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Nocturnal Vision and Landmark Orientation in a Tropical Halictid Bee

Eric J. Warrant,[1,*] Almut Kelber,[1] Anna Gislén,[1] Birgit Greiner,[1] Willi Ribi,[2] and William T. Wcislo[3]
[1]Department of Cell and Organism Biology
Zoology Building
University of Lund
Helgonavägen 3
S-22362 Lund
Sweden
[2]University of the Principality of Liechtenstein
P.O. Box 535
FL-9495 Triesen
Liechtenstein
[3]Smithsonian Tropical Research Institute
Apartado 2072 Balboa
Repubic of Panama

Summary

Background: Some bees and wasps have evolved nocturnal behavior, presumably to exploit night-flowering plants or avoid predators. Like their day-active relatives, they have apposition compound eyes, a design usually found in diurnal insects. The insensitive optics of apposition eyes are not well suited for nocturnal vision. How well then do nocturnal bees and wasps see? What optical and neural adaptations have they evolved for nocturnal vision?

Results: We studied female tropical nocturnal sweat bees (*Megalopta genalis*) and discovered that they are able to learn landmarks around their nest entrance prior to nocturnal foraging trips and to use them to locate the nest upon return. The morphology and optics of the eye, and the physiological properties of the photoreceptors, have evolved to give *Megalopta's* eyes almost 30 times greater sensitivity to light than the eyes of diurnal worker honeybees, but this alone does not explain their nocturnal visual behavior. This implies that sensitivity is improved by a strategy of photon summation in time and in space, the latter of which requires the presence of specialized cells that laterally connect ommatidia into groups. First-order interneurons, with significantly wider lateral branching than those found in diurnal bees, have been identified in the first optic ganglion (the lamina ganglionaris) of *Megalopta's* optic lobe. We believe that these cells have the potential to mediate spatial summation.

Conclusions: Despite the scarcity of photons, *Megalopta* is able to visually orient to landmarks at night in a dark forest understory, an ability permitted by unusually sensitive apposition eyes and neural photon summation.

Introduction

Bees and wasps are primarily day-active insects, renowned for their impressive repertoire of visually guided behaviors. The European honeybee—the great insect model in studies of visual behavior for almost 100 years [1]—uses her compound eyes to learn and distinguish landmarks [2], to navigate by using polarized skylight [3], to orient [4], and to discriminate the colors of flowers [1]. However, she can only do these things in bright daylight; by early dusk her small, insensitive apposition compound eyes (Figure 1A) capture insufficient light to allow foraging [5], and her activity ceases for the day [6, 7]. Apposition eyes are constructed of individual optical units called ommatidia. Each ommatidium contains a corneal facet lens that focuses incoming light onto the rhabdom, a rod-like structure composed of the photoreceptive elements (or rhabdomeres) of several photoreceptor cells. Because the ommatidia of apposition eyes are each sheathed in a sleeve of light-absorbing screening pigment, the only light that reaches the rhabdom enters through the small corneal lens—typically only 20 μm wide in honeybees [8]. This tiny aperture limits the use of apposition eyes in dim light, and not surprisingly, these eyes are typical of diurnal insects. Superposition eyes, a sensitive design based on the superposition of light rays entering hundreds, or even thousands, of ommatidia (Figure 1B), is the eye design typically found in nocturnal insects, including moths and beetles [9–11]. Remarkably, despite the consequences for vision, several groups of bees and wasps have independently evolved nocturnal activity [12–17] and have carried their apposition eyes with them. Many other nocturnally active insects, including cockroaches [18] and locusts [19, 20], are also known to have apposition eyes. A nocturnal lifestyle is hypothesized to have two major advantages [5, 12, 21, 22]. First, insects can take advantage of the abundant pollen and nectar resources available from nocturnally flowering plants. Second, the risk of predation and of parasitation of the brood may be lower [22, 23].

The nocturnal sweat bee *Megalopta genalis* (Hymenoptera: Halictidae) is a large halictid species native to the rainforests of Central and South America. The females are facultatively social and live in hollowed-out sticks with 1–10 females per nest [22, 24, 25]. When they prepare to forage in the darkness of a rainforest understory at night, the task that awaits them is not a trivial one. They negotiate the often dense vegetation that obscures their path and, more difficult still, must find their way home again, to their small stick concealed in the undergrowth. This task would be difficult enough in bright daylight, but at night the scarcity of photons makes the task particularly challenging.

Nevertheless, we have discovered that at the commencement of foraging, a female *Megalopta* uses vision to learn landmarks around the nest entrance. She later uses these landmarks to recognize her home upon return. The structure of her eyes [26] and the physiology of the photoreceptors have various adaptations that are suited for use in dimmer light, but these are not sufficient in themselves to explain her impressive nocturnal visual performance. Our conclusion is that higher neural pro-

*Correspondence: eric.warrant@cob.lu.se

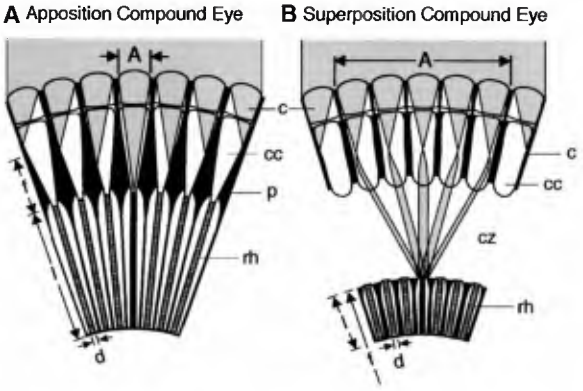

Figure 1. Schematic Diagrams of the Two Main Compound-Eye Designs

(A) Apposition Eye. (B) Superposition eye (of the refracting type, relying on crystalline cones with internal gradients of refractive index). The paths and fates of parallel light rays, incident on the external eye surface, are indicated in each (shaded area). For each design, the target rhabdom is shaded black. *A* = diameter of the aperture, *f* = focal length (which, in superposition eyes, is measured from the eye's center of curvature [not indicated]), c = corneal facet lens, cc = crystalline cones, *p* = screening pigment, *rh* = rhabdom, cz = clear zone, *l* = rhabdom length, and *d* = rhabdom diameter.

cesses must be active to intensify the visual signal by summing the incoming light both spatially and temporally, a conclusion supported by the discovery of laterally branching first-order interneurons in the first optic ganglion (the lamina ganglionaris) [27].

