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Visual ecology of Indian carpenter bees I: Light intensities and flight activity

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Abstract Bees are mostly active during the daytime, but nocturnality has been reported in some bee families. We studied temporal flight activity in three species of carpenter bees (genus *Xylocopa*) in relation to light intensities. *X. leucothorax* is diurnal, *X. tenuiscapa* is largely diurnal being only occasionally crepuscular, while *X. tranquebarica* is truly nocturnal. Occasional forays into dim light by *X. tenuiscapa* are likely to be due to the availability of richly rewarding *Heterophragma quadriloculare* (Bignoniaceae) flowers, which open at night. *X. tranquebarica* can fly even during the moonless parts of nights when light intensities were lower than 10^{-5} cd m^{-2}, which makes this species the only truly nocturnal bee known so far. Other known dim-light species fly during crepuscular or moonlit periods. We compare eye and body sizes with other known diurnal and dim-light bees. We conclude that while extremely large ocellar diameters, large eye size:body size ratio, large number of ommatidia and large ommatidial diameters are all adaptations to dim-light foraging, these alone do not sufficiently explain the flights of *X. tranquebarica* in extremely dim light. We hypothesise that additional adaptations must confer extreme nocturnality in *X. tranquebarica*.

Keywords Apoidea · Bees · Compound eyes · Nocturnality · *Xylocopa*

Introduction

Most bees are diurnal and foraging activity begins at dawn and ends at dusk. However, some bees begin foraging at dusk and are nocturnal to various degrees. Nocturnal or crepuscular activity has evolved independently several times in at least four bee families, presumably to exploit flowers that offer pollen and nectar rewards at night (Hopkins et al. 2000; Wcislo et al. 2004) or as a response to greater competition, parasitism and predation risk during the day (Bohart and Youssef 1976; Smith et al. 2003; Wcislo et al. 2004). Among dim-light foraging bees, some such as *Xylocopa tabaniformis, Xenoglossa fulva*, *Ptiloglossa guinnae* and *Megalopta genalis* are active only during crepuscular periods (Lindsley et al. 1955; Janzen 1964; Roberts 1971; Warrant et al. 2004). In others, such as *X. tranquebarica* in Thailand, *Apis dorsata*, *Apis mellifera* and *Lasioglossum* (Sphecodogastra) *texana*, nocturnal activity on moonlit nights has been reported (Kerfoot 1967a, b; Dyer 1985; Burgett and Sukumalanand 2000).

All bees, including the nocturnal species, have apposition compound eyes that are well adapted to vision in daylight. In apposition eyes, the photoreceptors of individual ommatidia receive incident light only through a small aperture representing a single facet. This eye design is therefore generally conducive to diurnality. This contrasts with the superposition compound eyes of insects such as moths, where each rhabdom receives light through a much wider aperture consisting of hundreds of facets. This increases the number of photons caught in dim-light conditions, and is typical of nocturnal insects (Warrant 2004). How do nocturnal bees with apposition eyes forage and return to their nests at night? Two of the authors (EJW and AK) have studied eye structure and visual adaptations in *M. genalis*, the only dim-light bee that has been studied in any detail (Greiner et al. 2004a; Warrant

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et al. 2004; Kelber et al. 2006). Apart from the large facet diameter, physiological and anatomical adaptations in the photoreceptors and lamina monopolar cells, as well as a probable strategy of neural summation, appear to facilitate vision under dim-light conditions in this species (Warrant et al. 2004; Greiner et al. 2005; Theobald et al. 2006).

Nocturnality among Indian carpenter bees was first reported a century ago (Bingham 1897), but remained uninvestigated until recently (Somanathan and Borges 2001; Somanathan et al. 2004). Due to their large and robust body, powerful flight and strong mandibles, *Xylocopa* can exploit a diversity of habitats, environmental conditions and floral resources often by nectar robbing via chewing into flowers without effecting pollination (Gerling 1989). This flexibility can facilitate the expansion of ecological space and specifically the evolution of nocturnality, because eye size is correlated with body size, and large eyes generally allow for greater sensitivity (Kirschfeld 1974; Land 1997; Kelber et al. 2006). Among the Old World *Xylocopa*, flight activity has been observed in *X. tranquebarica* on moonlit nights in Thailand (Burgett and Sukumalanand 2000) and in *X.* (*Proxylocopa*) *olivieri*, which forages during twilight hours (Gottlieb et al. 2005). In the New World, *X. tabaniformis* forages on flowers 30 min after sunset (Janzen 1964). We recently discovered extreme nocturnality in *X. tranquebarica* in India. Along with *X. tranquebarica*, at least two diurnal *Xylocopa* species, namely, *X.* (*Platynopoda*) *tenuiscapa* and *X.* (*Koptortosoma*) *leucothorax* co-occur in our study site. Here, we describe the temporal flight patterns in these three *Xylocopa* species in relation to light intensity. Next, we place nocturnality in these carpenter bees in the context of nocturnality in bees from other biogeographic regions, with special reference to *M. genalis*, the only nocturnal bee in which flight activity has been related to light levels (Kelber et al. 2006).

The advantage of this study system is the possibility of meaningful cross-species comparisons due to: (1) the presence of congeneric sympatric species exposed to the same resource and habitat conditions, (2) a range of exhibited temporal activity periods, and (3) the elimination of the confounding effect of body size variation on ecology and behaviour since all three species are large.

Materials and methods

Study site and species

The three *Xylocopa* species were studied in the Bhimashankar Wildlife Sanctuary (19°21′–19°11′N, 73°31′–73°37′E, 900 m asl), Maharashtra State, in the Western Ghats of India. The vegetation is heterogeneous and consists of a matrix of highly fragmented seasonal cloud forest, open grassy patches with rocky outcrops in the hillcrests, paddy fields and slash-and-burn agricultural land. We determined the bee subgenera using the key given in Michener (2000). The species were determined by C. D. Michener by comparing specimens in the collections of the Zoological Museum in Lund University, Smithsonian Institution and the Oregon State University.

Ambient light measurements

Ambient light intensities were measured on several mornings and evenings with an International Light IL1700 radiometer together with a highly sensitive silicon detector (SED033) at different nest sites both in open areas and under the partial cover of trees. The sensor was equipped with a filter allowing us to measure the luminance of a horizontally oriented white surface (reflectance approximately 90%) with the sensor held at a 45° angle. Astronomical data were obtained from the NASA database (http://aa.usno.navy.mil/). Using light measurements and the NASA database allowed us to relate flight observations to sunrise, sunset, moonrise and moonset, and to the onset and offset of astronomical twilight. After astronomical twilight, when the sun is more than 18° below the horizon, the sun does not contribute to light incident from the sky.

