

## Flight performance in night-flying sweat bees suffers at low light levels

Jamie Carroll Theobald<sup>1,\*</sup>, Melissa M. Coates<sup>1</sup>, William T. Wcislo<sup>2</sup> and Eric J. Warrant<sup>1</sup>

<sup>1</sup>Department of Cell and Organism Biology, Helgonavägen 3, Lund University, S-223 62, Lund, Sweden and

<sup>2</sup>Smithsonian Tropical Research Institute, Box 0843-03092, Balboa, Ancon, Republic of Panama

\*Author for correspondence at present address: University of California, Department of Physiological Science, 621 Charles E. Young Dr. South, Box 951606, Los Angeles, CA 90095-1606, USA (e-mail: jamiet@physci.ucla.edu)

Accepted 5 September 2007

### Summary

The sweat bee *Megalopta* (Hymenoptera: Halictidae), unlike most bees, flies in extremely dim light. And although nocturnal insects are often equipped with superposition eyes, which greatly enhance light capture, *Megalopta* performs visually guided flight with apposition eyes. We examined how light limits *Megalopta*'s flight behavior by measuring flight times and corresponding light levels and comparing them with flight trajectories upon return to the nest. We found the average time to land increased in dim light, an effect due not to slow approaches, but to circuitous approaches. Some landings, however, were quite fast even

in the dark. To explain this, we examined the flight trajectories and found that in dim light, landings became increasingly error prone and erratic, consistent with repeated landing attempts. These data agree well with the premise that *Megalopta* uses visual summation, sacrificing acuity in order to see and fly at the very dimmest light intensities that its visual system allows.

Key words: nocturnal vision, flight, apposition eye, visual summation, invertebrate, arthropoda, insecta.

### Introduction

Nocturnal behavior is a successful strategy in insects, but is noticeably rare in certain groups, such as bees (Michener, 2000). To see successfully in dim light, an insect usually requires either optical adaptations to increase light capture, such as superposition eyes, or a significant reduction in visually demanding behaviors, such as flight. Bees meet neither criterion; they all have apposition eyes, and they fly using visual navigation to forage and return to their nests. Nocturnal bees, therefore, are understandably rare. The large neotropical sweat bees of the genus *Megalopta* (Family Halictidae) are an exception. Not only do they fly exclusively at night, but they do so at light levels dimmer than starlight (Warrant et al., 2004; Kelber et al., 2006), and they have undergone an adaptive radiation with 30 described species (Moure and Hurd, 1987; Engel et al., 1997; Wcislo et al., 2004).

*Megalopta* experience such low light levels for several reasons. First, they forage only when the sun is down: shortly before sunrise, and shortly after sunset (Warrant et al., 2004; Kelber et al., 2006). Second, because the sun moves perpendicular to the horizon near the equator, light changes more quickly at sunrise and sunset than at higher latitudes. A mere 15 min before sunrise or after sunset, the sun is significantly below the horizon. Third, *Megalopta* nests are often found under thick canopy in the understory, which can make the forest seem dim even during bright daylight. During active flight times, the forest at nest sites is more than ten times dimmer than the forest edge (Kelber et al., 2006). Taken together, these factors produce what is, to a human observer, impenetrable darkness.

All eusocial bees, ants and wasps are in the monophyletic lineage Euculeata (Grimaldi and Engel, 2005), in which nesting evolved together with nest homing (Jander, 1997). Homing is accomplished in part using remembered visual landmarks (Collett et al., 2007; Collett and Zeil, 1998; Collett, 1992), such as by homing in on a goal (such as minute nest entrances) (Tinbergen, 1932) and piloting along a familiar route (Baerends, 1941), and *Megalopta* likely use both these techniques. During their brief flights from the nest, *Megalopta* undertake the same visual activities as diurnal bees and other insects: they learn landmarks around their nests using orientation flights, navigate to pollen sources, and return to inconspicuous nest entrances (Warrant et al., 2004). For landing at the nest, *Megalopta* use visual navigation to the exclusion of other senses such as olfaction, as they reject even their own nests located just a few cm away, when visual landmarks indicate that another nest is correct (Warrant et al., 2004).

For most animals, reliable vision in this environment requires a nocturnal eye design, which maximizes capture of scarce light, and *Megalopta* has several of the required adaptations. Female *Megalopta genalis* have relatively large eyes (Jander and Jander, 2002). Also, corneal facet diameters are 1.8 times larger, and rhabdom diameters 4–5 times larger, than those of diurnal halictids (Greiner et al., 2004a). These optical adaptations together make the eyes of *M. genalis* 27 times more sensitive than those of their diurnal counterparts (Greiner et al., 2004a; Warrant et al., 2004); this is a worthwhile improvement, but not enough to explain their visual behaviors, such as landing at the nest entrance at night (Warrant et al., 2004). What *Megalopta*

and all other bees lack is the classical nocturnal adaptation for compound eyes: superposition optics. Superposition eyes gather light from many facets, and superimpose it onto a single rhabdom (Land, 1981; Land and Nilsson, 2002). This is the predominant eye design of nocturnal insect groups, such as moths, and can increase light catch and sensitivity by up to several thousand-fold (Nilsson, 1989; Warrant and McIntyre, 1993; Land and Nilsson, 2002).

To bridge the gap between the tremendous light gathering ability of a superposition eye and their own modest optical enhancements, *Megalopta* may rely heavily on neural strategies. Both anatomical (Greiner et al., 2004b; Greiner et al., 2005) and theoretical (Theobald et al., 2006) evidence indicates the use of two forms of neural summation in *Megalopta* optic lobes. Spatial summation, which improves visual reliability by grouping signals from neighboring photoreceptors, and temporal summation, which does so by increasing integration times. These strategies can dramatically improve vision in conditions otherwise plagued by photon noise (Laughlin, 1990; Warrant et al., 1996; Warrant, 1999).

However, while the primary cost of optical strategies is just the extra size, mass and energy required by a bigger eye (Kirschfeld, 1976; Land, 1981; Laughlin et al., 1998), neural summation strategies are costly due to their degradation of visual acuity (Warrant, 1999). Size and mass are certainly important factors for small flying insects, but acuity is equivalent to visual information. The only reason to trade resolution for sensitivity is if it improves visual performance, which is the case in really dark conditions (Snyder et al., 1977a; Snyder et al., 1977b). Without nocturnal optics, a sharp, fast visual system must become blurry and slow, or face an image swamped with photon noise. This is conceptually analogous to the problems faced by a photographer in dim light without a large lens, who must use either grainy film or slow shutter speeds to get a proper exposure. Both techniques sacrifice quality to obtain an image that is otherwise impossible.