Results and Discussion

Periods of Nocturnal Activity

Seven nest sticks, each containing a single adult female *Megalopta*, were collected from the rainforests of Barro Colorado Island in Panama, and experiments were performed during the period from September 1 to 25, 2000. Nests were arranged in a row on a stand (see Figures 2B and 2C), about 1 m above the ground. These were observed by two observers who used image intensification apparatus over 15 consecutive nights and filmed the nests with digital video cameras and infrared illumination (cameras were mounted below and to the side of the nest entrance).

The bees left the nest to forage on only two occasions each day, each time for up to about half an hour. The first period started up to an hour before dawn, the second about 15–20 min after sunset. In other seasons, bees have been occasionally observed to fly from the nest at times outside the dusk and dawn activity windows described here [22]. However, by using a device that electronically recorded departures and returns from the nest, we failed to observe such flight behavior (A.K., unpublished data).

Records of 120 flights from the seven female bees showed that dawn flights lasted from 1–36 min. Most bees made only a single trip, but some made as many as four. Of 72 recorded first departures, eight occurred earlier than 50 min before sunrise (which was at 6.09 am), when light levels from the background foliage were less than 2×10^{-5} cd/m^2 (10–20 times dimmer than starlight illumination). During the following 15 min period (49–35 min before sunrise: 2×10^{-5} to 5×10^{-4} cd/m^2), 18 further departures were observed. The remaining 46 departures occurred 35–15 min before sunrise (greater than 5×10^{-4} cd/m^2). Of 70 recorded returns from first foraging trips, 45 occurred later than 30 min prior to sunrise, when light levels were at least 1×10^{-2} cd/m^2. Twenty-two bees returned between 40 and 31 min prior to sunrise (1×10^{-4} to 1×10^{-3} cd/m^2), and three returned less than 40 min prior to sunrise (less than 1×10^{-4} cd/m^2).

Lasting between 2 and 22 min, dusk foraging flights were, in general, slightly shorter than those occurring at dawn. Moreover, not all bees flew every evening, and with one exception, those that did fly did so only once. Of 68 recorded departures at dusk, 67 occurred 10–25 min after sunset. Of 69 recorded returns, 65 occurred 20–34 min after sunset (2×10^{-3} to 1×10^{-4} cd/m^2), and four occurred 35–39 min after sunset (less than 1×10^{-4} cd/m^2).

These results show that *Megalopta* is active in extremely low light levels, both at dawn and at dusk, with some individuals capable of flying at light levels less than the intensity of starlight (ca. 1×10^{-4} cd/m^2). For human observers, these intensities are extremely dim, and it was impossible to see flying bees without an image intensification apparatus.

Nocturnal Landmark Orientation Is Mediated Visually

Does *Megalopta* use vision during foraging? A first and very telling observation suggests that they do. Using the digital video camera and infrared illumination described above, we discovered that departing bees perform an "orientation flight," a behavior well known in diurnal bees [28–33]. As the bee leaves the nest, she turns to view the nest entrance and hovers back and forth in short arcs, these becoming increasingly wider as she backs away from the nest (Figure 2A). After a few seconds, she spirals upward and disappears from sight. Diurnal honeybees and solitary bees use orientation flights to visually learn the spatial arrangement of landmarks around the nest entrance and the landscape between the nest and the foraging site [31]. These landmarks are then used in homing. Presumably, *Megalopta* makes orientation flights for the same purpose. To test this possibility, we performed two landmark-manipulation experiments.

In the first (Figure 2B), we arranged five nests in a row, about 1 m above the forest floor. Of these, only one nest—the central one—was occupied (marked by a *star* in Figure 2B). In the example shown (of 13 similar experiments, with 13 different bees), the bee left the nest at 18:48 (16 min after sunset), when the light intensity was 0.002 cd/m^2. As she departed, she performed an orientation flight, presumably to learn the spatial arrangement of the five nests as well as other landmarks in the general vicinity. Several minutes after she had left, and without disturbing the previous spatial arrangement, we exchanged her nest with one of the outer nests. Upon her return at 18:58 (26 min after sunset

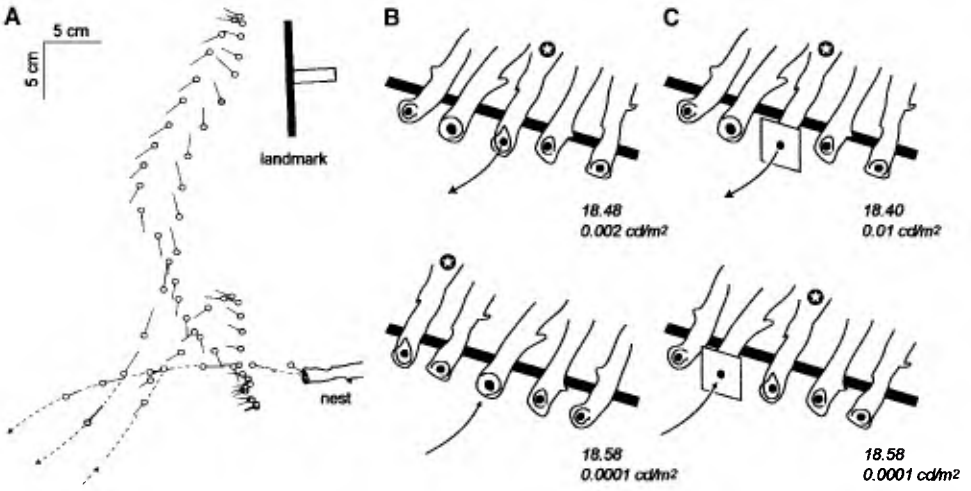

Figure 2. Nocturnal Landmark Orientation in *Megalopta*

(A) A typical nocturnal orientation flight, as seen from below. The bee leaves her nest and quickly returns to face the nest entrance. Flying in short arcs, she investigates the nest entrance and a neighboring landmark to learn their spatial arrangement before departing on her foraging trip. Each "ball-and-stick" represents the position of the head (*ball*) and body (*stick*) at 40 ms intervals.

