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Visual ecology of Indian carpenter bees II: adaptations of eyes and ocelli to nocturnal and diurnal lifestyles

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Abstract Most bees are diurnal, with behaviour that is largely visually mediated, but several groups have made evolutionary shifts to nocturnality, despite having apposition compound eyes unsuited to vision in dim light. We compared the anatomy and optics of the apposition eyes and the ocelli of the nocturnal carpenter bee, *Xylocopa tranquebarica*, with two sympatric species, the strictly diurnal *X. leucothorax* and the occasionally crepuscular *X. tenuiscapa*. The ocelli of the nocturnal *X. tranquebarica* are unusually large (diameter ca. 1 mm) and poorly focussed. Moreover, their apposition eyes show specific visual adaptations for vision in dim light, including large size, large facets and very wide rhabdoms, which together make these eyes 9 times more sensitive than those of *X. tenuiscapa* and 27 times more sensitive than those of *X. leucothorax*. These differences in optical sensitivity are surprisingly small considering that *X. tranquebarica* can fly on moonless nights when background luminance is as low as 10^{-5} cd m^{-2}, implying that this bee must employ additional visual strategies to forage and find its way back to the nest. These strategies may include photoreceptors with longer integration times and higher contrast gains as well as higher neural summation mechanisms for increasing visual reliability in dim light.

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Introduction

The apposition compound eye is the predominant eye design in most diurnal insects including bees (Land and Nilsson 2002). In these eyes, each visual unit, or ommatidium, consists of a corneal lens, a crystalline cone and photoreceptor cells surrounded by screening pigment that absorbs incoming off-axis light. The rhabdom, the light-sensitive portion of the photoreceptor cells, receives only light that passes through the small aperture defined by the corneal lens. This eye design results in low photon catch in dim light and is generally not well suited to insects active at night (Warrant 2004). The more sensitive superposition compound eye design, in which single rhabdoms receive light that enters through hundreds of ommatidia, is common in nocturnal insects such as moths and many beetles.

Bees, which are primarily diurnal insects, have apposition compound eyes. However, some bees have made evolutionary transitions to nocturnality, presumably because of lower predation and competition pressures, whilst retaining the apposition eye design that is best suited to bright light conditions (reviewed in Warrant 2008). It is, therefore, interesting to understand how the eyes of nocturnal bees have adapted to dim-light conditions. Prior to this study, the Panamanian sweat bee *Megalopta genalis* was the only nocturnal bee whose eyes had been studied in detail. In this bee, it is assumed that apart from possessing eyes that are many times more sensitive than diurnal bees, *M. genalis* also uses spatial summation strategies to improve vision at night (Greiner et al. 2004, 2008; Warrant et al. 2004; Frederiksen et al. 2008; Theobald et al. 2005).

We recently discovered flight activity at extremely low light intensities (below 10^{-5} cd m^{-2}) in an obligately nocturnal species of Indian carpenter bee, *Xylocopa* (*Nyctomellita*) *tranquebarica*, which forages even under the darkest conditions that prevail beyond the end of astronomical twilight and when there is no moon in the sky (Somanathan et al. 2008a). We have shown that this nocturnal carpenter bee uses vision to identify nests in the dark and can see colour in starlight (Somanathan et al. 2008b). In the current paper, we describe the eyes and ocelli of *X. tranquebarica* and compare them with those of two sympatric congeneric species, *X.* (*Mesotrichia*) *tenuiscapa* (largely diurnal but occasionally crepuscular) and *X.* (*Koptortosoma*) *leucothorax* (obligately diurnal). In addition, we compare the eyes of the nocturnal carpenter bee with those of the nocturnal halictid bee *M. genalis* and the diurnal European honeybee *Apis mellifera*. Our study system gives us the opportunity to compare the anatomy and optics of the visual organs of three congeneric bee species that live in the same habitat and are exposed to similar environmental conditions, yet differ in their temporal activity.

Methods

Study site and species

The eyes of the three diurnal and nocturnal *Xylocopa* species mentioned above were investigated as part of a larger study on their ecology and foraging behaviour in the Bhimashankar Wildlife Sanctuary (19°21′–19°11′N, 73°31′–73°37′E), Maharashtra State, situated in the Western Ghats of India. Only female *Xylocopa* have been used in this study. The biology and flight activity of these bees were detailed earlier (Somanathan and Borges 2001; Somanathan et al. 2008a).

Histological procedures

Light and electron microscopy was performed using standard methods. Bees were anaesthetized with ether, and whole eyes and ocelli were dissected from decapitated individuals. The ventral-most quarter of the eye was removed to allow the fixative to penetrate. Eyes and ocelli were placed in fixative (2% glutaraldehyde, 2% paraformaldehyde, 2% sucrose in 0.15 M sodium cacodylate buffer) for 12–24 h. After rinsing repeatedly in buffer, the eyes were fixed in 1% osmium tetraoxide for 1 h and embedded in epoxy resin. Longitudinal and transverse sections of 1 μm thickness were made with a Reichert Ultracut microtome using glass knives to slice the tissue embedded within the epoxy resin. The sections were placed on a slide, dried on a hot plate, stained with toluidine blue and photographed under a microscope. Ultrathin sections were stained with lead citrate and uranyl acetate and studied under a JEOL 1240 Transmission Electron Microscope.

Optics

Standard procedures were used to map interommatidial angles in the frontal part of the visual field (Land and Eckert 1985; Rutowski and Warrant 2002), but will be briefly reviewed here. The small end was cut from a plastic pipette tip leaving an opening large enough for the head of the bee to protrude through. To fix the bee in this position, its proboscis was glued to the tube using dental wax, and this preparation was then mounted at the centre of curvature of a Leitz goniometer. The goniometer was placed beneath an optical apparatus consisting of a Canon MD150 digital video camcorder and a Hasselblad Distagon 1:3.5 60-mm camera objective (which has an 80 mm back focal distance). The Hasselblad objective was connected to the Canon camera, front-lens to front-lens, and this assembly was then connected to a vertical post in a downward orientation: in this way, the rear face of the Hasselblad objective pointed downwards towards the goniometer, and gave a full 80 mm of working distance when focussed on the bee's head. This optical apparatus acted as a microscope that allowed single images to be captured from the Canon camcorder.

The bee's head was then manipulated so that the flat posterior eye edge was parallel to the plane of the goniometer stage. The head was further manipulated so that (1) the origin of the three goniometer axes was in the centre of the head and (2) the three goniometer axes were lined up with the dorsal–ventral (yaw), anterior–posterior (roll), and left–right (pitch) axes of the bee's head. With the stage horizontal, both eyes then looked vertically upwards into the lens of the Hasselblad objective, and when observed in this position, the eyes were oriented exactly anteriorly (from the animal's point of view). The goniometer allowed us to tilt the stage (and thus the head) in defined angular steps of latitude and longitude, with latitude = 0° and longitude = 0° (0°, 0°) defined as the anterior orientation.

