The Journal of Experimental Biology 212, 2448-2453
Published by The Company of Biologists 2009
doi:10.1242/jeb.031484

Resolution and sensitivity of the eyes of the Asian honeybees *Apis florea*, *Apis cerana* and *Apis dorsata*

Hema Somanathan[1,2,*], Eric J. Warrant[1], Renee M. Borges[2], Rita Wallén[1] and Almut Kelber[1]

[1]Department of Cell and Organism Biology–Zoology, Lund University, 22363 Lund, Sweden and [2]Centre for Ecological Sciences, Indian Institute of Science, Bangalore 560 012, India

*Author for correspondence (e-mail: hema.somanathan@cob.lu.se)

Accepted 10 May 2009

SUMMARY

Bees of the genus *Apis* are important foragers of nectar and pollen resources. Although the European honeybee, *Apis mellifera*, has been well studied with respect to its sensory abilities, learning behaviour and role as pollinators, much less is known about the other *Apis* species. We studied the anatomical spatial resolution and absolute sensitivity of the eyes of three sympatric species of Asian honeybees, *Apis cerana*, *Apis florea* and *Apis dorsata* and compared them with the eyes of *A. mellifera*. Of these four species, the giant honeybee *A. dorsata* (which forages during moonlit nights) has the lowest spatial resolution and the most sensitive eyes, followed by *A. mellifera*, *A. cerana* and the dwarf honeybee, *A. florea* (which has the smallest acceptance angles and the least sensitive eyes). Moreover, unlike the strictly diurnal *A. cerana* and *A. florea*, *A. dorsata* possess large ocelli, a feature that it shares with all dim-light bees. However, the eyes of the facultatively nocturnal *A. dorsata* are much less sensitive than those of known obligately nocturnal bees such as *Megalopta genalis* in Panama and *Xylocopa tranquebarica* in India. The differences in sensitivity between the eyes of *A. dorsata* and other strictly diurnal *Apis* species cannot alone explain why the former is able to fly, orient and forage at half-moon light levels. We assume that additional neuronal adaptations, as has been proposed for *A. mellifera*, *M. genalis* and *X. tranquebarica*, might exist in *A. dorsata*.

Key words: apposition compound eyes, ocelli, moonlight, nocturnal foraging, sensitivity, optics.

INTRODUCTION

The European honeybee, *Apis mellifera*, has been the object of considerable scientific curiosity and has served as the classical model organism in diverse fields such as behaviour, navigation, sensory biology, ecology and pollination biology for almost a century (Frisch, 1914; Frisch, 1927). *Apis mellifera* has eight congeners, all of which are Asian, and about which much less is known. Of the Asian honeybees, the cavity-nesting *Apis cerana* and the less well known species *Apis koshevnikovi*, *Apis nigrocincta* and *Apis nuluensis* are closely related to *A. mellifera* whereas the giant honeybees (*Apis dorsata*, *Apis dorsata binghami* and *Apis laboriosa*) and the dwarf honeybees (*Apis florea* and *Apis andreniformis*) form separate groups (Arias and Sheppard, 2005; Raffiudin and Crozier, 2007). Although *A. mellifera* is found in Africa, the Urals and Central Asia, it is allopatric to the south and south-east Asian species, many of which are sympatric in several tropical areas of Asia. For instance, in the Western Ghats in India, where we studied them, the most common *Apis* species, *A. cerana*, *A. florea* and *A. dorsata* co-occur.

All species of *Apis* are eusocial, building large nests with thousands of worker bees that collect nectar and pollen. Worker bees collect these resources from a large variety of flower species and can fly several kilometres between the hive and good nectar or pollen sources. From studies on *A. mellifera*, it is known that navigation and orientation as well as the detection and discrimination of suitable floral resources depend to a great deal on vision (Lehrer, 1998; Chittka and Spaethe, 2007). Like the vast majority of bees, *A. florea* and *A. cerana*, as well as the European *A. mellifera ligustica*, are restricted to foraging during the day. However, both the African honeybee, *A. mellifera adansonii*, and the Asian giant honeybee, *A. dorsata*, are reported to occasionally forage at night (Fletcher, 1978; Dyer, 1985). In both these species, nocturnal foraging has only been observed on moonlit nights, when the moon is at least half full, when there is no cloud cover and when no vegetation obscures this light source. By contrast, *A. cerana* is restricted to foraging during the day although it is able to fly at much lower temperatures (down to 10.5°C) than *A. dorsata* (Oldroyd et al., 1992).

Crepuscular or nocturnal foraging activity – requiring flight in rather dim light – has been reported from several genera in four families of the Apoidea (for a review, see Kelber et al., 2006), ranging from the minute *Perdita bequaertiana* to the large *Xylocopa tranquebarica* (Burgett and Sukumalanand, 2000; Somanathan et al., 2008). These bees, like all bees, possess apposition compound eyes. The transition to a dim-light environment is an apparent disadvantage to animals with apposition eyes because these eyes tend to have a very low absolute sensitivity, due to the small diameter of their facet lenses (see Warrant et al., 2004; Kelber et al., 2006; Somanathan et al., 2008). However, several reasons may have made this transition to a dim-light environment favourable for at least some species (see Wcislo et al., 2004). The matinal bees *Xenoglossa* and *Peponapis*, for instance, use nectar- and pollen-rich resources such as *Cucurbita* before other and often larger competitors take flight (Ordway et al., 1987). Species that fly during dusk, such as *Perdita* (*Xerophasma*) and *Lasioglossum* (*Sphecodogastra*), use equally rich pollen sources. These include the flowers of *Oenothera* spp., which these bees visit just after they open in the evening, and before the arrival of their obligate pollinators, the large hawkmoths (Kelber et al., 2006).