(B and C) Landmark learning. Bees leaving for a foraging trip learn the position of their nest relative to others (B) or learn the presence of a white square card attached to their nest (C). Upon return, bees enter the nest marked by the landmarks they have previously learned, not their actual nests (which are marked by *stars*). The rear side of the square card was attached to a Perspex cylinder that slipped neatly over the end of the nest stick to hold the card in place over the nest entrance. Times and light intensities at departure and return are also shown.

when light levels had fallen to 0.0001 cd/m^2), she flew rapidly and without hesitation into the middle nest, precisely as the learned spatial arrangement would have dictated. Within a couple of seconds, she flew straight back out again. After reinspection of the nests for a few seconds, she returned yet again to the central "spatially correct" nest, only to reemerge rapidly. This behavior persisted until her actual nest was replaced to its original position, after which she entered it and no longer reemerged. This simple experiment demonstrates that vision plays an important role in *Megalopta's* homing behavior, a fact reinforced by the second experiment (Figure 2C).

In a second experiment, a specific landmark—a white square of cardboard—was used to identify the nest entrance (Figure 2C). We performed seven repetitions (with seven different bees) of which one is shown in Figure 2C. All bees behaved in the same manner. After leaving her nest (marked by a *star* in Figure 2C) at 18:40, when the light intensity was 0.01 cd/m^2, *Megalopta* again performed an orientation flight, during which she presumably learned the presence of the white card and the arrangement of the other nests. After her departure, and without moving her real nest, we removed the card and placed it on the nest next to her real nest. Upon return at 18.58, when the light intensity had fallen to 0.0001 cd/m^2, she flew into the nest bearing the white card, not her real nest. Again, similar to the bee in the previous experiment, she reemerged rapidly. After reinspecting the nests for a few seconds, she again entered the nest bearing the card, only to reemerge rapidly. As before, she continued to enter the landmarked nest until the card was finally reattached to her original nest, after which she entered and no longer emerged.

Using apposition eyes, an eye design unsuited for the task, *Megalopta* learns landmarks near the nest in very dim light and uses them to find a 6-mm-wide hole in the end of a stick obscured by the tangled understory of a tropical rainforest. How is *Megalopta*'s visual system adapted for this task?

Are the Eyes of *Megalopta* Unusually Sensitive for Apposition Eyes?

In relation to eyes of other bees, the eyes of *Megalopta* (and of other nocturnal species) are large relative to body size [34], a firm indication that vision plays an important role in her behavior (Figure 3A). Larger eyes have the potential to capture more light [35–37] but are metabolically more expensive [38], a cost that again indicates the importance of vision to *Megalopta*.

At 350 μm long and 8.0 μm wide, the rhabdoms of females' eyes are very large compared to those in diurnal bees ([26], Figure 3B). This width is very large for an apposition eye. In the diurnal worker honeybee *Apis mellifera*, the rhabdoms are 2 μm wide and 320 μm long [26]. This represents a 16-fold-greater rhabdom cross-sectional area in *Megalopta* compared to *Apis*, an adaptation clearly suited to nocturnal activity because wider photoreceptors capture more light. Other apposition eye-bearing nocturnal insects, such as the cockroach [18], also have wide rhabdoms, although not as wide as those in *Megalopta*. Some insects with both nocturnal and diurnal activity have rhabdoms that double their width at night; such insects include locusts [20] and mantids [39].

Compared to those of the honeybee [8, 40, 41], the diameters of corneal facet lenses, the aperture through which light reaches the rhabdom, are also large. In both *Megalopta* and *Apis*, the largest diameters are found in the frontal part of the eye, where they reach 36 μm and 20 μm, respectively [26]. In *Megalopta*, this large value is reached via a smooth gradient from both the dorsal

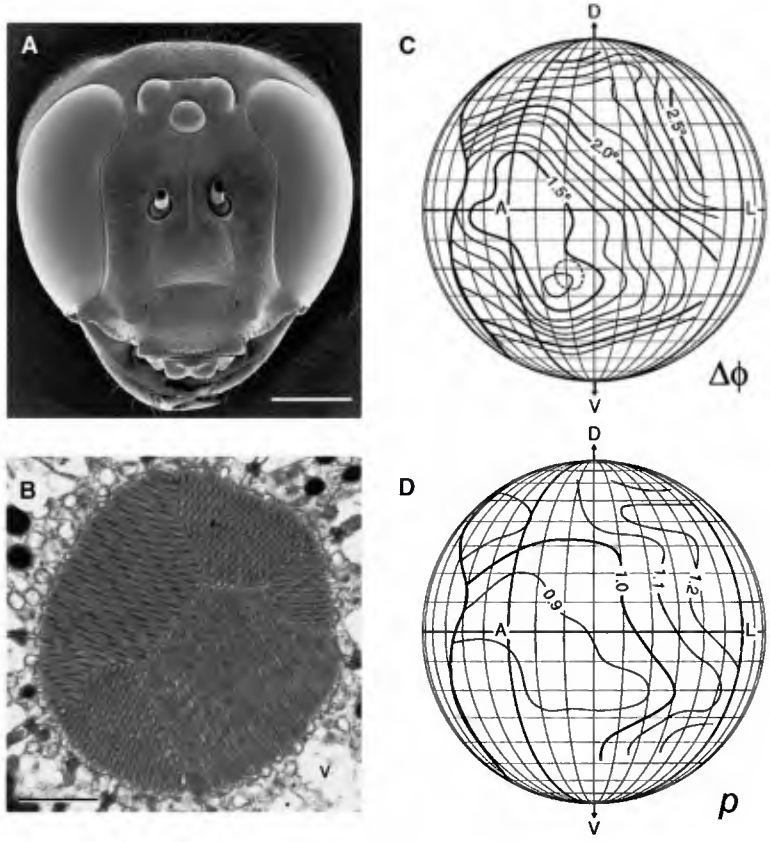

Figure 3. The Apposition Eyes of *Megalopta*

(A) A scanning electron micrograph of the head of a female *Megalopta genalis* (antennae removed for clarity). Note the three very large ocelli. Large ocelli are typical of nocturnal bees and wasps [12, 14, 72]. The scale bar represents 1 mm.

