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Compound eyes of *Camponotus compressus* (Fabricius, 1798) (Hymenoptera, Formicidae) reflects caste specific organisation and adaptation

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ABSTRACT: Caste polymorphs of the ant *Camponotus compressus* (Fabricius, 1798) are distinct by their morphology and life styles; the two distinct castes are largely nocturnal and rely on their visual sensory system to interpret their temporal niches. The compound eyes of the castes were explored through Light and Scanning Electron Microscopy (SEM) in order to delineate cast specific organisation and adaptations of the compound eye. The findings reveal that major workers of *C. compressus* possess a more sophisticated visual system in terms of its morphological features along with optical properties that enhances a better vision, which includes a greater number of ommatidia and high ommatidial density, a higher ommatidial diameter, low inter ommatidial angle and a more efficient pupillary mechanism to counter conditions where ambient light levels are high. This underlines the dependence of scouts (major workers) on visual system and the foragers on olfactory system in the species. © 2024 Association for Advancement of Entomology

KEY WORDS: Ommatidia, caste polymorphs, screening pigments, ommatidial angle

INTRODUCTION

Sensory systems of animals enable them to perceive vital sensory information from their surroundings- a process which is crucial for the success and survival of animals (Rössler, 2023). Social insects - particularly ants offer suitable and amenable systems to address questions about successful sensory adaptations and behaviours in diverse niches (Hölldobler and Wilson 1990). Finding mates, foraging, ovipositing, defending or communicating between conspecifics are notable behaviours of ants that are predominantly mediated by visual cues (Schwarz *et al*., 2011). Features of ommatidia constitute the fundamental design of the compound eye and determine their visual capabilities (Hunt *et al.*, 2018; Narendra *et al*., 2016b). Nocturnal, diurnal and crepuscular ant species have compound eye designs that match their lifestyle and behaviours (Greiner *et al*., 2007; Narendra *et al*., 2016a). Evolutionary, behavioural and ecological aspects of each ant species are reflected in the features of their compound eyes (Klotz *et al*., 1992; Knaden and Graham, 2016).

Camponotus compressus (Fabricius, 1798) (Hymenoptera, Formicidae) is common in evergreen forests, sholas, deciduous forests and plantations and are largely nocturnal. Like other ant societies caste-based division of labour is evident among individuals of these ants. Major (scouts) and minor (foragers) are the prominent caste polymorphs of

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the colony with characteristic morphological features and sizes. Though medium castes are also present in the society they are less in number. Major worker castes are the scouts of the colony and minor workers are foragers. It will be interesting to understand how the compound eyes are organised in the castes of *C. compressus* especially in a context when behaviour of the castes is notably different. Behaviour is driven by visual cues in these ants. In this study the morphological features of compound eyes of major (foragers) and minor (nurses) castes of *C. compressus* were examined to decipher caste specific designs. Castes of this ant species are largely nocturnal and are found to be active in similar temporal niches. Major workers are main defenders of the colony and are involved in foraging with the minors also (Mysore *et al.,* 2009). Foraging and defence are behaviours that put different levels of demands on the sensory systems especially the visual system. This hypothesis is tested by analysing the important optical features of the compound eye of the major workers (scouts) and minor workers (foragers) castes and the distribution pattern of the screening pigments as a reflection of the visual adaptation mechanism within their compound eyes.

MATERIALS AND METHODS

Major and minor castes of *C. compressus* were collected from the colonies found in the campus of St Berchmans College, Changanasserry Kerala (9.2702°N; 76.3219°E). Caste polymorphs are distinct by their size. Though queen, major workers media workers and minor workers are present in the colony, selected only major and minor workers. Major workers are larger (14–18mm) than minor workers (6–8mm). Majority of the major workers are foragers. Minor workers are nurses and forage occasionally. They were collected by forceps into a plastic bottle and brought to the laboratory. These ants were cold anesthetized by keeping in the refrigerator for the study.

Haematoxylin and Eosin staining: Heads of different castes of *C. compressus* were decapitated using a razor blade and were fixed in paraformaldehyde (4%) at room temperature in the morning (Decapitated heads of the castes were fixed in the morning and further processed to understand the distribution of the screening pigments in daylight conditions). Heads were then passed through ascending concentrations of alcohol grades (from 35% - 50% - 70%- 80% - 90% - 100%; 15 minutes each in each grade) and cleared in Xylene. The tissues were embedded in paraffin and then were taken onto a rotary microtome to take sections of 5 micrometre thickness. Sections were then stained in hemotoxylin for 3-5 minutes. The sections were then washed in running tap water until sections turned bluish by keeping it for 5 minutes or less and differentiated in 1per cent acid alcohol (1% Hl in 70% alcohol) for 5 minutes; Then washed in running tap water until the sections were again blue by dipping in an alkaline solution followed by tap water wash. Then they were stained in Eosin Y for 10 minutes and washed in tap water for 1-5 minutes and then dehydrated in increasing concentrations of alcohol and cleared in Xylene and mounted on a micro glass slide using DPX for observation under a microscope.

