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Adaptations for nocturnal vision in insect apposition eyes

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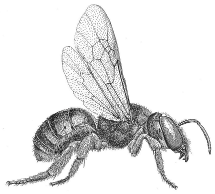
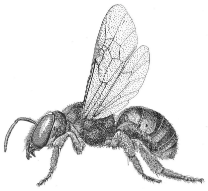
ADAPTATIONS FOR NOCTURNAL VISION IN INSECT APPOSITION EYES





Birgit Greiner

Doctoral Thesis
Lund December 2005

LUND UNIVERSITY
Department of Cell and Organism Biology

Academic thesis in the fulfilment of the degree of Doctor of Philosophy at the Faculty of Natural Science at Lund University. The thesis defence will take place in the Zoology Building, Helgonavägen 3, Lund, Sweden, at 10.00 am, December 16, 2005. Faculty opponent: Professor Ian A. Meinertzhagen, Life Sciences Centre, Dalhousie University, Halifax, Canada.

Für Mama, Papa & Gabi

Hur kan nattaktiva insekter se i mörker med fel sorts ögon?

Majoriteten av alla dagaktiva insekter har en sorts facettöga som kallas appositionsöga. Appositionsögon är anpassade till ett liv med god tillgång på ljus – nattaktiva djur har därför i princip alltid andra sorters ögon (superpositionsögon). Anledningen till att appositionsögon fungerar bäst på dagen är att de har små linser vilket gör dem mycket okänsliga och opålitliga i mörker. I avhandlingens introduktion behandlar jag insekternas synsinne och anledningen till att vissa ändå använder dåligt anpassade ögon för nattseende. Men det är bara i en enda insekt, ett väldigt speciellt nattaktivt bi, som hittills alla aspekter undersökts – såväl beteende som optiska och neurala anpassningar och teorin bakom det hela.

De flesta bin i världen är dagaktiva och har appositionsögon. Ändå finns det bin i Panama som flyger runt på natten. Teoretiska beräkningar visar att dessa bin inte borde kunna se någonting så fort det börjar skymma, men Panamabina flyger från sitt bo (ett 5 mm stort hål i en liten pinne), genom grenar och lövverk i djungeln, letar reda på nektargömmor långt borta från sitt hem och hittar sedan tillbaka till boet utan att flyga vilse. Dessutom, som visas i första artikeln, kan de även urskilja och använda landmärken för att orientera i mörker. Men hur klarar de det med sina okänsliga appositionsögon?

Den andra och tredje artikeln visar att appositionsögon hos nattaktiva bin och getingar har specifika optiska och anatomiska anpassningar som gör dem 30 gånger ljuskänsligare än deras dagaktiva släktingar. Mest effektivt är otroligt stora ljuskänsliga fotoreceptorer som absorberar ljus från en mycket bredare infallsvinkel än i ett normalt appositionsöga. En känslighetsförbättring på 30 gånger är inte att förakta, men inte tillräcklig om man tänker på att ljusintensitetsskillnaden är 100 millioner gånger mellan dag och natt. Därför undrade jag vilka andra möjliga anpassningar som måste finnas.

Enligt vår teoretiska modell (artikel VI), skulle nattaktiva bin använda sig av fotonsummering (i rum och tid) för att kunna se i svag belysning. Om Panamabina summerar signaler från flera facetter kan de få tillräckligt med ljus för att även kunna se under natten. Som jag visar i artiklarna IV & V, finns det stora, sidoförgrenade celler i den optiska delen av deras hjärna. Alla förgreningarna är mycket större jämfört med motsvarande celler i dagaktiva bin – en tendens som finns också i andra insektgrupper – och täcker en stor yta av 10-18 facetter. Vår modell förutsäger detta, vilket förstärker hypotesen att dessa celler förmedlar spatiell fotonsummering.

I min avhandling visar jag att nattaktiva insekter med appositionsögon har utvecklat specifika optiska och neurala anpassningar vilka möjliggör för dem att vara aktiva under natten i en tryggare miljö med förminskad konkurrens.

Main References:

I. Warrant EJ, Kelber A, Gislén A, Greiner B, Ribi WA, Wcislo WT (2004) Nocturnal vision and landmark orientation in a tropical halictid bee. *Current Biology*, 14:1309-1318

II. Greiner B, Ribi WA and Warrant EJ (2004a) Retinal and optical adaptations for nocturnal vision in the halictid bee *Megalopta genalis*. *Cell and Tissue Research*, 316:377-390

III. Greiner B (2005) Visual adaptations in the night active wasp *Apoica pallens*. *The Journal of Comparative Neurology*, accepted

IV. Greiner B, Ribi WA, Wcislo WT and Warrant EJ (2004b) Neural organisation in the first optic ganglion of the nocturnal bee *Megalopta genalis*. *Cell and Tissue Research*, 318:429-437

V. Greiner B, Ribi WA and Warrant EJ (2005) A neural network to improve dim-light vision? Dendritic fields of first-order interneurons in the nocturnal bee *Megalopta genalis*. *Cell and Tissue Research*, in press

VI. Theobald JC, Greiner B, Wcislo WT and Warrant EJ (2005) Visual summation in night-flying sweat bees: a theoretical study. *Vision Research*, submitted

The thesis introduction will be published in revised form as an invited review article: Greiner B (2006) Adaptations for nocturnal vision in insect apposition eyes. *International Review of Cytology: A Survey of Cell Biology* (Editor: Kwang W. Jeon).