Temporal flight activity patterns

All three species nest inside tunnels that they construct in the dead wood of large trees. Nests within a few feet from the ground were chosen for observations, since these could be observed easily. During a pilot study in 2005, we observed nests over several 24 h cycles and over several months to determine the extent of nocturnal or diurnal activity in the three *Xylocopa* species. Once the temporal windows of flight activity in the three species were established, we observed nests during the dry months in 2006 and 2007. Nests were observed from the time the first daily flight commenced until after the last flight ended, and the nest exits and entries by bees were recorded. This was done for several days in the two diurnal species *X. tenuiscapa* (140 h 45 min, 18 days, 6 nest trees, 45 nests) and *X. leucothorax* (156 h, 13 days, 6 nest trees and 12 nests), and nights in the nocturnal *X. tranquebarica* (199 h, 31 nights, 12 nest trees and 40 nests).

Exits from, and returns to, nests during the night in *X. tranquebarica* were determined by listening to their distinct buzzing, or by using infrared-sensitive night vision equipment, when the weather permitted. We also recorded flight behaviour in several *X. tranquebarica* individuals using an infrared-sensitive Sony camcorder (TRV310E). The camera was directed towards the nest entrance to capture the orientation behaviour of bees after they exit their nests. Flight paths in front of the nest were reconstructed in two dimensions.

The number of hours, days or nights for which we observed nests was unequal in the three species. To correct for this, we expressed flight activity in our analyses as the number of exits or returns per observation hour for those analyses in which the total hours of observation during a time slot could bias results.

Landmark orientation experiments

To determine if the nocturnal *X. tranquebarica* used visual cues while returning to nests, we attached small visual landmarks to the entrances of four nests. These were white plastic squares (5 cm × 5 cm) with a hole cut out in the centre for the nest entrance. We allowed the bees to get used to the new landmarks. A week later, after the bees had left the nest for the first time, we displaced the landmark from its original position by a distance of about 10–20 cm to the side or above and re-attached it to the tree trunk. We then observed whether returning bees would approach the nest entrance or pay attention to the landmark, indicating that they used this obvious visual landmark for nest orientation. One nest that was inhabited by three bees was filmed during the experiment.

Eye and body measurements

Measurements of body size, eye size and median ocellus size were made for the three *Xylocopa* species. We used intertegular width (distance between wing bases), which was considered the most appropriate measure of body size in other studies (Spaethe and Chittka 2003), as a surrogate for body size. The number of ommatidia was determined using corneal nail polish replicas, following the methods adopted by Praagh et al. (1980) and Kelber et al. (2006). The ommatidial diameter was determined in the fronto-ventral region of the eye where they were found to be the largest in other bees (Greiner et al. 2004a; Kelber et al. 2006). Ocellar and ommatidial diameters were measured using light microscopy and scanning electron microscopy (SEM) photographs. Using these body and eye measurements, as well as published data for other bees (Kelber et al. 2006), we examined differences in the relationship between body size (intertegular width) and eye and ocellar size in several diurnal and dim-light bee species.

Results

General nest observations

Nests may be found singly or in aggregations on a dead tree. Nest entrance holes were just large enough to accommodate the body of a bee. Mean entrance hole diameters were 1.0 ± 0.3 cm ($n = 4$) in *X. leucothorax*, 2.34 ± 0.1 cm ($n = 5$) in *X. tenuiscapa* and 1.5 ± 0.6 cm ($n = 5$) in *X. tranquebarica*. A nest may be inhabited by one bee or may house up to eight bees at a given time. A nest branch is usually occupied by only one *Xylocopa* species and interspecific sharing of a dead wood substrate was uncommon. In 2 out of the 24 nest trees that we monitored, we found that *X. tranquebarica* and *X. leucothorax* shared the nest branch, but inhabited separate nests. Flight activity was mostly confined to the dry months from October to May, which was also the major flowering season. Flights were rare during the monsoon season (June–September), which is characterised by a community-wide scarcity of flowers except for some monsoon annuals (HS et al., unpublished data).

Ambient light intensities

Light levels as a function of time of day are given in Fig. 1. Under weather conditions with a mostly blue sky, light levels

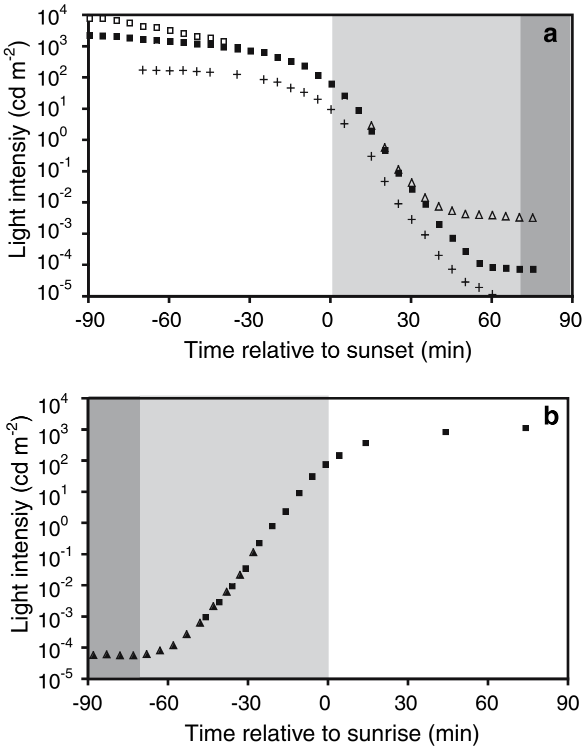


Fig. 1 Light intensities at the study site. **a** Change of light intensities in the evening, with a clear sky. *Filled squares*: data from the moonless evening of the 9 March 2005, measured in the shadow; *open squares*: same evening, but measured in a directly sunlit spot; *crosses*: moonless evening of 10 March 2005, at a nest site under a partial tree cover; *open triangles*: data from 16 March 2005, with a half moon high up in the sky. **b** Change of light intensities in the morning, on clear moonless mornings. Measurements were taken at 5 min intervals, and the time is given relative to sunrise. *Squares*: data from 13 March 2005, *triangles*: data from 16 March 2005; *light grey shading*: twilight hours between sunset and the end of astronomical twilight, and between the beginning of astronomical twilight and sunrise; *dark grey shaded areas*: time periods when the sun does not contribute to sky illumination, before the beginning and after the end of astronomical twilight

reflected from a white surface increased from less than 10^{-4} at night to 10^4 cd m^{-2} during the morning, in the open areas. The opposite happened in the evenings. Under partial canopy cover, where some nests were positioned, light intensities were lower by a factor of ten, and with a half moon in the sky, light levels did not drop below 10^{-3} cd m^{-2}.