Compound eye replica: The ant was mounted on an insect pin and then the colourless nail polish (Lakme) was applied uniformly as a thick film over the ant eye by placing a small drop of the fast-drying colourless nail polish and quickly allowing it to spread over the eye. The nail polish was out brushed throughout the compound eye area to get a full replica of the compound eye once it is peeled off as a thin layer from the compound eyes. Once it was fully set at the room temperature, a fine insect pin was used to gently lift the replica from the head capsule surrounding the eye. A fine pair of clean forceps was used to lift the replica. The replica was placed on a glass slide for observation. A razor blade was used to trim the replica by carefully removing excess material around the eye. A needle or a pair of forceps was used to prevent the replica from moving. A cover slip was placed gently on the eye replica and sealed the cover slip using very little nail polish on four corners. The slide was imaged on a compound microscope.

Scanning Electron Microscopy (SEM): Heads of the ants were decapitated using a razor blade and fixed with paraformaldehyde (4%) at 40^0C overnight. On the next day, heads were washed with phosphate buffered saline (PBS) and dehydrated in ascending series of ethanol. The dehydrated heads were then mounted on a stub having a double adhesive carbon tape with the help of a stereo microscope. Heads were coated with gold for three minutes with the help of a sputter coater (Q150RES, quorum Tech). Coated heads were imaged under SEM/ Energy Dispersive Spectrometer (Jeol JS-6390LV/JED-2300) to study the detailed morphological features of the compound eyes of the major and minor workers. All the images obtained were exported to Image J to calculate the various parameters of the eye.

Morphometry: The length (Cl) and width (Cw) of the compound eyes and the thorax length (Thl) of the major and minor castes of *C. compressus* were measured from (the compound eye replicas obtained after applying nail polish). After drying, the polish was removed from their eyes and photographed and observed under light microscope (Magnus MLX) equipped with a digital camera (CatCam E-Series). Images were then digitized in a computer for quantification of the total number of ommatidia (TO), which was obtained from a direct count on a lateral view of the eye. The total surface area of the compound eye (A, µm^2) was calculated using the formula for the area A of an ellipse with length Cl and width Cw (Moser *et al.,* 2004). All measurements in micrometers (µm).

$$A = \pi \left[\frac{Cl}{2} \times \frac{Cw}{2} \right]$$

Yilmaz *et al.* (2014) procedure was followed for obtaining all the optical parameters of the compound eyes; length Cl and width Cw of the compound eye were measured using the Image J software from the images; mean inter ommatidial angle (Õ), which describes the cornea sampling density, mean ommatidial diameter (D) (ìm), which provides a measure of the sensitivity to light (Land, 1997). Ramirez-Esquivel *et al*. (2017) method was adopted to calculate the inter ommatidial angle (Õ). The mean inter ommatidial angle was obtained for each ant (after repeating three times this calculation, in different areas of the compound eye that were chosen randomly). The ommatidial diameter was measured by drawing and measuring a line going through a row of 5–10 ommatidia in the horizontal or the vertical plane, and dividing that length by the number of ommatidia crossed. The mean ommatidial diameter was obtained for each ant (after repeating three times this calculation, in different areas of the compound eye that was chosen randomly). The eye parameter, P gives the relationship between the sensitivity and resolution of the eye. It was calculated as,

$$P = D.\Delta\varphi$$

Where D is the mean ommatidial diameter and “ `” is the mean inter ommatidial angle

Morphological data between two groups of castes were compared using a student’s unpaired t test with 0.001 as the significance level. At least five samples were used for statistical analysis. Microsoft Excel was used for calculations.

RESULTS AND DISCUSSION

Morphometrics:

Compound eyes of *C. compressus* were elliptical in shape (Fig.1). Important parameters of the eye which are crucial for optics and visual perception, varied significantly between the major and minor worker ants. Compound eyes of the two castes were at varying distances with a significant difference in the number of ommatidia (Major: 900±; minors: 630±). Noticed differences in the shape and size of ommatidia in different areas of the compound eye of both the castes (Figs. 3a-e, 4 a-f). Mean ommatidial diameter and total surface area of the compound eye showed significant variation between major workers and minors. The number of ommatidia per unit area (µm^2) in both the castes varied significantly with the major workers having a significantly high density of ommatidia (399 ommatidial facets/ µm^2). Mean inter ommatidial angles were also found to be less in the major eye in contrast to the minor workers. Ommatidial angles between the ommatidial facets are reliable indices of the resolution of the compound eye. Major worker with a lesser number of ommatidial angle is likely to possess a between the castes (Table 1). Interestingly the major workers had a high parameter P value relatively high-resolution power of compound eyes. The eye parameter P also varied significantly.