The combination of apposition eyes and nocturnal behaviors makes *Megalopta* the ideal candidate to exploit neural summation strategies. If they are, in fact, relying on visual summation to fly at the very darkest limits of their nocturnal activity, do they suffer from reduced acuity? Our goal was to determine whether visual summation, while lowering the light levels at which bees are able to fly, also degrades their capacity for precise flight. After foraging, the return flight to the nest culminates in an approach and landing at the nest entrance. In brighter light this is direct and accurate. We expected that nest returns would become slow and inexact in darker conditions. To assess this we measured light levels at the nest, and reconstructed three-dimensional flight paths of bees returning to the nest.

## Materials and methods

### *Field sites and study species*

We performed experiments and measurements on Barro Colorado Island (BCI), in the Barro Colorado Nature Monument (9°9'N, 79°51'W) in the Republic of Panama, between 15 February and 15 March, 2006; for site details see Rau (Rau, 1933) and Leigh (Leigh, 1999). *Megalopta* is a Neotropical genus of nocturnal and crepuscular sweat bees (Hymenoptera:

Halictidae) (Moure and Hurd, 1987; Engel et al., 1997). *Megalopta genalis* Meade-Waldo is distributed throughout Panama and northern Colombia, and *Megalopta ecuadoria* Friese is found in Panama, Colombia, Ecuador and Brazil (Moure and Hurd, 1987).

### *Light measurement*

To measure photometric light levels we used an International Light IL700 (International Light, Inc., Newburyport, MA, USA) photometer (detector: SHD033 #234) fitted with a lens and filter (Y #21496) to read  $\text{cd m}^{-2}$ . The detector was pointed at the nest site of interest, at a distance of 5 m to avoid disturbing the bees and to include a sample of the scene surrounding the nest. A feature of extended fields is that luminance stays constant with distance; the drop in intensity with distance exactly offsets the extra area included by the projected solid angle. Sampling different directions revealed that at this site, brightness was fairly constant with detector angle. Horizontal directions varied between 0.9 and 1.4 times the brightness pointed at the nest; upwards at the canopy was about 10 times as bright, downwards at the ground was 0.2 times as bright, and these ratios were constant as ambient light changed. We took readings every 2 min during videotaping for approximately 30 min before and after sunrise and sunset. Although *Megalopta* are known to fly earlier in the morning and later at night, at this site they were never observed to do so. All readings were taken with a 1 min integration time.

### *Three dimensional flight paths*

We included both *M. genalis* and the closely related *M. ecuadoria* for this experiment. They were pooled because their nests cannot be reliably distinguished in the field without damaging them and disturbing the bees. We videotaped eight different nests from which bees emerged to forage. Of these, three were indigenous to our site and videotaped where they were discovered. The other five were transported to our site, placed at the locations of old nests, and allowed to settle for at least 3 days before data were taken. Each nest site was 1–1.5 m above ground, and was flanked by at least one hemisphere of open space. This made the nests convenient to locate and videotape. The site was approximately 40 min hike from the nearest buildings on BCI, chosen to minimize light pollution from non-natural sources.

To compute flight paths, we used a pair of consumer video-cameras in 'nightshot' infrared recording mode (Sony Corporation, Tokyo, Japan), which recorded at 30 frames  $\text{s}^{-1}$ , at  $720 \times 576$  pixels. Videotapes provided just over 1 h of recording time, which began approximately 30 min before sunrise or sunset, and ended 30 min after. These limits were chosen after preliminary observations indicated that, at this site, no flights usually took place outside of this time window. We placed video-cameras on tripods at right angles, each 1 m from the entrance of an identified nest and oriented horizontally, with the nest entrance centered in each frame. Cameras were leveled using a level bubble on the tripods, set perpendicular to one another with a known right angle pointed towards each simultaneously, and moved 1 m from the nest entrance (measured with a meter stick). Zoom settings were held constant and absolute lengths were calibrated each time by holding a

ruler at the nest towards each camera in turn, approximately 10 min before taping began (to avoid disturbing the bees).

*Megalopta* monitors the light level from the nest before flying in the morning, and artificial lights near the nest entrance can cause bees to exit earlier than they ever do naturally (Kelber et al., 2006). To avoid this, for each morning recording we set up tripods on the previous night. In the morning, cameras were set up and started in the dark. Likewise, in the evening after recording, cameras were taken down in the dark.

We used auxiliary infrared lights to illuminate bees from below. This produced a bright image, but nightshot is always quite blurry. So although the bee's position could be measured reliably, body axis could not, and we measured only her position in these experiments.

Before each recording session, we synchronized the camera clocks to within 1 s of each other. However, at  $30 \text{ frames s}^{-1}$ , recordings might still be tens of frames off. We used the redundant dimension, up and down, to correct this asynchrony. In other words, in a  $(x, y, z)$  coordinate scheme, one camera captured  $x$  and  $z$  motion, while the other captured  $y$  and  $z$  motion. After data were digitized, we interpolated the paths with a line that presumed each bee's jerk – the derivative of acceleration – to be constant between points. The two views were then synchronized by adding a time offset to the front camera recording that maximized the correlation between the front and side camera  $z$  motion. This adjustment was always small, never more than 0.17 s (five frames), and in over half our recordings it was less than 0.03 s (one frame).

Every video frame at this point had a complement taken at the same moment, but at a  $90^\circ$  angle. A point in an image represents an object in space, relative to the entrance pupil of the camera. In a single image this position is ambiguous, as it could represent any point along a line in that direction, but with two images taken from known, different locations, the three-dimensional coordinates of a point are determined by the intersection of lines projected at the two angles. This is illustrated in Fig. 1. With this set-up we could compute flight paths in a volume of space slightly larger than a 1 m cube.

In three dimensions, non-parallel lines are not guaranteed to intersect, and in practice with our video data, they never did. We used the unique point in space that is simultaneously closest to both lines to estimate a bee's position. This is the midpoint of the line segment that is perpendicular to both these lines. And although in our analysis the line projections never crossed exactly, they never missed by more than a few cm. In particular, the length of the line segment that connected the projected lines was always smaller than 3.5 cm, and always smaller than 1.4 cm when a bee was within 25 cm of the nest entrance. Despite the combination of limited camera resolution, blurry infrared images, and some imprecision of camera positions, we still found test objects could be located within 1 cm of their independently measured positions. The test objects were static, however, and in actual recordings motion blur may have increased the error.