The only species of bee known to fly all night is the Indian carpenter bee *X. tranquebarica*, which seems to avoid competition

with several equally large and much more abundant sympatric congeners such as *Xylocopa tenuiscapa* (Somanathan and Borges, 2001; Somanathan et al., 2008). The latter facultatively nocturnal species, however, has also been found to collect pollen from a nocturnally flowering tree, *Heterophragma quadriloculare*, and is able to fly in dim light to do so. However, unlike *X. tranquebarica*, *X. tenuiscapa* can only fly on full-moon nights. Accordingly, Somanathan et al. found that the eyes of this species lack some adaptations for nocturnal vision that are found in *X. tranquebarica* (Somanathan et al., 2009). We expected that *A. dorsata* and *A. m. adansonii* may be similar cases because they extend foraging activity into the night if at least a half-moon is present in the sky (Fletcher, 1978) and if favourable food sources can be exploited at such times.

While Kerfoot (Kerfoot, 1967) has recognised that nocturnal bees have unusually large ocelli, the adaptations of the eyes and the visual system for a nocturnal lifestyle have only recently been the focus of research. Warrant et al. (Warrant et al., 2004) gave a detailed report of the visual activity of the halictid bee *Megalopta genalis*, and Somanathan et al. (Somanathan et al., 2009) have described the eyes of *X. tranquebarica* and compared them with the eyes of sympatric diurnal congeners. Common features of nocturnal bees – and other nocturnal hymenopterans – are their huge ocelli and relatively large eyes with reasonably large facets and, most importantly, their unusually wide rhabdoms (Kerfoot, 1967; Menzi, 1987; Greiner et al., 2004a; Warrant et al., 2004; Greiner, 2006; Kelber et al., 2006; Greiner et al., 2007; Somanathan et al., 2009) (for a review, see Warrant, 2008).

To better understand the importance of visual sensitivity and resolution for the feeding ecology of the Asian species of *Apis*, we have undertaken a study of the eyes and ocelli of the three most common species of Asian honeybees: *A. dorsata*, *A. cerana* and *A. florea*, with the goal of comparing their visual systems with those of the well-studied European honeybee, *A. mellifera.* Our expectation was that *A. dorsata* would possess visual adaptations that increase the sensitivity of their eyes, enabling flights on moonlit nights whereas the strictly diurnal *A. cerana* and *A. florea* would possess less sensitive eyes.

MATERIALS AND METHODS

Field site and foraging activity

We observed and obtained individuals of *Apis dorsata* Fabricius 1793, *Apis cerana* Fabricius 1793 and *Apis florea* Fabricius 1793, from areas adjoining the Bhimashankar Wildlife Sanctuary in the Pune district of Maharashtra, India (19°21′–19°11′N, 73°31′–73°37′E). During dry season flowering from November 2007 to April 2008, we observed flowers of 71 plant species in this plant community for honeybee visitation. Over several days, the timing of honeybee visits was observed for a single day for each plant species from 06:00 h to 11:00 h and from 15:00 h to 21:00 h. Additionally, we also made anecdotal observations of flight and foraging outside these windows of time. From these data, we obtained the earliest and latest daily foraging times for the three *Apis* species. Ambient light intensities measured on several mornings and evenings in different areas of the study site with an International Light IL 1700 radiometer (Peabody, MS, USA) have been reported by us in an earlier paper [see fig. 1 in Somanathan et al. (Somanathan et al., 2008)]. Solar and lunar data were obtained from the NASA website (http://aa.usno.navy.mil/).

Eye and body measurements

The eyes of the bees were studied using standard methods described in detail elsewhere (e.g. Somanathan et al., 2009). Here, we give a short description of these methods. Measurements of body size, eye size and median ocellus size were made for workers of the three *Apis* species that were captured while visiting flowers. We used intertegular width, the distance between the wing bases, as an estimate of body size (e.g. Spaethe and Chittka, 2003; Somanathan et al., 2008). The number of ommatidia was determined using corneal nail polish replicas, following the methods adopted by Praagh (Praagh et al., 1980). Ommatidial diameters were determined in the fronto–ventral region of the eye where they were found to be the largest in other bees (Ribi et al., 1989; Greiner et al., 2004a; Kelber et al., 2006). Ocellar and ommatidial diameters were measured using light microscopy and scanning electron microscope (SEM) photographs.

Histological procedures

The following histological procedures were performed to measure rhabdom diameters and lengths for the three species of *Apis*. After anaesthetising and decapitating bees, whole eyes were dissected and placed in fixative (2% glutaraldehyde, 2% paraformaldehyde, 2% sucrose in 0.15 mol l^{-1} sodium cacodylate buffer) for 12–24 h. After rinsing repeatedly in buffer, the eyes were fixed in 1% osmium tetraoxide for one hour and embedded in epoxy resin. Longitudinal sections 1 μm thick were placed on a slide, dried on a hot-plate, stained with toluidine blue and photographed under a microscope. Ultrathin transverse sections were stained with lead citrate and uranyl acetate and studied under a JEOL 1240 Transmission Electron Microscope (Tokyo, Japan).

