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RESEARCH ARTICLE

Light and dark adaptation mechanisms in the compound eyes of *Myrmecia* ants that occupy discrete temporal niches

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ABSTRACT

Ants of the Australian genus *Myrmecia* partition their foraging niche temporally, allowing them to be sympatric with overlapping foraging requirements. We used histological techniques to study the light and dark adaptation mechanisms in the compound eyes of diurnal (*Myrmecia croslandi*), crepuscular (*M. tarsata*, *M. nigriceps*) and nocturnal ants (*M. pyriformis*). We found that, except in the day-active species, all ants have a variable primary pigment cell pupil that constricts the crystalline cone in bright light to control for light flux. We show for the nocturnal *M. pyriformis* that the constriction of the crystalline cone by the primary pigment cells is light dependent whereas the opening of the aperture is regulated by an endogenous rhythm. In addition, in the light-adapted eyes of all species, the retinular cell pigment granules radially migrate towards the rhabdom, a process that in both the day-active *M. croslandi* and the night-active *M. pyriformis* is driven by ambient light intensity. Visual system properties thus do not restrict crepuscular and night-active ants to their temporal foraging niche, while day-active ants require high light intensities to operate. We discuss the ecological significance of these adaptation mechanisms and their role in temporal niche partitioning.

KEY WORDS: Crystalline cone, Pupil, Rhabdom, Screening pigment

INTRODUCTION

Ants of the genus *Myrmecia* rely heavily on vision for prey capture (Eriksson, 1985; Via, 1977) and navigation (Narendra et al., 2013b; Reid et al., 2011; Zeil et al., 2014). This is reflected in the size of their eyes, with some species having more than 3000 ommatidia per eye (Gronenberg, 2008; Narendra et al., 2011), which is unusually high for ants. For instance, the desert ants, which are well known for their navigation capacities, have about 500 (in *Melophorus bagoti*; Schwarz et al., 2011) to 1000 ommatidia (in *Cataglyphis fortis*; Menzi, 1987) in each eye. Within the genus *Myrmecia*, four congeneric and sympatric species are active at distinctly different and barely overlapping times of the day, ranging from strictly diurnal to diurnal–crepuscular, crepuscular–nocturnal and strictly nocturnal (Fig. 1; Greiner et al., 2007; Jayatilaka et al., 2011; Narendra et al., 2010; Reid et al., 2013). Correlated with these distinct foraging schedules are specific adaptations of the visual system in each of the four species to the ambient light conditions at which they operate (Greiner et al., 2007; Narendra et al., 2011). This temporal niche partitioning may have evolved and may be maintained by predation pressure, temporal distribution of food resources and competition (Kronfeld-Schor and Dayan, 2003). Abiotic factors such as temperature and light can also dictate the time at which animals are active (Greenaway, 1981; Narendra et al., 2010). Hence, the animal's physiological ability to cope with the variations of temperature and light along a diel and annual cycle could play a role in determining their time of activity. However, in this particular group of *Myrmecia* ants, neither temperature tolerance nor competition between the day-active and the night-active species can explain why they restrict their activity to their specific temporal niche (Jayatilaka et al., 2011). What then locks these species into their respective activity patterns? One possibility, which we investigate here, is that the compound eye design itself limits animals to be active in specific temporal niches.

Hymenopteran insects such as ants, bees and wasps have apposition compound eyes, an eye design that – in contrast to the optical superposition eye design of most night-active insects (e.g. Nilsson, 1989, 1990) – typically limits vision to bright daylight conditions because each photoreceptor receives light through one small facet lens only (Greiner, 2006; Schwarz et al., 2011; Warrant et al., 2004). However, several hymenopteran insects are active in dim light conditions (Greiner, 2006; Greiner et al., 2007; Kelber et al., 2006, 2003; Klotz and Reid, 1993; Narendra et al., 2010; Somanathan et al., 2008, 2009; Wolda and Roubik, 1986) and have modified apposition compound eyes to increase photon capture: larger lenses, wider and longer rhabdoms and spatial and temporal pooling of photoreceptor signals across neighbouring ommatidia (Stöckl et al., 2016; Warrant and Dacke, 2011). Such highly sensitive eyes have then to be protected against bright light. In apposition compound eyes, light flux to the rhabdom is regulated in two ways (reviewed by Autrum, 1981; Stavenga, 1989; Stavenga et al., 1979): through screening pigment migration within the retinular cells towards and away from the rhabdom at a time frame of seconds (Kirschfeld and Franceschini, 1969; Menzel and Knaut, 1973; Stavenga and Kuiper, 1977; Stavenga et al., 1977), and through a diaphragm pupil formed by the two primary pigment cells that constricts the proximal crystalline cone to form a narrow tract at a time frame of tens of minutes (e.g. Home, 1976; Kolb and Autrum, 1972; Lüdtke, 1953; Mclean and Horridge, 1977; Meyer-Rochow, 1972, 1999; Wada and Schneider, 1967, 1968; Walcott, 1971; Williams, 1980). In the light-adapted eyes of ants active in dim light, such a constriction forms a narrow aperture of 0.5–1.0 µm, which opens up to nearly 5 µm in the dark-adapted state (Menzi, 1987; Narendra et al., 2013a). In the strictly day-active ants, the only light adaptation mechanism that has been observed is the radial migration of retinular cell screening pigment granules wherein the pigments tightly ensheath the rhabdom in the light-adapted state and move away from the rhabdom in the dark-adapted state (Brunnert and Wehner, 1973; Menzel and Knaut, 1973; Menzi, 1987). This migration of the retinular cell pigment granules