Flight activity in *X. leucothorax*

Flights in *X. leucothorax* were almost exclusively restricted to the day and to light intensities of more than 10^{-1} cd m^{-2} (Figs. 2, 3, 4). First flights from the nest were highly synchronous with most bees leaving within a short period of time (Fig. 2a). The earliest time when observed flight activity commenced was 0547 hours, or 27 min before sunrise. The latest time when observed flight activity ceased was 1912 hours, or 13 min after sunset. Such crepuscular activity was, however, rare and flight activity usually ceased 10–20 min before sunset (Fig. 3a). Flights declined around midday, between 1100 and 1300 hours, and peaked in the morning between 0600 and 0700 hours and again in the evening between 1600 and 1800 hours (Fig. 2a).

Flight activity in *X. tenuiscapa*

Flight activity in *X. tenuiscapa* was largely diurnal with occasional extensions of flight activity into late evening periods (Figs. 2, 3, 4). First flights from the nest were highly synchronous and most females in a nest tree left within a few minutes of each other (Fig. 2b). The number of exits per hour peaked in the morning between 0600 and 0900 hours and dropped around midday as with *X. leucothorax*, but between 1300 and 1400 hours (Fig. 2b). Flights started as early as 22 min before sunrise (0537 hours), and the latest time at which flights ended was 2022 hours. The latest observed foraging trip occurred after the end of astronomical twilight on one evening when there was a full moon in the sky (24 February 2005; Fig. 3b). However, on other evenings, flights ceased much earlier, usually no more than 45 min after sunset, when light levels were still above10^{-3} cd m^{-2}. On one occasion, when the moon rose only 20 min after the last bee had returned to the nest, bees did not initiate flight activity but stayed in the nest for the remainder of the night. During another moonlit night, bees did not extend flight activity. This was at a darker nest site situated under partial tree cover. When we looked at foraging activity as a function of light intensity, it became obvious that on all evenings the last bees returned to their nest at about the same light level. *X. tenuiscapa*, possibly extends foraging activity into crepuscular time periods when night-opening flowers of *Heterophragma quadriloculare* (Bignoniaceae) are available. This tree is a common species on the mountain crests and usually flowers from late January to March (Somanathan and Borges 2001). During other times of the year, late evening flights were never observed.

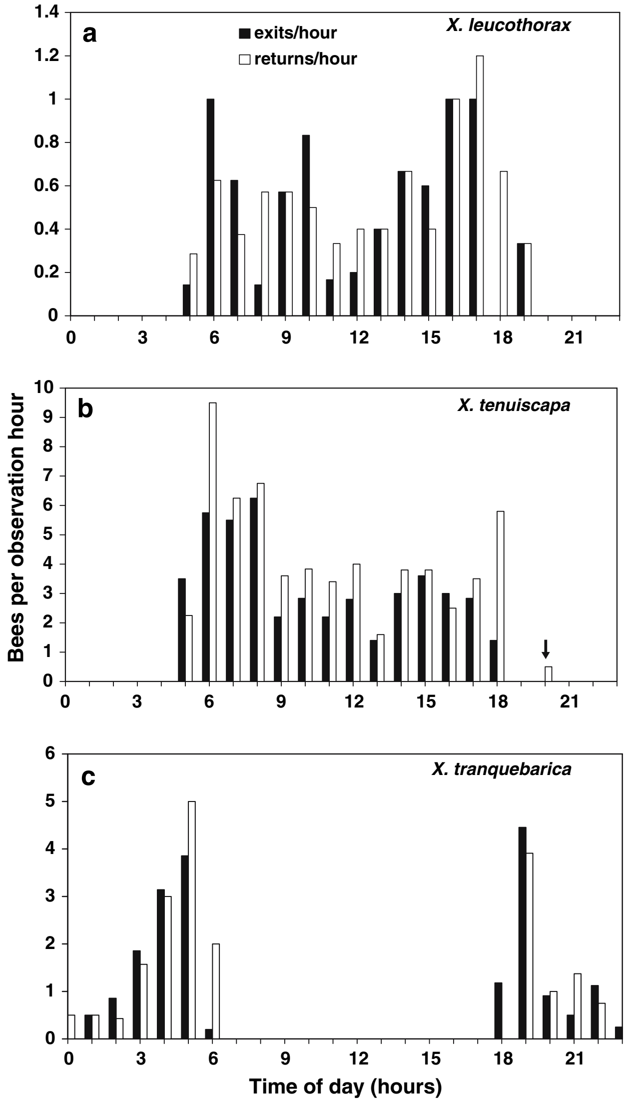


Fig. 2 Flight activity of (**a**) *X. leucothorax*, (**b**) *X. tenuiscapa* and (**c**) *X. tranquebarica* as a function of the time of day. Nest exits (*black bars*) and entries (*white bars*) are normalised for the number of observation days for each 1 h time slot. The large differences in absolute numbers are due to differences in the numbers of nests found in the same nest tree. Nest congregations of *X. tenuiscapa* are largest, followed by *X. tranquebarica* and *X. leucothorax*. The *arrow* indicates the single late flight activity in *X. tenuiscapa*. For details see text

Flight activity in *X. tranquebarica*

At the other extreme of temporal activity was *X. tranquebarica* in which flight activity was largely restricted to the night and light levels lower than 10^{-1} cd m^{-2} (Figs. 2, 3, 4). In October 2006, immediately after the end of the 4-month monsoon season, while flowers were still very scarce in the study site, this species was observed to leave nests much

