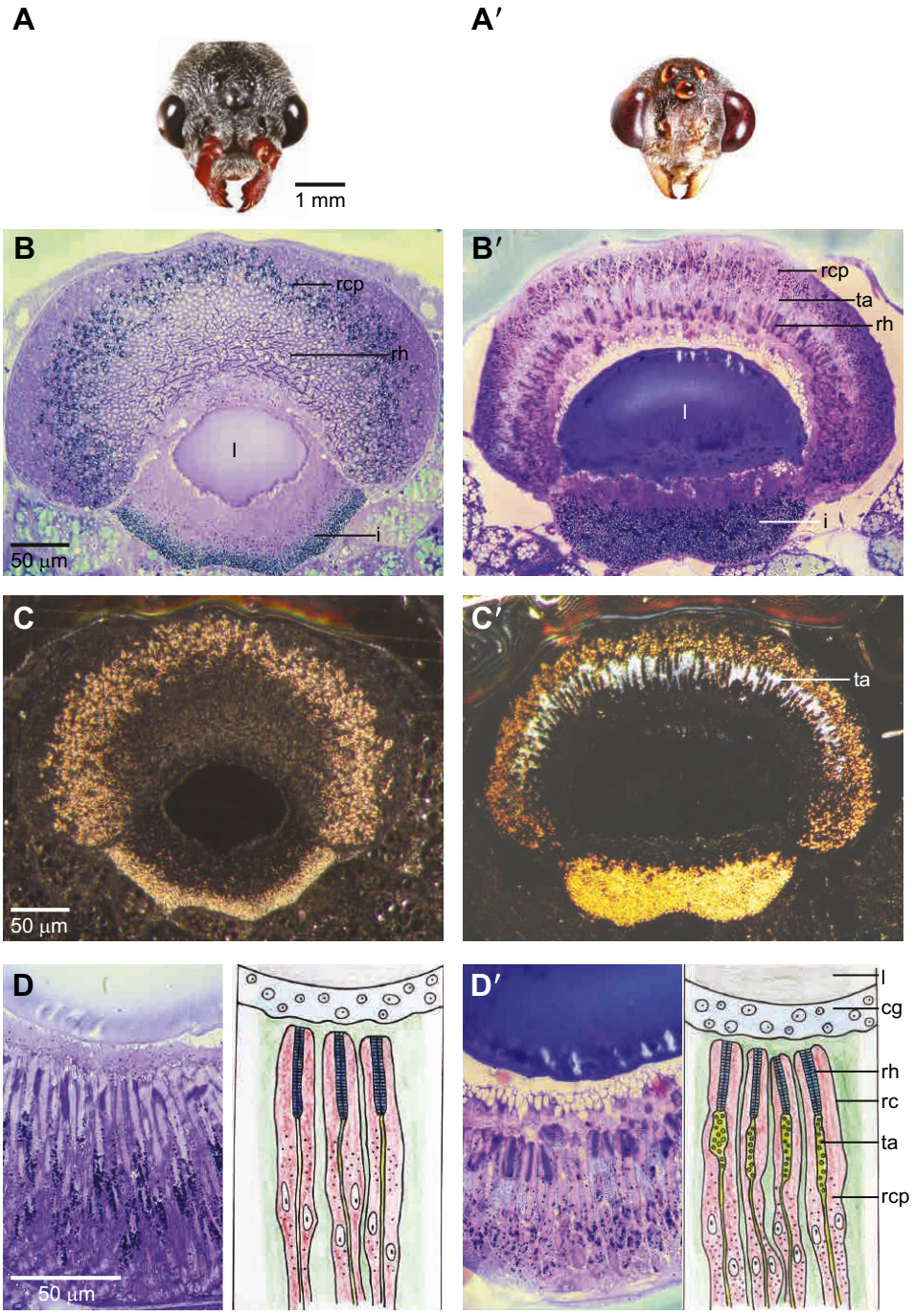

Fig. 6. Ocellar retina of day- and night-flying *Myrmecia* male ants. Left column: day-flying *M. pyriformis*; right column: night-flying *M. nigriceps*. (A,A′) Dorsal view of the head showing the three ocelli. (B,B′) Cross-section of the median ocellus as viewed under a transmission light microscope. (C,C′) Cross-section of the median ocellus in B and B′ viewed using a phase-contrast microscope, showing the presence of a reflective tapetum in the night-active species only. (D,D′) Left: longitudinal section of the median ocellus at the same level as the respective cross-section in B and B′. Right: schematic diagram to illustrate the organisation of the rhabdoms and tapetum in the longitudinal plane. Scale bar for each row is shown in panels on the left. cg, corneagenous layer; i, iris; l, lens; rc, retinula cell; rcp, retinula cell pigments; rh, rhabdom; ta, tapetum.