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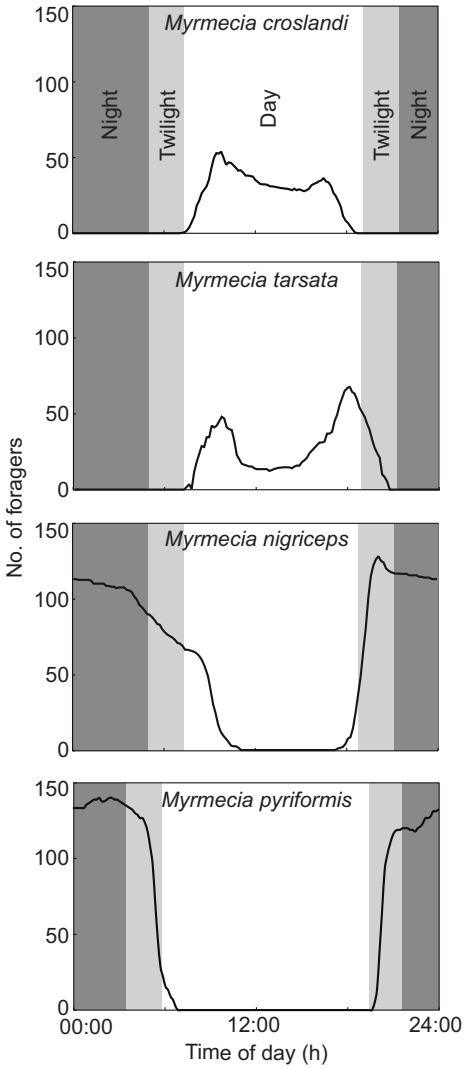

Fig. 1. Worker activity schedules and temporal niche partitioning in four sympatric congeneric *Myrmecia* ants.

towards and away from the rhabdom alters the refractive index of the surrounding medium, resulting in a change of the mode-dependent properties (Jonson and Nilsson, 1994; Jonson et al., 1998; Kirschfeld and Franceschini, 1969; Snyder and Horridge, 1972; Stavenga, 2004a,b).

We asked here whether and to what extent the eyes of day-active, crepuscular and night-active *Myrmecia* ants retain the ability for short-term light and dark adaptation that would allow foragers in principle to operate at a broader range of light intensities than the ones they experience during their normal and distinctly different activity schedules. In short, we aimed to determine whether the design of their visual systems restricts animals to discrete temporal niches.

MATERIALS AND METHODS

We located several nests for each of the four *Myrmecia* species, *Myrmecia croslandi* Taylor, *Myrmecia tarsata* Fr. Smith, *Myrmecia nigriceps* Mayr and *Myrmecia pyriformis* Fr. Smith in Canberra, Australia. These ants are usually sympatric and often found foraging on *Eucalyptus* trees. All four species of ants capture a variety of arthropods to feed their larvae and gather liquid food produced by aphids and coccids. Daily and seasonal activity patterns have been reported earlier (Greiner et al., 2007; Jayatilaka et al., 2011; Narendra et al., 2010; Reid et al., 2013).

Histology

Ants were collected individually during their activity periods, brought to the laboratory and immobilised on ice; their mandibles were removed and the head capsules were opened. Optimal retinal fixation was achieved by making a cut in the ventral-most rim of the eye. Red light was used for dissecting the nocturnal animals. The effect of dark adaptation versus light adaptation was studied in the laboratory. All four species were dark adapted from 3 h after sunset for 24 h (stored individually in a jar placed in a light-tight container) and dissected under red light. For the light-adapted state, animals were exposed to room-light conditions (~300 lx or 4.3×10^{-5} W cm^{-2}) from 3 h after sunrise for 24 h and dissected. Specimens were fixed for 2 h in 2.5% glutaraldehyde and 2% paraformaldehyde in phosphate buffer (pH 7.2–7.5), followed by a series of buffer washes and post-fixation in 2% OsO_4 in distilled water for 2 h. Samples were then dehydrated in an ethanol series, transferred to propylene oxide or acetone and embedded in Epoxy resin (Fluka).

To identify and track the compound eye changes in the ambient light environment, we carried out an additional experiment exclusively with the truly nocturnal ant *M. pyriformis*. Under clear skies, workers of *M. pyriformis* were dissected in the field at natural light intensities using red light at 20:00 h, 21:30 h, 23:30 h, 05:30 h and 08:00 h on a single December day in Austral summer. The activity pattern of the ant nest was recorded simultaneously. Samples were immediately fixed, stored in glass scintillation vials and left for 2 h at the field site to preserve the actual state of the eye in natural light conditions. Samples were then processed as described earlier.

In the night-active ant *M. pyriformis*, we tested the degree to which pigment migration is driven by ambient light intensity and/or by an endogenous circadian rhythm. For this, we captured ants 30 min past sunset and fixed their eyes after exposing them to (a) ambient light levels of 1 lx for 10 min, (b) bright light intensities of 300 lx for 10 min or (c) ambient light intensities for 960 min (i.e. roughly until the afternoon of the following day), followed by 10 min of darkness (0 lx). In addition, in the day-active ant *M. croslandi*, we investigated the effect of light intensity on retinular cell pigment migration. For this, we light adapted animals in room-light for 24 h (from 3 h after sunrise) and dark adapted another group of animals by keeping them in complete darkness for 24 h (from 3 h after sunset). Samples were then processed as described above.