Focal length measurements

The focal lengths of compound eyes have been measured in many studies and the procedure has been described elsewhere in detail (Greiner et al., 2004a). Here, we provide a brief description of the methods involved. A small piece of cornea (containing 100–200 facets) was cut from the surface of the eye in the fronto–ventral region, placed in a Petri dish of saline, cleaned to remove pigments and tissues and placed external side outwards in a tiny drop of physiological saline (refractive index=1.34) in the centre of a cover slip. An o-ring was waxed to a microscope glass slide, after which the upper surface of the o-ring was lightly greased with petroleum jelly. The cover slip was then turned upside down and placed onto the greased o-ring thus creating an air-tight chamber containing the saline drop and the downward-pointing piece of cornea. The slide was mounted on the stage of a conventional light microscope (Leica, Wetzlar, Germany) with the condenser removed. A single facet lens was chosen for focal length measurements. Objects of known size (typically patterns of dark stripes on translucent tracing paper) were placed on the foot of the microscope, over the lamp aperture. Images of these objects were focused by the facet lens within the saline drop. The images were viewed with the ×40 objective lens and photographed with a digital camera fitted to the microscope. The focal length f of each facet lens was calculated according to the following equation:

$$f = s_o \frac{\lambda_i}{\lambda_o}, \quad (1)$$

where s_o is the distance between the striped object and the lens (127 mm), λ_o is the spatial wavelength of the striped pattern (the distance between the centre of one stripe and the centre of the next: 4.53 mm) and λ_i is the spatial wavelength of the image of the striped pattern (mm).

Calculation of acceptance angles and optical sensitivity

The spatial resolution of eyes is largely limited by the acceptance angle (half width of the photoreceptor's receptive field, in radians)

of each ommatidium (Warrant and McIntyre, 1993). For apposition compound eyes, the acceptance angle $\Delta\rho$ can be approximated by the ratio of the rhabdom diameter d and the focal length of the ommatidium f: $\Delta\rho \approx d/f$ (Stavenga, 2003; Frederiksen and Warrant, 2008).

The optical sensitivity of an eye to an extended source of broad-spectrum light, S (expressed in units of $\mu m^2 sr$), can be approximated by (Kirschfeld, 1974; Land, 1981; Warrant and Nilsson, 1998):

$$S = \left(\frac{\pi}{4}\right)^2 D^2 \Delta\rho^2 \left(\frac{kl}{2.3 + kl}\right), \quad (2)$$

where, in an apposition eye, D is the maximum ommatidial diameter, l the length of the rhabdom and k is the peak absorption coefficient of the visual pigment (taken as $0.0067\,\mu m^{-1}$) (see Warrant et al., 2004). This equation predicts that good sensitivity to an extended scene results from a facet of large area ($\pi D^2/4$) as well as photoreceptors that each view a large solid angle of visual space ($\pi\Delta\rho^2/4$ steradians) and are long enough to absorb a substantial fraction of the incident light [$kl/(2.3+kl)$].

RESULTS AND DISCUSSION

Foraging activity in the three Asian *Apis* species

Observations of activity are summarised in Fig. 1. *Apis cerana* usually foraged earliest of all three species, confirming observations on this species by Oldroyd et al. (Oldroyd et al., 1992) in Thailand. The onset and offset of the foraging periods did not have constant relations to sunset and sunrise times, and thus light intensity, indicating a dependence of flight activity on temperature. In the warmer months (March and April), all three species started foraging around or even before sunrise. The minimum temperatures at night during the flowering season can be as low as 2°C in this site (range 2–14°C) (Somanathan and Borges, 2001). During this study, we recorded early morning temperatures between 07:00 h and 08:30 h that ranged between 16°C and 23°C during the flowering period. Nocturnal foraging by *A. dorsata* was observed on three occasions, during February and April. All three observations were made during the first few nights after full moon, when more than 70% of the moon was visible. In February 2008, *A. dorsata* were observed visiting flowers of the supra-annual mass-flowering species *Memecylon umbellatum* (Melastomataceae). These flowers open between 13:00 h and 19:00 h and *A. dorsata* were observed visiting them on a bright night, one day after full moon. Again in April 2008, we observed *A. dorsata* visiting flowers of *Bridelia retusa* (Euphorbiaceae) and *Randia dumetorum* (Rubiaceae), during the hours after midnight. These observations confirm earlier reports (Dyer, 1985; Kirchner and Dreller, 1993; Rao et al., 2001) that the species forages on moonlit nights. Like the cavity-nesting species *A. cerana* and *A. mellifera* but unlike *A. florea*, *A. dorsata* adds an acoustic component to its nocturnal dances, wherein length of sound signal was also correlated with distance to the food source (Kirchner and Dreller, 1993; Dreller and Kirchner, 1994). Because *A. dorsata* communicate on open combs, the acoustic component is thought to be correlated with regular nocturnal activity.