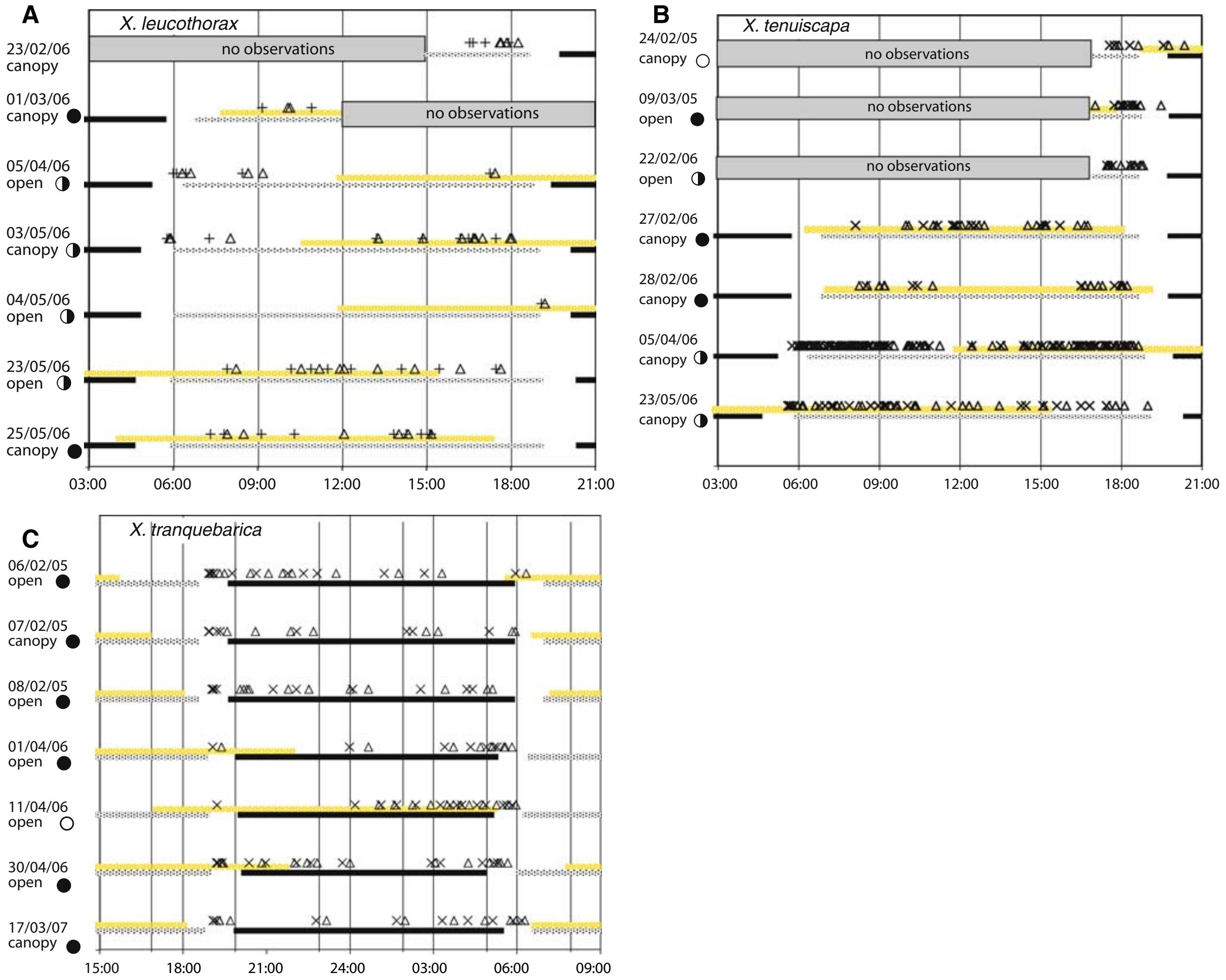


Fig. 3 Examples for flight activity patterns of (**a**) *X. leucothorax*, (**b**) *X. tenuiscapa* and (**c**) *X. tranquebarica* during several days or nights. *Each row* in *each figure* represents one observation day or night. *Triangles*: nest exits; *crosses*: nest entries; *shaded lines*: time between sunrise and sunset; *black lines*: time when the sun is more than 18° below the horizon; *Yellow lines*: time when the moon is in the sky. Observation date, nest location (open or under partial canopy) and moon phase are indicated on the *left side* of the figures, with a *white circle* for a full moon, a *black circle* for a new moon and *intermediate shape* indicating any in-between lunar phase

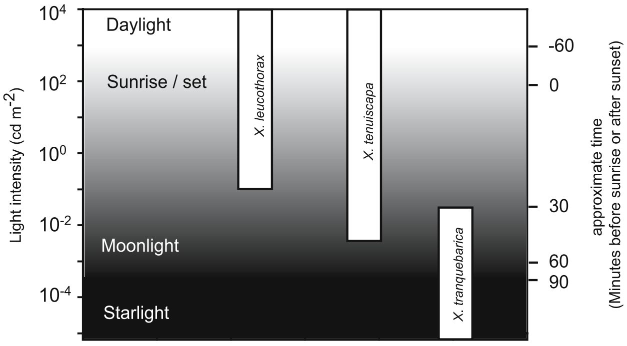


Fig. 4 Flight activity in all three species as a function of light intensity (*left ordinate*) and time relative to sunrise and sunset (*right ordinate*: 0 min corresponds to sunrise or sunset)

earlier in the evening (1700 hours) for only a single trip after which there was no more flight activity for the remainder of the night. *X. tranquebarica* were active within nests during the day, and engaged in activities such as nest construction, nest maintenance and defence against ants. These bees flew out of their nests during the day only when seriously disturbed. When a nest tree fell down during the monsoons, two bees were seen flying from nests, one of these entered a tree cavity about 50 m away and did not emerge from it till it was dark. The other occasions when this species was seen to fly out of the nest during the day were when a nest tree was cut by local wood collectors and when the small green barbet (*Megalaima viridis*), a common predatory bird, attempted to peck open nests during the day.

First flights in the evenings were highly synchronous, as also observed with the first morning flights of the two diurnal species (Fig. 2c). The earliest time when flights started was 1830 hours in the evening, which was half an hour before sunset, but usually flights started approximately 20 min after sunset (13 evenings). The latest time at which flight activity ceased was at 0620 hours the following morning, and generally, bees stopped flying between 10 and 48 min before sunrise (eight mornings). Bees left and returned to the nests all through the night (Figs. 2c, 3c). The number of flights per hour peaked between 1900 and 2000 hours, and there was a second, smaller peak between 0300 and 0600 hours. However, bees continued flying even when light intensities fell to starlight levels, during the darkest parts of the nights, and also after the end of astronomical twilight and without the moon (Figs. 3c, 4). On one of these occasions, the nest was under the cover of the canopy, where light intensities were below 10^{-5} cd m^{-2}. To our knowledge, this is the lowest light intensity at which any bee species has ever been observed to forage and return to its nest. Nocturnal flight activity in *X. tranquebarica* was negligible in May, which marks the end of the community-wide flowering season at this site. Although flights were rare, bees were active within nests and were seen to engage in activities such as nest excavation and defence. During this time, many bees were seen to block the nest entrance with their abdomens for several hours at a stretch.