Longitudinal and serial cross-sections, 1 µm thick, as well as 50 nm thin sections from selected regions, were cut on an ultramicrotome (Leica EM UC7 or Reichert Ultracut) using glass and diamond knives. Sections for light microscopy were stained with Toluidine Blue and digitally photographed with either a Zeiss Axioskop compound microscope equipped with a Spot Flex 16 megapixel colour camera or an Olympus BX53 compound microscope equipped with a DP26 digital camera. Sections for transmission electron microscopy were stained with 6% saturated uranyl acetate (25 min) and lead citrate (5 min) before being viewed with a Hitachi transmission electron microscope (HA 7100).

RESULTS

Light and dark adaptation of the compound eye

In the light-adapted state, the primary pigment cells constrict the proximal crystalline cone to form a narrow tract, which has a diameter of 0.8±0.05 µm (mean±s.d.) in *M. tarsata*, 0.8±0.05 µm in *M. nigriceps* and 1.6±0.05 µm in *M. pyriformis* (Figs 2A and 3A; *N*=3 for all species) and causes the crystalline cone to elongate. In

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the dark-adapted state, the primary pigment cells move away from the crystalline cone, increasing the diameter of the proximal crystalline cone to 1.6±0.05 µm in *M. tarsata*, 5.1±0.05 µm in *M. nigriceps* and 5.3±0.05 µm in *M. pyriformis* (Figs 2B and 3C; *N*=3 for all species). The diameter of the proximal crystalline cone tract in the diurnal ant *M. croslandi* is narrow (1.2±0.05 µm) and remains unchanged in both the light- and dark-adapted state (Fig. 2). In the diurnal–crepuscular *M. tarsata*, the light-adapted cone tract is clearly visible (Fig. 2A), but the opening of the pupil in the dark-adapted state of the eye is less pronounced (Fig. 2B).

In all four species, the retinular cell pigments move close to the rhabdom in the light-adapted state, but are withdrawn from the rhabdom in the dark-adapted state, leaving an enlarged and clear vacuole palisade surrounding the rhabdom (Figs 3 and 4A).

Pigment migration during natural foraging activity

How do these light and dark adaptation changes in the compound eye relate to natural foraging activity patterns? As the constriction of the crystalline cone tract was extreme in the crepuscular and nocturnal species, we investigated this in the nocturnal *M. pyriformis*. We fixed the eyes of *M. pyriformis* under natural light conditions at periods when foragers were active. Animals typically leave the nest around sunset (Fig. 4B). At this time, the primary pigment cells constrict the crystalline cone to form a narrow cone tract (20:00 h in Fig. 4A). As light levels drop after sunset, the primary pigment cell pupil opens gradually, remains open throughout the night, and begins to close at the start of the morning twilight (05:30 h in Fig. 4A) when the majority of the animals return home. After sunrise, the primary pigment cell again constricts the crystalline cone to form a narrow tract (08:00 h in Fig. 4A). These changes are accompanied by distinct distribution patterns of retinular cell pigments (Fig. 4A). Close to sunset, the retinular cell pigments hug the rhabdom and as night falls, they gradually move away from the rhabdom, leaving it surrounded by a clear vacuole palisade. In the morning, as light intensity increases, these pigments form dense clusters in direct contact with the rhabdom. We note that this is true for six of the eight retinular cells that contribute microvilli to the distal rhabdom (Fig. 4A, bottom left and bottom right images), indicating that under natural conditions, retinular cells are light or dark adapted depending on their spectral sensitivity (e.g. Bernard and Wehner, 1980; Menzel and Blakers, 1976; Ribi, 1978b,c, 1987; Roebroek and Stavenga, 1990).