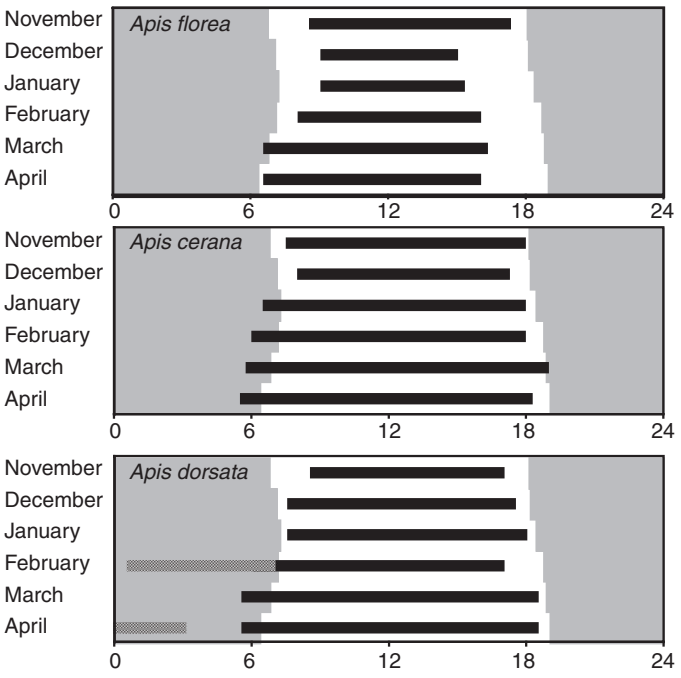

Fig. 1. Foraging activity of three Asian species of *Apis* at the field site in the Indian Western Ghats. Black bars indicate regular feeding periods, hatched bars mark the nocturnal observation of *Apis dorsata*, all on moonlit nights. Light grey shading indicates the time between sunset and sunrise on the 15th of each month, at the field site. Nocturnal observations of *A. dorsata* (hatched bars) were made on three occasions during nights one and four days after full moon, in February and April 2008. The mean and s.d. of monthly daytime temperatures (°C) from November 2007 to April 2008 were 22±2.12, 20.2±0.79, 16.4±2.06, 17.3±1.77, 19.6±2.05 and 23.0±1.84, respectively.

Eye–body size relationship and external eye anatomy

As indicated by their names, the dwarf honeybee *A. florea* and the giant honeybee *A. dorsata* differ in body size from the other two, similarly-sized species *A. mellifera* and *A. cerana* (Table 1). Dorso–ventral eye length increases linearly with body size but the scaling factor is considerably smaller than 1 (0.68) whereas the tongue length, as another example, increases with a scaling factor higher than 1 (1.7).

The eyes of all species of *Apis* are very similar in appearance: they are oval-shaped and have large numbers of sensory hairs between the ommatidia (see Fig. 2). These hairs differ in length between species: *A. florea* has the shortest (50 µm) and *A. cerana* the longest (125 µm) hairs, although even these hairs are shorter than those of *A. mellifera* (275 µm) (Neese, 1965).

As expected from their different body and eye sizes, the three study species differ with respect to ommatidial diameter and number of ommatidia (Table 1; Fig. 2D,F,H), both of which are parameters that are known to vary with body size (see Kelber et al., 2006). *Apis cerana* has a similar number of ommatidia compared with the similarly-sized *A. mellifera* (in the range of 5000), but it has smaller eyes and smaller ommatidia, indicating a lower sensitivity to light than its European congener. The ommatidia of *A. dorsata* are 29.5 µm in diameter and thus almost 10 µm wider than those of *A. florea*. The latter species also has fewer facets (<3500) than the former (>6300). A thicker cornea and longer crystalline cones in *A. dorsata* (Table 1) are possibly due to their larger eyes.

Acceptance angles in the compound eye

In most bees that have been studied so far, the highest spatial resolution has been found in the fronto–ventral eye region, where both interommatidial angles and the acceptance angles of each ommatidium are smallest (Warrant et al., 2004; Somanathan et al., 2009). In our study species, we estimated the acceptance angles in this eye region from the focal length and the rhabdom diameter and found similar results for *A. cerana* and *A. florea*, which have very

Table 1. Ecological data, body, ocellus and eye measurements, and the acceptance angles and optical sensitivities of single ommatidia, in four species of honeybees of genus *Apis*

	Symbol	*A. mellifera*	*A. cerana*	*A. florea*	*A. dorsata*
Ecological data					
Temperature range (°C)		>11[7]	>10.5[1]	18–43[1]	>15[1]
Light intensity		Half-moon in some subspecies[7]	Daylight	Daylight	Half-moon[8]
Body and eye measurements					
Intertegular width (mm)		3.2	3.0±0.05	2.2±0.07	3.9±0.2
Tongue length (mm)		5.8–7.1[3]	4.53±0.01[2]	3.41±0.02[2]	6.14±0.04[2]
Ocellus diameter (mm)		0.27[4]	0.26	0.24	0.38±0.02
Eye length (mm)		2.6[4]	2.1±0.1	1.7±0.05	2.8±0.1
Length of sensory hairs on the eyes (μm)		275[9]	125±14	50±6	100±7
Facet number per eye		5432[6] 4752[5]	5004	3484	6394
Max. ommatidial diameter (μm)	*D*	20[5]	24.5	19.5	29.5
Corneal thickness (μm)		27–38[4]	27-35	25	46
Crystalline cone length (μm)		55	50	36	55
Focal length (μm)	*f*	66[5]	84	79	104
Distal rhabdom diameter (μm)	*d*	2.0[5]	1.8	1.5	3.2
Acceptance angle (deg.)	$\Delta\rho$	1.7[5]	1.2	1.1	1.8
Rhabdom length (μm)	*l*	320[5]	270	190	260
Optical sensitivity to white light (μm^2 sr)	*S*	0.11[5]	0.07	0.03	0.21