(B) A transmission electron micrograph showing a distal transverse section through the large rhabdom. Eight photoreceptor cells are visible, each of which contributes microvilli to the rhabdom. The scale bar represents 2 μm.

(C) A map of interommatidial angle $\Delta\phi$ in the bee's left eye. Data are plotted onto a sphere that represents the three-dimensional space around the bee. Lines of latitude and longitude are shown in intervals of 10°. The boundary of the eye's visual field is also shown. *D* = dorsal, *V* = ventral, *A* = anterior, and *L* = lateral. The *dashed circle* encloses a region of the eye from which the recordings shown in Figure 4 were made.

(D) A map of eye parameter $p = D\Delta\phi$ (μm rad) in the bee's left eye, where *D* is the local corneal facet diameter. Other conventions as in (C).

and ventral parts of the eye, where facet diameters are smaller, around 28 μm. In *Apis* a similar situation is found, the dorsal and ventral facet diameters also being smaller (18 μm).

The packing density of ommatidia in a compound eye, represented by the angle between neighboring ommatidia, or the interommatidial angle $\Delta\phi$, determines the anatomical spatial resolution of the eye [37, 42]. The greater the density (or the smaller $\Delta\phi$), the greater the potential resolution. However, as in all eyes, greater resolution tends to come at the cost of sensitivity, and insects active in dim light (especially those with apposition eyes) tend to have less densely packed ommatidia (greater $\Delta\phi$) with larger facets. In fact, the product of these two parameters—the interommatidial angle $\Delta\phi$ (in radians) and the facet diameter *D* (in μm)—is the well-known "eye parameter" *p* [43]: $D\Delta\phi$ (μm·rad). The eye parameter can tell us a great deal about the trade-off between resolution and sensitivity in an apposition eye. Slowly moving insects that are active in bright light (e.g., mantises and hovering sphecid wasps) have a value of *p* less than 0.45 μm·rad. Flying diurnal insects that experience high angular velocities require greater sensitivity; for example, in the house fly *Musca*, $p \approx 1.3$ μm·rad [43]. Insects active in dimmer light also require greater sensitivity, and this too leads to larger eye parameters (typically $p > 2$ μm·rad [43]).

What is the situation in *Megalopta*? Using optical methods, we have found that the local averaged interommatidial angle $\Delta\phi$ in females decreases in a smooth gradient toward the frontal-ventral part of the eye and reaches an average minimum value of 1.4° (Figure 3C). These values of $\Delta\phi$ are surprisingly small for a nocturnal insect and even indicate the presence of an "acute zone" of high spatial resolution in the part of the eye that is used to view the nest entrance. In the honeybee, averaged frontal values of $\Delta\phi$ are much greater, around 1.9° [44]. In both species, however, these averaged values of $\Delta\phi$ mask an ommatidial packing that characterizes "oval eyes" [45]: in bees, $\Delta\phi$ values in the vertical direction are smaller than in the horizontal direction (see Experimental Procedures). Nonetheless, in terms of ommatidial packing, *Megalopta* has an eye design adapted for high spatial resolution, more so even than in the diurnal honeybee, a paradoxical result indeed. However, her eyes are large, and this has allowed a simultaneously larger facet diameter, so sensitivity may not have been sacrificed as much as $\Delta\phi$ on its own might suggest. If we examine this trade-off with the eye parameter *p*, we find values of around 0.9 μm·rad in the frontal eye, and these become larger elsewhere (Figure 3D). These values suggest activity in dimmer light or flight at higher velocities, but probably not both. Nevertheless, the eye parameter is still much lower than one would expect for a flying nocturnal insect (in which case $p > 2$ μm·rad).

Thus, *Megalopta's* large eyes, rhabdoms, and corneal facets are clearly adapted for vision at night, but the eye's dense packing of ommatidia and sharp frontal acute zone are paradoxically better suited to an insect active in bright light. Perhaps this paradox is overcome

by the spatial and temporal properties of the photoreceptors, the topic to which we turn next.

Are the Spatial and Temporal Properties of the Photoreceptors Optimized for Photon Capture?

Using intracellular electrophysiology, we measured the spatial receptive fields and temporal impulse responses of dark-adapted photoreceptors from a frontal-ventral region of the female eye (enclosed by the *dashed circle* in Figure 3C). Wider receptive fields and slower impulse responses are both adaptations for improved vision in dim light [46], but only at the expense of spatial and temporal resolution, respectively.

The spatial receptive fields (or "angular-sensitivity functions") of photoreceptors set the limit of spatial resolution in a compound eye, irrespective of the interommatidial angle [9]. In *Megalopta* they were found to be large relative to diurnal bees (Figure 4A). The half-width of the angular-sensitivity function, or the "acceptance angle" $\Delta\rho$, is a good indicator of receptive-field width (Figure 4A). Larger values of $\Delta\rho$ indicate poorer spatial resolution and, when $\Delta\rho$ is increased by the use of wider photoreceptors, a greater sensitivity to light. In a sample of the most reliable recordings from six cells in two bees, we found $\Delta\rho = 5.6° \pm 0.8°$. In the single receptive field shown in Figure 4A, $\Delta\rho = 6.3°$. Note also that this receptive field is "squarer" than the Gaussian shape typical [9] of angular-sensitivity functions (*dashed* function in Figure 4A). This certainly reflects *Megalopta's* very wide rhabdoms. The receptive field's squarer shape and considerable width are both clear adaptations for greater light capture at the expense of resolution, a conclusion reinforced by the extent of receptive-field overlap ($\Delta\rho/\Delta\phi$). At the same location at which we made our recordings, $\Delta\phi = 1.4°$ (Figure 3C), implying an overlap of $5.6°/1.4° = 4$. Thus, the fine ommatidial matrix (Figure 3C) is clearly coarsened by the spatial properties of the photoreceptors, an adaptation that fits well with nocturnal activity (see Table 2 in [47]). In comparison, the diurnal honeybee has clearly favored resolution. Its Gaussian receptive fields have $\Delta\rho = 2.6°$ in the dark-adapted state [48, 49], and with $\Delta\phi = 1.9°$ [44], this represents an extent of receptive-field overlap of only 1.4, a value not unusual in a diurnal apposition eye.