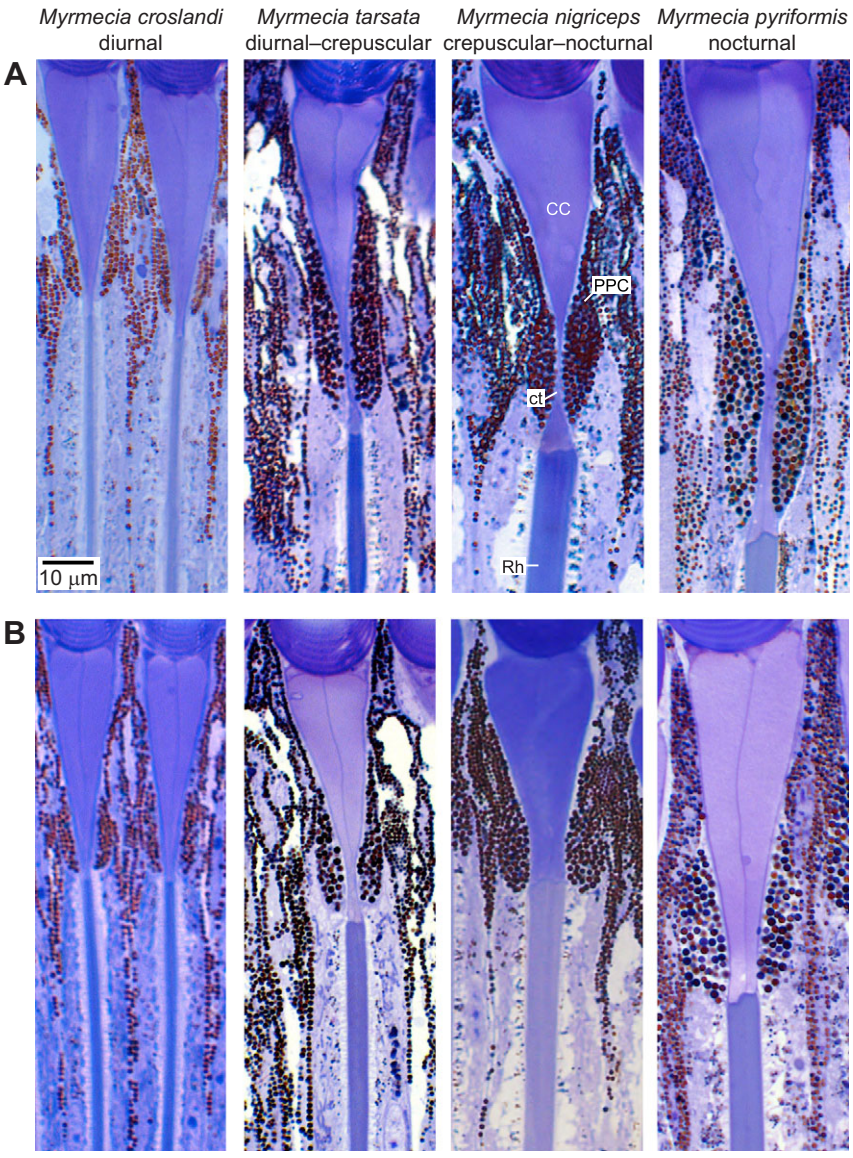

Fig. 2. Light-microscopy images of longitudinal sections of the ommatidia of four species of ants: diurnal *Myrmecia croslandi*, diurnal–crepuscular *Myrmecia tarsata*, crepuscular–nocturnal *Myrmecia nigriceps* and nocturnal *Myrmecia pyriformis*. (A) Light-adapted and (B) dark-adapted state. CC, crystalline cone; ct, crystalline cone tract; PPC, primary pigment cell; Rh, rhabdom.

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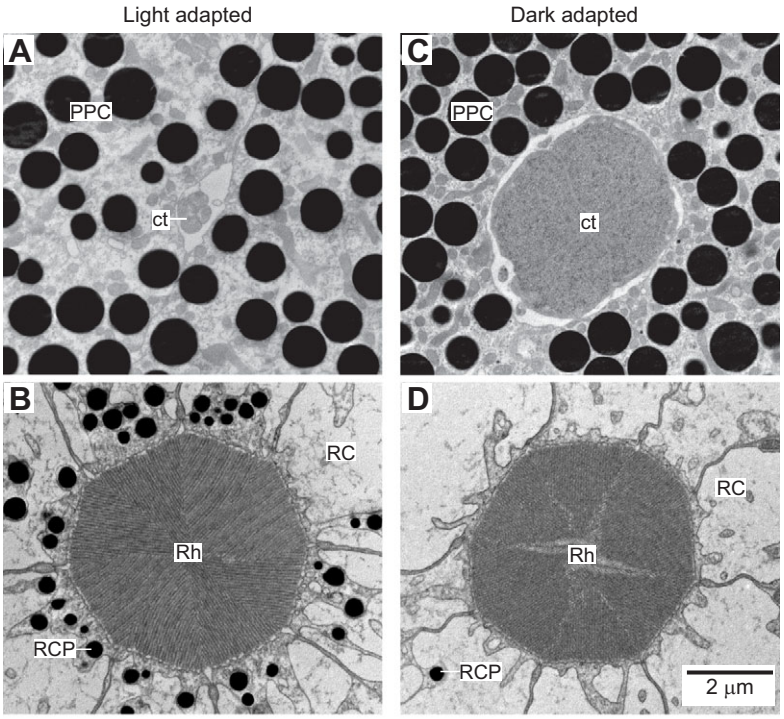

Fig. 3. Transmission electron micrographs of cross-sections of the ommatidia of the crepuscular–nocturnal ant *M. nigriceps*. (A,B) Light-adapted state and (C,D) dark-adapted state. (A,C) Cross-section of the crystalline cone tract (ct) at the level of primary pigment cells (PPC). In the light-adapted state, the primary pigment cells constrict the proximal crystalline cone to form a cone tract of less than 1 µm in diameter; in the dark-adapted state, the cone tract opens to a diameter of approximately 5 µm. (B,D) Cross-section at the level of the distal rhabdom (Rh), showing the retinular cells (RC) and retinular cell pigments (RCP). In the light-adapted state, retinular cell pigments tightly ensheath the rhabdom; in the dark-adapted state, pigment granules are away from the rhabdom. Panel B is reproduced with permission from Greiner et al. (2007).