TABLE OF CONTENTS

I. Introduction

Insects with apposition eyes are generally day-active since this eye design has significant limitations in dim light. Apposition eyes have small lenses and therefore a poor photon capture, resulting in unreliable visual signals at low light intensities. Nevertheless, some insects have managed the transition from a diurnal to a nocturnal lifestyle whilst retaining apposition eyes.

As in the words of Autrum (1981), " the term *adaptation* denotes all those events that change the structure, form, function, or behaviour of organisms in such a way that they are better adjusted to their surroundings." Thus, the most intriguing question of this review is: what are the adaptations for nocturnal vision that have evolved in insect apposition eyes?

The structures of the major eye designs present in terrestrial insects will be introduced in section II, illustrating why apposition eyes are highly unsuited for nocturnal vision. Section III will then describe dim-light foraging behaviour in bees and other insects that use apposition eyes at night. Vision plays an important role in many of these insects, and this is demonstrated by the ability of nocturnal bees to use landmarks for their orientation. The advantages of a nocturnal life will also be discussed. Section IV will point out the main limitations for vision in dim light, allowing us to understand the following paradox: insects are able to see at low light intensities despite using apposition eyes. Section V will finally attempt to solve this paradox. The evolution of unique optical adaptations in insect apposition eyes significantly enhances sensitivity for nocturnal vision. Wide apertures, large photoreceptors and pupillary pigment migrations are only some of the mechanisms used to improve photon capture. However as the gain of these optical adaptations is limited, additional neural mechanisms are necessary. The well-established hypothesis of temporal and spatial summation is explained and supported by anatomical findings of wide laterally-branching neurons that potentially mediate spatial summation. A neural summation model demonstrates that the size of these dendritic fields is sufficient in nocturnal bees to markedly improve sensitivity while still allowing them to spatially resolve their environment.

So far, only one insect, the nocturnal bee *Megalopta genalis*, has been thoroughly studied with respect to all aspects of vision and visual navigation at night. This remarkable species will thus be in focus throughout this review.

II. The structure and design of compound eyes

The compound eye's basic structure follows a general theme. The eye consists of repetitive visual units called ommatidia, each of which contains a dioptric apparatus that focuses light onto the layer of photoreceptors. The evolution of specific variations in either the optics of the eye or the neural wiring between the eye and the first optic ganglion (lamina) have led to the classification of three major compound eye types in insects: apposition, neural superposition and refracting superposition compound eyes (Fig. 1).

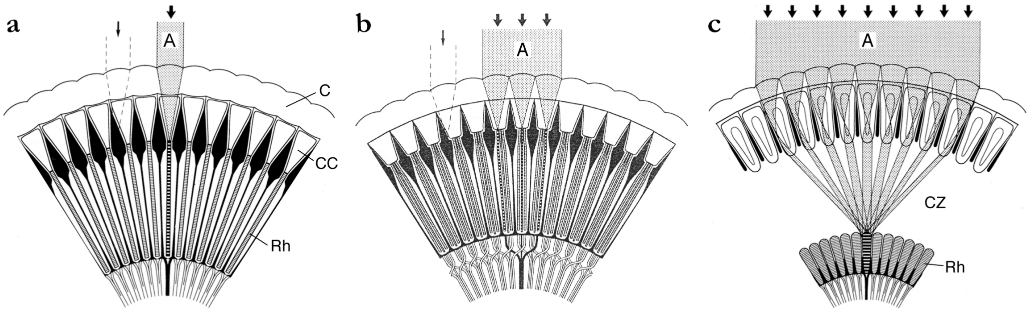


Fig. 1: Schematic longitudinal sections of the three major compound eye designs, (a) apposition, (b) neural superposition and (c) refracting superposition eye, showing the axial ray paths of light absorbed by the photoreceptor (*shaded grey*) and off-axis light absorbed in the screening pigment (*dashed lines*). The aperture sizes (A) reflect the differences in sensitivity between the eye types. For further explanations see text. C – cornea, CC – crystalline cone, CZ – clear zone, Rh – rhabdom (adapted from Nilsson, 1989).

The need for spatial resolution dictates the optical design of an eye and the light intensity of the environment limits its application. As a general guideline, apposition and neural superposition eyes are designs typically found in day-active animals, while most nocturnal insects make use of the more sensitive superposition eye. Why this is the case, and how animals with less sensitive apposition eyes nevertheless manage to see in dim light, will be discussed in the following sections.