et al., 2011; Warrant and Dacke, 2011). We found a similar pattern in the ocelli of *Myrmecia* workers: nocturnal ants had the widest ocellar rhabdoms, which potentially leads to increased optical sensitivity (Table 1), suggesting that gathering more light is a crucial function of the ocelli for crepuscular and nocturnal worker ants. A similar increase in the size of the ocellar rhabdoms has been found in the nocturnal carpenter bees (Somanathan et al., 2009). Among males, the night-flying *M. nigriceps* had the widest rhabdom and in addition had a reflective sheath, the tapetum, proximal to the rhabdom (Fig. 6). The tapetum reflects light back to the rhabdom, allowing light to be absorbed a second time, thus further increasing the optical sensitivity of the ocelli. The tapetum may also act as a polariser, as seen in the secondary eyes of the gnaphosid spider *Drassodes cupreus* (Dacke et al., 1999). Males of *Myrmecia* tend to leave the nest and fly to nearby hill tops where they locate females to mate with. The ability to detect polarised skylight may allow ants flying at night to maintain a straight course during flight. It also has the potential to generate sufficient contrast between the terrestrial landmark panorama and the horizon (Schultheiss et al., 2016; Zeil et al., 2014) to control for head stabilisation (roll and pitch axes) during flight (Stange, 1981). A tapetum made up of reflective crystalline deposits has been described in a variety of insects including locusts (Goodman, 1970), cicadas (Ribi and Zeil, 2015), dragonflies (Stange et al., 2002), beetles (Simmons and Ridsdill-Smith, 2011) and jumping bristletails (Böhm and Pass, 2016). The nocturnal carpenter bee *Xylocopa tranqueberica* (Somanathan et al., 2009) has a tracheolar tapetum, where tracheoles between photoreceptor cells optically insulate them from each other. The

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tapetum is formed of urate or xanthine nanocrystals, which are thought to cause the observed reflection (Böhm and Pass, 2016). The tapetum was clearly absent in the slow-moving pedestrian ants, suggesting that improving optical sensitivity through a tapetum is crucial for flight mode (but not for walking) in dim light.

There was a nearly 3-fold increase in the number of rhabdoms in males compared with workers (Table 1). The day-active workers had slightly fewer rhabdoms compared with the nocturnal animals, but their relationship to activity time was unclear as rhabdom numbers did not vary by much between day- and night-flying males. The retinula cell numbers also did not show a clear trend between the pedestrian and flying ants. But, the number of retinula cells in both castes was high in the day-active animals and gradually decreased as animals became nocturnal. Hence, it is not surprising that the number of retinula cells required to make up an ocellar rhabdom was higher in the workers than in the males and that more retinula cells contributed to form an individual ocellar rhabdom in the day-active animals.

The organisation of the ant ocellar retina is strikingly different from that in other hymenopterans. In both bees and wasps, each retinula cell contributes a rhabdomere and two paired retinula cells contribute microvilli in opposite directions to form a rhabdom, with the direction being perpendicular to the long axis of the rhabdom (Ribi et al., 2011; Taylor et al., 2016; Zeil et al., 2014). In ants, typically, multiple retinula cells contribute to the formation of a single rhabdom and it was rare to find rhabdoms made up of only two retinula cells. While the need and constraints to build a rhabdom with more retinula cells are unclear at this stage, there are some distinct patterns in the organisation of the ocellar retina. In the primarily day-active worker ants (*M. croslandi* and *M. tarsata*) and in both the diurnal and nocturnal males, the orientation of the microvilli in most of the rhabdoms is perpendicular to the long axis of the rhabdom. In a few of these ocellar rhabdoms, a single retinula cell contributes microvilli in a direction parallel to the long axis of the rhabdom. This organisation is typical of polarisation detectors as it allows an animal to compare e-vector intensities from the same spot. Among ants, the physiological properties of the ocelli have been investigated only in the diurnal *Cataglyphis bicolor* (Mote and Wehner, 1980), where ocellar rhabdoms were shown to be polarisation sensitive, but to the best of our knowledge the ocellar structure of these ants is unknown. In worker ants active in low light (*M. nigriceps* and *M. pyriformis*), the organisation of the retina was markedly different. In these ants too, typically more than two retinula cells contributed to the formation of a rhabdom. But in the majority of the rhabdoms, each retinula cell contributed microvilli in more than one direction (e.g. the two retinula cells, in red and yellow, in Fig. 3C), thus destroying polarisation sensitivity. However, these ants have wide rhabdoms. Hence, it is likely that the ocelli have two different functions: in workers, ocellar rhabdoms in day-active species are polarisation sensitive, whereas in nocturnal species they are involved in increasing optical sensitivity. Males, both day and night fliers, clearly have a requirement to be sensitive to the pattern of polarised skylight, which is reflected in the organisation of the ocellar retina.

We have shown here that closely related species have the need and the ability to develop anatomical structures that allow them to modify their information-processing capacities. Ocellar lenses in a few hymenopterans have the potential to resolve images (e.g. Kelber et al., 2011; Ribi et al., 2011; Taylor et al., 2016; Somanathan et al., 2009). At present, we do not know whether the ant ocelli can resolve images and this needs to be addressed with the knowledge that the ocellar lens is not homogeneous and that ocelli have a dorsal- and ventral-facing retina.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: A.N.; Methodology: A.N., W.A.R.; Formal analysis: A.N.; Investigation: A.N., W.A.R.; Writing - original draft: A.N.; Writing - review & editing: A.N., W.A.R.; Visualization: A.N., W.A.R.; Funding acquisition: A.N.