Role of light intensity and circadian rhythm in pigment migration

We asked to what degree pigment migration is triggered directly by light intensity or driven by an endogenous circadian rhythm. We fixed the eyes of the nocturnal *M. pyriformis* in three conditions: ambient light at night, after light adapting eyes at night and after dark adapting eyes in the day. In ambient light conditions at night, the primary pigment cell pupil is open, with the proximal crystalline cone diameter measuring 2.4 µm (Fig. 5A). Retinular cell screening pigments are mainly

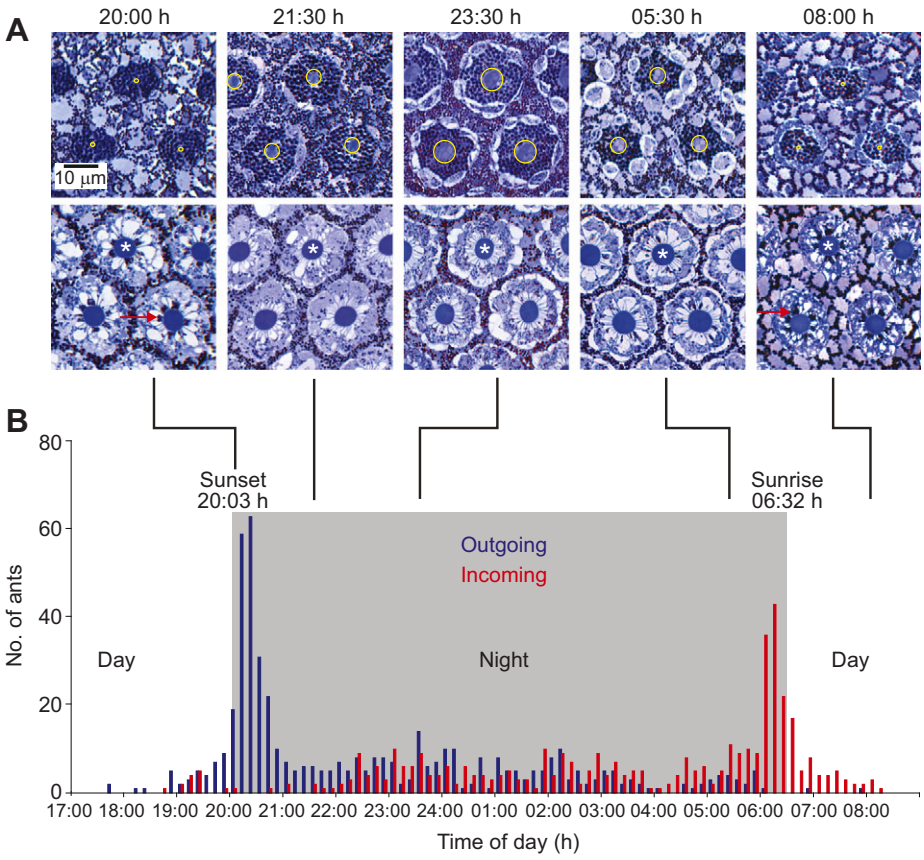

Fig. 4. Light and dark adaptation under natural foraging conditions in the eyes of the nocturnal *M. pyriformis*. (A) Light-microscopy images of cross-sections of crystalline cone tract (top panels) and distal rhabdoms (bottom panels). The crystalline cone tract is outlined in yellow; the red arrows point to the retinular cell pigments; the rhabdom is indicated by a white asterisk. (B) The simultaneously recorded foraging activity on a single summer day in December.

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located outside the palisade, but in some cases they are still close to the rhabdom. Secondary screening pigment granules form a dense pigment screen around the ommatidium (Fig. 5B). In light-adapted eyes at night, the primary pigment cells constrict the crystalline cone to form a narrow tract of 0.5 µm diameter (Fig. 5C). Retinular cell screening pigment is located close to the rhabdom, but the pigments in the secondary pigment cells appear scattered (Fig. 5D; see also Fig. 4A, bottom right image). In the dark-adapted eye during the day, the primary pigment cell pupil does not open fully, having a diameter of 1.7 µm (Fig. 5E), indicating that a light-driven response is moderated by a circadian rhythm. In contrast, the movements of retinular cell screening pigment granules are fully determined by ambient light intensity: the pigments are fully retracted from the rhabdom after 10 min of dark adaptation during the day (Fig. 5F), compared with the naturally light-adapted state in the morning (Fig. 5F, inset). Retinular cell pigment migration is also light dependent in the day-active *M. croslandi*, which lacks a variable primary pigment cell pupil (Fig. 2A,B): retinular cell pigments hug the rhabdom in the light-adapted state and are farther from the rhabdom in the dark-adapted state (Fig. 6).

The opening and closing mechanism of the primary pigment cell pupil

We note three features that may be relevant for elucidating the mechanisms underlying the opening and closing of the primary pigment cell pupil in *Myrmecia* ants: (1) in the dark-adapted eye of *M. tarsata*, the cone tract (Fig. 7A,B) is filled with granules that resemble glycogen vesicles (Fig. 7C,D); (2) microtubuli are present in the cone tract of *M. tarsata* eyes (Fig. 7D,E); and (3) the extensions of the four crystalline cone cells, the Semper cells, reach all the way to the basement membrane (data not shown), which therefore may serve as mechanical anchors supporting shape changes of the crystalline cone (Walcott, 1975).