A. Apposition eyes

Like in all compound eyes, the surface of the apposition eye consists of an array of tiny corneal lenses known as facets. Underneath each facet lies the crys-talline cone, generally formed by four Semper cells. Together, the corneal lens and the crystalline cone build up the dioptric apparatus of the compound eye (Fig. 2). Each ommatidium usually contains 8-9 photoreceptors known as retinula cells, where the photon-absorbing, visual pigments are arranged within microvilli (rhabdomeres) of the light-sensitive rhabdom. These rhabdomeres can be either fused together as a rod-shaped rhabdom, or open (separated) throughout their length. In addition, several cell types containing screening pigments can be found: two primary pigment cells surround the crystalline cone, a varying number of secondary pigment cells ensheath the entire ommatidium, and retinula cell pigments are present inside the retinula cells (Fig. 2). In general, a thick layer of pigments contained in the secondary pigment cells and the crystalline cone extensions covers the basement membrane to absorb stray light (Fig. 2).

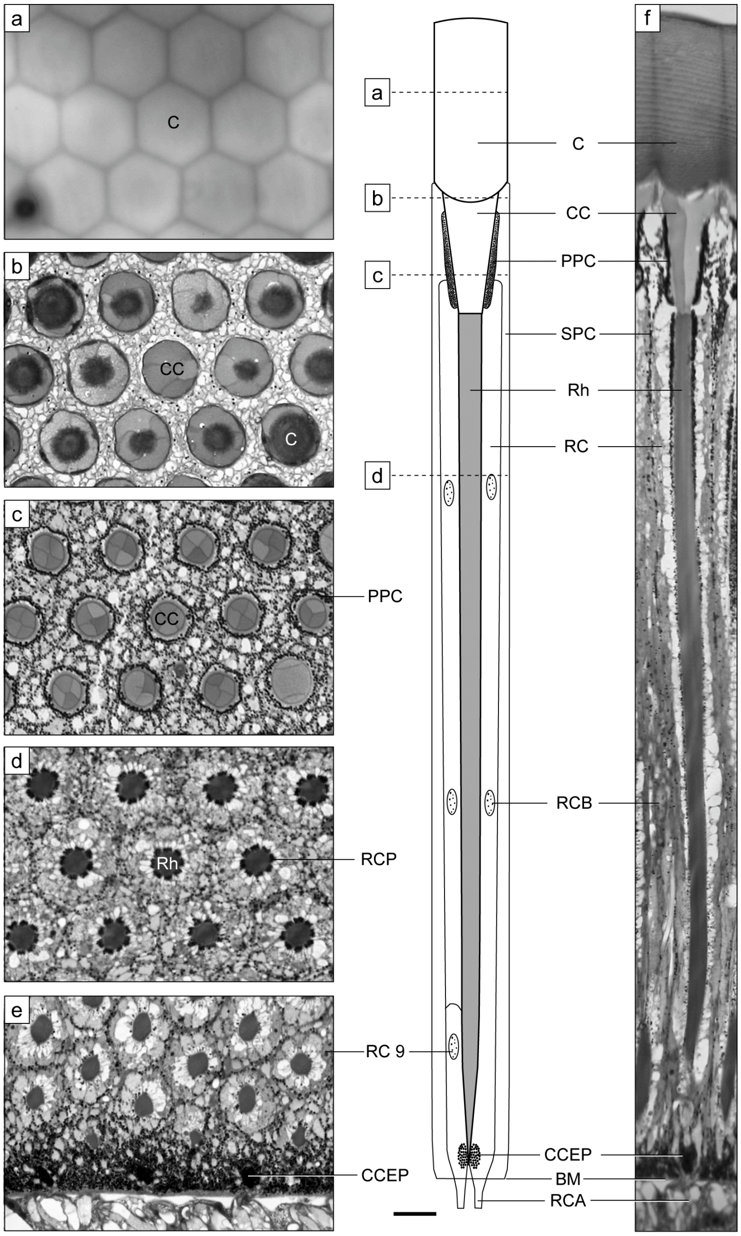


Fig. 2: The apposition eye of the nocturnal wasp *Apoica pallens*. (a,b) The dioptric apparatus consists of the corneal facet (C) and the crystalline cone (CC). (c,f) Two primary pigment cells (PPC) and several secondary pigment cells (SPC) surround the crystalline cone. (d,e) The fused rhabdom (Rh) contains nine retinula cells (RC). (e) The ninth retinula cell (RC9) appears only in the proximal third. (e,f) The pigments inside the four crystalline cone extensions (CCEP) surround the most proximal end of the rhabdom. (f) The retinula cell axons (RCA) pass as axon bundles through the basement membrane (BM). (d) In the light-adapted state, the pigments of the retinula cells (RCP) tightly surround the rhabdom. (a-e) 1 μm thick transverse and (f) longitudinal sections. 15 μm scale accounts for (a-f) and the drawing (*center*) (from Greiner 2005).

A light-reflecting tapetal mirror proximal to the retina, formed by a well-developed tracheal system or reflective pigment granules, would be of great benefit for nocturnal insects. Via reflection of the light at the tapetum, photons get a second chance to be absorbed within the rhabdom, thus enhancing the eye's sensitivity. Tapeta are commonly found in the eyes of butterflies (Ribi, 1980) and dark-adapted crustacean apposition eyes (Debaisieux, 1944). However, tapeta have so far not been described in the apposition eyes of nocturnal insects.