Landmark orientation experiments in the nocturnal *X. tranquebarica*

Similar to other bees and wasps (Zeil et al. 1996), *X. tranquebarica* often performed orientation flights immediately after leaving a nest for a foraging flight. Two such short recorded orientation flights are presented in Fig. 5a, b. We observed the behaviour of bees at four nests to which we had attached artificial landmarks a week earlier. For two of the nests to which landmarks had been attached, we could not observe the response to moving the landmarks because one nest was abandoned during the week, and at the other nest, only a single bee flew out and returned before we displaced the landmark. At the remaining two nests, we were able to observe the behaviour of six bees reacting to landmark displacement. All except one bee seemed confused by the displaced landmark. Four of the bees approached the centre of the displaced landmark and searched for the nest entrance before finally finding the real entrance. One bee approached and landed between the nest entrance and the landmark before inspecting the landmark and finally finding the nest. The flight paths of two bees returning to the nest after the landmark was displaced are shown in Fig. 5c and d, respectively. In addition to the bees from nest with artificial landmarks, some bees from neighbouring nests on the same nest tree also seemed to be confused by the displaced landmark when they returned from foraging trips.

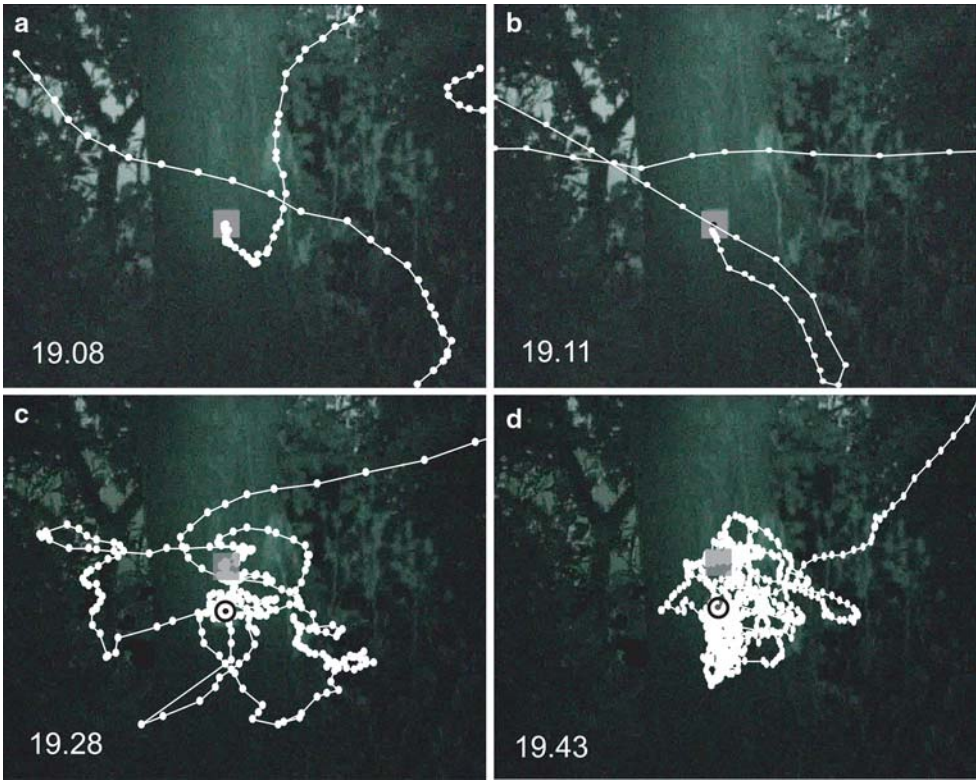


Fig. 5 Two-dimensional reconstruction of flight paths of *X. tranquebarica* at a nest site. The *dots* indicate bee positions every 40 ms, the *square* indicates the position of the white landmark square attached to the nest tree. **a**, **b** Two bees leave the nest and perform very short orientation flights in front of the nest entrance. **c**, **d** The bees return after the landmark position was changed. The nest position is indicated by a *black ring*. The bees approach the landmark before they find the nest entrance, clearly indicating that they used the landmark for orientation

Eye and body measurements

The eyes and ocelli of the three *Xylocopa* species taken with SEM are presented in Fig. 6. Eye and body size measurements are presented in Table 1. *X. tenuiscapa* is the largest, and *X. leucothorax* the smallest, of the three *Xylocopa* species we studied. Eye lengths were very similar in *X. tenuiscapa* and *X. tranquebarica* even though the intertegular width of the latter is smaller than the former by almost 1 mm. The ratio of eye length to intertegular width was largest in *X. tranquebarica* (0.75) followed by *X. tenuiscapa* (0.68) and smallest in *X. leucothorax* (0.55). The number of ommatidia in one eye was also largest in *X.* *tranquebarica* (18,808) and smallest in *X. leucothorax* (12,716). The maximum ommatidial diameter in the frontoventral region of the eye was the same in *X. tenuiscapa* and in *X.* *tranquebarica*, but considerably smaller in *X. leucothorax*. In contrast, the ocelli of *X. leucothorax* and *X. tenuiscapa* are similar in size, whereas those of *X. tranquebarica* have twice the diameter. Thus, with respect to the compound eyes, *X. tenuiscapa* is similar to the nocturnal species, but with respect to the ocelli, this species is similar to the diurnal species of the genus.

We next related intertegular widths to eye measurements in several diurnal, crepuscular and nocturnal bees from our study and from previous studies (Fig. 7). The most obvious difference between nocturnal bees and diurnal or crepuscular bees was in the diameter of the median ocellus. For a given body size, all nocturnal bees have larger ocelli than the diurnal and crepuscular species. In general, for a given body size, ommatidial diameters are also larger in nocturnal bees than in diurnal and crepuscular species (Fig. 7c), but the difference is less pronounced.