Table 1. External parameters of the compound eye of the workers of *Camponotus compressus*

Workers	Cl (μm)	D (μm)	" `" = angle(^{0})	Cw (μm)	Between the eyes (μm)	TO	A (μm^2)	P (μm.rad)
Minor	21.875	16.75±1.8	5.168	1773.3	1506±2.46	630±0.3	197317 313OF	0.28±.83
Major	23.6	21.5±.2.9*	3.71*	5260	3120*± 1.8	900±0.6*	359868* 399OF*	1.38±.76*

Cl = Length of facet; D = Ommatidial diameter; " `" = Inter ommatidial angle; Cw = Head width; A = TSA of Eye; TO = Ommatidia no. P = Eye parameter; OF = ommatidial facets; mean ± standard deviation; *significant at P= 0.001

Histological studies:

Screening pigments (pigments inside the primary pigment cells and retinula cells) play a crucial role in the pupillary mechanism of the compound eyes of insects. Contrasting results in the distribution of screening pigments in major and minor castes were noted which convey a different mode of sensory adaptation. Major workers had a dense distribution of the pigments in the junction of the crystalline cone and rhabdom area in daylight conditions (Fig 4a), whereas the minor workers had a feeble distribution of the pigments (Fig 4b). Pupillary mechanism is an important adaptation to counter the photons the compound eye encounter when ambient light is more. Being nocturnal ants, they are supposed to possess adaptive measures to counter light abundance in the niches.

Major and minor castes of *C. compressus* were distinct by external features and behaviours, though they have the same genotype. The study indicated that external features that influence optic properties of compound eyes differ significantly between major and minor castes. Major and minor castes of *C.compressus* pereceive light differentially which is evident in their morphological features of compound eyes. Castes of *C. compressus* rely on a a spectrum of olfactory and visual sensory sensory cues in accordance with their contrasting life styles and tasks they are engaged in. The findings indicated that differences in the external and internal features of compound eyes of the castes correlate with their lifestyles. Major workers who are the scouts seems to rely on the visual system more compared to the minor workers, but in such a way that they seem to compromise on some aspects of the visual perception for example the parameter P value of their compound eyes. The study revealed that compound eyes of minor castes were less sophisticated in external visual features compared to major workers. This was evident in the lesser number of ommatidia count they possess, lesser surface area of compound eye with less number of ommatidia per unit area of the compound eye and small ommatidial facet diameter and variations in other crucial parameters inter ommatidial angle and parameter P that are important factors which influence optical properties of the compound eyes (Table 1). A compound eye with less surface area and less number of ommatidial facets is likely to be less efficient in some important aspect of visual perception. Compound eyes with a greater count of ommatidia obviously has more number of lenses for the capture of photons. Ommatidial angles between the ommatidial facets are reliable indices of the resolution capacity of the compound eye. Minor workers with a higher ommatidial angle seemed to have a relatively poor resolution power of compound eyes and their visual capability was further hindered by a lesser number of ommatidia. However it is interesting to note that minor worker posses a low P value which is suggestive of its better optical sensitivity

Further analysis of compound eyes of castes

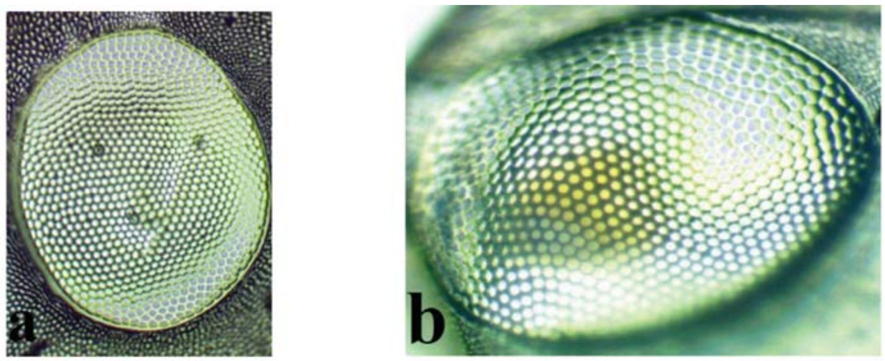

Fig. 1 Light microscopic images of replicas of compound eye of – a) major; b) minor worker of *Camponotus compressus*