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Data availability
Myrmecia ocelli data are available at: https://ecologicalneuroscience.com/datasets/

Supplementary information
Supplementary information available online at
http://jeb.biologists.org/lookup/doi/10.1242/jeb.159392.supplemental

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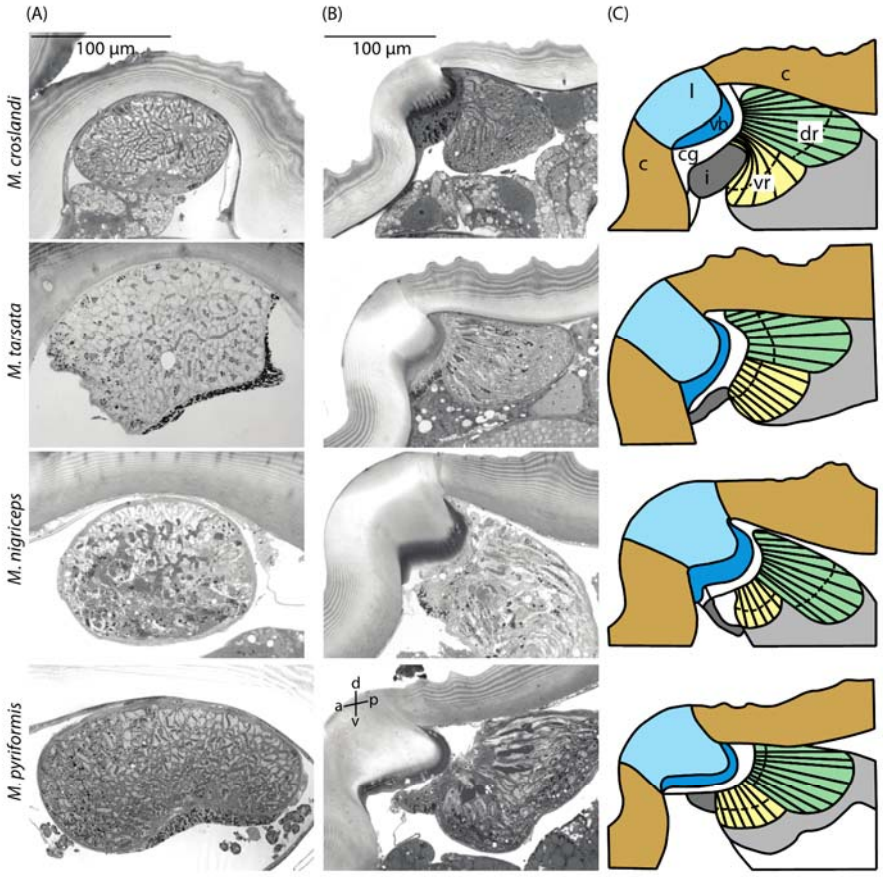
Figure S1



Ocelli structure in *Myrmecia* ants

Figure S1. Structure of the lateral ocellus in workers of *Myrmecia* ants. (A) Cross-section of the lateral ocellus with rhabdoms seen in dark grey in colour. (B) Longitudinal section of the lateral ocellus. (C) Schematic of the longitudinal section of the lateral ocellus showing the different optical components: c: cuticle; l: lens; cg: corneagenous layer; vb: vitreous body; i: iris; vr: ventral retina looking at the sky; dr: dorsal retina looking at the horizon. Orientation of the sections and illustrations is shown: a-anterior, p-posterior, d-dorsal and v-ventral. Scale bar for each panel is shown in the top panel of the column.

Figure S2

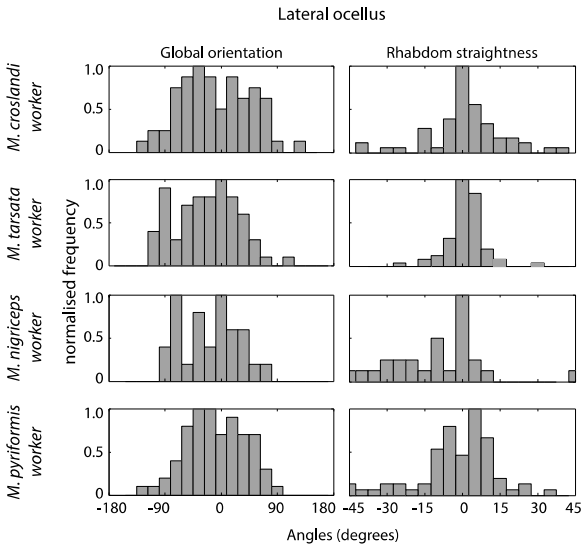


Figure S2. Histogram showing frequency distribution of rhabdom straightness in the lateral ocellus in workers of *Myrmecia* ants. For rhabdom straightness, 0° indicates least deviation from the straight line. See methods for details.