Discussion

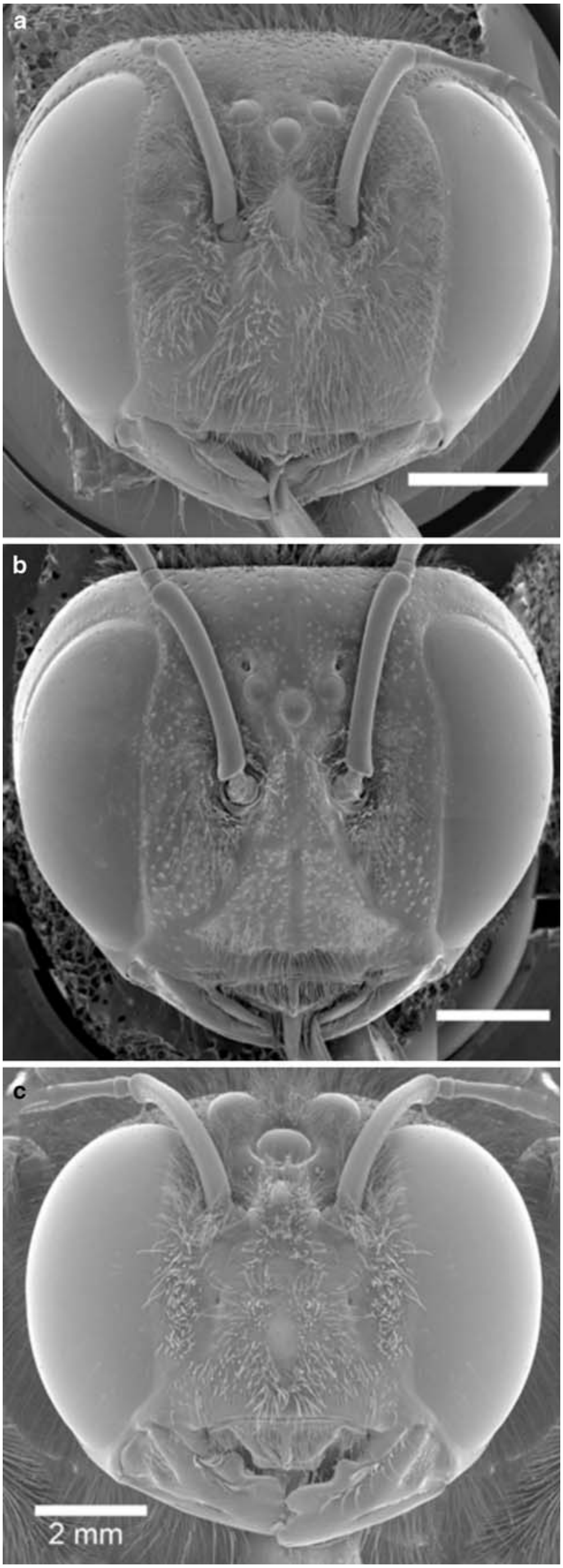
Ambient light intensities and flight activity in the three species

Our observations indicate that flight activity in *X. tranquebarica* commences during the early twilight period. The evening and morning peaks in flight activity may be attributed to bees taking a cue from a certain decrease or increase in light levels to commence or stop foraging in order to arrive at newly opened flowers before other diurnal flower visitors in the morning, or to bees attempting to arrive early in the evening at flowers that have already been depleted by diurnal flower visitors. Early morning peaks in flight activity were also seen in the diurnal *X. tenuiscapa* and *X. leucothorax*.

A recent study on *M. genalis* from Panama in which exact light intensities at nest sites were measured (Warrant et al. 2004; Kelber et al. 2006) shows that they flew during short windows of time, during dawn and dusk, and returned to their nests at least 25 min before the end of astronomical twilight which is about 70 min after sunset in Panama (Warrant et al. 2004; Kelber et al. 2006). In other studies, *X. tranquebarica* (Apidae; Burgett and Sukumalanand 2000), *A. dorsata* (Apidae, Dyer 1985), *A. mellifera* (Apidae; Fletcher 1978) and *L.* (*Sphecodogastra*) *texana* (Halictidae; Kerfoot 1967a) were all active only during moonlit nights. Other dim-light bees such as *Lasioglossum* (*Sphecodogastra*) *galpinsiae* (Bohart and Youssef 1976), *Xenoglossa fulva* (Apidae; Hurd and Linsey 1970), *Ptiloglossa jonesi* (Collettidae; Shelly et al. 1993), *Ptiloglossa arizonensis* (Shelly 1993), *X. olivieri* (Apidae; Gottlieb et al. 2005) and *X. tabaniformis* (Apidae; Janzen 1964) were all active only during dawn or dusk. In this study, we found that *X. tranquebarica* was active even after the end of astronomical twilight when the sun does not contribute to skylight, when the light intensities are down to starlight levels, and when there is no moon in the sky. Light levels during a moonless night are at least 100 times darker than on a moonlit night with a clear sky and under the open sky (Fig. 1). *X. tranquebarica* flew at extremely low light intensities, below 10^{-5} cd m^{-2}.

X. tranquebarica in Thailand has been observed to fly on moonlit, but not on moonless nights (Burgett and Sukumalanand 2000). The extreme nocturnality observed in our population of *X. tranquebarica* may, therefore, be the result of local variation. One sub-generic characteristic of *Nyctomelitta* described by Hurd and Moure (1963) is the large ocellus size. This makes it very likely that all species within this sub-genus are active in dim light. *X. (Nyctomelitta) myops* is also reported to be a pollinator of plants at night in Sarawak (Kato 2006); however, unfortunately little is known about diel flight pattern in the other species in the sub-genus *Nyctomellita*.

It has been hypothesised in the case of *M. genalis* that it is more challenging for dim-light bees in the forest to locate their nests on returning than it is to find flowers that are often bright and release floral odours at night (Warrant et al. 2004; Kelber et al. 2006). *M. genalis* in the same study returned to their nests 45 min before astronomical twilight ended in the evening. Bees that flew out early in the morning during astronomical twilight always returned to their nests at much higher light intensities. In addition, both in the morning and evening, *M. genalis* returned to their nests at approximately the same light intensities. This offers support to the hypothesis that threshold light intensities at the time of returning to nests limit foraging flights in *M. genalis* (Kelber et al. 2006). In comparison, *X. tranquebarica* in our study can find their nests under extremely low light intensities, as on moonless nights and when the sun is lower than 18° below the horizon.



◀ **Fig. 6** Scanning electron micrograph of female heads: **a** *X. leucothorax*, **b** *X. tenuiscapa*, and **c** *X. tranquebarica*. Note the extremely large ocelli of the obligately nocturnal *X. tranquebarica* compared to the two diurnal species. Enlarged ocelli are typical of nocturnal bees; however, *X. tranquebarica* have the largest ocelli measured in any bee so far

X. tenuiscapa are largely diurnal bees with some bees occasionally extending flights into the crepuscular periods. Crepuscular flights in *X. tenuiscapa* mostly result from bees returning late after a long foraging flight that started while the sun was present in the sky. Very few bees left the nest after sunset (see Fig. 3b). These "late" bees always returned to their nests before the end of astronomical twilight in the evening. Only on one occasion (24 February 2005) did a bee remain in flight after astronomical twilight ended in the evening, and this was during a night with a full moon. The crepuscular flights of *X. tenuiscapa* coincided with the availability of *H. quadriloculare* flowers, which open at dusk and stay open all night, providing copious nectar and pollen. These flowers are available for about 2 months during the dry season and *Xylocopa* are their main pollinators (Somanathan and Borges 2001). Night-opening flowers are rare at our study site (Somanathan and Borges, unpublished data) as compared to the neotropics where a large number of flowers are typically bat-pollinated (Wcislo et al. 2004). However, after the end of astronomical twilight, *X. tenuiscapa* stops flying even though flowers still actively produce nectar and pollen and continue to be visited by *X. tranquebarica*. Thus, threshold low light intensities probably limit the degree to which *X. tenuiscapa* can forage under dim-light conditions. *X. leucothorax*, on the other hand, is rarely crepuscular and almost all flight activity occurred during the day at high light intensities.