The major characteristics of apposition eyes are the tight apposition of the crystalline cone and the rhabdom, as well as the pigment sheath of the secondary pigment cells. These pigments optically isolate the ommatidia from each other by absorbing light reaching the eye off-axis (Fig. 1a). Thus, only axial light from a single facet, representing the aperture of the apposition eye, is focused onto the respective rhabdom underneath. Due to this small aperture, the apposition eye design works best at bright light intensities, usually restricting the animal to a diurnal lifestyle. Low light intensities result in a poor photon catch and unreliable visual signals, as will be explained in section IV.

B. Neural superposition eyes

The neural superposition eye of advanced flies (brachycerans) is similar to the apposition eye design, where each ommatidium receives light only through its own facet. However, instead of possessing a fused rhabdom, the rhabdomeres are open (separated) throughout their entire length and each of them receives light from a slightly different angle (Fig. 1b). This is also the case in most other flies (dipterans), hemipteran bugs, earwigs and many beetles, but the unique characteristic found in brachycerans lies in the neural connections of the photoreceptors from the retina to the first optic ganglion (lamina) of the brain. In conventional apposition eyes all photoreceptor axons originating in one ommatidium project to a single neural unit (cartridge) in the lamina (Ribi, 1974; Meinertzhagen, 1976). In advanced flies, however, the retinula cell axons from those rhabdomeres in six adjacent ommatidia, which all have the same field of view, converge together onto the same cartridge (Fig. 1b) (Vigier, 1909; Trujillo-Cenóz, 1965; Braitenberg, 1967; Kirschfeld, 1967). Thus, without sacrificing any spatial resolution, sensitivity can be increased 6-fold (Gemperlein and Smola, 1972; Strausfeld and Nässel, 1981). Necessary requirements for the function of a neural superposition eye are the optical alignment of the converging rhabdomeres and the exact retina-lamina projection of their axons to the appropriate neighbouring cartridges. Neural superposition is present already in the most primitive brachycerans and several attempts have been made to trace the origin of this principle (Shaw and Meinertzhagen, 1986; Shaw, 1989; Shaw and Moore, 1989; Shaw, 1990; Meinertzhagen, 1991; Nilsson and Ro, 1994; Melzer et al., 1997). Their closest relatives are the nematocerans (e.g. mosquitoes, crane flies and midges). Indeed, variations of the trapezoidal pattern in advanced flies (Fig. 3a) (Kirschfeld, 1967) can be found in the next-but-one cartridge arrangement of male bibionids and diurnal mosquitoes (Fig 3b) (Zeil, 1979, 1983; Land and Horwood, 2005), as well as in chaoborid midges (Fig. 3c) (Melzer et al., 1997). These examples of asymmetric branching in nematocerans most likely represent the ancestral pattern and precursor to neural superposition in advanced flies (Nilsson and Ro, 1994; Melzer et al., 1997).

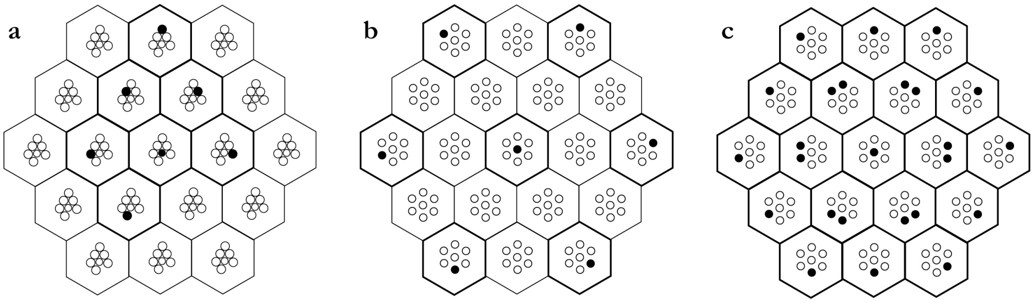


Fig. 3: Schematic cross-section of an ommatidial array with open rhabdomeres illustrating neural superposition in (a) advanced flies, (b) male bibionids and (c) chaoborid midges. The black rhabdomeres view the same point in space and project to the same lamina cartridge (a, b redrawn from Nilsson, 1989; c, redrawn from Melzer et al., 1997).