DISCUSSION

Four congeneric and sympatric *Myrmecia* ant species partition their niche temporally such that each species is active at specific times of the day (Fig. 1). Here, we investigated the light and dark adaptation strategies in the compound eyes of these ants. We found that the crepuscular–nocturnal (*M. tarsata*, *M. nigriceps*) and the truly nocturnal species (*M. pyriformis*) possess a variable primary pigment cell pupil, which is absent in the strictly day-active ant (*M. croslandi*). In *M. pyriformis*, the closing of the

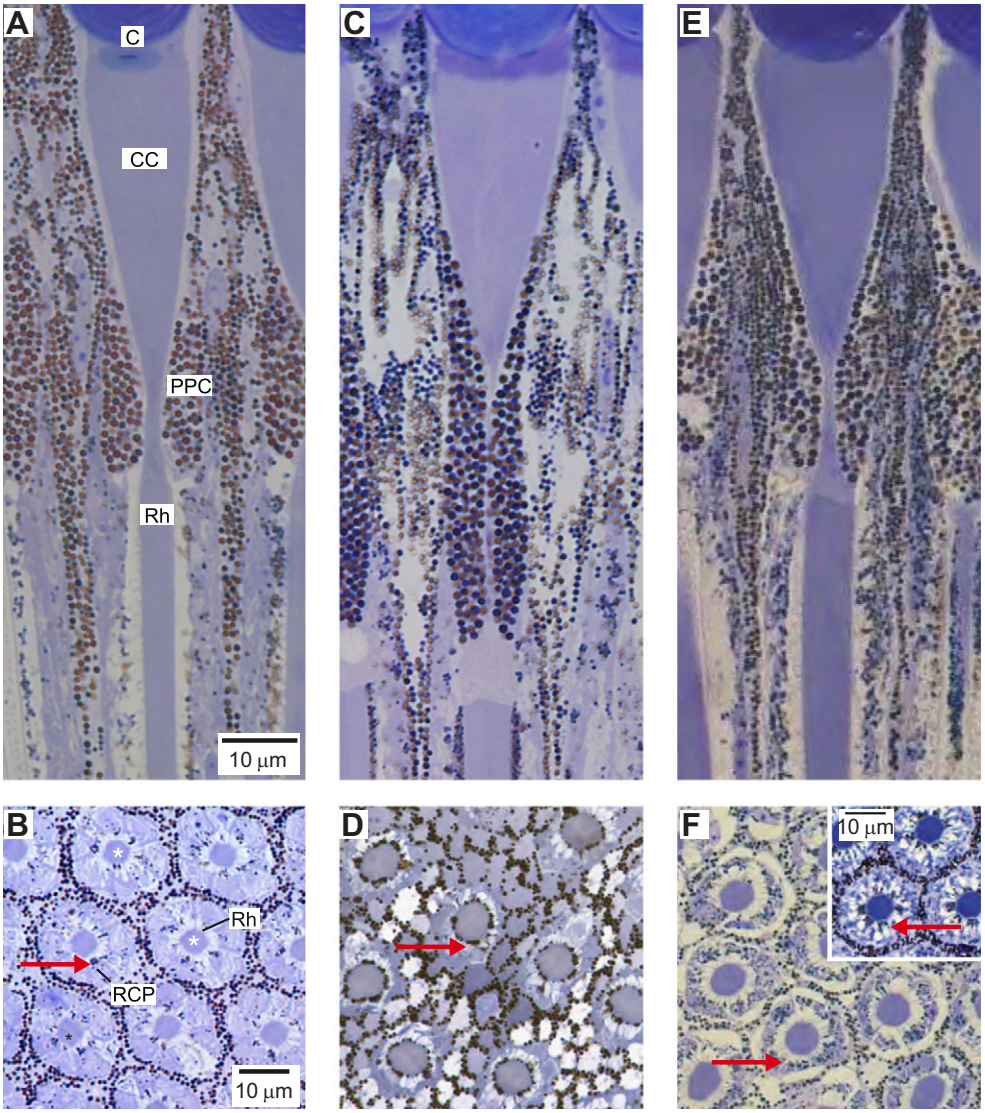

Fig. 5. Role of circadian rhythm and light intensity in the pupillary mechanism of the nocturnal ant *M. pyriformis*. Eyes of ants captured 30 min past sunset were fixed in three conditions: (A,B) ambient light levels (1 lx for 10 min), (C,D) light adaptation at night (300 lx for 10 min) or (E,F) dark adaptation in the day (ambient light for 960 min followed by 10 min of darkness, 0 lx). (A,C,E) Longitudinal sections of an ommatidium and (B,D,F) cross-sections of distal rhabdoms. Inset in F is a cross-section of distal rhabdoms of an eye exposed to ambient light conditions and fixed in the day, indicating that in bright light the retinular cell pigments move close to the rhabdom. The red arrow indicates the retinular cell pigments (RCP; B,D, inset in F) close to the rhabdom (Rh) and (F) away from the rhabdom; the rhabdom is indicated by a white asterisk. C, cornea; CC, crystalline cone; PPC, primary pigment cell.

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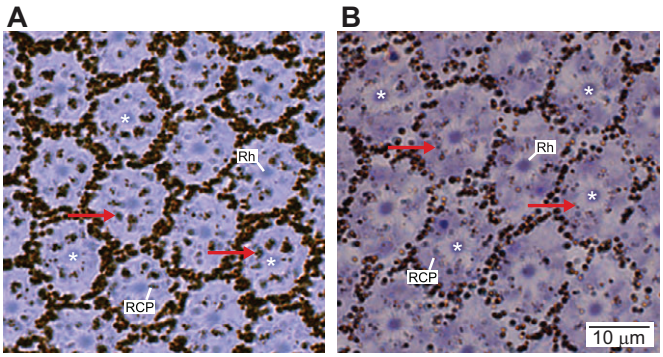

Fig. 6. Role of light intensity in retinular cell pigment migration in the day-active ant *M. croslandi*. Cross-section of the distal rhabdoms in (A) the light-adapted and (B) the dark-adapted state. For light adaptation, animals were kept in room-light for 24 h (from 3 h after sunrise); for dark adaptation, animals were kept in complete darkness for 24 h (from 3 h after sunset). In the light-adapted state, the retinular cell pigments (RCP) hug the rhabdom (Rh); in the dark-adapted state, the retinular cell pigments move away from the rhabdom, creating a vacuole palisade around the rhabdom.

pupillary mechanism is driven by ambient light, whereas the opening of it appears to be constrained during the day by a circadian rhythm. In all four species, retinular cell pigments migrate to and away from the rhabdom depending on light conditions, and for the day-active (*M. croslandi*) and the night-active species (*M. pyriformis*) this migration is driven directly by ambient light intensities.