## Results

### *Light levels*

We averaged morning and evening light levels for approximately 1 month (Fig. 2) and found both sunrise and sunset alter the light level by almost 4 orders of magnitude in

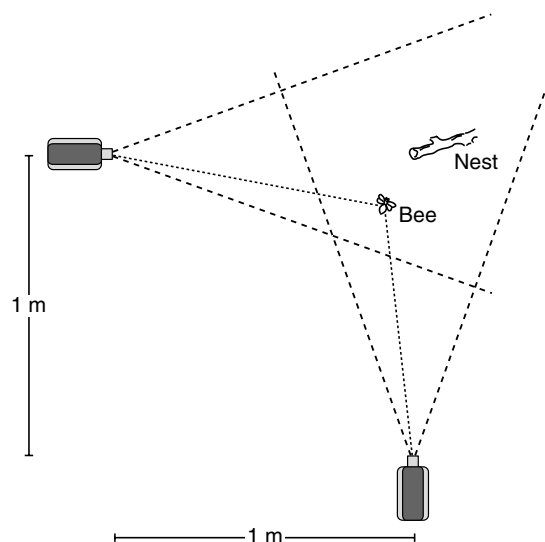


Fig. 1. A top-down schematic of video-camera placement. The cameras were on tripods, set to the same height, and oriented horizontally, as determined by a level. Cameras were at right angles to one another, and both 1 m from the entrance of an identified nest, positioned at the center of each frame. Each camera had its own infrared (IR) light source, and an external array of IR light-emitting diodes lit the area from below; these lights are invisible to humans and bees.

just over 1 h. At the steepest part of this slope, intensity changes by a factor of 10 in only 10–15 min. This rapid change in the available light was coupled with large variation. The 95% confidence intervals of this data show 1–2 orders of magnitude uncertainty in the light level at any time relative to sunrise or sunset.

Notably, this variability was not simply the result of bright days and dim days, which varied, for example, with the phase of the moon. Rather, much of the variability was on a minute-to-minute basis, which is illustrated by several sample curves in Fig. 2 (gray lines). A seemingly bright evening sometimes became much darker than average in just a few minutes. This was largely the result of clouds, which could alter brightness in short time periods. Clouds appearing overhead had a complex effect, and could produce local dimming, for example when they blocked the moon, or brightening, for example when they reflected light from the horizon. Sometimes wind moving the canopy leaves also had a small effect.

Although mean light levels were fairly symmetrical between morning and evening, we found a curious increase in light variability after sunset, which had no counterpart in the early morning. We do not know the cause, and it may have been just a peculiarity of our sample, but one striking difference between mornings and evenings is air temperature at the ground. Since cloud formation and position is driven by temperature and humidity, and clouds probably caused most of the variation in light level at a given time of day, temperature is a good candidate to explain this variability.

### *Flight trajectories and duration*

Our reconstructed flight paths were videotaped with simultaneous light measurements at the site of the nest. For the

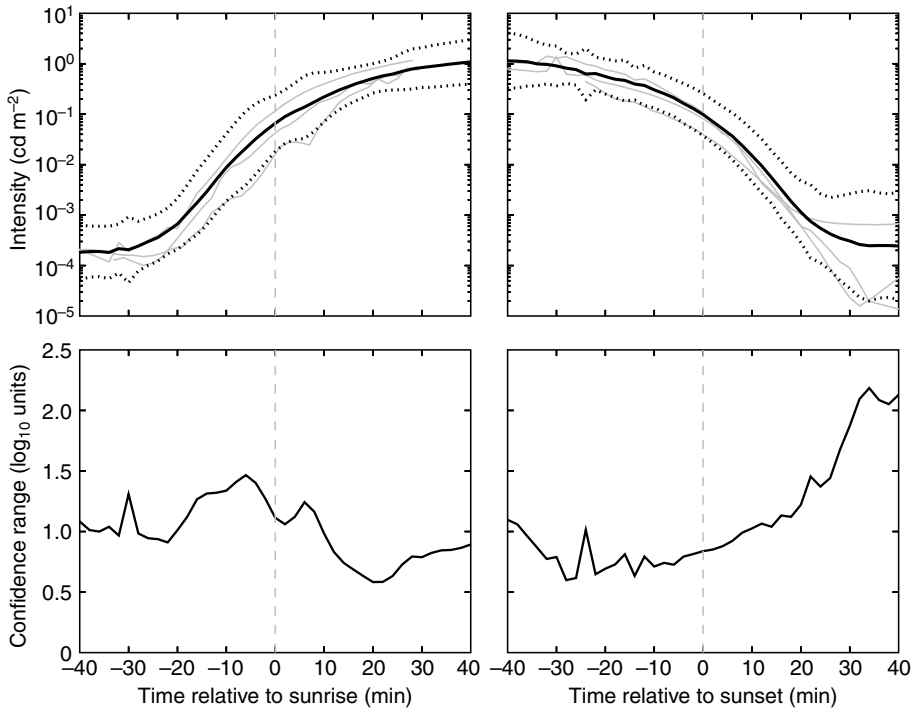


Fig. 2. Nest site light levels at dawn and dusk, in human photometric light units. The upper plots show light levels as a function of time relative to the sun at the horizon. The black trace is the mean, dotted lines show 95% confidence intervals, and gray lines show several sample traces. The lower plots show the magnitude of the 95% confidence intervals. Light changes most when the sun is just below the horizon, by about an order of magnitude in just 10 min. The uncertainty in light intensity is around an order of magnitude for any time around this window.