To illuminate the eyes, we used a small but powerful hand-held LED torch. This torch illumination revealed (through the microscope) a dark pseudopupil whose position on the surface of the eye was not affected by different directions of torch illumination. Trial-and-error manipulation of the torch revealed the angle of illumination that gave the best contrast and visibility of the pseudopupil. The eyes of *X. tenuiscapa* and *X. leucothorax* are light coloured, whilst *X. tranquebarica* have dark eyes. Using finely ground barium sulphate dust sprinkled lightly on the eye to

provide landmarks, and using the methods outlined in Rutowski and Warrant (2002), we took a series of photographs of the dark pseudopupil in the left eye at 10° intervals of latitude and longitude. Due to the structure of the apparatus, we could not go beyond latitudes of +70° or −70° or a longitude of 100°. Hence, our observations of the appearance and location of the pseudopupil were restricted to the frontal region of the eye, which is, in any event, the region of greatest interest.

From each photograph, we were able to determine the coordinates of the facet found at the centre of the pseudopupil, using the landmarks as a guide. Using established formulae that correct for latitude distortions in the projection (Rutowski and Warrant 2002), for each combination of latitude and longitude, we calculated the average local interommatidial angle $\Delta\phi$ which reflects the density of ommatidia in that region. These data were plotted on a sphere representing three-dimensional space around the animal, and contours were interpolated to connect regions of space viewed by parts of the eye with the same $\Delta\phi$. Contour plots of the angular separations of x, y and z facet rows were made separately to control for the fact that the eyes of carpenter bees, and indeed the eyes of all bees, are highly non-spherical (plots not shown). Since the ommatidia are hexagonally packed, the x and y rows are oriented at about 60° to the equator of the eye: the x rows run frontal–ventral and the y rows frontal–dorsal. The z facet rows run roughly dorso-ventrally. Spherical plots of facet diameter D were also made and used together with plots of average $\Delta\phi$ to calculate the eye parameter p at each point in the eye. The eye parameter is the product of D and $\Delta\phi$ ($p = D\Delta\phi$, μm rad) and indicates how closely the eye is constructed to the limits imposed by diffraction (Snyder 1977, 1979). These limits are expected to set a lower bound on how small the eye, and in particular the diameter of individual ommatidia, can be made and still provide good resolution. As such, p indicates something about the trade-offs between resolution and sensitivity that have been made during evolution, both between species of different sizes and between eye regions. Small values of p generally mean that the eye, or that region of the eye, has been constructed in a way that maximizes acuity at the expense of sensitivity (Snyder 1977, 1979).

Focal length measurements

The focal lengths of corneal facet lenses and back focal distances of ocellar lenses were measured using a modification of Homann's (1924) hanging drop method. For ocelli, a small piece of cuticle containing either a lateral or median ocellus was carefully dissected from the head capsule, placed in a petri dish of saline and lightly cleaned using a small paintbrush to remove tissue and pigment. It was then placed external side outwards in a tiny drop of physiological saline (refractive index = 1.34) that was placed in the centre of a cover slip. An o-ring was waxed to a conventional microscope glass, 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 its downward pointing ocellus. The microscope slide was mounted on the stage of a conventional light microscope (Leica) with condenser removed. 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 focussed by the ocellus within the saline drop. These images were then viewed with the 40× objective, and photographed with a digital camera fitted to the microscope.

For the corneal facet lenses (from the compound eyes), the preparation procedure was identical, except that a small piece of cornea (containing 100–200 facets) was cut from the surface of the eye in the fronto-ventral region where facets tend to be the largest, and cleaned to remove pigments and tissues in the manner described above. A single facet lens was then chosen for focal length measurements. 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).

For ocellar lenses, the optical back focal distance was measured. This is the distance from the back of the ocellar lens to the plane of best focus, and was measured by first focussing upon small particles of debris attached to the back of the lens. The back focal distance was determined by focussing upwards until the best image of the striped object was obtained. The change in focus (in μm) was measured using a micrometer gauge attached to the microscope stage. This procedure was repeated at least ten times and the values averaged. This mean value was corrected for the refractive index of the saline by multiplication by 1.34.

Calculation of optical sensitivity

The optical sensitivity (S) of an eye to an extended source of broad-spectrum light (expressed in units of μm^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 diameter of the corneal facet lens, l is the length of the rhabdom, k is the peak absorption coefficient of the visual pigment (taken as 0.0067 μm^{-1}; see Warrant et al. (2004)) and $\Delta\rho$ is the acceptance angle (half width of the photoreceptor's receptive field, in rad). For many apposition compound eyes, $\Delta\rho$ 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). 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 ($\pi\Delta\rho^2/4$ sr) and are long enough to absorb a substantial fraction of the incident light ($kl/(2.3 + kl)$).

Results

Compound eyes

Anatomy

Comparative eye measurements for the three *Xylocopa* species, as well as for the nocturnal Panamanian halictid bee *M. genalis* and the European honeybee *A. mellifera*, are given in Table 1. The large compound eyes of *Xylocopa* consist of several thousand ommatidia, each of which consists of a corneal facet lens, a crystalline cone and receptors forming a fused rhabdom (Fig. 1). The eyes of all three species have large visual fields (see Figs. 2, 3). Moreover, in a single individual, the visual fields of the two eyes display considerable frontal binocular overlap (ca. 20°), which may be important for improving distance estimations and/or signal-to-noise ratio in the frontal visual field.

All three species have large facets, but even the largest (found in *X. tranquebarica*: 39 μm on average) are only slightly larger than those of the much smaller nocturnal bee *M. genalis*. The large size of the eyes is mirrored by the number of facets per eye, ranging from over 12,000 in the smallest species *X. leucothorax* to almost 19,000 in *X. tranquebarica*. The most remarkable anatomical difference between the eyes of the three *Xylocopa* species is the extremely wide rhabdoms of *X. tranquebarica* (6 μm). These are 3 times wider than those of the diurnal *X. leucothorax* or *A. mellifera* and 2.4 times wider than those of *X. tenuiscapa* (Figs. 1d–f; Table 1). However, the rhabdoms of *X. tranquebarica* are narrower than those of the nocturnal *M. genalis* (8 μm). Corneal thickness in the obligately diurnal *X. leucothorax* is similar to the nocturnal

Table 1 Optical and physiological parameters in the compound eyes and ocelli of nocturnal and diurnal female bees

Parameter	*A. mell*[a] (D)	*M. gena*[a] (N)	*X. leuc* (D)	*X. tenu* (D/C)	*X. tran* (N)
Intertegular width (mm)	3.2	2.8	7.5 ± 0.8[b]	8.8 ± 0.4[b]	7.1 ± 0.7[b]
Eye length (mm)	2.6[c]	3.2[c]	4.5 ± 0.6[b]	5.7 ± 0.3[b]	6.7 ± 0.3[b]
Number of facets	4,752	4,883	12,716[b]	15,994[b]	18,804[b]
Maximum corneal facet diameter (μm)	20	36	34[b]	37[b]	39[b]
Cornea thickness (μm)	28	102	100	150	130
Length of crystalline cone (μm)	55	48	80	105	80
Distal rhabdom diameter, d (μm)	2	8	2	2.5	6
Rhabdom length, l (μm)	320	350	380	440	490
Focal length, f (μm)	66	97	142	129	127
Acceptance angle (theory), $\Delta\rho_t$ (°)	1.7	4.7	0.8	1.1	2.7
Acceptance angle (experimental), $\Delta\rho_{ex}$ (°)	2.6	5.6	–	–	–
F number, F	3.3	2.7	4.2	3.5	3.2
Optical sensitivity[d], S (μm^2 sr)	0.1	2.7	0.1	0.3	2.7
Median ocellus diameter (mm)	0.27[e]	0.49[c]	0.40 ± 0.04[b]	0.50 ± 0.02[b]	0.95 ± 0.07[b]