The impulse response of a photoreceptor is its response to a very brief and dim flash of light (Figure 4B). The time course of this response, particularly its "time-to-peak" τ_p and its "integration time" Δt [43], are good indicators of the speed of vision (Figure 4B, *inset*). A slower response, and longer values of τ_p and Δt, indicates slower vision (and lower temporal resolution). Slower vision in dim light increases the signal-to-noise ratio and improves contrast discrimination by suppressing photon noise at temporal frequencies that are too high to be reliably resolved [46]. In *Megalopta*, the dark-adapted impulse response, with $\tau_p = 41 \pm 8$ ms and $\Delta t = 32 \pm 8$ ms (six cells, two bees), is slower than we have measured for the worker honeybee *Apis*: $\tau_p = 27 \pm 2$ ms and $\Delta t = 18 \pm 3$ ms (five cells, two bees). These values indicate that the *Megalopta* photoreceptors, being almost twice as slow as those of *Apis*, are better adapted for nocturnal vision. *Megalopta* also has considerably slower photoreceptors than other diurnal bees [50]; however, compared to those of many diurnal insects, the photoreceptors of *Megalopta* are not exceptionally slow [51].

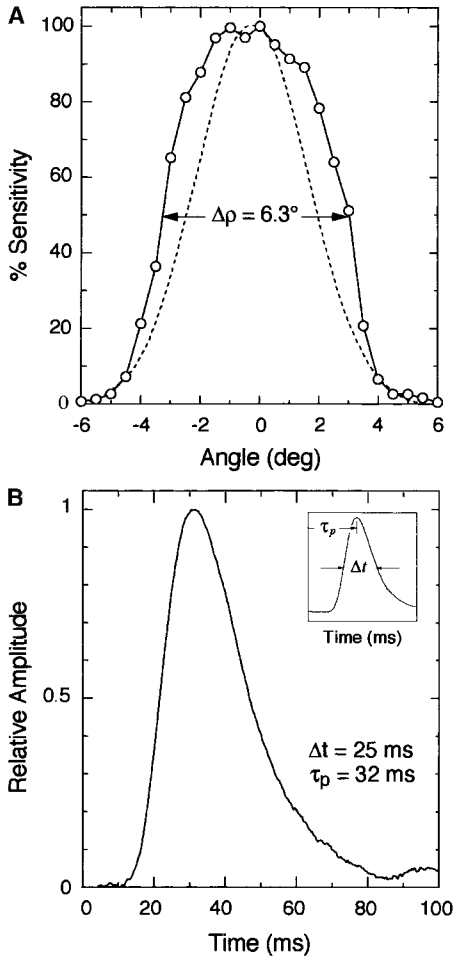

Figure 4. The Dark-Adapted Spatial and Temporal Properties of Photoreceptors

Measurements were made in the eye location enclosed by the *dashed circle* in Figure 3C.

(A) A typical angular-sensitivity function (*circles*), whose half-width (the acceptance angle $\Delta\rho$) is 6.3°. This function is much squarer than a Gaussian of the same angular base-width (*dashed function*), most likely the result of the eye's very wide rhabdoms.

(B) A typical impulse response, with the definition of time-to-peak τ_p and integration time Δt [43] (*inset*). Just as it is impossible to specify the precise location of a distant point source because of the finite width of the angular-sensitivity function, it is also impossible to specify the precise time that a light stimulus occurs because of the finite width of the impulse response [43]. Thus Δt is defined as the half-width of the impulse response [47], in direct analogy to $\Delta\rho$ being the half-width of the angular-sensitivity function [43]. For this cell, $\tau_p = 32$ ms and $\Delta t = 25$ ms.

Thus, the spatial and temporal properties of *Megalopta's* photoreceptors are well adapted to vision at night. The question that now remains is whether these properties, together with the morphology and optics of the eye, are together sufficient to explain *Megalopta's*

Table 1. Optical and Physiological Parameters in the Eyes of Bees

Parameter	Symbol	Units	*Apis*	*Megalopta*
Acceptance angle	$\Delta\rho$	radians	0.0454	0.0978
Corneal facet diameter	D	μm	20	36
Rhabdom length	l	μm	320	350
Integration time	Δt	s	0.018	0.032
Quantum efficiency of transduction	κ	unitless	0.5	0.5
Transmission fraction of the optics	τ	unitless	0.8	0.8
Absorption coefficient of the rhabdom	k	μm^{-1}	0.0067	0.0067

Values for *Apis mellifera* workers, and the chosen values of k, τ and κ, are explained and referenced in [5] and [26]. Values in both species are for the frontal eye region in the dark-adapted state. Those specific to *Megalopta genalis* females are from the present study.

ability to navigate by landmarks at night. To answer this question, we must rely on theory.

How Well Does Megalopta's Eye Capture Photons at Night?

So far, we have seen that the eyes of *Megalopta* have morphological, optical, and electrophysiological characteristics that better suit them to a nocturnal life than would the eyes of diurnal honeybees. But how can we quantify these differences?