How different are the three *Xylocopa* eyes?

From our experiments using high contrast, artificial landmarks, we conclude that *X. tranquebarica* uses visual cues to identify and return to their nests. What visual adaptations allow this species to continue flying from, and returning to, nests at extremely low light levels? Moreover, what makes the eyes of this nocturnal species so sensitive that it can continue flight activity when closely related sympatric species cannot? These are questions that we are currently addressing in greater detail. From the eye measurements we present here, we can conclude that large body size, greater eye:body size ratio, unusually large ocelli and large ommatidial diameters are all factors that contribute to increased sensitivity in dim light. The higher eye length to intertegular-width ratio in *X. tranquebarica* compared to *X. tenuiscapa* (which is larger in body size by approximately 1 mm) indicates the important role of vision in the former. While ommatidial diameters are comparatively small in the

Table 1 Body and eye measurements of females of the three *Xylocopa* species

	n	Intertegular width (mm)	Eye length (mm)	Number of ommatidia	Maximum ommatidial diameter (µm)	Diameter of median ocellus (mm)
X. leucothorax	3	7.5 ± 0.8	4.5 ± 0.62	12,716	34.2 ± 2.5	0.40 ± 0.04
X. tenuiscapa	4	8.8 ± 0.4	5.67 ± 0.30	15,994	37.3 ± 3.9	0.50 ± 0.02
X. tranquebarica	4	7.1 ± 0.7	6.6 ± 0.30	18,803	38.7 ± 1.3	0.95 ± 0.07

Values are mean ± standard deviation for all measurements except for the number of ommatidia, which was counted in only one female of each species

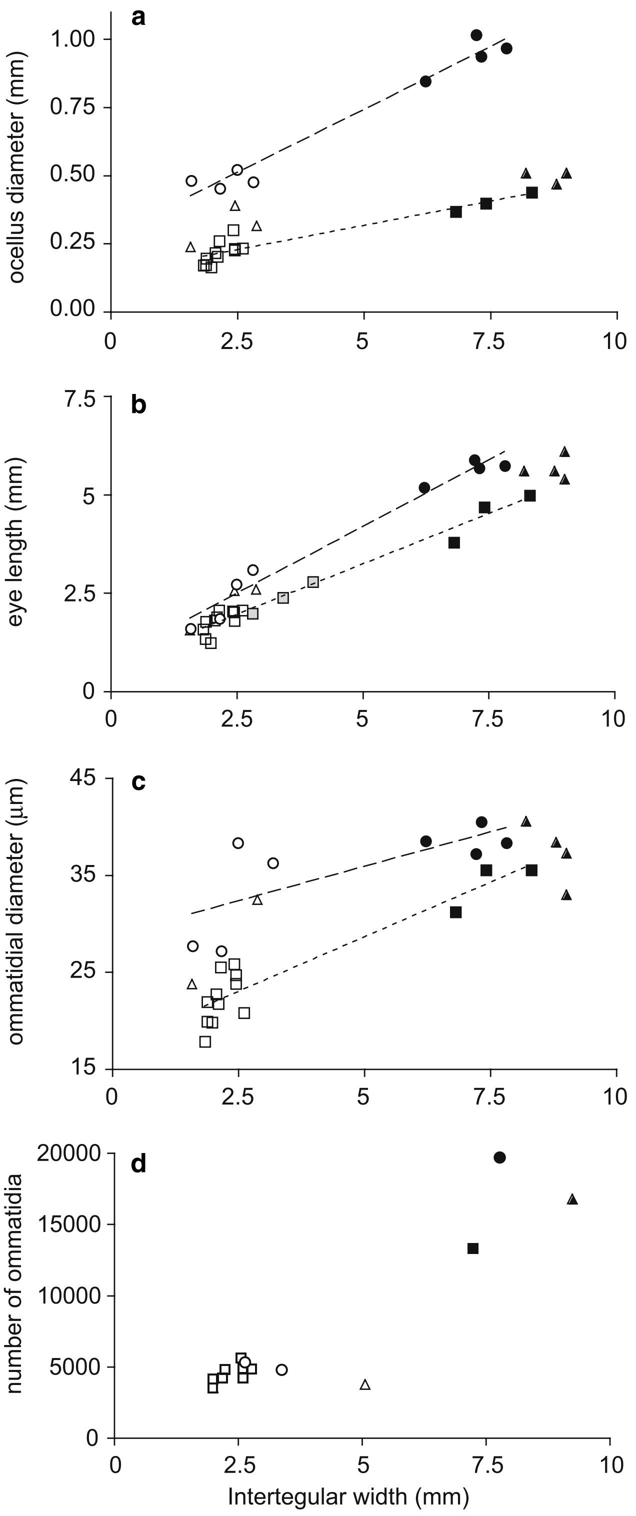
diurnal *X. leucothorax*, the large ommatidial diameters seen in *X. tenuiscapa* and *X. tranquebarica* can improve sensitivity, since large apertures allow greater photon capture under dim light conditions (Warrant 2004). The ocelli of *X. tranquebarica* are the largest among the bees that we compared (Fig. 7) and also probably the largest that have been measured in any bee so far. Nocturnal bees typically have larger ocelli than diurnal bees (Fig. 7; Kerfoot 1967a; Kelber et al. 2006; Warrant et al. 2006). Bee ocelli are badly focussed (Warrant et al. 2006) and thought to be involved in flight stabilisation (Mizunami 1995). Larger ocelli allow a greater amount of light to be collected by the retina (Warrant et al. 2006), although the size difference between the diurnal and nocturnal species is too small (a factor of 4 in area) to fully explain the ability to fly at starlight levels. In contrast to the ocelli, compound eyes have to make a compromise between absolute sensitivity (favoured by large ommatidia) and spatial resolution (favoured by a large number of ommatidia). As a result of their large body size, the numbers of ommatidia are largest in the three *Xylocopa* species of our present study compared to other bees included in this analysis (Fig. 7).