C. Superposition eyes

In superposition eyes, unlike the eye types already described, a wide pigment-free 'clear zone' spatially separates the optics and the light-absorbing rhabdom layer (Fig. 1c). The rhabdoms are usually wider and shorter compared to those of apposition eyes and the optical isolation provided by screening pigments is absent (at least during dark adaptation). Through specialised optics, commonly via refractive index gradients in the crystalline cones (refracting superposition, Fig. 1c), parallel light rays from a large number of facets can be focussed or 'superimposed' across this clear zone onto a single rhabdom (Exner, 1891; Nilsson, 1989). Hence, each rhabdom receives light through wide superposition apertures comprising typically several hundreds or even thousands of facets, which greatly improves photon catch and thus sensitivity. The gain in sensitivity is approximately equivalent to the number of superimposed facets and due to this enhanced photon capture, superposition eyes are typically found in nocturnal insects. Interestingly, the benefits of improved sensitivity are not restricted to nocturnal insects. Some diurnal insects also possess superposition eyes (Warrant et al., 1999) and often have a similar resolving power to diurnal insects with apposition eyes (Land, 1984). It is mainly at night that the huge advantage of the superposition eye becomes evident: while apposition eyes have to pay for each increase in photon capture with a proportional loss in resolution, in superposition eyes improved sensitivity often only causes a modest degradation of resolution (Caveney and Mcintyre, 1981; McIntyre and Caveney, 1985). Interestingly, superposition optics is restricted to eyes of certain size and beetles with small eyes tend to depend on apposition optics instead (Meyer-Rochow and Gál, 2004).

D. Implications of eye design for nocturnal vision

The small apertures of apposition eyes clearly limit insects to vision at bright light intensities while superposition eyes are the most effective design for an insect active in dim light (Warrant, 2001; Warrant, 2004). Thus, the driving question is how some insects have managed the transition to a nocturnal lifestyle whilst retaining their insensitive apposition eyes. Why have they not simply evolved a superposition eye? As beautifully explained by Nilsson (1989) a direct transition from an apposition to a superposition design is not as simple as one may think. Apposition eye optics generates multiple inverted images, whereas superposition optics creates a single upright image. A direct evolution from an apposition to a superposition eye would therefore need to convert this visual imaging from inverted to upright, which involves a passage through an unfocussed system. As there exists no selective pressure for unfocussed intermediate eye designs, superposition eyes cannot directly evolve from conventional apposition eyes. This has puzzled scientists for many years until the discovery of afocal apposition optics in butterflies presented an evolutionary pathway where every step results in improved vision (Nilsson et al., 1984, 1988). In butterfly eyes a strong lens within the crystalline cone works in a similar manner to the telescope system of refractive superposition eyes. Hence, moths might have achieved their transition to a nocturnal lifestyle, and the evolution of superposition eyes, via these afocal apposition optics (Nilsson et al., 1984, 1988). Although such an optical system may also exist in other apposition eyes, this has not yet been reported.

III. Nocturnal life with apposition eyes

Dim light severely limits nocturnal insects with apposition eyes during foraging and visual navigation. A crepuscular or nocturnal lifestyle has nevertheless evolved repeatedly within bees and also other insects with apposition eyes, like wasps, ants, mosquitoes and bugs. A major cost for enhanced sensitivity at low light intensities is decreased spatial resolution. In bees, this trade-off needs to be well balanced: finding a food source is known to be strongly guided by olfaction, however, upon return to their nest vision plays a large role in navigation. Reliable navigational cues (e.g. landmarks, sky polarization, canopy patterns and path integration) are available even at low light intensities. The nocturnal bee *M. genalis* is in fact the first insect known to orient using landmarks at very low light intensities. However, when spatial acuity becomes too low, such demanding tasks can no longer be achieved. In the apposition eyes of nocturnal mosquitoes, for instance, optical adaptations have evolved in favour of sensitivity (section V-A). Having sacrificed almost their entire spatial resolution, nocturnal mosquitoes need to rely on simpler visual behaviours (e.g. phototaxis) and in addition use other specialised sensory systems (e.g. olfaction, mechanoreception) to guide them through the night.

A. Crepuscular and nocturnal bees

Worldwide there are more than 16,000 described bee species (Michener, 2000) and a large majority of them are only active during the day. Nevertheless, crepuscular or nocturnal species occur in at least four of the seven currently described bee families, the Apidae, Andrenidae, Colletidae and Halictidae. Some of these bees are able to extend their foraging period into twilight or even throughout the night when the moon is present, and a few exceptional species have adopted a strictly nocturnal lifestyle.

1. Foraging in dim light

Most Apidae, like the European honeybee *Apis mellifera carnica*, forage only during the day despite their ability to retain achromatic vision down to moonlight intensities (Menzel, 1981; Rose and Menzel, 1981; Warrant et al., 1996). This capability of the visual system may explain why some honeybee species, including the African race of the honeybee *A. mellifera adansonii* and the Asian giant honeybee *A. dorsata*, will actively forage into the night when at least a half-moon is present and temperatures are mild (Fletcher, 1978; Dyer, 1985). A similar situation can be found in the carpenter bees (*Xylocopa,* Apidae), where dim-light foraging has been reported for some species in India, Thailand and Mexico (Janzen, 1964; Burgett and Sukumalanand, 2000; Somanathan and Borges, 2001). A particularly interesting example is the occurrence of three *Xylocopa* species in the Western Ghats of India: *X. ruficornis* is strictly diurnal, *X. tenuiscapa* occasionally also forages in the evening and *X. proximata* is strictly nocturnal (Somanathan and Borges, unpublished data). Carpenter bees are large bees and their ability to forage despite low temperatures and strong winds, conditions in which not even moths are able to fly, makes them important pollinators for night flowering plants (Somanathan and Borges, 2001). Similarly, the predawn flights of *Xenoglossa fulva* (Apidae) are of considerable importance for the pollination of its sole pollen host *Curcubita* sp. in Central and North America (Linsley et al., 1955).