Data that were not obtained in this study are from [1]Corlett, 2004; [2]Oldroyd et al., 1992; [3]Böttcher, 1977; [4]Ribi et al., 1989; [5]Greiner et al., 2004a; [6]Seidl and Kaiser, 1981; [7]Fletcher, 1978 on *A. m. adansonii*; [8]Dyer, 1985; [9]Neese, 1965. The acceptance angle $\Delta\rho$ was calculated from the ratio of the distal rhabdom diameter *d* to the focal length *f*, and optical sensitivity *S* was calculated using Eqn 2.

small acceptance angles of 1.2 deg. and 1.1 deg. (Table 1). This is in contrast to the larger *A. dorsata* with acceptance angles of 1.8 deg., similar to the European honeybee (1.7 deg.). We assume that, as with other diurnal bees (Greiner et al., 2004a; Somanathan et al., 2009), acceptance angles follow interommatidial angles in the two diurnal species and thus can be used as indicators for spatial resolution. By contrast, although *A. dorsata* has a considerably larger number of facets than both smaller bees, its larger acceptance angle indicates a lower spatial resolution, which mostly results from the large distal rhabdom diameter (Fig. 3). With a diameter of 3.2 μm, its rhabdom is clearly larger than that of all other congeners: 1.5 μm in *A. florea*, 1.8 μm in *A. cerana* and 2.0 μm in *A. mellifera* (Table 1). As a comparison, the halictid *M. genalis*, flying in the dim twilight of the Panamanian rainforest, has rhabdoms that are 8 μm wide (Warrant et al., 2004) whereas the obligately nocturnal *X. tranquebarica* has rhabdoms that are 6 μm wide (Somanathan et al., 2008). Both these species have correspondingly larger acceptance angles and lower spatial resolution (Warrant et al., 2004; Somanathan et al., 2008).

Sensitivity of the eyes to white light

The sensitivity of apposition compound eyes depends on three parameters: the facet diameter, the acceptance angle of each ommatidium and the length of the rhabdom (see Eqn 2). The rhabdom length, together with the absorption coefficient of the rhabdom *k* (assumed to be similar in all species), determines the

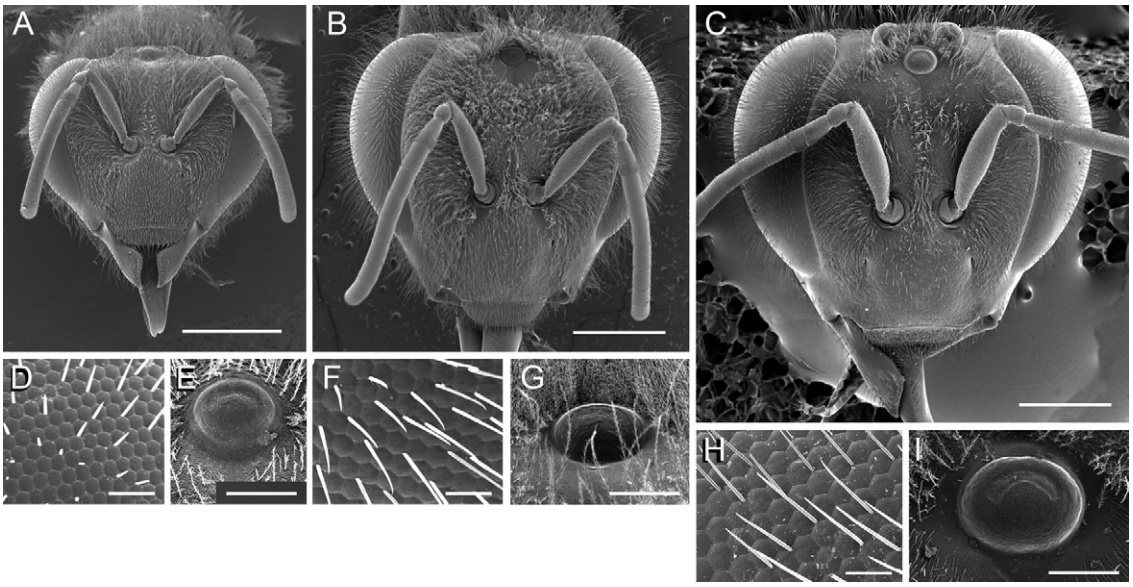

Fig. 2. The eyes (A–C), the ommatidia in the frontal–horizontal eye region (D,F,H) and the median ocelli (E,G,I) of *Apis florea* (left column), *Apis cerana* (middle column) and *Apis dorsata* (right column), viewed under the scanning electron microscope. Scale bars are 1 mm for the eyes (A–C), 50 μm for ommatidia (D,F,H) and 200 μm for the ocelli (E,G,I).

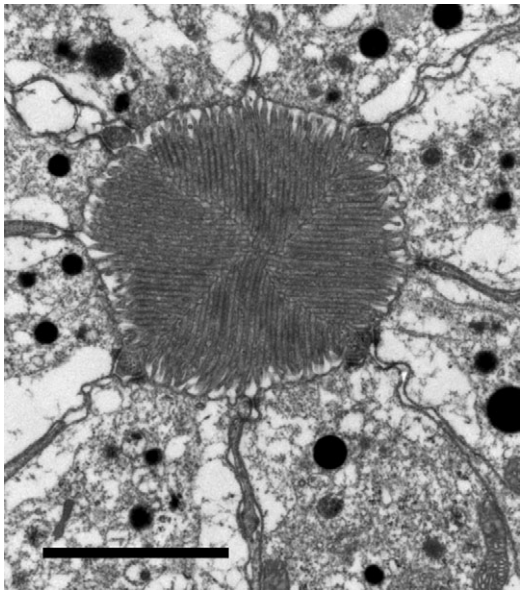

Fig. 3. A transmission electron micrograph of a cross-section through the distal rhabdom in a frontal ommatidium of the compound eye of *Apis dorsata*. Scale bar, 2 μm.