These eye measurements alone are insufficient to explain the extreme nocturnality of *X. tranquebarica*. For example, *X. tenuiscapa* and *X. tranquebarica* vary only slightly in eye length, body size, ommatidial diameters and the number of ommatidia. The difference in the ocellar diameters between these species is more pronounced. Another species *X. latipes* has 16,000 ommatidia (Jander and Jander 2002), approximately 3,000 fewer than *X. tranquebarica*, but is diurnally active (Raju and Rao 2006). This leads us to believe that the eyes of *X. tranquebarica* should have evolved very distinct specialisations for extreme nocturnality. Eye and ocellar size are constrained by energetic costs, and ommatidial diameters could only be further increased at the cost of spatial resolution. In *M. genalis*, the large rhabdom diameter increases both the acceptance angle of the photoreceptors and the probability of photon capture (Greiner et al. 2004a; Warrant et al. 2004). In addition, neuronal processes that possibly intensify visual signals by summing incoming light both spatially and temporally are implicated in their dim-light vision (Warrant et al. 2004; Greiner et al. 2004b). We plan to study additional mechanisms involved in the extreme dim-light vision of *X. tranquebarica*.

Reasons for being nocturnal

What are the benefits of a nocturnal life in bees that have apposition eyes? Hypotheses for the evolution of nocturnality revolve around the two major themes of competition for resources and escape from predators. However, it is difficult to reconstruct past evolutionary processes that were involved in the transition to nocturnality. Hypotheses regarding the evolution of nocturnality have mostly been advanced for bats (Park 1940; Baker and Harris 1957; Luckow and Hopkins 1995). How relevant are these to nocturnal bees? There is some evidence to show that the nocturnal bee *M. genalis* suffers lesser brood parasitism than closely related diurnal species (Wcislo et al. 2004). In our study, the nocturnal species *X. tranquebarica* spends considerable time plugging nest entrances with their abdomens, even during the day, probably to protect their brood from ant predators, indicating the importance of diurnal predation risk in this species. This behaviour was not observed in the two diurnal species. However, little is known about ant predation and protective behaviour in these species.

Another possible explanation for nocturnality in our study species is the role of floral resources. *X. tenuiscapa* and *X. leucothorax* overlap temporally to a large extent and could be potential competitors for floral resources. Observations of diurnal flower visitation on the tree *Randia dumetorum* (Rutaceae) show that both *X. leucothorax* and *X. tenuiscapa* visit these flowers, but were never observed foraging simultaneously on the same tree (HS, unpublished data). We did not see aggressive interactions between the two species at flowers; however, *X. leucothorax* (which is smaller) left a tree as soon as *X. tenuiscapa* arrived. This indicates that these two species, which overlap temporally and utilise common food resources, probably mitigate competition by avoiding simultaneous foraging and also minimise spatial proximity by never sharing a common nesting tree. However, since *X. leucothorax* and *X. tranquebarica* are temporally completely segregated, and probably never encounter each other at floral resources, competition for resources may be mitigated and proximity of nests is



◀ **Fig. 7** Body size correlates in bees. **a** Median ocellus diameter, **b** Eye length, **c** ommatidial diameter, and **d** number of ommatidia as a function of body size in the three *Xylocopa* species (*filled symbols*), *Bombus terrestris* (*grey squares*, data from Späthe and Chittka 2003, Fig. 4a) and a number of other bee species (*open symbols*). *Squares*: diurnal bee species, *filled squares*: *X. leucothorax*, *triangles*: crepuscular or facultatively nocturnal species, *filled triangles*: *X. tenuiscapa*, *circles*: nocturnal bee species, *filled circles*: *X. tranquebarica*. Eye and ocellus data for the other species are from Kelber et al. (2006), but their intertegular widths were determined in this study. Apart from the three *Xylocopa* spp., taxa include *Perdita bohartorum*, *Lasioglossum sisymbrii*, *Lasioglossum leucozonium*, *Neocorynura* sp., *Augochloropsis hebescens*, *Caenaugochlora costariciensis*, *Augochlora nigrocyanea*, *Augochloropsis fuscognatha*, *Pseudaugochloropsis graminea* (all diurnal), *Sphecodogastra lusoria*, *Sphecodogastra aberrans*, *Rhinetula dentricus*, *Megalopta equadoria*, *Megalopta genalis*, *Megalopta* sp, *Perdita bequaertiana* and *Sphecodogastra texanum* (all crepuscular or nocturnal). *Dashed lines* are regression curves for nocturnal species, and *dotted lines* for diurnal species. Correlation coefficients for different measurements and intertegular width in diurnal and nocturnal bees are: 0.98 and 0.99 (eye size), 0.93 and 0.77 (ommatidial diameter) and 0.94 and 0.98 (ocellar diameter)

tolerated. Among nest trees we monitored periodically, we have recorded concurrent sharing, although uncommon, of nest trees by *X. leucothorax* and *X. tranquebarica.* In some other crepuscular and nocturnal bees, there is some evidence that the availability of resources plays a role in the temporal partitioning of activity. Non-tropical bees including *Sphecodogastra* forage on *Oenothera* pollen very efficiently during crepuscular periods (Kelber et al. 2006 and references therein). Moreover, short and efficient foraging trips can reduce the length of time away from the nest at a stretch and thus improve defence against nest parasites. The richness of nighttime floral resources utilised by bats could be related to nocturnal foraging in *M. genalis* in Panama (Wcislo et al. 2004). At our site, facultative nocturnal foraging on *H. quadriloculare* flowers by *X. tenuiscapa* indicates that the seasonal availability of richly rewarding floral resources at night can cause seasonal shifts in temporal activity to crepuscular periods (Somanathan and Borges 2001).

A plausible proximate cause for nocturnality is related to physiology and thermoregulation. Some *Xylocopa* are capable of thermoregulating: thoracic temperatures can be raised for flight at low ambient temperatures (Gerling et al. 1989). Similarly, at high ambient temperatures, the smooth, flat, relatively large abdomen of glabrous species can dissipate heat much more efficiently than that of furry species (Willmer 1988). Nocturnal *X. tranquebarica* is furry and would probably overheat if it flew during the high ambient daytime temperatures found at our study site, while nighttime temperatures can go to as low as 2°C at this site (Somanathan and Borges 2001), when such pubescence might be advantageous. Future studies will focus on visual adaptations, resource partitioning as well as flight performances in these bees.

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