Dim-light foraging within the Adrenidae is only known from the crepuscular bee *Perdita bequaertiana* (Cockerell, 1923). This oligolectic species collects pollen from the evening primrose *Oenothera* sp., which opens its flowers just after sunset. Within the family of the Colletidae, pre-dawn foraging is found in *Ptiloglossa* sp. and *Caupolicana* sp. (Linsley, 1962; Linsley and Cazier, 1970; Roberts, 1971), which close their burrows during the night. Within a population, the opening of the nests occurs simultaneously in the early morning, indicating that an internal circadian rhythm may play a role in the onset of their foraging (Linsley and Cazier, 1970).

Dusk and dawn foraging has also been reported for *Sphecodogastra galpinisiae*, a member of the large family Halictidae. *S. galpinisiae* starts to forage early on warm mornings and has a second period of evening flights lasting until late twilight (Bohart and Youssef, 1976).

This foraging period is even further extended in *S. texana*, which is crepuscular, but continues to forage for as long as the moon is present at night (Chandler, 1961; Kerfoot, 1967b). Foraging at low light intensities has been thoroughly studied in the halictid bees *M. genalis* and *M. equadoria* (Fig. 4) (Warrant et al., 2004; Kelber et al., 2006). Both species are native to parts of Central and South America with the main study site located on Barro Colorado Island, Panama (Rau, 1933; Leigh, 1999). Using their strong mandibles, they hollow out small wooden branches to construct nests and brood chambers (Fig. 4b), which they provision with nectar and pollen from a large variety of canopy and understorey flowers (Sakagami, 1964; Janzen, 1968; Roulston, 1997; Wcislo et al., 2004). Although predominantly solitary, *M. genalis* and *M. equadoria* display facultatively social behaviour (Arneson and Wcislo, 2003; Smith et al., 2003).

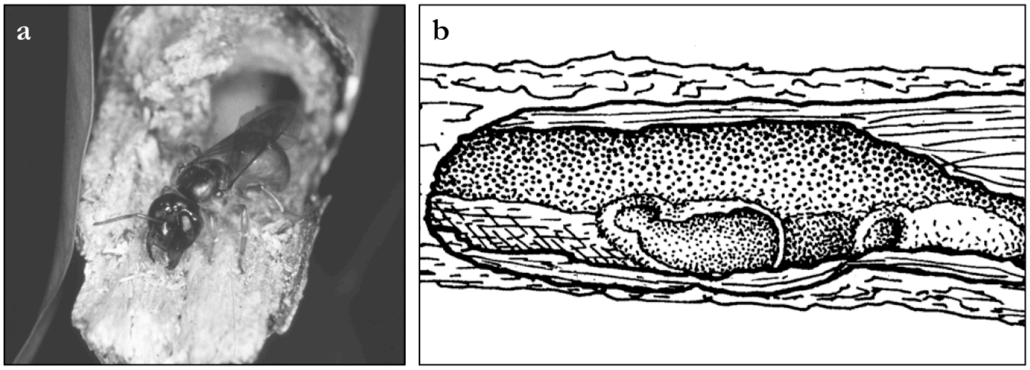


Fig. 4 (a) A female *Megalopta genalis* in front of its nest entrance (photograph courtesy of M. Pfaff). (b) Cut-away view from the nest showing the brood chamber (drawing courtesy of D. Conlon).

Amazingly, these bees are able to forage and navigate at extremely low light intensities (Warrant et al., 2004). Both species have been caught in light traps throughout the night (Wolda and Roubik, 1986; Roulston, 1997), however, recent behavioural studies show two distinct foraging periods during early dawn and late dusk, when light levels underneath the forest canopy can reach less than starlight intensities (Warrant et al., 2004; Kelber et al., 2006). Although such extremely low light intensities should theoretically render their apposition eyes blind, these nocturnal bees are able to find their nest entrances with the aid of surrounding landmarks (Warrant et al., 2004).

2. Landmark navigation

Many insects are able to memorise visual landmarks around their nests and along the routes to their foraging sites (Collett and Collett, 2002). A variety of features (e.g. colour, size, shape and symmetry) can be used to learn and subsequently recognise visual landmarks. Two different classes of landmarks are likely to be available to insects: landmarks close to the goal that are used for determining its exact position, and larger and more distant landmarks for guiding the insect to the approximate position of the goal. Bees and wasps perform so-called orientation flights to acquire landmark information near to the nest site (Zeil, 1993) and around feeders (Collett and Lehrer, 1993; Lehrer, 1993). The shape of these flights resembles a series of increasing, semicircular arcs that are kept at constant angular velocity whilst the insect is backing away from the goal (Zeil, 1993).