fraction of light captured by the rhabdom that is absorbed by the visual pigment. Although rhabdom length differs between the four species of *Apis*, it has a minor influence, with the percentage of white light absorbed by the rhabdom [$kl/(2.3+kl)$, see Eqn 2] ranging from 36% in *A. florea* to 48% in *A. mellifera* (with 43% in *A. cerana* and 44% in *A. dorsata*). Taken together, it is mostly the larger facet diameters and acceptance angles that make the eyes of *A. dorsata* (S=0.21 μm^{2}sr) twice as light-sensitive as those of *A. mellifera* (S=0.11 μm^{2}sr), three times as sensitive as those of *A. cerana* (S=0.07 μm^{2}sr) and seven times as sensitive as those of *A. florea* (S=0.03 μm^{2}sr). However, even the eyes of *A. dorsata* are 10 times less sensitive than those of the dim light specialists *M. genalis* and *X. tranquebarica* (both with S=2.7 μm^{2}sr). In the bumblebee, *Bombus terrestris*, Kapustjansky et al. (Kapustjansky et al., 2007) found that large-sized workers and drones had larger facet diameters and ocelli and were able to fly at slightly lower experimental illumination levels compared with smaller-sized individuals.

A trade-off between sensitivity and spatial resolution in the eyes of crepuscular bees

The rather modest differences in optical sensitivity of the eyes to white light does not explain how *A. dorsata* and *A. m. adansonii* can fly and forage on moonlit nights, when light intensities are 100 times dimmer than the lowest intensities at which we observed *A. cerana f*oraging and 1000 times lower than those at which *A. florea* was found foraging. Neither does the 10 times higher sensitivity of the truly nocturnal bees *M. genalis* and *X. tranquebarica* explain their ability to forage at even dimmer light intensities. The large rhabdom diameters (that compromise spatial resolution) and the anatomy of the lamina monopolar cells, the interneurones that receive signals from the photoreceptors, indicate that *M. genalis* most probably uses spatial pooling of the receptor signals to improve the signal-to-noise ratio and to allow reliable vision at these dim light levels (Warrant et al., 2004; Theobald et al., 2005; Warrant, 2008). Warrant et al. (Warrant et al., 1996), in a study on the spatial resolution and sensitivity of *A. m. ligustica*, proposed that even honeybees use spatial and temporal pooling of receptor signals to improve the signal-to-noise ratio, thereby enabling them to see reliably even in dim light. The dendritic trees of lamina monopolar cells of *A. mellifera* do not have equally wide branches as those of *M. genalis* but they do have wider branches than those in other diurnal insects (Greiner et al., 2004b). Even in bright light, the behaviourally measured resolution of *A. m. ligustica* is coarser than expected from the optics of the eyes, an adaptation that allows for extremely good colour discrimination and probably also higher sensitivity. By contrast, bees of the closely related genus *Bombus* have coarser colour discrimination but higher spatial acuity (Dyer et al., 2008). We assume that adaptations similar to those in *A. m. ligustica* are found in *A. m. adansonii*, and possibly also in *A. dorsata*, and may enable these species to forage on moonlit nights. It is not impossible that the eyes of *A. m. adansonii* have enlarged rhabdom diameters making their eyes just as sensitive as those of *A. dorsata*. Behavioural studies of the spatial and colour resolution of the facultatively nocturnal African and Asian honeybees should thus prove interesting.

Ocelli

Ocellar diameters in the two smaller Asian species are similar to those of the European race of *A. mellifera*: 0.24 mm in *A. florea* and 0.26 mm in *A. cerana* (Table 1; Fig. 2E,G). The ocelli are considerably larger in *A. dorsata* where they measure 0.38 mm in diameter (Table 1; Fig. 2I). The optical sensitivity of the ocellus is proportional to the square of the ocellar diameter (Warrant et al., 2006); thus, endowing *A. dorsata* with ocelli that are twice as sensitive as any of the other three species. Again, compared with the ocelli of *M. genalis* (0.49 mm) and *X. tranquebarica* (0.95 mm) (Warrant et al., 2006; Somanathan et al., 2009), even the ocelli of *A. dorsata* appear relatively small. The ocelli of all bees that have been studied earlier are under-focused and do not generate images of any useful resolution (Warrant et al., 2006; Somanathan et al., 2008). We did not expect an exception in the Asian species of *Apis* and we thus did not obtain focal lengths.

To conclude, our results indicate that of all species of *Apis* studied so far, *A. dorsata* has the most sensitive visual organs: both the eyes and the ocelli give this species the best preconditions to fly and orient visually in dim light. Yet, both visual organs of this species are only twice as sensitive as those of the European race of *A. mellifera* whereas those of the African race *A. m. adansonii* have never been studied. Compared with bee species such as *X. tranquebarica* or *M. genalis* that are active only in dim light, *A. dorsata* has eyes of lower sensitivity that cannot sufficiently explain its ability to fly, orient, recognise rewarding flowers and find its way back to the hive when only a half-moon is present in the sky (and light levels are a million times lower than during the day). We need to therefore assume additional adaptations at the neuronal level, specifically temporal and spatial summation as proposed for the European honeybee (Warrant et al., 1996) and *M. genalis* (Warrant et al., 2004; Theobald et al., 2005; Warrant, 2008) to explain this behaviour. The truly diurnal bees, *A. florea* and *A. cerana*, have less sensitive eyes but the potential for a higher spatial resolution than both *A. mellifera* and *A. dorsata*.