With foraging schedules ranging from daylight hours to beyond astronomical twilight (Fig. 1), the crepuscular and nocturnal foragers of *M. pyriformis* and *M. nigriceps* in particular would have a need to dynamically adjust the absolute light sensitivity of their eyes. In addition, the crepuscular and nocturnal ants often defend their nest from trespassers and predators during the day. It is perhaps for these reasons that the crepuscular and the nocturnal species possess both a variable pupil formed by the two primary pigment cells and a 'longitudinal pupil' formed by retinular cell screening pigments. The primary pigment cell pupil diameter does not appear to be variable in the strictly day-active *M. croslandi* and its 'dynamic range' appears to be restricted in the diurnal–crepuscular *M. tarsata*, compared with the crepuscular–nocturnal (*M. nigriceps*) and the nocturnal (*M. pyriformis*) ant (Fig. 2). Variable primary pigment cell pupils have also been found in other ants capable of being active in dim light such as *Camponotus ligniperda*, *Camponotus irritans* (Menzi, 1987) and *Polyrhachis sokolova* (Narendra et al., 2013a). Similar to our findings in the strictly day-active *M. croslandi*, such a primary pigment pupil mechanism is absent in other day-active ants such as *Cataglyphis bicolor* (Brunnert and Wehner, 1973) and *Formica polyctena* (Menzel and Knaut, 1973). Do compound eye properties restrict *Myrmecia* ants to their respective temporal foraging niches? From what we have shown here, it would appear that this is not the case, with the exception of the day-active ant, *M. croslandi*, which is restricted by its narrow rhabdoms and relatively small lenses to forage only in bright-light conditions, unless it employs spatial and temporal summation (e.g. Klaus and Warrant, 2009; van Hateren, 1993; Warrant, 1999). The diurnal–crepuscular *M. tarsata*, the crepuscular *M. nigriceps* and the nocturnal *M. pyriformis* all have compound eye modifications that enable them to operate at low light levels, but in addition they possess pupillary mechanisms which allow them to adjust the sensitivity of their eyes to bright light (Fig. 2). When forced to, the nocturnal *M. pyriformis* can indeed navigate visually during the day (Narendra et al., 2013c). Vision is clearly not a factor that constrains crepuscular and nocturnal ants to their discrete temporal activity niches.

The mechanism enabling the changes in diameter of the primary pigment cell pupil in insects is still unclear, but it may lie within the crystalline cone itself. We found granules resembling glycogen vesicles in the cone tract (Fig. 7C,D) that have also been described in the crystalline cones of wasps and butterflies in the dark-adapted state (Ribi, 1978a,b). These vesicles are thought to be 'energy packets' which could possibly assist in bringing about the shape changes of the crystalline cone. In line with this is the presence of microtubuli in the cone tract of the eyes of *M. tarsata* (Fig. 7D,E) and the fact that the extensions of the four crystalline cone cells reach all the way to the basement membrane, as has been documented in the ant *C. ligniperda* (Menzi, 1987), the butterfly *Pieris rapae* (Ribi, 1978b) and the digger wasp *Sphex cognatus* (Ribi, 1978a). These mechanical

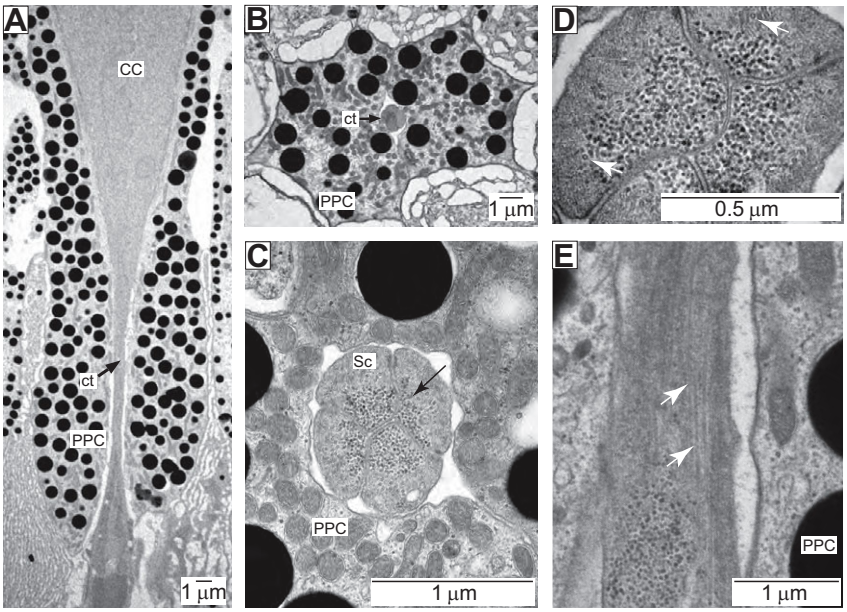

Fig. 7. Ultrastructure of the cone tract in the crepuscular *M. tarsata*. (A) Longitudinal section of the crystalline cone tract (ct). CC, crystalline cone; PPC, primary pigment cell. (B–D) Cross-section of the crystalline cone tract showing the four Semper cells (Sc) with primary pigment cells surrounding the tract. Glycogen vesicles are indicated by the black arrow and microtubuli by the white arrows. (E) Longitudinal section of the crystalline cone tract showing microtubuli (white arrows).