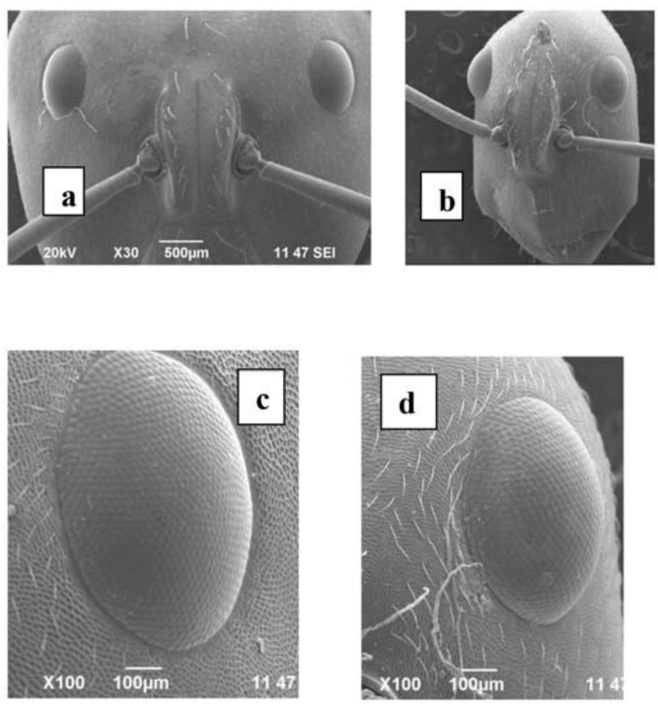

Fig. 2 a-d. SEM images of Compound eyes - a, c major worker; b, d - minor worker

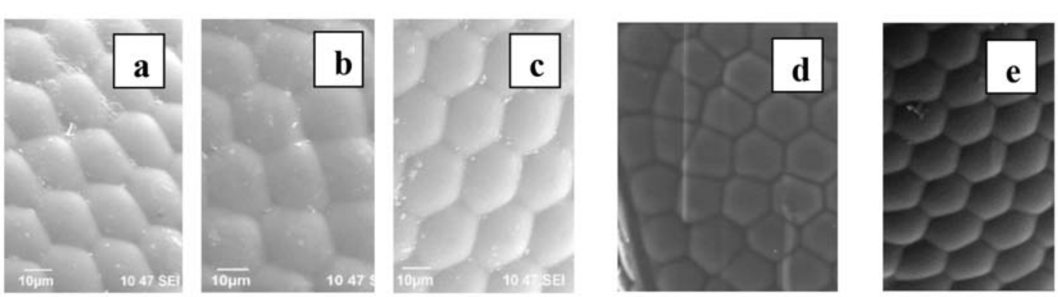

Fig. 3 a) SEM images of different regions of the compound eyes of major workers, showing the differences in the shape of ommatidia at different regions. a-c) middle area, d-e) dorsolateral

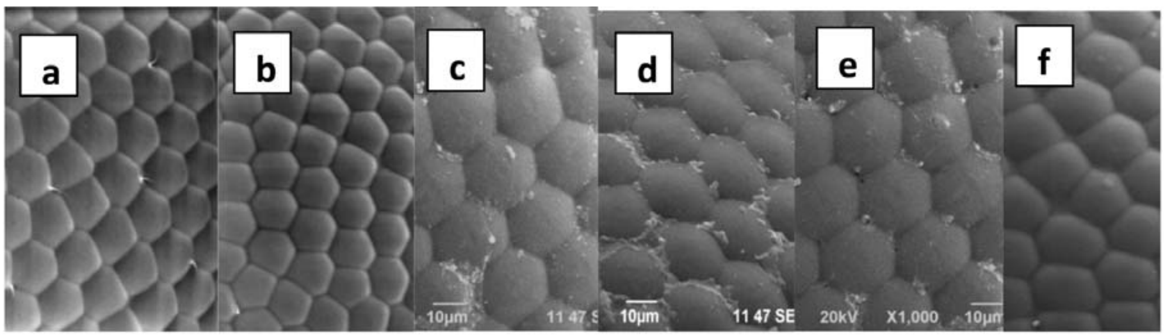

Fig. 4 a –f. SEM images of different regions of the compound eyes of minor workers showing the differences in the ommatidia shape. a-b) central area, c-f) dorsolateral area