LIST OF ABBREVIATIONS

d	distal rhabdom diameter
D	maximum ommatidial diameter
f	focal length of the facet lens
k	peak absorption coefficient of the visual pigment
l	rhabdom length

S	optical sensitivity to white light
s_o	distance between striped object and lens
λ_i	spatial wavelength of the image of the striped pattern
λ_o	spatial wavelength of the striped pattern
$\Delta\rho$	acceptance angle

We wish to thank Kalu Kurade, Ganpat Lohakare, Subhash Vangere, Narayan Chikhale and Vittal Lohakare for assistance in the field and Carina Rasmussen and Eva Landgren for laboratory assistance. We are grateful to the Forest Department of Maharashtra State for research permissions. This study was generously funded by the Swedish International Development Agency (SIDA), the Swedish Research Council (VR) and the Wenner-Gren Foundation, all in Stockholm. We declare that the experiments comply with the 'Principles of animal care' and also with the current laws of the countries in which the experiments were performed.

REFERENCES

Arias, M. C. and Sheppard, W. S. (2005). Phylogenetic relationships of honey bees (Hymenoptera:Apinae:Apini) inferred from nuclear and mitochondrial DNA sequence data. *Mol. Phylogenet. Evol.* **37**, 25-35.

Böttcher, F. K. (1977). Vergleichende Untersuchungen der Rüssellänge von Arbeiterinnen, Königinnen und Drohnen der Honigbiene (*Apis mellifica* L.). *Apidologie* **8**, 169-204.

Burgett, D. M. and Sukumalanand, P. (2000). Flight activity of *Xylocopa* (*Nyctomelitta*) *tranquebarica*: a night flying carpenter bee (Hymenoptera: Apidae). *J. Apic. Res.* **39**, 75-83.

Chittka, L., Spaethe, J. (2007). Visual search and the importance of time in complex decision making by bees. *Arthropod Plant Interact.* **1**, 37-44.

Corlett, R. T. (2004). Flower visitors and pollination in the Oriental (Indomalayan) region. *Biol. Rev.* **79**, 497-532.

Dreller, C. and Kirchner, W. H. (1994). Hearing in the Asian honeybees *Apis dorsata* and *Apis florea*. *Insectes Soc.* **41**, 291-299.

Dyer, A. G., Spaethe, J. and Prack, S. (2008). Comparative psychophysics of bumblebee and honeybee colour discrimination and object detection. *J. Comp. Physiol. A* **194**, 617-627.

Dyer, F. C. (1985). Nocturnal orientation by the Asian honeybee, *Apis dorsata*. *Anim. Behav.* **33**, 769-774.

Fletcher, G. J. C. (1978). The African bee, *Apis mellifera adansonii*, in Africa. *Annu. Rev. Entomol.* **23**, 151-171.

Frederiksen, R. and Warrant, E. J. (2008). The optical sensitivity of compound eyes: theory and experiment compared. *Biol. Lett.* **4**, 745-747.

Frisch, K. v. (1914). Der Farbensinn und Formensinn der Biene. *Zool. Jahrb. Abt. Zool. Physiol. Tiere* **35**, 1-188.

Frisch, K. v. (1927). *Aus dem Leben der Bienen*. Berlin: Springer-Verlag.

Greiner, B. (2006). Visual adaptations in the night-active wasp *Apoica pallens*. *J. Comp. Neurol.* **495**, 255-262.

Greiner, B., Ribi, W. A. and Warrant, E. J. (2004a). Retinal and optical adaptations for nocturnal vision in the halictid bee *Megalopta genalis*. *Cell Tissue Res.* **316**, 377-390.

Greiner, B., Ribi, W. A., Wcislo, W. T. and Warrant, E. J. (2004b). Neural organisation in the first optic ganglion of the nocturnal bee *Megalopta genalis*. *Cell Tissue Res.* **318**, 429-437.

Greiner, B., Narendra, A., Reid, S. F., Dacke, M., Ribi, W. A. and Zeil, J. (2007). Eye structure correlates with distinct foraging bout timing in primitive ants. *Curr. Biol.* **17**, R879-R880.

Kapustjansky, A., Streinzer, M., Paulus, H. F. and Spaethe, J. (2007). Bigger is better: implications of body size for flight ability under different light conditions and the evolution of alloethism in bumblebees. *Funct. Ecol.* **21**, 1130-1136.

Kelber, A., Warrant, E. J., Pfaff, M., Wallén, R., Theobald, J. C., Wcislo, W. T. and Raguso, R. A. (2006). Light intensity limits foraging activity in nocturnal and crepuscular bees. *Behav. Ecol.* **17**, 63-72.

Kerfoot, W. B. (1967). Correlation between ocellar size and the foraging activities of bees (Hymenoptera, Apoidea). *Am. Nat.* **101**, 65-70.

Kirchner, W. H. and Dreller, C. (1993). Acoustical signals in the dance language of the giant honeybee, *Apis dorsata*. *Behav. Ecol. Sociobiol.* **33**, 67-72.