A simple method is to ask how many photons N are absorbed by a single photoreceptor within its integration time Δt, when each species experiences the same nocturnal intensity I. The above measurements of integration time, facet diameter D, rhabdom length l, and acceptance angle $\Delta\rho$ are all important parameters because larger values of these will increase N [52–54]:

$$N = 1.13\left(\frac{\pi}{4}\right)\Delta\rho^2 D^2 \kappa\tau\Delta t\int(1 - e^{-kR(\lambda)l})I(\lambda)d\lambda \qquad (1)$$

Other parameters important for photon absorption are the quantum efficiency of transduction κ, the transmission of the optics τ, and the absorption coefficient of the rhabdom k. Values for these and all other parameters are given in Table 1 for *Megalopta* and the honeybee *Apis*. The integral term describes the number of photons that will be absorbed in a photoreceptor of spectral sensitivity $R(\lambda)$ when a bee views an illumination spectrum of quantal intensity $I(\lambda)$, where λ is wavelength. For *Megalopta*, which views a rainforest, $I(\lambda)$ was taken as the spectrum obtained from green foliage [53]. The terms before the integral simply determine the number of these photons that the optics of the eye allow to reach the photoreceptor. $R(\lambda)$ is calculated with the Stavenga-Smits-Hoenders rhodopsin template [55] with peak spectral sensitivity at 540 nm. The integral is calculated between two wavelength limits: λ_1 and λ_2 [52]. λ_1 is set at 280 nm, the lowest wavelength likely to be seen by any animal. λ_2 is the wavelength at which the spectral sensitivity $R(\lambda)$ falls to 1% of its maximum at its long wavelength end. In the Stavenga-Smits-Hoenders template, $\lambda_2 = 1.231\lambda_{max}$, where λ_{max} is the absorbance peak wavelength of the visual pigment. In our calculation, $\lambda_{max} = 540$ nm, and thus $\lambda_2 = 665$ nm.

Our measurements show that *Megalopta* can find its nest when as few as 0.01 photons/μm^2/sec/sr ($\lambda = 540$ nm) are incident on the eye. At this intensity, Equation 1 reveals that 0.15 photons are absorbed by a single green receptor in *Megalopta* during one integration time (i.e., $N = 0.15$). In *Apis* at the same intensity, $N = 0.0053$ photons. Thus, the eyes of *Megalopta* are indeed better adapted to nocturnal vision than those of *Apis*; they are 28.3 times more sensitive (0.15/0.0053). Can this difference alone account for *Megalopta's* nocturnal visual behavior?

We can answer this question by considering the difficult task of locating the nest entrance upon return from a dusk foraging trip. *Megalopta* must first recognize and negotiate leaves and branches in the vicinity of the nest. Using these landmarks to find the nest stick, she must then locate the small entrance hole. Sometimes she lands on the stick and simplifies the task by walking to the hole, but we have often observed bees flying directly into the nest without landing. The entrance hole appears darker than the wood that surrounds it, and a light meter shows that the brightness difference (or contrast c) between the hole and the stick for two sticks was 0.72 and 0.97, implying considerable variation between sticks. This variation is due to the coloration of the wood, older nest entrances being more darkly stained by dirt and mold. These contrasts are nevertheless quite high, and other objects in the general vicinity, such as foliage, would be expected to have much lower contrast. According to Land [37], $2(1.96/c)^2$ photons must be absorbed in each receptor during one integration time to just allow a brightness difference c to be distinguished with 95% reliability. With $c = 0.72$, this implies that 14.8 photons must be absorbed per integration time. With $c = 0.97$, 8.1 photons must be absorbed. This is respectively 100 and 55 times as many photons as *Megalopta* actually absorbs when approaching her nest entrance! An even greater photon catch would be required for distinguishing the surrounding low-contrast foliage. Thus, the light-gathering capacity of the eye's optics and the physiology of single photoreceptors are simply unable on their own to account for her behavior. What then can?

Neural-Image Enhancement: Spatial and Temporal Summation

When the optics and physiology of the eye are unable to collect sufficient photons for each visual channel, there is one final neural strategy that can be used to increase sensitivity [43, 53, 56]. This strategy – which resides in the cellular circuits processing the incoming visual signal – involves the neural summation of light in space and time.

We have already seen that a long integration time

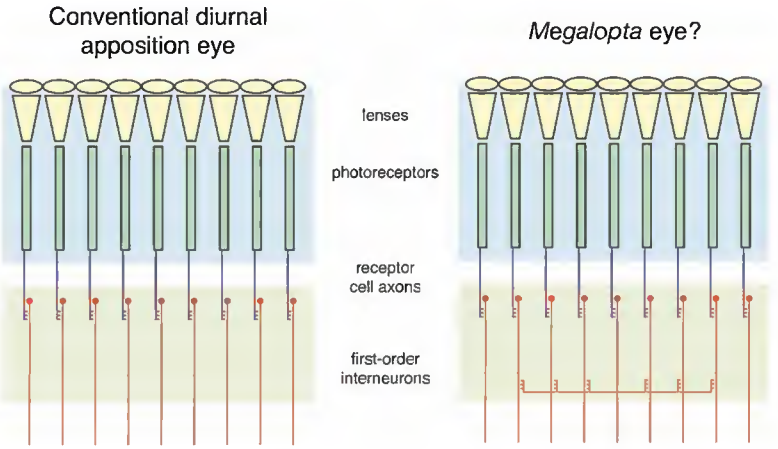

Figure 5. A Possible Mechanism for Spatial Summation in *Megalopta*'s Eye

In a conventional diurnal apposition eye, such as that of a dragonfly (*left*), the photoreceptors of each ommatidium send their axons to the first optic ganglion, the lamina ganglionaris, where they synapse with first-order interneurons. The first-order interneurons then send the signal farther to the next optic ganglion, the medulla. In bright light, summation is not necessary, and the visual channels defined by each ommatidium can remain isolated from each other. In *Megalopta* (*right*), with ommatidia insufficiently sensitive to generate a reliable visual signal in dim light, spatial summation of ommatidial signals is a viable strategy for improving sensitivity. One possibility is that one or more classes of first-order interneurons, with modified morphologies, provide the neural wiring that couples neighboring visual channels together. In this scenario, first-order interneurons branch to a group of neighboring lamina cartridges, each cartridge having cells that process information arriving from a single overlying ommatidium. Thus, if properly arranged, these first-order interneurons could connect a group of ommatidia together, and provided that the necessary circuitry exists, this might allow spatial summation.

improves the reliability of contrast vision in dim light. If higher neural mechanisms that lengthen this integration time beyond the value inherent in the photoreceptors exist, then contrast vision can be further enhanced. However, despite its benefits, this temporal summation only comes at a price: quickly moving objects are seen less reliably.