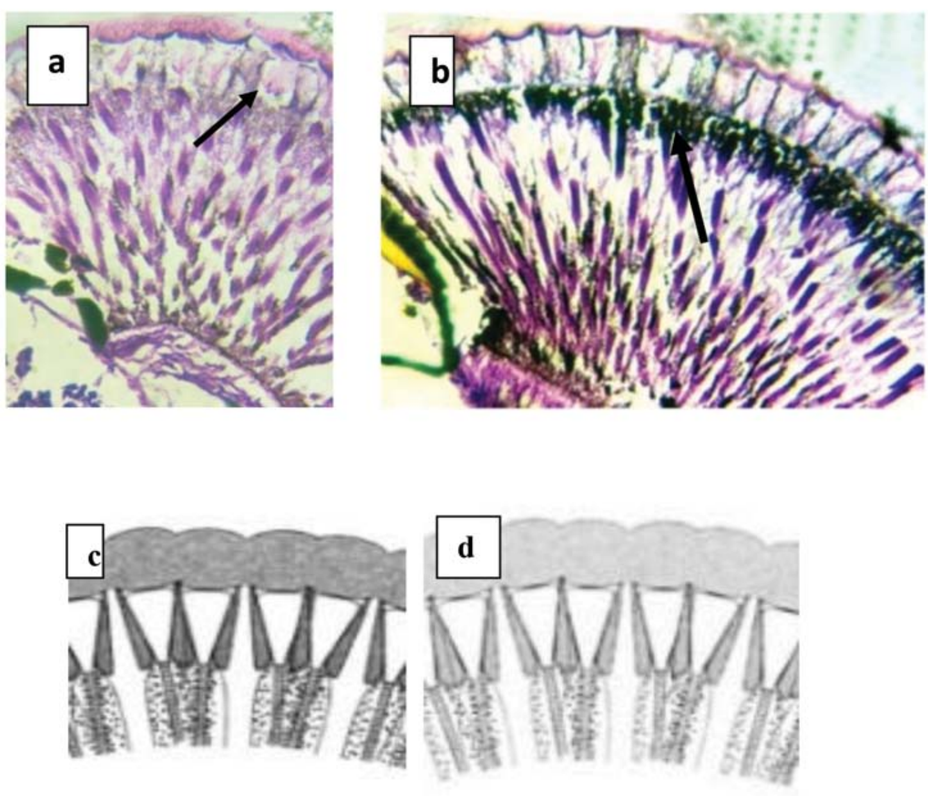

Fig. 4a-d Haemotoxylin and Eosin-stained sections of the compound eyes of (a) major workers, (c) minor workers, (b, d) schematic diagrams of the same sections, the white oval dot indicates the crystalline cone area of the compound eye. Black arrow indicates the screening pigments distribution. Note the difference in the pattern of distribution between the major (a) and minor (b) and schematically represented (c) and (d) indicated contrastive visual adaptive mechanisms between major and minor workers of *C. compressus*. Visual screening pigments were differentially distributed in the compound eyes of castes; Major castes exemplify radial distribution of visual screening pigments towards rhabdoms in daylight conditions, which seemed to be a typical light adaptation pattern observed in nocturnal ants (where a dense population of screening pigments migrate towards rhabdoms during daylight conditions a phenomenon what is known as pupillary mechanism). The compound eyes of the major workers showed a dense and clustered pattern within the eye at the time of daylight conditions when they were fixed. However a different pattern of distribution was observed in minor castes where primary pigments were diffuse and lesss dense with scarce distribution. Pupillary mechanism is an effective adaptation in insect apposition eyes where primary pigments through the enveloping mechanism cover the rhabdoms and effectively protected the light reception through each lens in ambient conditions, where light is abundant. The findings suggested that minor workers were with comparatively poor pupillary mechanism consistent with lesser amount of time they were active in daylight conditions. Further they were more reliant on the olfactory cues for the foraging duties. Minor workers relied predominantly on olfactory sensory cues for meeting their foraging tasks. Peripheral component of olfactory system, antennal lobes and mushroom body areas of the brain concerned with olfactory sensory processing and perception were found more prominently organised in minor worker castes of *C. compressus* than in majors (Mysore *et al*., 2009) Quite interestingly this a pattern which contradicted the general pattern observed in many studies, where the large sized individuals like the major workers of many ant societies have a more elaborate organisation of sensory systems (Babu *et al*., 2011). The findings are in line with the general trend in that large individuals like major workers possess a more sophisticated compound eye. Contrasting designs of olfactory and visual sensory systems of major and minor castes makes *C. compressus* an interesting insect model system. Minor castes who seem to be more competent in perceiving chemosensory cues possess a more elaborate olfactory system and associated brain areas. However, the study highlights the more superior visual system of major workers.

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