Values for all species are from the frontal eye region in the dark-adapted state

A. mell, *Apis mellifera*; *M. gena*, *Megalopta genalis*; *X. leuc*, *Xylocopa leucothorax*; *X. tenu*, *Xylocopa tenuiscapa*; *X. tran*, *Xylocopa tranquebarica*; *N* nocturnal, *D* diurnal, *C* crepuscular

[a] Mean ± standard deviations from Greiner et al. (2004)

[b] Mean ± standard deviations from Somanathan et al. (2008a)

[c] Data from Kelber et al. (2006)

[d] Based on theoretical acceptance angles

[e] From Kerfoot (1967)

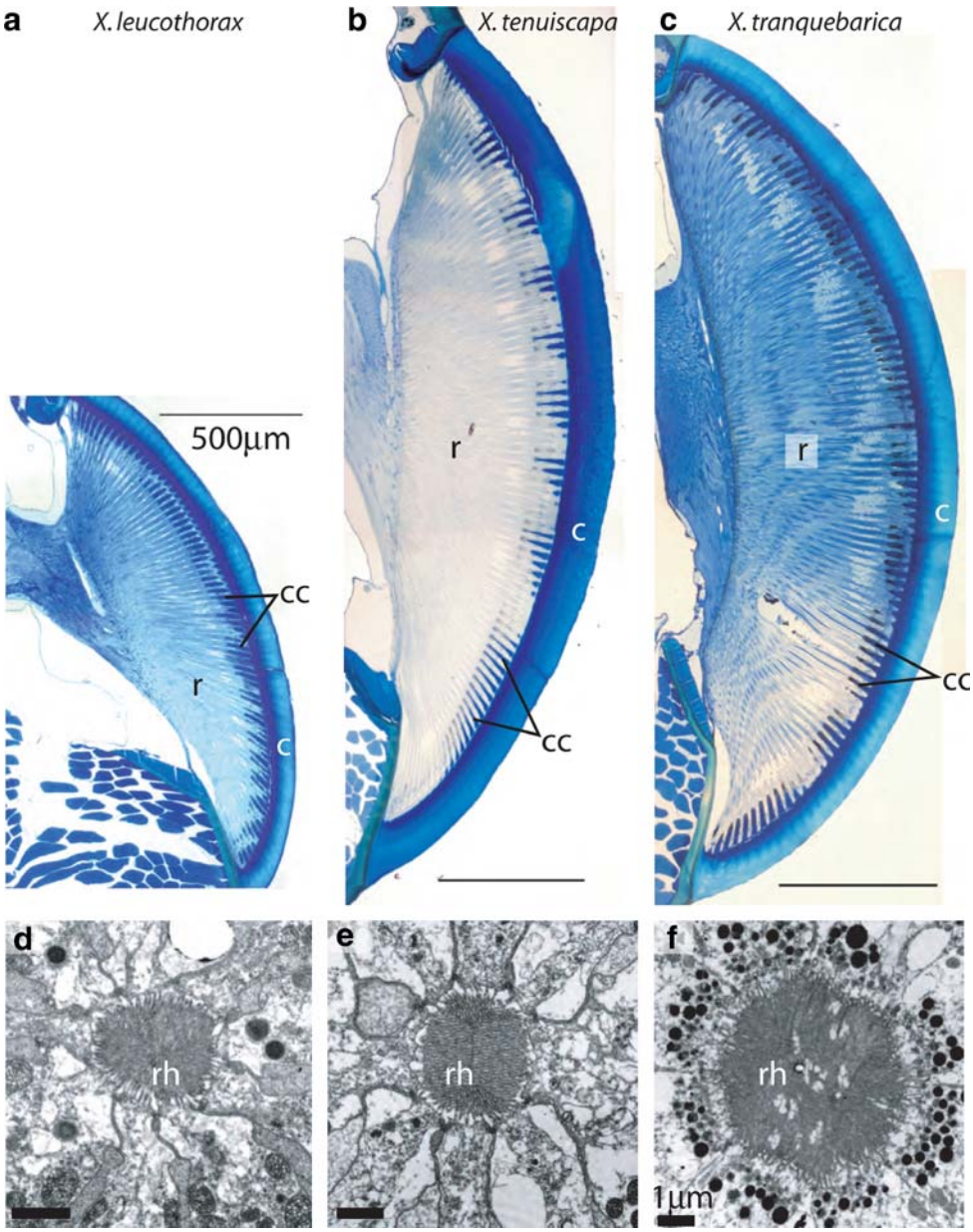

Fig. 1 Eyes of the two diurnal female carpenter bee species, *Xylocopa leucothorax* (**a**, **d**) and *X. tenuiscapa* (**b**, **e**), and the nocturnal female *X. tranquebarica* (**c**, **f**). **a–c** Horizontal sections through the eyes, showing the cornea (*c*), crystalline cones (*cc*), and the retina (*r*). Scale bar for **a–c**: 500 µm. **d–f** Transmission electron micrographs of transverse sections through the distal rhabdoms (*rh*) of ommatidia in the fronto-lateral eye region. *Scale bars* for **d–f**: 1 µm

M. genalis and is much thicker than in *X. tenuiscapa* and *X. tranquebarica*. The lengths of the crystalline cones are similar in all three *Xylocopa* species, although they are much longer than in *M. genalis* or *A. mellifera*. This is most likely due to the larger eye sizes of the *Xylocopa* bees.

Optics

Corneal focal length, which was measured for a single individual in all three species, is the longest in the diurnal *X. leucothorax* (142 µm), whilst focal lengths are shorter and very similar in *X. tenuiscapa* (129 µm) and *X. tranquebarica* (127 µm). The focal lengths of *A. mellifera* and *M. genalis* are much shorter (66 and 97 µm, respectively), no doubt due to their smaller body sizes. We could not measure acceptance angles for *Xylocopa* experimentally; however, theoretically estimated acceptance angles (the ratio of the rhabdom diameter d to the focal length f of the ommatidium) are widest for the nocturnal *X. tranquebarica* (2.7°) and smallest in the strictly diurnal *X. leucothorax* (0.8°). Facet diameters D are quite large, and similar (maximum D in large *Xylocopa* specimens = 40 µm), in the two *Xylocopa* species that are regularly or occasionally active in dim light (*X. tenuiscapa* and *X. tranquebarica*: Fig. 2c, e), but are considerably smaller in the strictly diurnal *X. leucothorax* (maximum D = 28 µm: Fig. 2a). In all three species, facet diameters are largest in the equatorial regions of the eye, with a distinctly frontal bias