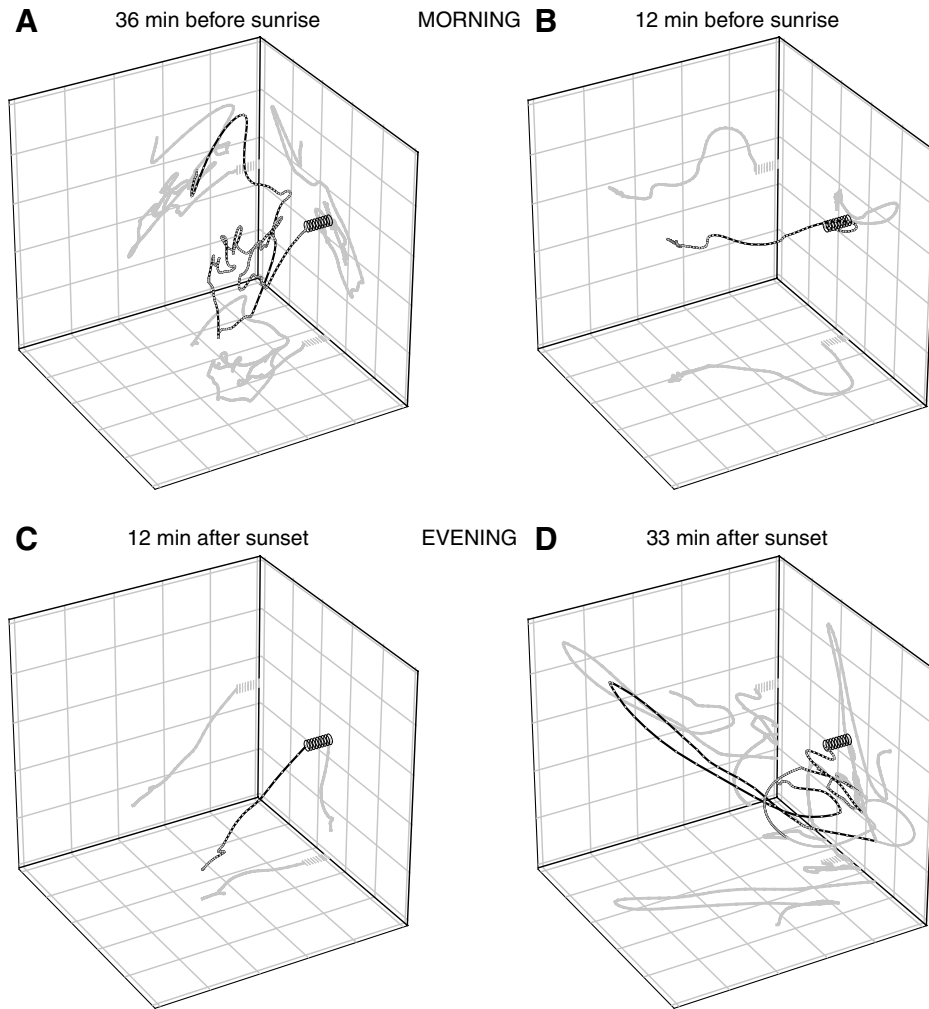


Fig. 3. Example paths of four return flights at early and late times relative to sunrise and sunset. In each plot, the flight path is in black, with white markers to indicate the sample points. The nest entrance is shown as a cylinder on the right wall, and two-dimensional projected flight paths are shown as gray shadows on the right, left and bottom walls. Luminance in the early morning was  $1.1 \times 10^{-4} \text{ cd m}^{-2}$  and the landing lasted 11.4 s; late morning was at  $1.9 \times 10^{-3} \text{ cd m}^{-2}$  and the landing lasted 4.7 s; early evening was at  $3.9 \times 10^{-3} \text{ cd m}^{-2}$  and the landing lasted 1.8 s; and late evening was at  $3.9 \times 10^{-4} \text{ cd m}^{-2}$  and the landing lasted 16.2 s. Each grid square is 10 cm per side.

month of our measurements, no bees flew while the sun was up, with the single exception of an orientation flight that occurred just 4 min before sunset.

We recorded and analyzed the flight paths from 37 nest returns. These approaches usually occurred from the same direction, the front of the nest, facing open air space. Most striking were the long, circuitous flights that occurred only in darker conditions, and never when the sun was near the horizon (Fig. 3A,D). In contrast, bees in brighter conditions invariably took short and quick routes to the nest entrance (Fig. 3B,C). The figure shows example landings that involved longer paths and more time in darker conditions. These winding flights were present in the darkest flights of both mornings and evenings. However, flights in the dark were not exclusively long. Fig. 4 plots the duration of each return flight against light intensity. Although the longest returns occurred only in darker conditions, many quick returns took place in the dark as well.

To determine the underlying cause of the longer duration flights, we examined two factors that would slow a bee's approach to the nest: speed and path length. In other words, a bee would spend more time approaching the nest if it either flew more slowly, or took a more circuitous route (or both). Fig. 5A traces speed through the course of two sample morning landings (which are also plotted in Fig. 3). Although there may be qualitative differences between the two, such as the spikes in speed early in the longer flight, the mean speeds are nearly identical. Fig. 5B shows the mean speeds for all the landings, which do not vary significantly with duration of landing. The cumulative path lengths of the same example flights (Fig. 5C), which are the integrals of the speed traces, show that the dim

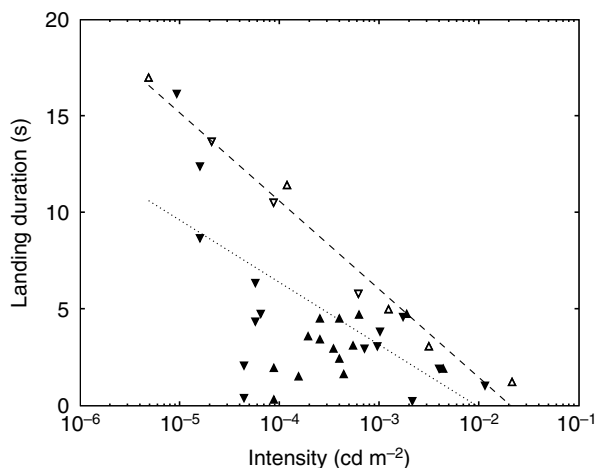


Fig. 4. The time required to land on the nest, relative to appearing in the camera view, for bees at different light levels. Upward triangles denote morning flights, downward triangles denote evening flights. The dotted line is a linear regression for all the data (on log-transformed intensity values: slope =  $-3.22$ , intercept =  $-6.54$ ), and although it is significantly different from 0 ( $t=5.01$ , d.f. = 35,  $P < 0.001$ ) it explains little of the variance ( $r^2=0.41$ ). The broken line is a regression of only the maximal flight lengths at each intensity (denoted by open triangles), where the range of intensities was divided into 8 half log unit bins. The variance of this upper edge of data is explained well by linear regression (slope =  $-4.57$ , intercept =  $-7.69$ ,  $r^2=0.97$ ) and is statistically significant ( $t=14.97$ , d.f. = 6,  $P < 0.001$ ).

light landing covered more than twice the distance of the bright light landing. Fig. 5D shows the total path lengths of all the landings, and the significant linear relationship they have with landing duration. This implies the longer flights under darker conditions resulted from longer, not slower, approaches.

To determine whether these longer approaches were the result of smoothly edging towards the nest (such as a spiral approach), or something more erratic, we chose an arbitrary distance of 15 cm from the nest entrance, and recorded how often each bee entered and left this imaginary threshold sphere. Fig. 5E shows the distance from the nest for the sample flights as a function of time, and a horizontal threshold marker. The derivative of this distance trace is not velocity, since it is a scalar distance between the bee and its nest. For example, a bee could move quite quickly, but orbit at a constant nest distance. The final plot (Fig. 5F) shows for each flight, the number of approaches crossing this threshold, against landing duration. The number of approaches is significantly related to flight duration, supporting the notion that the longer landings are erratic.