Eyes can also improve sensitivity by summing photons in space [43, 53]. Instead of each visual channel (or ommatidium in *Megalopta*) collecting photons in isolation (as in a diurnal eye: Figure 5A), animals active in dim light may have specialized neurons that couple the channels together into groups. In this way each group—themselves now defining the channels—could collect many more photons over a wider visual angle, that is, with a greatly enlarged receptive field (Figure 5B). Unfortunately, this improved photon catch is accompanied by a simultaneous and unavoidable loss in spatial resolution. Despite being brighter, the image becomes necessarily coarser. The significant overlap of photoreceptor visual fields we mentioned earlier ($\Delta\rho/\Delta\phi = 4$) suggests that some degree of summation is warranted. Because the ommatidial matrix is anyway coarsened by this overlap, it would pay to sum to at least the same extent.

For *Megalopta*, spatial and temporal summation would allow a brighter view of the rainforest habitat, albeit a coarser and slower one. But this is undoubtedly better than seeing nothing at all, which is the only other alternative.

Good evidence for spatial summation has been found in the motion-detecting pathways of flies and crabs. Threshold optomotor responses in tethered flies viewing a wide-field, moving, grating stimulus occur when individual photoreceptors are responding to single photons with "bumps" at an average rate of only 1.7 $\pm$ 0.7 bump responses/receptor/s [57]. In the shore crab *Leptograpsus variegatus*, optokinetic threshold to a moving point source occurs at an even lower bump rate: 0.4 bumps/receptor/s [58]. In flies, such weak photoreceptor signals are eventually used by several classes of wide-field cells in the lobula plate of the optic lobe to process motion [59]. In bright light, the elementary motion detectors calculate motion by using signals generated in neighboring ommatidia, and processing thus occurs at the highest possible acuity. But as light levels fall, motion acuity falls in a manner consistent with spatial summation [60]: the elementary motion detectors calculate motion by comparing signals generated in successively more distant neighbors, up to two, three, or even four ommatidia apart [61]. This increase in spatial summation is accompanied by a decrease in lateral inhibition [62].

Is there any evidence for summation in *Megalopta*? As yet, we have no evidence for temporal summation.

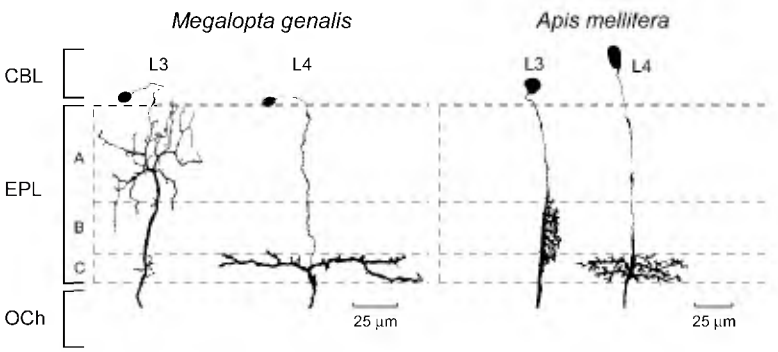

Figure 6. Comparison of the First-Order Interneurons, L-fiber types L3 and L4, of the *M. genalis* female (*left*) and the worker honeybee *A. mellifera* (*right*)

Compared to the worker honeybee (*right*), the horizontal branches in the nocturnal halictid bee (*M. genalis*, *left*) are more than twice as wide, suggesting a possible role in spatial summation. Reconstructions are from Golgi-stained frontal sections. *CBL* = cell body layer, *EPL* = external plexiform layer or first optic ganglion, *OCh* = outer chiasm, and *A*, *B*, and *C* = layers of the first optic ganglion. Adapted from [27] and [73].

We are, however, beginning to find possible evidence of spatial summation. In the first optic ganglion, the lamina, Golgi studies reveal widely branching first-order interneurons (Figure 6). Compared to the first-order interneurons of honeybees [63], those of *Megalopta* feature similar branching patterns but are of much wider extent [27]. As suggested previously [64], these wide lateral branches have the potential to couple the neural cartridges of several ommatidia together and thus mediate spatial summation. Laterally spreading monopolar cells have also been found in other arthropods active in dim light; examples include cockroaches [65], fireflies [66], deep-sea amphipods [67], and hawkmoths [68]. Whether the laterally spreading cells of *Megalopta* are actually mediating spatial summation remains to be seen, but their morphology is definitely suited to the task.

Conclusions

A large proportion of the world's animals are active in dim light, either at night or in the depths of the sea. Many of them see surprisingly well; some are even able to distinguish colors [54]. The nocturnal sweat bee *Megalopta genalis* reinforces this view. Even though *Megalopta* has compound eyes that are 30 times more sensitive than those of diurnal bees, this is not sufficient for visually guided behavior in the rainforest understory at night. However, laterally branching first-order interneurons in the animal's first optic ganglion suggest that *Megalopta*'s visual sensitivity could be explained by spatial summation. Our future work will determine whether this is the case.

Experimental Procedures

Behavioral Experiments

Twenty bee nests were collected in the forests of Barro Colorado Island, Panama, and set up on a stand in a position that was far from artificial-light sources but easily accessible for observers in the evenings and mornings. Of these nests, seven turned out to be inhabited. The canopy at the site had a density normal for the island and had an even coverage of small gaps that exposed the sky. No clearings that exposed a large patch of sky were present. For collecting data on activity periods and flight times, two observers watched nests for 15 days in a row by using an image intensification apparatus. At the same time, several nests were filmed with an infrared-sensitive Sony Video camcorder. Videotapes were analyzed frame by frame for the reconstruction of orientation flights. Light intensities are given for the test site.

Histology

Light and electron microscopy was performed via standard methods. Whole eyes were placed for 2 hr at 4°C in standard fixative (2.5% gluteraldehyde and 2% paraformaldehyde in phosphate buffer [pH 7.2]). After a buffer rinse, eyes were then added to 2% OsO_4 for 1 hr. Dehydration was performed in an alcohol series, and eyes were embedded in Araldite. Ultrathin sections for electron microscopy were stained with lead citrate and uranyl acetate.