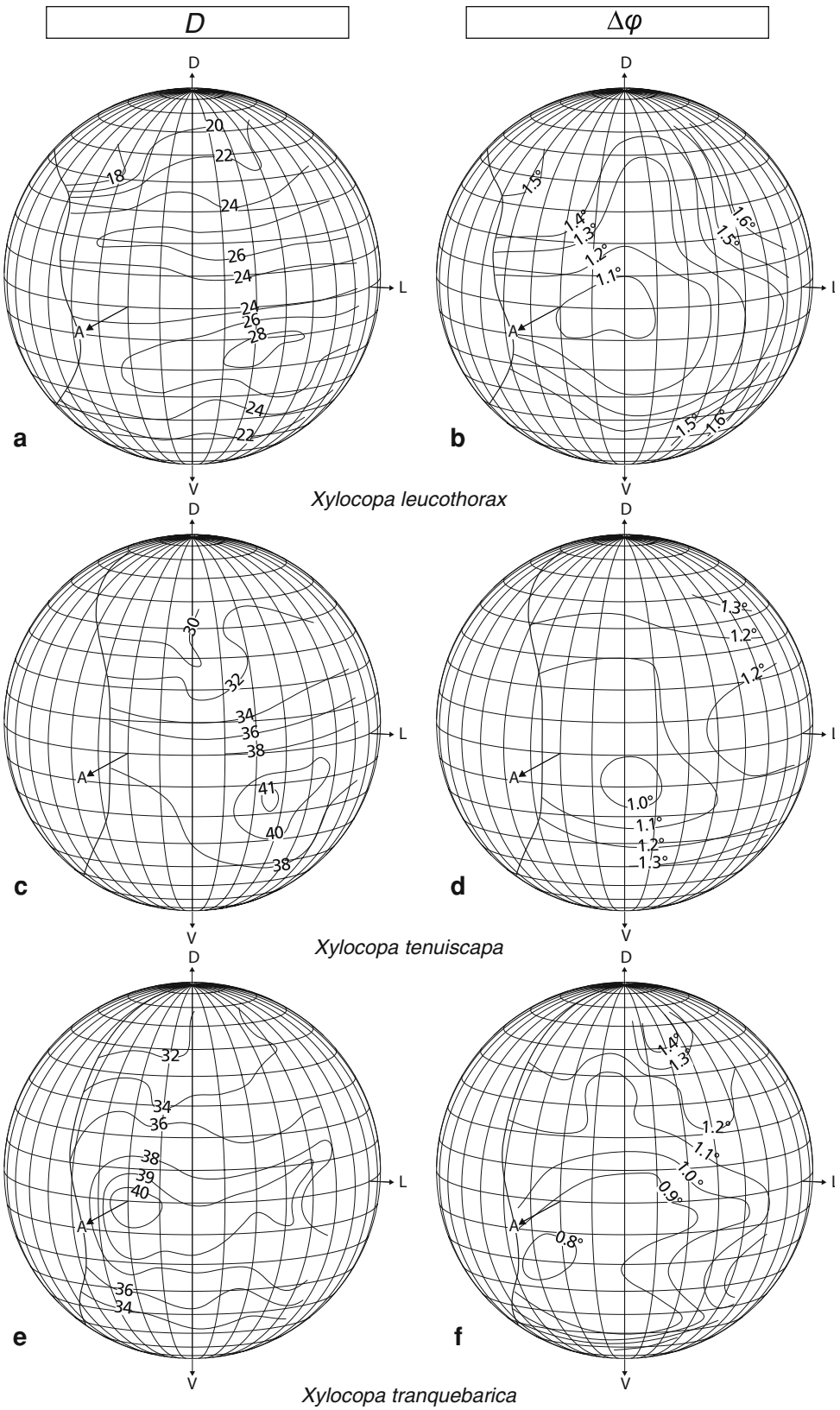

Fig. 2 Facet diameters (D) and interommatidial angles ($\Delta\phi$) in female *Xylocopa* eyes (for one individual of each species). Contour maps showing facet diameters in the frontal part of the eye of female *X. leucothorax* (**a**), *X. tenuiscapa* (**c**) and *X. tranquebarica* (**e**). Contour maps of interommatidial angles in the same specimens of *X. leucothorax* (**b**), *X. tenuiscapa* (**d**) and *X. tranquebarica* (**f**). *The thick lines* indicate the visual field borders. Dorsal ("*D*") corresponds to a latitude of +90°, ventral ("*V*") to a latitude of −90°, and lateral ("*L*") to a latitude of 0° and a longitude of +90°. Facet diameters are indicated as *isolines*. As in other bees, the eyes of *Xylocopa* are not spherical and the calculated interommatidial angles are shown as local averages of angles between *x* facet rows, *y* facet rows and *z* facet rows (see "Methods" for definitions of these rows). In a spherical eye, these values would be identical, but in bee eyes they are distinctly different: angles between *x* facet rows, *y* facet rows and *z* facet rows are, respectively, 0.90°, 1.82° and 1.28° in *X. leucothorax* (at 0°, +10°), 0.82°, 1.82° and 1.28° in *X. tenuiscapa* (at −10°, +10°) and 0.67°, 1.12° and 0.81° in *X. tranquebarica* (at −30°, +10°). The minimum interommatidial angle is smallest in *X. tranquebarica* at 0.8°, whilst it is close to 1° in the other two *Xylocopa* species

towards large facets in the nocturnal *X. tranquebarica*. The *F* number (*f*/*D*) of the dioptric apparatus is lowest in the nocturnal *X. tranquebarica* (3.2) and highest in the strictly diurnal *X. leucothorax* (4.2; Table 1). This indicates that, of the three *Xylocopa* species, the ommatidial optics of *X. tranquebarica* form the brightest image on the retina but

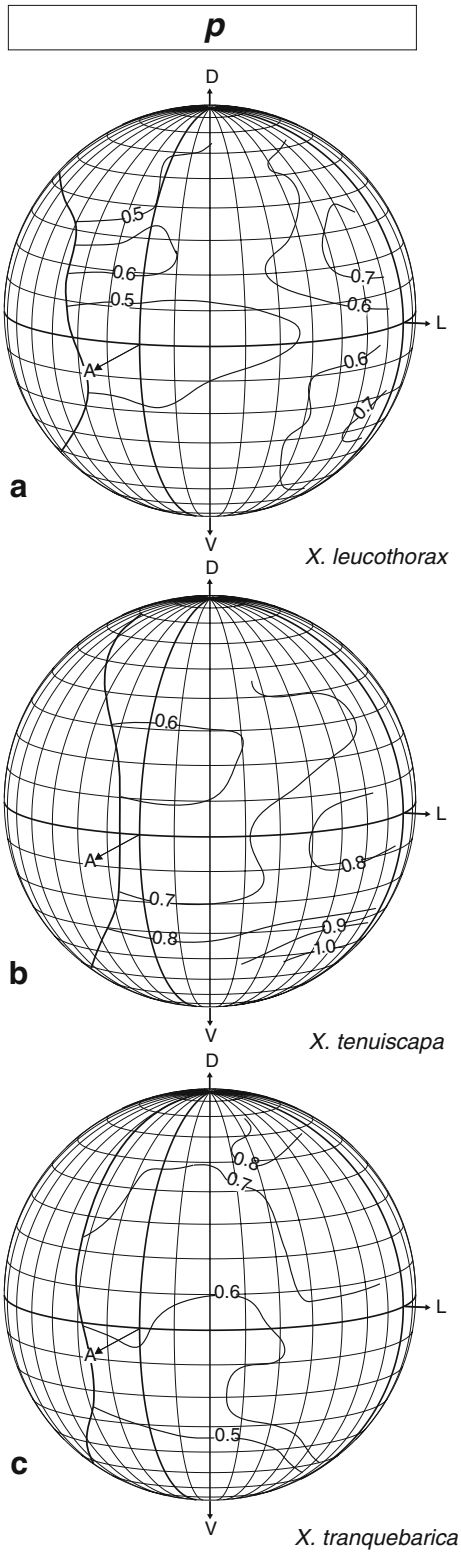

Fig. 3 Eye parameters ($p = D\Delta\phi$, μm rad), indicated as *isolines*, in the frontal part of the eye in the same individual female specimens of *X. leucothorax* (**a**), *X. tenuiscapa* (**b**) and *X. tranquebarica* (**c**) as in Fig. 2. Eye parameters are rather similar in all three species. Conventions as in Fig. 2