To understand the structure of these flights, we examined the changes in flight speeds as they varied with nest distance. Fig. 6A shows a box plot of speeds (both dark and light flights) as they varied with distance from the nest entrance. The median speeds near the nest ( $<10$  cm) dropped to about a third of the speeds from farther out ( $>50$  cm). More notable, however, was the range of speeds farther out, nearly an order of magnitude greater than the range near the nest. Put another way, the nearer the nest, the more restricted flight speed became. We then broke these motion vectors into components parallel and perpendicular to the nest axis (Fig. 6B). Most of this variation – not necessarily most of the motion – is from flight parallel to the nest axis.

## Discussion

### *Landing in the dark is sometimes quick*

The longest bee landings occurred in the dimmest light, but darker conditions also included many short landings, some as short as the landings in bright light. This accounts for the somewhat triangular spread of the data points in Fig. 4: bright light landings were always quick, but low light landings included both quick and slow samples. The quick landings in the dark were not obviously different from the quick landings in the light, so what caused some landings in the dark to last much longer than others? Put another way, why were some landings quick and accurate despite so little light?

We had anticipated that bees might advance more slowly in the dark, temporally integrating visual information as they homed in on their nests. Low flight speeds would benefit any onlooker faced with an unreliable scene, or low acuity caused by spatial and temporal pooling. Nevertheless, the bees that landed in dim light did not move slowly.

Two considerations might explain why they did not. First, slow flight and hovering are difficult skills even in bright light, but they pose special problems at night (O'Carroll et al., 1996). They require sensitivity to low velocity motion, which is coded in a visual scene in high spatial frequencies and low temporal frequencies (the velocity of any sinusoidal component of a scene is  $f_t/f_s$ , the temporal divided by the spatial frequency) (O'Carroll et al., 1997). Diurnal hovering insects, such as dragonflies, have

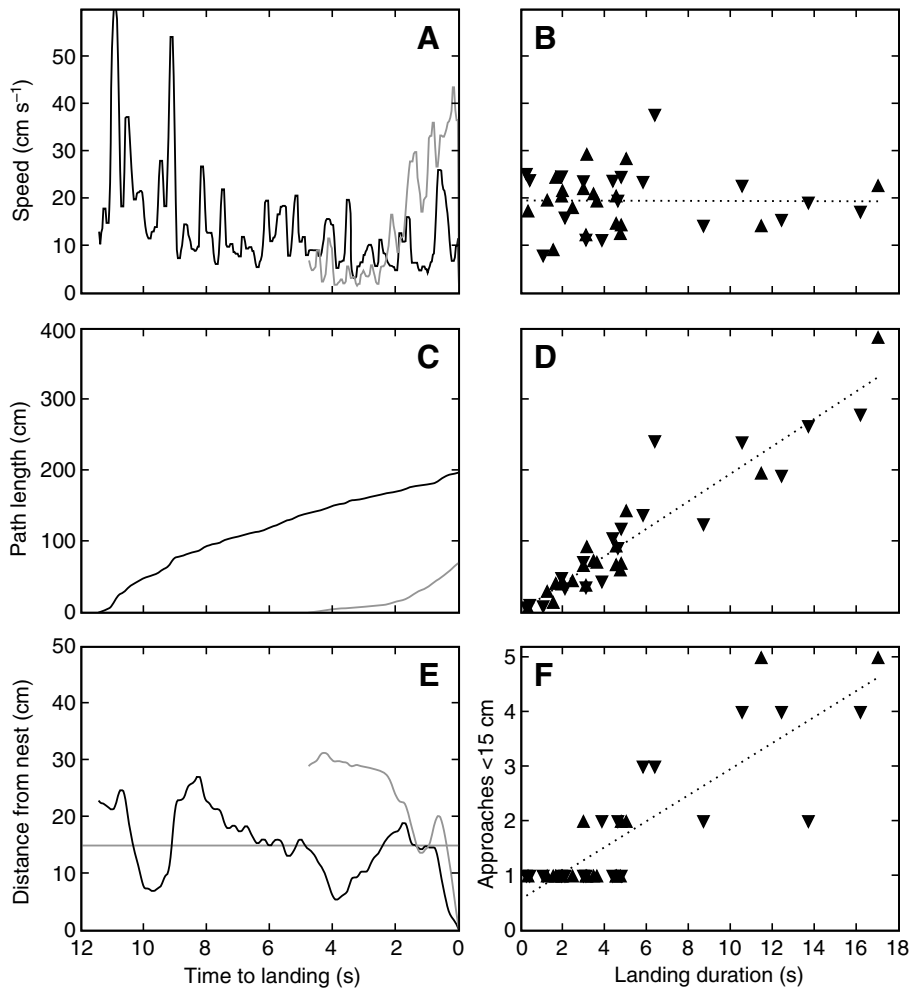


Fig. 5. Bee speed and relative position. (A) Flight speed of the two landing bees shown in Fig. 3 (morning plots: black line, 36 min before sunrise; gray line, 12 min before sunrise). (B) Mean speed of each flight plotted in Fig. 4 relative to landing duration, and a linear regression (slope= $-0.01$ , intercept= $19.65$ ) that explains little of the variation ( $r^2 < 0.001$ ) and is not significantly different from no slope ( $t = 0.04$ , d.f. = 35,  $P = 0.48$ ). (C) Cumulative path lengths of the same sample flights from A; the value at landing (time = 0) is the total path length. (D) Total path lengths of each flight relative to landing duration, and a linear regression (slope =  $19.37$ , intercept =  $2.75$ ) that explains most of the variation ( $r^2 = 0.88$ ), and is significantly different from no relationship ( $t = 16.08$ , d.f. = 35,  $P < 0.001$ ). (E) Absolute distance from the nest entrance as the sample flights from A progress, showing multiple crossings of the 15 cm threshold (gray horizontal line). (F) The number of approaches closer than this threshold versus landing duration, for each flight in all sampled bees. The regression (slope =  $0.23$ , intercept =  $-0.58$ ) is significant ( $r^2 = 0.73$ ,  $t = 9.67$ , d.f. = 35,  $P < 0.001$ ). In each figure, upward triangles mark morning flights, downward triangles mark evening flights.

unusually high acuity compound eyes. Nocturnal hovering insects, such as hawkmoths, lose acuity to spatial pooling, and so must be sensitive to very low temporal frequencies. All things considered, night hovering may actually be more difficult than night nest finding. Second, it has been shown that bees (Cartwright and Collett, 1979) and wasps (Zeil, 1993) learn the speed at which landmark images move across the retina as they fly towards their goal. It is possible that, even as light levels and acuity drop, bees cannot slow their approaches without compromising landmark recognition near the nest.