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anchors may be supporting shape changes of the crystalline cone (Walcott, 1975).

As in other insects, the radial migration of retinular cell screening pigments in *Myrmecia* ants is under ambient light control (Meyer-Rochow, 1999), while the opening and closing of the primary pigment cell pupil is in addition controlled by a circadian rhythm (reviewed by Fleissner and Fleissner, 2006). Radial migration of retinular cell pigment granules has been documented in the day-active desert ant *C. bicolor*, which was either light adapted or dark adapted for 18 h (Brunnert and Wehner, 1973). In the diurnal ant *F. polyctena*, the retinular cell screening pigment movement depends on the spectral sensitivity of each individual retinular cell (Menzel and Knaut, 1973). Under natural conditions, retinular cell pigments initially move radially towards the rhabdom during the morning twilight but at dawn they move away (1000–10,000 lx) and only once it is brighter (>10,000 lx) do the pigments move closer to the rhabdom. This biphasic radial migration of screening pigments is thought to be related to the chromatic light distribution in the sky during sunrise, which causes a decrease in the concentration of unbleached photopigments (Menzel and Knaut, 1973).

Regarding the primary pigment cell pupil, our results indicate that there are subtle variations in the extent to which dynamic adjustments are driven by ambient light conditions and by a circadian rhythm (Fleissner and Fleissner, 2006; Ventura et al., 1976). It appears that light adaptation through the primary pigment pupil can occur at any time of the day, with light intensity overriding endogenous rhythm: the crystalline cone tract forms within 10–15 min of light exposure (this study and Menzi, 1987). However, for dark adaptation, it is evident from both *M. pyriformis* (this study) and *Camponotus* (Menzi, 1987) that the circadian rhythm constrains the range of pupil action: if eyes are dark adapted for 10 min during the day, the primary pigment cell pupil does not open fully (Menzi, 1987). The full opening of the primary pigment cell pupil occurs only at night and in dark conditions (Figs 4 and 5; Menzi, 1987).

As such, the adaptation mechanisms in the compound eyes of ants are similar to those described in beetles, in the common backswimmer *Notonecta* and in the giant water bug *Lethocerus* (Ro and Nilsson, 1993a, 1994, 1995; Walcott, 1971): light adaptation via the primary pigment cell pupil is slow (in the range of tens of minutes) against the 'predictive nature' of a circadian rhythm, while retinular cell pigment movements respond to changes in ambient light intensity within seconds (e.g. Bernard and Wehner, 1980; Jonson and Nilsson, 1994; Kirschfeld and Franceschini, 1969; Stavenga and Kuiper, 1977).

The primary pigment cell pupil with its range of diameters between 0.5 and 5 µm in crepuscular and night-active ants decreases or increases light flux to the rhabdom about 100-fold (because the number of photons absorbed by a rhabdom is proportional to the square of aperture diameter; Warrant, 2004), and this may be needed to protect the large rhabdoms from too much light. However, in addition, light flux is regulated by retinular cell pigment movement and it is interesting to note that the mechanism by which retinular cell pigments control light absorption in rhabdoms differs in day-active and night-active ants. The large diameter rhabdoms in crepuscular and nocturnal ants (>5 µm in *M. nigriceps* and *M. pyriformis*; Greiner et al., 2007) function as light guides, with light contained within the rhabdom by internal reflection. In these ants, the pigment movement towards the rhabdom in the light-adapted state decreases the refractive index difference between the rhabdom and cytoplasm and therefore reduces reflection at the boundary (meaning that light leaves the light guide; see Kirschfeld and Franceschini, 1969). In the dark-adapted state, pigments move away from the rhabdom, thus creating a high refractive index–low refractive index rhabdom–palisade interface that increases internal reflection. In contrast, small diameter rhabdoms (1.3 µm in *M. croslandi* and 2.9 µm in *M. tarsata*; Greiner et al., 2007) function as wave guides in which a large fraction of light travels outside the rhabdom and can be absorbed by retinular cell screening pigments (e.g. Stavenga, 2004a,b). The very different dynamics of these light adaptation processes, the 'field-stop' primary pigment pupil and the radial migration of retinular cell pigments, may reflect the fact that the range over which fast changes of ambient light intensity occur and drive retinular cell pigment movements is much smaller than slow diurnal changes that are compensated for by the primary pigment cell pupil. A comparative study of the dynamical properties of adaptive processes in these ants would require the use of a non-invasive, *in vivo* optical method such as infrared reflectometry (Ro and Nilsson, 1993b).

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Competing interests
The authors declare no competing or financial interests.

Author contributions
A.N., W.A.R. and J.Z. conceptualized the study; A.N., B.G. and W.A.R. performed the experiments; A.N. carried out data analysis and wrote the first draft of the manuscript; A.N., W.A.R. and J.Z. revised the manuscript.

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