due to the small differences in focal length and facet diameters, values are rather similar for all three *Xylocopa* species.

All three species have interommatidial angles close to 1°, again reflecting the large eye size and elevated number of ommatidia (Fig. 2b, d, f). Unexpectedly, the minimum interommatidial angle is smallest in the nocturnal *X. tranquebarica* (at 0.8°). Moreover, if we consider "smaller" interommatidial angles less than 1.1°, these are maintained in a much larger part of the visual field in *X. tranquebarica* than in the other two *Xylocopa* species (Fig. 2b, d, f). In all three species, interommatidial angles are smallest in the equatorial regions of the eye, with the smallest angles (and highest anatomical spatial resolution) located in the frontal visual field.

The eye parameter, p ($D\Delta\phi$, μm rad), in the three *Xylocopa* species was remarkably similar despite their very different preferred light intensities (Fig. 3), with values in most parts of the visual field in the range 0.5–0.8.

Optical sensitivity

Optical sensitivities calculated from Eq. 2 reveal that *X. tranquebarica* has eyes that are at least 27 times more sensitive than those of *X. leucothorax* and 9 times more sensitive than those of *X. tenuiscapa* (with $S = 2.7$, 0.3 and 0.1 μm^2 sr, respectively; Table 1). The eyes of *X. tranquebarica* have a similar optical sensitivity to those of *M. genalis* (Table 1). The slightly larger facet diameters and acceptance angles contribute to the increased sensitivity of *X. tranquebarica* eyes when compared with the other two *Xylocopa* species.

Ocelli

With a diameter of almost 1 mm, the ocelli of *X. tranquebarica* are larger than those of the other two *Xylocopa* species (0.4 and 0.5 mm in *X. leucothorax* and *X. tenuiscapa*, respectively; Fig. 4), and they are also the largest measured in any bee so far (Table 1). The ocelli of all three *Xylocopa* species are slightly oval and show dorso-ventral asymmetry. All three species have asymmetric lenses and a pigmented area adjacent to the ventral side of the lens that is larger than that on the dorsal side. The two diurnal species also have dark screening pigment in the retina, basal to the rhabdoms of the photoreceptors. This pigment is absent in the nocturnal *X. tranquebarica*. Instead, the ocelli in this species appear to possess a tracheal tapetum. Figure 5 shows large numbers of tracheoles below and even between photoreceptor cells in the retina.

Optical ray traces obtained from anatomical sections of the ocelli and from hanging drop determinations of back

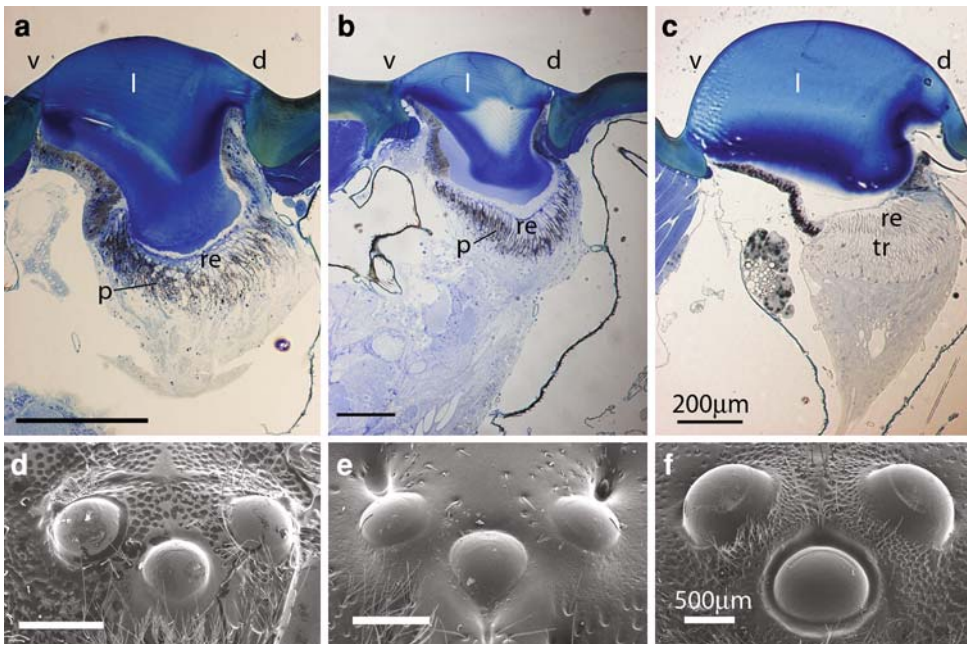

Fig. 4 Ocelli of female *X. leucothorax* (**a**, **d**), *X. tenuiscapa* (**b**, **e**) and *X. tranquebarica* (**c**, **f**). **a–c** Longitudinal dorso-ventral sections of median ocelli (*v* ventral, *d* dorsal) showing the lens (*l*), retina (*re*), and in the diurnal species (**a**, **b**), the pigmented region below the retina (*p*). Additional black-pigmented regions, dorsal and ventral to the retina, are not marked. In the nocturnal species (**c**) no pigment is seen below the retina but a dense network of tracheae (*tr*) is found in this region (see Fig. 5). *Scale bar* for **a–c**: 200 µm. **d–f** Scanning electron micrographs of the ocelli of the three *Xylocopa* species, *scale bars* 500 µm

focal distances reveal that the plane of best focus is proximal to the retina in all three *Xylocopa* species (Fig. 6), and that optical quality is poor. Of the three species, the ocellar focal plane is closest to the retina in the strictly diurnal *X. leucothorax* indicating that in this species the optical image quality is the least degraded.