Instead, the advancing and retreating flights noted in Fig. 5F and Fig. 6B offer the compelling interpretation that each advance is a landing attempt. *Megalopta* land with certainty in the bright light, but as it darkens the chance of a successful landing the first time drops, and the average number of attempts increases. This accounts for the variable flight time in the dark by probability; some landings worked on the first try, others took multiple passes. It is also consistent with visual summation; bees faced with blurry images of their nests might often miss the landing. Finally, it is consistent with orientation behavior in diurnal bees, where perturbations to previously learned landmarks induce multiple approaches and retreats from a nest entrance (Tinbergen, 1972; Weislo, 1992).

When navigating towards a close goal, insects continually

compare their current retinal image with memory 'snapshots' of landmarks and move to reduce discrepancies between the two (Cartwright and Collett, 1983; Junger, 1991). Whether in the morning or evening, *Megalopta* leaves and returns to the nest at different light intensities. Matching an image remembered from a different light level could be problematic because dim light produces a mismatch between viewed and remembered images (Zeil et al., 2003). This problem is minimized, however, since optimal vision in the dark favors low frequency spatial information (Warrant, 1999), which corresponds to the highest contrasts in natural scenes (Burton and Moorhead, 1987; Ruderman, 1994). Further, natural images are easily transformed to reduce the effects of illumination (Sturzl and Zeil, 2007).

*Megalopta's* landing attempts can be roughly compared to the familiar task of finding a light switch in the dark. We might study the wall with night vision and gradually locate the switch, or simply start groping around. Surely we use both strategies to light up the room as quickly as possible. *Megalopta* probably uses both strategies as well, but the attempted landings may dominate the obvious structure of the flight. Occasional quick landings in the dark may be the pay-off for taking a guess even when uncertain. One difference that favors a bee over a light switch hunter is that as a bee moves in, she gets a closer view

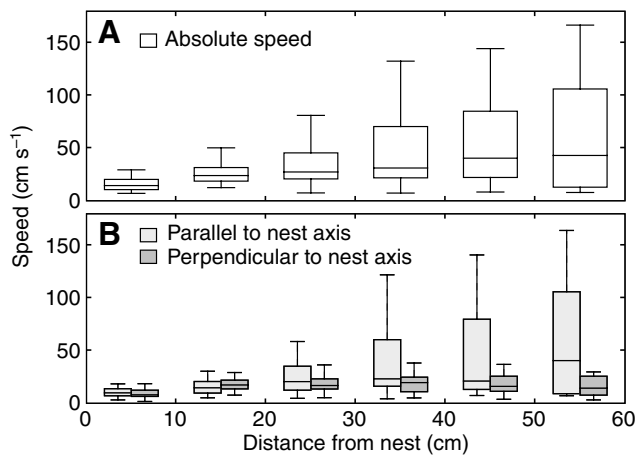


Fig. 6. A box plot showing the distribution of flight speeds as they vary with distance from the nest. In (A) the flights are pooled and divided into bins of 10 cm increments increasingly far from the nest. Each box extends between the lower and upper quartiles of flight speeds, with a line at the median and whiskers showing the range. Near the nest the median flight speed was low (14.5 cm s<sup>-1</sup>, while closer than 10 cm) about a third compared to speeds far from the nest (43.1 cm s<sup>-1</sup> while between 50 and 60 cm). (B) These speeds broken down into vector components parallel and perpendicular to the axis of the nest. Each measured absolute speed in A is the square root of the sum of squares of the components in B.

of her target. A difference that works against a bee, in contrast, is that without feet on the ground, she can use only visual information to estimate her own motion. This could potentially lead to unintended collisions with surrounding sticks, but we didn't observe this. As noted before, the nests we videotaped had wide, open spaces around at least a hemisphere of the entrance, and bees approached from this direction for landing.

#### *Sometimes bees failed to return during our videotaping*

We restricted our analysis to flights that ended in a successful landing. However, occasionally in the evenings we observed flights that did not end with a landing (Fig. 7). Often a bee had not returned even 20 min after the attempt, at which point the

videotape ended. In every case bees were present the next morning, but when and how they returned is unknown. One possibility is that they made more attempts later in the night, although it would be no brighter before the morning. Another possibility is that bees landed nearby and walked back, and although this would be an inherently risky strategy in the crowded rainforest, there is at least one example of a diurnal neotropical bee that walks to her nest after foraging (Cameron and Whitfield, 1996). It remains uncertain how well a bee that navigates by vision and usually approaches her nest in the air, can find her nest on foot. Possibly olfactory cues, although not part of regular landing (Warrant et al., 2004), come into play in this situation (Wcislo, 1992).

On some mornings we observed early returning bees unsuccessfully attempt to land, but they simply left and returned later. Since light levels often increased by an order of magnitude in just 10 min, a bee struggling to locate her nest might have no problems just minutes later.

In this sense, morning flights are less risky than evening flights. *Megalopta* are in some sense, risk-averse bees, guarding their nests almost full time. This is important, since unprotected nests are highly prone to ant predation (Smith et al., 2003). The observation that bees seem sometimes unable to return from evening flights is not in line with this general strategy. Further, the degradation of their flight performance in the dark is risky as well, since swooping bats were videotaped near the nest on an almost daily basis.

#### *Other sources of variation*

Light level is only one of several factors potentially affecting the accuracy of bee flights. In this study we pooled *M. genalis* and *M. ecuadoria*, but *M. genalis* has larger eyes, which therefore collect more light. Both species have large eyes relative to body size, and this shows the evolutionary importance of vision for these groups (Jander and Jander, 2002). But absolute eye size determines how much light is collected, and thus limits resolution and sensitivity. Even eye size variations between individuals of the same species might matter when resolving a dark image.