Golgi staining of first-order interneurons in the first optic ganglion was performed according to standard methods. See [26, 27, 69] for a full description of the methods used.

Optics

The procedure used to map interommatidial angles in the frontal part of the visual field follows standard procedures [70, 71] but will be briefly reviewed here. The small end was cut from a plastic pipette tip so that an opening large enough for a bee's head remained. We fixed the bee in position by gluing the proboscis to the tube with dental wax, and this preparation was then mounted at the center of curvature of a Leitz goniometer. The goniometer was placed onto the foot-plate of an Askania macroscope. It was then manipulated so that the flat posterior eye edge was parallel to the plane of the stage. The head was further manipulated so that (1) the origin of the three goniometer axes was in the center 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, respectively, of the bee's head. With the stage horizontal, both eyes then looked vertically upwards into the objective of the macroscope, 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° defined as the anterior orientation described above ("A" in Figures 3C and 3D). 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°.

To illuminate the eyes, we introduced a half-silvered mirror, angled at 45°, just beneath the objective of the macroscope. Collimated white light (from a halogen source) was directed laterally to the mirror so that the eyes were illuminated and viewed along the same axis ("orthodromic illumination"). This type of illumination reveals a luminous pseudopupil. This is displayed by many species of insects [45], including *Megalopta*. Using chalk dust sprinkled lightly on the eye to provide landmarks, and using the methods outlined in [70] and [71], we took a series of photographs of the luminous pseudopupil in the left eye at 10° intervals of latitude and longitude. Because of the structure of the apparatus, we could not go beyond latitudes of +70° or −70° or a longitude of 80°. 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 facet coordinates of the facet found at the center of the psuedopupil by using the landmarks as a guide. Using established formulae that correct for latitude distortions in the projection [70], we calculated the average local $\Delta\phi$ for each combination of latitude and longitude. 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 $\Delta\phi$. We made contour plots of the angular separations of *x*, *y*, and *z* facet rows separately to control for the fact that the eyes of *Megalopta*, and indeed the eyes of all bees, are highly nonspherical. We found that in the frontal part of the eye where the average $\Delta\phi$ is about 1.4° (Figure 3C), the *x* rows (which run frontally to ventrally) are separated by 1.9°, the *y* rows (which run almost horizontally) by 1.1°, and the *z* rows (which run frontally to dorsally) by 1.3° (plots not shown).

We also created a spherical plot of facet diameter *D* (not shown) and used this together with the plot of average $\Delta\phi$ (Figure 3C) to calculate the eye parameter $D\Delta\phi$ at each point in the eye (Figure 3D).

Electrophysiology

A bee was inserted into a plastic pipette tip whose end had been sliced off to allow the bee's head to pass through. A small quantity of bee wax was used to secure the head to the pipette tip. The bee was then mounted onto a small holder, and a tiny hole (5–10 facets wide) was cut near the dorsal margin of the left compound eye. The hole was sealed with Vaseline to prevent it from drying out. An indifferent electrode of thin silver wire was inserted into the other eye. A glass microelectrode (borosilicate glass, filled with 2 M potassium acetate, 200–300 MΩ in vivo) was inserted through the hole and advanced ventrally into the eye with a Märzhäuser piezo-driven manipulator. Intracellular penetrations of photoreceptors were distinguished by resting potentials between −40 and −50 mV and depolarizing responses to flashes of light. Responses were amplified on a Biologic microelectrode amplifier and digitized online with a Macintosh computer and LabVIEW software. White light from a xenon arc lamp was directed to the eye though a 100-μm-wide quartz light guide whose exit aperture subtended 0.05° at the eye (i.e., point-source illumination). Light intensity was controlled by quartz neutral density filters. The end of the light guide was held in a cardan

arm device that allowed the point source to be placed at any location on an imaginary sphere centered on the bee's eye. The point source could thus be moved in known angular steps throughout the visual field of the eye. When a photoreceptor was penetrated, the point source could be positioned on the visual axis of the cell (the direction from which the maximum response is generated), and the latitude and longitude of the cell's axis could thus be determined.

Bees were kept on a 12:12 light-dark cycle, and all electrophysiology was performed no earlier than 2 hr after lights off during the dark phase. Experiments in the dark-adapted state were performed at a laboratory temperature of 24°C. For light adaptation, we switched on the roof lights in the laboratory. Dark adaptation was at least half an hour, but usually longer. After penetration, the visual axis of the photoreceptor was located. The response of the cell to a series of 40 ms flashes of increasing light intensity was then measured (the V-LogI curve). After this, an intensity was chosen that gave a response about 60% of maximum. The point source was then displaced from the cell's axis and swept across the receptive field in angular steps of 0.5°. At each step, a flash was delivered, and the response was recorded. These responses were converted to equivalent intensities through the V-LogI curve, and sensitivity values at each angular step were calculated. The resulting "angular-sensitivity function" (sensitivity as a function of angular position) is the photoreceptor's spatial receptive field. After these measurements, the point source was repositioned on the axis of the cell, and the flash length was reduced to 2 ms. An intensity that gave a response from the cell having an amplitude no greater than 3 mV was chosen. The response of the cell to this impulse of light—the "impulse response"—was recorded 100 times and averaged.

Light Measurements

Light was measured with an International Light IL1700 photometer together with a highly sensitive silicon detector. In the forest close to the nests, we measured the intensity of light reflected at an angle of 45° from a horizontally held gray card (18% reflectance). We took measurements on several days. We used these measurements, together with the light spectrum of the forest, to calculate the number of photons available for vision.

The contrast of nest entrance holes was measured with an Ocean Optics S2000 Spectrometer. Reflected-light intensities were measured with a fiber-optic cable of 200 μm diameter, an aperture small enough to allow measurements from the 6 mm wide hole. Contrast was defined as $(I_{out} - I_{in})/(I_{out} + I_{in})$, where I_{in} is the intensity of light reflected from the hole and I_{out} is the intensity of light reflected from the surrounding wood.

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