Discussion

The compound eyes and ocelli of the nocturnal *X. tranquebarica* exhibit the same general features found in diurnal bees. All three species of *Xylocopa* are large, and large bees tend to have large eyes. In addition, the eyes of nocturnal bees tend to be larger relative to body size (Jander and Jander 2002; Kelber et al. 2006) and this was also found to be the case in *X. tranquebarica* (Table 1). The ratio between eye length and a measure of body size (intertegular width) is larger in *X. tranquebarica* than in *X. tenuiscapa* or *X. leucothorax* (0.94, 0.64 and 0.60, respectively). Large eyes favour sensitivity because they allow for large corneal facet diameters and thus greater photon capture (D^2 in Eq. 2), which is crucial in dim light. Even though all three *Xylocopa* species have large facet diameters, they are all in the same range as those in the eyes of the much smaller nocturnal bee *M. genalis* (Table 1). Since facet diameters in the three *Xylocopa* species and in *M. genalis* are similar, but their eye sizes vary considerably, the numbers of ommatidia in the four species must, therefore, vary markedly, which they do: ca. 13,000–19,000 in the three *Xylocopa* species compared with only 4,800 in *M. genalis*.

All three *Xylocopa* species have very thick corneas compared to *A. mellifera*. Corneal thickness in the obligately diurnal *X. leucothorax* (100 µm) is similar to the nocturnal *M. genalis* (102 µm) but it is much thicker in *X. tenuiscapa* and *X. tranquebarica* (150 and 130 µm, respectively). The adaptive significance for vision of possessing a thick cornea is unknown, but this feature may be related to maintaining the structural stability of the eye during wood boring, which is required for constructing nest tunnels in dead wood in *Xylocopa*. In contrast to *A. mellifera* (corneal thickness: 28 µm), all the other species discussed in this study excavate nests in dead wood using their mandibles. The length of the crystalline cone does not vary much between *Xylocopa* species, although it is much longer than in *M. genalis* or in *A. mellifera* reflecting the fact that *Xylocopa* are much larger bees. Amongst all the parameters we measured (Table 1), the most striking difference between the retinae of the diurnal and nocturnal *Xylocopa* species is the very wide rhabdom of the nocturnal *X. tranquebarica* (Fig. 1c), an adaptation that enhances photon capture in dim light. Amongst all nocturnal bees, ants and wasps that have been studied so far, wide rhabdoms are the most typical feature of their nocturnality: rhabdoms have a diameter of 6 µm in *X. tranquebarica* and in the nocturnal bull ant *Myrmecia pyriformis* (Greiner et al. 2007), 8 µm in *M. genalis* (Warrant et al. 2004) and in the nocturnal wasp *Apoica pallens* (Greiner 2006), and about 10 µm in the nocturnal ant *Camponotus irritans* (Menzi 1987). The latter species is interesting as it shows circadian structural changes in the ommatidia that have so far not been observed in bees. These changes include minor changes in rhabdom length and diameter but, most notably,

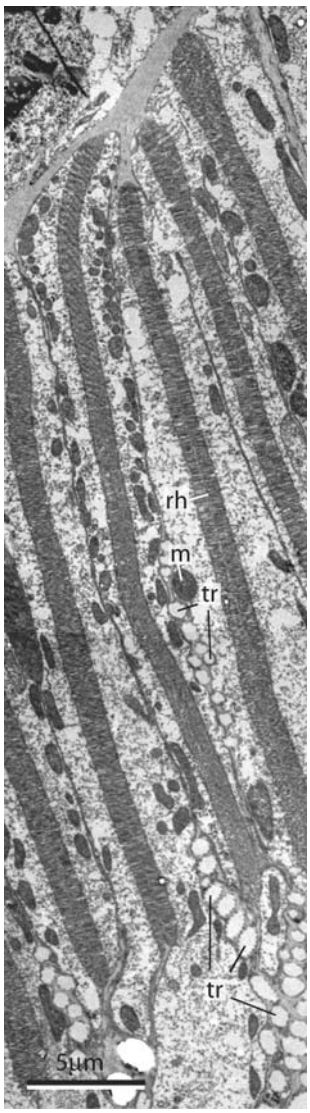

Fig. 5 Longitudinal electron micrograph section of the retina in the median ocellus of the female nocturnal carpenter bee, *X. tranquebarica*, showing the wide rhabdoms (*rh*), mitochondria (*m*) and pronounced tracheal branches (*tr*) extending between the receptor cells. *Scale bar* 5 µm

the crystalline cone diameter is the same as the distal rhabdom diameter at night whereas, in bright light, the cone builds a narrow tract of only 1 µm diameter that reduces sensitivity considerably. We have not studied the dark-adapted eyes of *X. tranquebarica* but, even in the light-adapted state, we did find that the proximal crystalline cone diameter equals the large distal rhabdom diameter. In *M. genalis*, the most prominent difference between light- and dark-adapted ommatidia is in the position of screening pigments (Greiner et al. 2004) and we expect similar differences in *X. tranquebarica*. Due to the migration of screening pigments in the retinula cells, and the wide rhabdoms, the angular sensitivity function narrows considerably in the nocturnal *M. genalis* during light adaptation, whilst in the diurnal *Lasioglossum leucozonium* it narrows only slightly (Frederiksen et al., unpublished results).

When similar-sized species were compared, we found that rhabdoms tended to be longer in the nocturnal *X. tranquebarica* than in the other two *Xylocopa* species. They were also longer in the nocturnal *M. genalis* compared to the diurnal *A. mellifera*. However, these differences do not change sensitivity to a large degree. The acceptance angle is geometrically approximated by the ratio of rhabdom diameter to the focal length, *d*/*f* (Stavenga 2003). Here, the fact that theoretical acceptance angles may underestimate the actual acceptance angles has to be taken into account. For instance, if we compare theoretically and experimentally determined acceptance angles in *A. mellifera* and *M. genalis* (Table 1), we find that this is clearly the case. Thus, we cannot exclude this possibility for *Xylocopa*. We will therefore base our comparisons on the theoretically calculated values in all species. Focal lengths are similar in *X. tenuiscapa* and *X. tranquebarica*, but are longer in the smaller and strictly diurnal *X. leucothorax*. In *X. leucothorax*, this longer focal length combined with narrow rhabdoms leads to a very small acceptance angle and low optical sensitivity (Table 1). This situation is similar to the diurnal *L. leucozonium* and *A. mellifera* (Greiner et al. 2004). The occasionally crepuscular *X. tenuiscapa* has a slightly shorter focal length, somewhat wider rhabdoms and thus an optical sensitivity that is three times higher than that of the strictly diurnal *X. leucothorax*. This is a surprisingly small difference considering that the facultatively nocturnal *X. tenuiscapa* is able to fly and collect pollen from the flowers of *Heterophragma quadriloculare* at light levels almost 100 times dimmer than the lowest light levels at which *X. leucothorax* has been observed foraging (Somanathan et al. 2008a). The larger theoretical acceptance angle found in the nocturnal *X. tranquebarica* is due mostly to its wider rhabdoms, and this endows their eyes with many times the sensitivity found in the other two species.

How does this situation compare to another insect group with apposition eyes, the butterflies? In the crepuscular butterflies *Caligo memnon* (Frederiksen and Warrant 2008) and *Melanitis leda* (Land and Osorio 1990), the acceptance angles are only marginally larger than in the diurnal butterfly *Morpho peleides*, but the eyes of the crepuscular butterflies are considerably more sensitive, largely because they possess wider facets (Land and Osorio 1990, Frederiksen and Warrant 2008). As we will see below, this is due to the fact that these butterflies probably need high spatial resolution.