As mentioned above, bees probably use memorized landmark images, or 'snapshots', on the outward and inward routes, which

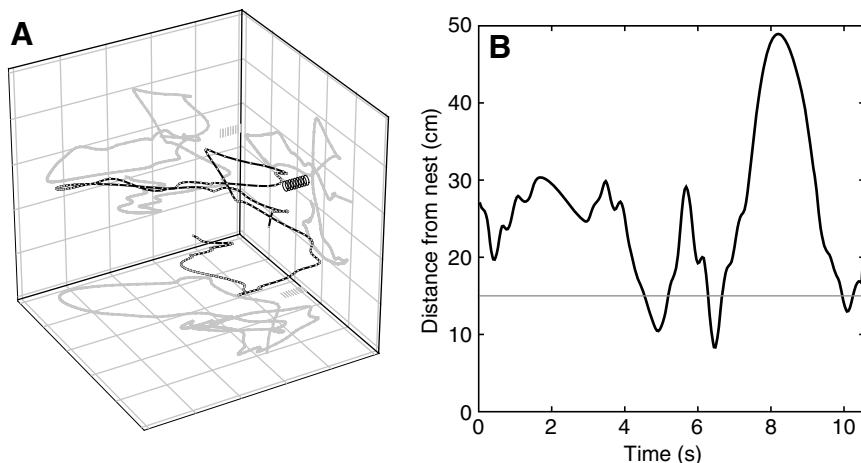


Fig. 7. A returning bee that does not land during the videotaping period. (A) The three-dimensional flight path of a returning bee 33 min after sunset, having left the nest 14 min earlier (19 min before sunset). The light level during this return was  $1.1 \times 10^{-4}$  cd m<sup>-2</sup>. (B) Running distance from the nest while the bee is visible to both cameras. The horizontal gray line shows the 15 cm threshold, for comparison with Fig. 5D,E. Each grid square in A is 10 cm per side.

are of large size objects, for example the canopy patterns, which some ants are known to use for orientation (Hölldobler, 1980). These probably get bees close to the vicinity of the nest, and the quality of these snapshot memories may vary from bee to bee. Bees with better memories and better snapshots could find the nest more quickly.

The terrain itself may also provide better snapshots. An area with conspicuous landmarks might be fundamentally more navigable than one without features noticeable to a bee. For example, honeybee navigation utilizes visual odometry and landmarks, but will favor landmarks in situations of ambiguity (Vladusich et al., 2005). Further, odometry varies with the properties of the terrain (Tautz et al., 2004). Even if bees began with good, recognizable nest locations, the nests are built in detached, dead sticks, which can be moved by wind, gravity and larger animals (biologists, for example).

Finally, many environmental and physiological issues might affect the quality of any single landing, such as wind, temperature, pollen load, energy level or bee age.

#### *Predictions from information theory*

If evolution produces eyes to maximize information capacity, then the optimal resolution depends on both light level and image speed (Snyder et al., 1977a; Snyder et al., 1977b). This means animals that often view dim or fast visual scenes need lower visual acuity to collect more information. This study demonstrates that *Megalopta* view a wide range of brightnesses and probably a wide range of image speeds, making them prime candidates to vary their acuity with their immediate conditions. This study measured light levels and bee speeds, but determining image velocity on the retina requires body and head orientation, which we could not measure (the video camera is also limited to blurry images in the dark). Better cameras and more infrared light might allow this level of analysis.

However, comparative behavior would also offer insight into this problem. As noted above, visual acuity is limited by absolute eye size, meaning that smaller bees with smaller eyes have much lower spatial resolution, no matter what light levels and image speeds they view (Jander and Jander, 2002). Tellingly, smaller bees also have more erratic nest approaches (Decelles and Laroca, 1979), and we surmise that low acuity from small size and low acuity from dim light vision have parallel effects on nest approaches. A direct comparison of flight paths from nocturnal bees and small diurnal bees could test this experimentally.

#### *Conclusions*

*Megalopta* have evolved into a niche rarely occupied by bees, and thus presumably enjoy reduced predation and competition (Wcislo et al., 2004). However, they have moved into this niche without the most powerful optical adaptations of their nocturnal competitors. This study shows that *Megalopta* flies in light so dim that flight performance is compromised, and suggests the benefits of a nocturnal lifestyle may have pushed them to fly near the very limit of their visual ability.

We wish to thank Juan Carlos Di Trani and Dr Adam Smith, for their consummate ability to find *Megalopta* nests for these experiments, and the gracious STRI staff, in Panama City and

BCI, who helped us with countless logistic details. We thank Dr Wendy Theobald, along with two anonymous reviewers, who greatly improved this manuscript by providing insightful comments. This work was funded by the National Science Foundation International Research Fellowship Program grant 0401906 to J.C.T.

#### References

- Baerends, G. P. (1941). Fortpflanzungsverhalten und Orientierung der Grabwespe, *Ammophila campestris*, Jur. Tijdschr. Entomol. **84**, 81-275.
- Burton, G. J. and Moorhead, I. R. (1987). Color and spatial structure in natural scenes. *Appl. Opt.* **26**, 157-170.
- Cameron, S. and Whitfield, J. B. (1996). Use of walking trails by bees. *Nature* **379**, 125.
- Cartwright, B. A. and Collett, T. S. (1979). How honey-bees know their distance from a near-by visual landmark. *J. Exp. Biol.* **82**, 367-372.
- Cartwright, B. A. and Collett, T. S. (1983). Landmark learning in bees: experiments and models. *J. Comp. Physiol.* **151**, 521-543.
- Collett, T. S. (1992). Landmark learning and guidance in insects. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **337**, 295-303.
- Collett, T. S. and Zeil, J. (1998). Places and landmarks: an arthropod perspective. In *Spatial Representation in Animals* (ed. S. Healy), pp. 18-53. Oxford: Oxford University Press.
- Collett, T. S., Graham, P. and Harris, R. A. (2007). Novel landmark-guided routes in ants. *J. Exp. Biol.* **210**, 2025-2032.
- Decelles, P. and Laroca, S. (1979). Behavioral interactions among solitary foraging bees (hymenoptera: Apoidea). *J. Kans. Entomol. Soc.* **52**, 483-488.
- Engel, M. S., Brooks, R. W. and Yanega, D. (1997). New genera and subgenera of augochlorine bees (Hymenoptera: Halictidae). *Sci. Pap. Univ. Kansas Nat. Hist. Mus.* **5**, 1-21.
- Greiner, B., Ribí, W. and Warrant, E. (2004a). Retinal and optical adaptations for nocturnal vision in the halictid bee *Megalopta genalis*. *Cell Tissue Res.* **316**, 377-390.
- Greiner, B., Ribí, W., Wcislo, W. and Warrant, E. (2004b). Neural organisation in the first optic ganglion of the nocturnal bee *Megalopta genalis*. *Cell Tissue Res.* **318**, 429-437.
- Greiner, B., Ribí, W. and Warrant, E. (2005). A neural network to improve dim-light vision? Dendritic fields of first-order interneurons in the nocturnal bee *Megalopta genalis*. *Cell Tissue Res.* **322**, 313-320.
- Grimaldi, D. and Engel, M. (2005). *Evolution of the Insects*. Cambridge: Cambridge University Press.
- Hölldobler, B. (1980). Canopy orientation: a new kind of orientation in ants. *Science* **210**, 86-88.
- Jander, R. (1997). Macroevolution of a fixed action pattern for learning: the exploration flights of bees and wasps. In *Comparative Psychology of Invertebrates: the field and laboratory study of insect behavior* (ed. G. Greenberg and E. Tobach), pp. 79-99. New York: Garland.
- Jander, U. and Jander, R. (2002). Allometry and resolution of bee eyes (Apoidea). *Arthropod Struct. Dev.* **30**, 178-193.
- Junger, W. (1991). Waterstriders (*Gerris paludum* F.) compensate for drift with a discontinuously working visual position servo. *J. Comp. Physiol. A* **169**, 633-639.
- 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.
- Kirschfeld, K. (1976). The resolution of lens and compound eyes. In *Neural Principles of Vision* (ed. F. Zettler and R. Weiler), pp. 354-370. Berlin: Springer.
- Land, M. F. (1981). Optics and vision in invertebrates. In *Comparative Physiology and Evolution of Vision in Invertebrates, Handbook of Sensory Physiology*. Vol. VII/6B (ed. H. Autrum), pp. 471-592. Berlin: Springer.
- Land, M. F. and Nilsson, D. E. (2002). *Animal Eyes (Oxford Animal Biology Series)*. Oxford: Oxford University Press.
- Laughlin, S. B. (1990). Invertebrate vision at low luminances. In *Night Vision* (ed. R. F. Hess, L. T. Sharpe and K. Nordby), pp. 223-250. Cambridge: Cambridge University Press.
- Laughlin, S. B., de Ruyter van Steveninck, R. R. and Anderson, J. C. (1998). The metabolic cost of neural information. *Nat. Neurosci.* **1**, 36-41.
- Leigh, E. G., Jr (1999). *Tropical Forest Ecology: A view from Barro Colorado Island*. New York: Oxford University Press.
- Michener, C. D. (2000). *The Bees of the World*. Baltimore: Johns Hopkins University Press.
- Moore, J. S. and Hurd, P. D. (1987). *An Annotated Catalog of the Halictid Bees of the Western Hemisphere*. Washington, DC: Smithsonian Institution Press.