Kirschfeld, K. (1974). The absolute sensitivity of lens and compound eyes. *Z. Naturforsch.* **29C**, 592-596.

Land, M. F. (1981). Optics and vision in invertebrates. In *Handbook of Sensory Physiology* (ed. H. Autrum), vol. VII 6B, pp. 471-592. Berlin: Springer.

Lehrer, M. (1998). Looking all around: honeybees use different cues in different eye regions. *J. Exp. Biol.* **201**, 3275-3292.

Menzi, U. (1987). Visual adaptation in nocturnal and diurnal ants. *J. Comp. Physiol. A* **160**, 11-21.

Neese, V. (1965). Zur Funktion der Augenborsten bei der Honigbiene. *Z. Vgl. Physiol.* **49**, 543-585.

Oldroyd, B., Rinderer, T. and Wongsiri, S. (1992). Pollen resource partitioning by *Apis dorsata*, *A. cerana*, *A. andreniformis* and *A. florea* in Thailand. *J. Apic. Res.* **31**, 3-7.

Ordway, E., Buchmann, S. L., Kuehl, R. D. and Shipman, C. W. (1987). Pollen dispersal in *Cucurbita foetidissima* (Cucurbitaceae) by bees of the genera *Apis*, *Peponapis* and *Xenoglossa* (Hymenoptera: Apidae, Anthophoridae). *J. Kans. Entomol. Soc.* **60**, 489-503.

Praagh, J. P. v., Ribi, W., Wehrhahn, C. and Wittmann, D. (1980). Drone bees fixate the queen with the dorsal frontal part of their compound eyes. *J. Comp. Physiol. A* **136**, 263-266.

Raffiudin, R. and Crozier, R. H. (2007). Phylogenetic analysis of honey bee behavioural evolution. *Mol. Phylogenet. Evol.* **43**, 543-552.

Rao, S. P., Atluri, J. B. and Subba Reddi, C. (2001). Intermittent mass blooming, midnight anthesis and rockbee pollination in *Pterocarpus santalinus* (Fabaceae). *Nord. J. Bot.* **21**, 271-276.

Ribi, W. A., Engels, E. and Engels, W. (1989). Sex and caste specific eye structures in stingless bees and honey bees (Hymenoptera: Trigonidae, Apidae). *Entomol. Gen.* **14**, 233-242.

Seidl, R. and Kaiser, W. (1981). Visual field size, binocular domain and the ommatidial array of the compound eyes in worker honey bees. *J. Comp. Physiol. A* **143**, 17-26.

Somanathan, H. and Borges, R. M. (2001). Nocturnal pollination by the carpenter bee *Xylocopa tenuiscapa* (Apidae) and the effect of floral display on fruit set of *Heterophragma quadriloculare* (Bignoniaceae) in India. *Biotropica* **33**, 78-89.

Somanathan, H., Borges, R. M., Warrant, E. J. and Kelber, A. (2008). Visual ecology of Indian carpenter bees I: light intensity and flight activity. *J. Comp. Physiol. A* **194**, 97-107.

Somanathan, H., Kelber, A., Wallén, R., Borges, R. M. and Warrant, E. J. (2009). Visual ecology of Indian carpenter bees II: adaptations of eyes and ocelli to nocturnal and diurnal lifestyles. *J. Comp. Physiol. A* **195**, 571-583.

Spaethe, J. and Chittka, L. (2003). Interindividual variation of eye optics and single object resolution in bumblebees. *J. Exp. Biol.* **206**, 2447-3453.

Stavenga, D. G. (2003). Angular and spectral sensitivity of fly photoreceptors. I. Integrated facet lens and rhabdomere optics. *J. Comp. Physiol. A* **189**, 1-17.

Theobald, J. C., Greiner, B., Wcislo, W. T. and Warrant, E. J. (2005). Visual summation in night-flying sweat bees: a theoretical study. *Vision Res.* **46**, 2298-2309.

Warrant, E. J. (2008). Seeing in the dark: vision and visual behaviour in nocturnal bees and wasps. *J. Exp. Biol.* **22**, 1737-1746.

Warrant, E. J. and McIntyre, P. D. (1993). Arthropod eye design and the physical limits to spatial resolving power. *Prog. Neurobiol.* **40**, 413-461.

Warrant, E. J. and Nilsson, D. E. (1998). Absorption of white light in photoreceptors. *Vision Res.* **38**, 195-207.

Warrant, E., Porombka, T. and Kirchner, W. H. (1996). Neural image enhancement allows honeybees to see at night. *Proc. R. Soc. Lond. B Biol. Sci.* **263**, 1521-1526.

Warrant, E. J., Kelber, A., Gislen, A., Greiner, B., Ribi, W. and Wcislo, W. T. (2004). Nocturnal vision and landmark orientation in a tropical halictid bee. *Curr. Biol.* **14**, 1309-1318.

Warrant, E. J., Kelber, A., Wallén, R. and Wcislo, W. T. (2006). Ocellar optics in diurnal and nocturnal bees and wasps. *Arthropod. Struct. Dev.* **35**, 293-305.

Wcislo, W. T., Arneson, L., Roesch, K., Gonzalez, V., Smith, A. and Fernández-Marín, H. (2004). The evolution of nocturnal behavior in sweat bees, *Megalopta genalis* and *M. ecuadoria* (Hymenoptera: Halictidae): an escape from competitors and enemies? *Biol. J. Linn. Soc.* **83**, 377-387.