Again, compared to the differences in light intensities experienced by the three sympatric *Xylocopa* species, the

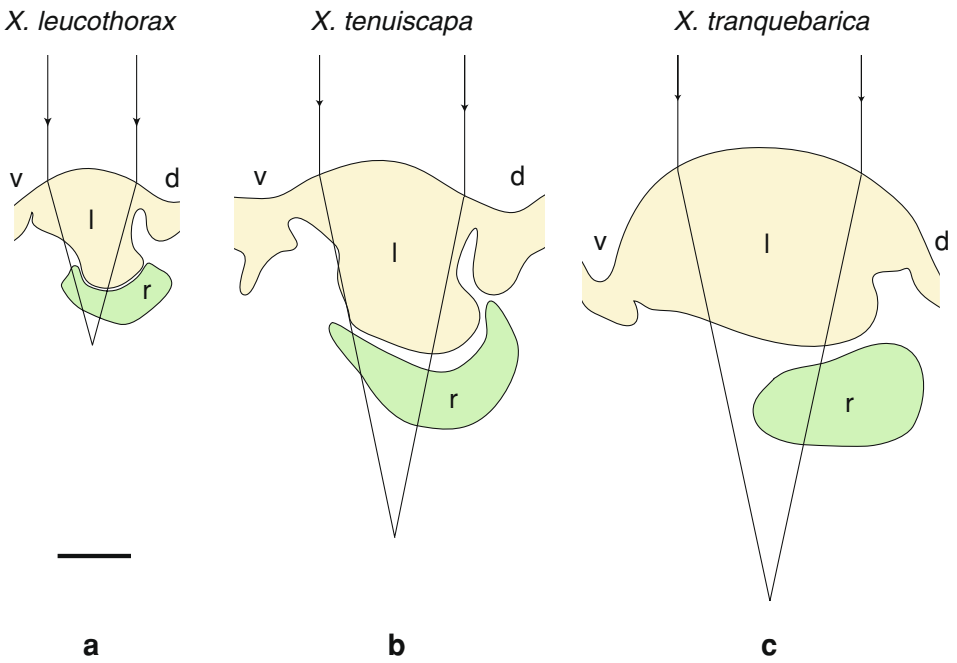

Fig. 6 Schematic optical ray-tracing diagrams of the ocelli in *X. leucothorax* (**a**), *X. tenuiscapa* (**b**) and *X. tranquebarica* (**c**) showing the positions of the focal planes. Schematic ocellar sections were obtained from Fig. 4. *v* ventral, *d* dorsal, *l* lens, *r* retina. *Scale bar* 200 μm

differences in optical sensitivity (*S*) are small. The eyes of the nocturnal *X. tranquebarica* are only about 27 times more sensitive than the eyes of the diurnal *X. leucothorax* and only about 9 times more sensitive than the eyes of the occasionally crepuscular *X. tenuiscapa*. Moreover, *X. tranquebarica* has roughly the same optical sensitivity as the nocturnal Panamanian sweat bee, *M. genalis*. Clearly, these modest differences in optical sensitivity do not adequately account for the moonless night flights in *X. tranquebarica* at light intensities as low, or lower, than 10^{-5} cd m^{-2}. *X. tranquebarica* must, therefore, possess additional adaptations for vision in dim light. One possible adaptation is that the photoreceptors of *X. tranquebarica* have longer integration times and higher contrast gains than those of the diurnal bees, increasing the sensitivity accordingly (Frederiksen et al. 2008). In the nocturnal sweat bee *M. genalis*, photoreceptor responses are much slower and contrast gains much higher than in the closely related diurnal sweat bee, *L. leucozonium* (Frederiksen et al. 2008). A second possible adaptation is that spatial and temporal summation of photoreceptor signals takes place at a later stage of neural processing, significantly improving sensitivity and visual reliability (Warrant 1999), a strategy believed to be employed by *M. genalis* (Warrant et al. 2004; Greiner et al. 2004; Frederiksen et al. 2008).

Spatial resolution of *Xylocopa* eyes

All three species of *Xylocopa* have large eyes and achieve minimum interommatidial angles in the vicinity of 1° in the frontal part of the visual field (Fig. 2), a value which indicates good resolving power. A surprising result was the fact that the larger eyes of the nocturnal *X. tranquebarica* do not simply accommodate similar numbers of ommatidia with larger (and thus more sensitive) facets, as was found in the eyes of *M. genalis* (Greiner et al. 2004). Instead, in contrast with the similarly large *X. tenuiscapa*, *X. tranquebarica* has a considerably larger number of facets (Table 1). Thus, of the three species, *X. tranquebarica* has the smallest interommatidial angles: 0.8° in its fronto-ventral eye region. The minimum interommatidial angle is around 1.4° in *M. genalis*, which is much larger than in *X. tranquebarica*. Moreover, compared to the other two carpenter bees, the smallest interommatidial angles in *X. tranquebarica* occur over a much larger area of the eye (Fig. 2).

Interestingly, in all three bee species, the region of the eye where the largest facet diameters (*D*) occur does not exactly coincide with the region of the eye where the smallest interommatidial angles ($\Delta\phi$) occur. In apposition eyes, a region of coincidence typically indicates an "acute zone", a region of high spatial resolution (Wehner 1981; Land 1981, 1997). Nonetheless, in the carpenter bees, the two regions *almost* overlap: the largest *D*s overlap with amongst the smallest $\Delta\phi$s, suggesting an acute-zone-like arrangement. The fact that the two regions do not exactly overlap may be due to their intimate relationship with the local radius of curvature of the eye surface, *R* ($D = R\Delta\phi$). Due to the peculiar geometry of the bee eye, it may not be easy to satisfy this relationship whilst at the same time ensuring total overlap between the largest *D*s and the smallest $\Delta\phi$s. Another explanation for the lack of coincidence may be that the zones in question are not acute zones, but rather are "bright zones". These are regions of

the eye where D is maximal but $\Delta\phi$ is not minimal (i.e. angular sensitivity can still be wide and resolution lower). In many insects, the regions of the eye with the largest Ds (i.e. the bright zone) and the smallest $\Delta\phi$s can be widely separated (although this is not the case in carpenter bees). Bright zones are found in some flies and are used to increase local sensitivity, and thus signal-to-noise ratio, in bright light (van Hateren et al. 1989; Straw et al. 2006). These regions increase the contrast sensitivity to small moving targets seen in this part of the eye (Straw et al. 2006) and are associated with improved detection of mates and prey. The separation of eye regions of largest D and smallest $\Delta\phi$ is most obvious in the two diurnal species, with the regions of largest D displaced laterally and ventrally. It is possible that these regions are indeed bright zones in the two diurnal species. Males of both species have a quite conspicuous sit-and-wait strategy for detecting females and rivals (after which a high-speed chase ensues: Somanathan et al., unpublished results), and a bright zone may be useful in that context. However, the bees used in this study were all females, and it is thus unclear whether the separation of eye regions with largest D and smallest $\Delta\phi$ is truly indicative of a bright zone or whether they have a distorted acute zone due to the ocular geometrical constraints mentioned earlier.