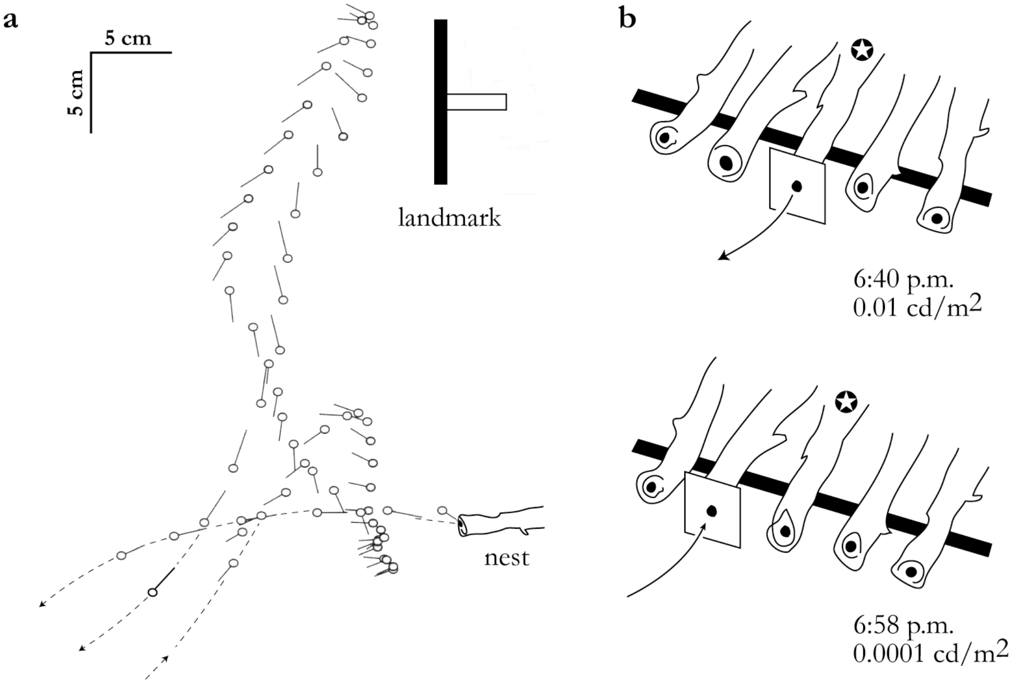


Fig. 5 (a) A reconstructed arc-shaped learning flight of the nocturnal bee *M. genalis* with the circle representing the head and the line the body of the bee at 40 ms intervals. (b) Landmark experiment, where the bees were given white square cards to learn, and which they use as reference upon return instead of other sensory cues from her original nest marked by the star. Time of the day and light intensities are shown, where 0.0001 cd/m^2 is equivalent to an intensity of less than starlight (from Warrant et al., 2004).

Such orientation flights around the nest entrance have recently been shown to occur even at very low light intensities (Warrant et al., 2004). The nocturnal bee *M. genalis* performs arc-shaped learning flights that are very similar to those in day-active bees (Fig. 5a). Using these, *M. genalis* is able to navigate by means of natural as well as artificial landmarks near the nest site to locate the nest entrance (Fig. 5b) (Warrant et al., 2004). Unlike some ground-nesting bees and wasps that perform learning flights each morning upon their first departure (Zeil, 1993; Brünnert et al., 1994), *M. genalis* displays these flights occasionally both early in the morning and in the evening. To prove that it really is vision and not olfaction that guides these bees close to the nest, Warrant et al. (2004) performed a series of landmark experiments. By means of an artificial landmark mounted around the nest entrance, the bee was first allowed to learn the new structure during its orientation flights. The bee was usually given several days to get used to the landmark. Then after a first flight on one evening, the landmark was moved to an unoccupied nest alongside and indeed the bee was 'fooled' to fly into the now newly marked but wrong nest entrance (Fig. 5b). Once inside the wrong nest it discovers its mistake and leaves the nest quickly. However, instead of searching the surrounding area for its true nest, the bee was so convinced by the visual landmarks that it would fly back into the same nest and out again continuing with this behaviour until the landmark was returned to the bee's own original nest.

An analysis of their collected pollen shows that these nocturnal bees are largely generalists and visit flowers both in the canopy as well as on understorey shrubs (Wcislo et al., 2004) with foraging flights lasting up to 34 min (Kelber et al., 2006). How can they navigate over the large spatial range of a long foraging trip? In addition to the described close-range landmark navigation, preliminary results suggest that polarisation vision may also play a role in the long-distance foraging flights of *M. genalis* (Greiner et al., in preparation). A major limitation for solving most behavioural questions in these nocturnal bees is that they are difficult to study outside their nest area. One looses sight of them quickly after their orientation flights and due to their densely vegetated natural environment within the rainforest, harmonic radar, as used to elucidate the long-distance flight path of honeybees (Menzel et al., 2005), is not an option. Furthermore unlike social bees, *M. genalis* forages only once or twice during each activity period, which makes it hard to train them even in laboratory conditions. Thus, no behavioural data are currently available regarding their long distance navigation.