- Nilsson, D.-E.** (1989). Optics and evolution of the compound eye. In *Facets of Vision* (ed. D. G. Stavenga and R. C. Hardie), pp. 30-73. Berlin: Springer-Verlag.
- O'Carroll, D., Bidwell, N., Laughlin, S. and Warrant, E.** (1996). Insect motion detectors matched to visual ecology. *Nature* **382**, 63-66.
- O'Carroll, D., Laughlin, S., Bidwell, N. and Harris, R.** (1997). Spatio-temporal properties of motion detectors matched to low image velocities in hovering insects. *Vision Res.* **37**, 3427-3439.
- Rau, P.** (1933). *The Jungle Bees and Wasps of Barro Colorado Island*. St Louis: Von Hoffman Press.
- Ruderman, D. L.** (1994). The statistics of natural images. *Network Comput. Neural Syst.* **5**, 517-548.
- Smith, A. R., Wcislo, W. T. and O'Donnell, S.** (2003). Assured fitness returns favor sociality in a mass-provisioning sweat bee, *Megalopta genalis* (Hymenoptera: Halictidae). *Behav. Ecol. Sociobiol.* **54**, 14-21.
- Snyder, A., Laughlin, S. and Stavenga, D.** (1977a). Information capacity of eyes. *Vision Res.* **17**, 1163-1175.
- Snyder, A., Stavenga, D. and Laughlin, S.** (1977b). Spatial information capacity of compound eyes. *J. Comp. Physiol. A* **116**, 183-207.
- Sturzl, W. and Zeil, J.** (2007). Catchment areas of panoramic snapshots in outdoor scenes. *Biol. Cybern.* **96**, 519-531.
- Tautz, J., Zhang, S., Spaethe, J., Brockmann, A., Si, A. and Srinivasan, M.** (2004). Honeybee odometry: performance in varying natural terrain. *PLoS Biol.* **2**, E211.
- Theobald, J. C., Greiner, B., Wcislo, W. T. and Warrant, E. J.** (2006). Visual summation in night-flying sweat bees: a theoretical study. *Vision Res.* **46**, 2298-2309.
- Tinbergen, N.** (1932). Ueber die orientierung des bienenwolfes (*Philanthus triangulum* Fabr.). *Z. Vergl. Physiol.* **16**, 305-335.
- Tinbergen, N.** (1972). On the orientation of the digger wasp *Philanthus triangulum* Fabr. I. In *The Animal in its World*. Vol. I (ed. A. Snyder), pp. 103-127. Cambridge, MA: Harvard University Press.
- Vladusich, T., Hemmi, J., Srinivasan, M. and Zeil, J.** (2005). Interactions of visual odometry and landmark guidance during food search in honeybees. *J. Exp. Biol.* **208**, 4123-4135.
- Warrant, E.** (1999). Seeing better at night: life style, eye design and the optimum strategy of spatial and temporal summation. *Vision Res.* **39**, 1611-1630.
- Warrant, E. and McIntyre, P.** (1993). Arthropod eye design and the physical limits to spatial resolving power. *Prog. Neurobiol.* **40**, 413-461.
- Warrant, E. J., Porombka, T. and Kirchner, W.** (1996). Neural image enhancement allows honey bees to see at night. *Proc. R. Soc. Lond. B Biol. Sci.* **263**, 1521-1526.
- Warrant, E., Kelber, A., Gislén, A., Greiner, B., Ribí, W. and Wcislo, W.** (2004). Nocturnal vision and landmark orientation in a tropical halictid bee. *Curr. Biol.* **14**, 1309-1318.
- Wcislo, W. T.** (1992). Nest localization and recognition in a solitary bee, *Lasioglossum figueresi* wcislo (Hymenoptera: Halictidae), in relation to sociality. *Ethology* **92**, 108-123.
- Wcislo, W. T., Arneson, L., Roesch, V. K. and Gonzalez Smith, A. and Fernández, H.** (2004). The evolution of nocturnal behaviour in sweat bees, *Megalopta genalis* and *M. ecuadoria* (Hymenoptera: Halictidae): an escape from competitors and enemies? *Biol. J. Linn. Soc. Lond.* **83**, 377-387.
- Zeil, J.** (1993). Orientation flights of solitary wasps (*Cerceris*; Sphecidae; Hymenoptera). II: Similarities between orientation and return flights and the use of motion parallax. *J. Comp. Physiol. A* **172**, 207-222.
- Zeil, J., Hofmann, M. I. and Chahl, J. S.** (2003). Catchment areas of panoramic snapshots in outdoor scenes. *J. Opt. Soc. Am. A* **20**, 450-469.