The eye parameter p ($D\Delta\phi$, μm rad) is a good indicator of the trade-off between resolution and sensitivity in an apposition eye. Eyes requiring higher sensitivity tend to have larger eye parameters. For instance, insects active in dimmer light often have eye parameters greater than 2 μm rad (Snyder 1977, 1979). Flying diurnal insects experiencing high angular velocities, such as the house fly *Musca*, also require greater sensitivity, and in *Musca*, $p \approx 1.3$ μm rad (Snyder 1977, 1979). Slowly moving insects active in bright light (e.g. mantises and hovering sphecid wasps) tend to have a value of p less than 0.45 μm rad (Snyder 1977). The minimum theoretical value of p which is set by the diffraction limit is 0.25 (Horridge 1978). Interestingly, p is both reasonably small and quite similar (ca. 0.5–0.8 μm rad) in all three *Xylocopa* species, despite their faster flight speeds and their quite different preferred light intensities (Fig. 3). As a comparison, in the nocturnal halictid bee *M. genalis*, p varies from 0.9 to 1.2 μm rad (Warrant et al. 2004). However, even these values are considered low for a nocturnal apposition eye. In three species of relatively slowly flying diurnal nymphalid butterflies, p varies from 0.5 to 0.7 μm rad, in reasonable agreement with theoretical predictions (Rutowski et al. 2009). In a fourth nymphalid—the crepuscular *Caligo eurilochus*—p is higher, varying from 0.7 to 1.3 μm rad, an increase that concurs well with its dimmer lifestyle (Rutowski et al. 2009). The same conclusion can be drawn for the eyes of the crepuscular satyrid butterfly *Melanitis leda*, in which $p \approx 0.9$ μm rad (Land and Osorio 1990).

Even though the small interommatidial angles and lower eye parameters found in the three *Xylocopa* species suggest an eye design biased towards higher spatial resolution and reduced sensitivity, this might not be the case. In the nocturnal *X. tranquebarica*, this higher spatial resolution is achieved only if a subsequent neural summation of signals from groups of neighbouring ommatidia does not occur. Rather than using the large number of ommatidia for high spatial resolution, these bees may achieve higher signal-to-noise ratios by spatial summation. This may be advantageous for several reasons. First, summation of signals from several smaller receptors leads to better signal-to-noise ratios since noise (which is uncorrelated) is cancelled out whilst signals are summed (Warrant 1999). Second, because the acceptance angles of the photoreceptors are large (due to the large rhabdom diameter: $\Delta\rho = 2.7°$), spatial resolution is anyway compromised: spatial summation to at least this extent would enhance visual performance without further losses in resolution (Warrant et al. 2004). As in *Xylocopa*, the nocturnal wasp *Apoica pallens*, although smaller in body size than its diurnal relative *Polistes occidentalis*, also has a larger number of more densely packed ommatidia and a very wide acceptance angle ($\Delta\rho = 7.2°$, Greiner 2006). This large discrepancy between interommatidial angle and acceptance angle in both *Xylocopa* and *Apoica* strongly implicates neural summation as a mechanism for enhancing sensitivity: rather than possessing fewer ommatidia with larger facet lenses, it could be a common strategy in nocturnal Hymenoptera to sum photoreceptor signals from groups of neighbouring ommatidia, each with a wide visual field.

How does this compare to the crepuscular butterflies that we discussed above? The crepuscular owl butterfly *C. memnon* has similar interommatidial angles to those found in the diurnal *Morpho peleides*, a butterfly with the same body size but with much smaller eyes (Frederiksen and Warrant 2008). Like nocturnal ants, bees and wasps, the crepuscular butterfly has wider rhabdoms than its diurnal relative (4 μm as compared to 2 μm in *Morpho*) but this only results in slightly wider acceptance angles. Instead, the crepuscular species has much larger eyes with much larger facet lenses (48 μm compared to 34 μm in *Morpho*) and probably longer focal lengths. Butterflies, including owl butterflies, have mate detection strategies that are largely visual, where high resolution is important even at dusk (Srygley and Penz 1999). This need for high resolution in dim light has driven them to evolve huge eyes with larger facets, and this may have hindered them from becoming nocturnal. Bees, in contrast, can possibly tolerate lower spatial resolution, allowing *X. tranquebarica* to fly at much lower light levels than its congener *X. tenuiscapa*.

Ocelli

The most conspicuous feature of nocturnal bees—their large ocelli—has already been described by Kerfoot (1967). In *X. tranquebarica*, they measure almost 1 mm in diameter (Fig. 4c, f), a size comparable to the highly resolved lens eyes of visually hunting nocturnal spiders (Blest and Land 1977). Like the ocelli of *M. genalis*, those of all three species of *Xylocopa* show a dorso-ventral asymmetry and have a half-circular ridge in the dorsal half (Fig. 4), the optical function of which is unknown. For the ocelli to have good spatial resolution, the plane of best focus must lie on the retina. However, ray-tracing diagrams produced from the anatomical sections of the ocelli of the three *Xylocopa* species indicate that the plane of best focus is below the retina in all three *Xylocopa* species (Fig. 6). This result confirms that *Xylocopa* ocelli do not resolve sharp images, and in this respect they are similar to other bee ocelli that have been studied (Warrant et al. 2006). In addition, the rhabdoms (in cross-section) are long and slim (Fig. 5), but with a considerable cross-sectional area that indicates a high sensitivity to light. Similar rhabdoms are found in the ocelli of the nocturnal halictid bee *M. genalis*, and this may be characteristic of ocelli in nocturnal Hymenoptera: in the ocelli of diurnal bees and wasps the rhabdoms are considerably smaller (Warrant et al. 2006). As has been stated earlier (e.g. Warrant et al. 2006), bee ocelli are most likely involved in flight control, and their large size in nocturnal species indicates a need for high quantum capture in order to produce a strong signal.

Strong signals from the ocelli may be especially important in dim light. In contrast to the compound eyes, which must find a balance between sufficient spatial resolution and adequate sensitivity, the three single ocelli have gone much further to increase sensitivity. For species that fly in dim light, when the signals from the compound eyes could be very noisy, the ocelli may have a more important function in flight control than in diurnal species (Wellington 1974). Our surprising finding of a tracheal tapetum in the ocelli of *X. tranquebarica* (Fig. 5) points in this direction. Tracheal tapeta are missing in the other two *Xylocopa* species, as well as in *A. mellifera* and *M. genalis*, all of which instead have dark pigmentation below the ocellar retina. As far as we know, tracheal tapeta have not previously been described in any ocellus.

In summary, the apposition compound eyes of the nocturnal *X. tranquebarica* are as sensitive as the eyes of the nocturnal Panamanian sweat bee *M. genalis* and about nine times more sensitive than the eyes of the congeneric *X. tenuiscapa*, which is occasionally crepuscular. Despite being the only known obligately nocturnal bee, *X. tranquebarica* is able to forage and distinguish colour like a diurnal bee, even on moonless nights (Somanathan et al. 2008b). Extreme nocturnality in *X. tranquebarica* has possibly evolved due to increased competition for floral resources or predation during the day (Roubik 1989; Wcislo et al. 2004; Somanathan et al., unpublished results). However, *X. tranquebarica*, with apposition compound eyes, is likely to possess additional visual adaptations for seeing well at night, including photoreceptors with longer integration times and higher contrast gains, and more efficient neural mechanisms for increasing visual reliability via spatial and temporal summation.

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