B. Other nocturnal insects with apposition eyes

Bees are not the only insects that use apposition eyes at low light intensities. Other hymenopterans (i.e. wasps and ants) are also active at night. In two genera of social wasps, *Apoica* and *Provespa*, nocturnal behaviour has been documented (Hunt et al., 1995; Matsuura, 1999). The nocturnal wasp *A. pallens*, for example, swarms during dusk and forages only at night (Vesey-FitzGerald, 1938; Schremmer, 1972; Hunt et al., 1995; Howard et al., 2002). Despite the importance of chemical cues in ant societies, specific optical adaptations to enhance sensitivity are present in the apposition eyes of night-active *Camponotus* species (Menzi, 1987). This suggests that at least in these nocturnal ants, vision plays a role in their orientation. Nocturnal flights are present in male army ants of the genus *Neivamyrmex* sp. (Baldridge et al., 1980). However, the potential role of their unusually large eyes and wide rhabdoms (B. Greiner, unpublished data) in nocturnal foraging or mating flights is unknown. Activity periods of mosquitoes can range from nocturnal blood-feeding species, like *Anopheles gambiae*, to the diurnal plant-feeder *Toxorhynchites brevipalpis*. *A. gambiae* is strictly nocturnal and shows behavioural optomotor responses down to light levels between moon- and starlight (Clements, 1963; Gibson, 1995). As the apposition eyes of nocturnal mosquitoes trade spatial resolution for enhanced sensitivity, specialisations of their olfactory system play a dominant role during host-finding (Takken and Knols, 1999). The nocturnal bug *Triatoma infestans* also possesses a highly sensitive visual system, which is mainly used to find refuges and to avoid predator exposure at night (Lazzari, 1992; Reisenman et al., 1998).

C. Advantages of being nocturnal

What are the benefits of a nocturnal life for insects with apposition eyes? The current hypothesis is that the cover of night provides protection from diurnal predators and the reduced density of competitors secures early access to food sources. Bats discovered foraging on night-blooming flowers as a new niche and these animals act as important pollinators of nocturnal flora (Park, 1940; Baker, 1961; Marshall, 1983; Machado and Vogel, 2004).

Is this well-established hypothesis also true for insects? Wcislo et al. (2004) recently showed that these advantages are most likely responsible for the transition to nocturnal life in tropical bees and future work may further support the general validity of this hypothesis in other insects. Foraging at low light intensities is particularly common in tropical areas, an environment of severe food-competition and a high risk of predation. As temperature is no limitation, even small species are able to exploit food sources early in the morning or late at night when most competitors are inactive. By hiding during the day, these bees are able to protect themselves, and also their brood, when most predators and parasites are active (Smith et al., 2003). However, for bees the major limitation on their flight activity at night is light intensity (Kelber et al., 2006).

IV. Limitations to vision at low light intensities

While spatial resolution determines eye designs in bright daylight, extracting information from unreliable signals is the main limitation for vision in dim light. Light intensity can be defined as the average number of photon arrivals per unit area and time, where photon arrival is a random event (Fig. 6). Consequently, the fewer photons a photoreceptor absorbs per integration (or sampling) time, the larger the unreliability (noise) in the average number of photons sampled. Minimising the noise and simultaneously maximising the signal is the major task of an eye in order to function efficiently at low light intensities (Laughlin, 1990). Another limitation for eyes with small apertures may be diffraction. Parallel light passing through small apertures will suffer an angular spread, thus leading to a blurry image (Airy disc) and a loss in resolution. However, nocturnal apposition eyes generally have large lenses and wide rhabdoms where diffraction has little effect (Warrant and McIntyre, 1993).

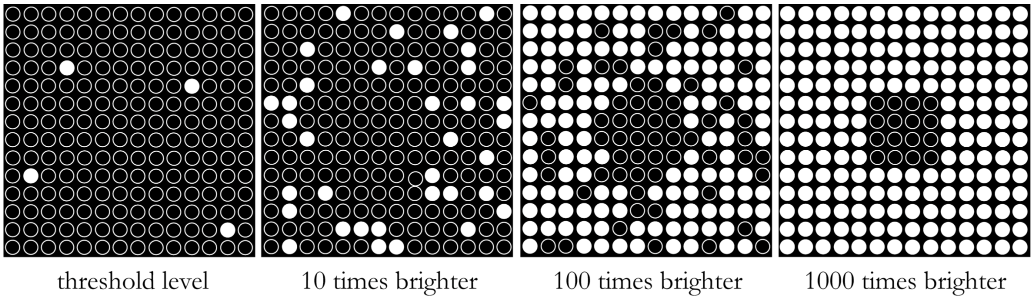


Fig. 6: The random arrival of photons on an array of photoreceptors demonstrates the unreliability of the visual system at low light intensities. The black square in the centre can only be distinguished with certainty at light levels 3 log units brighter than threshold level (redrawn from Pirenne 1967).

A. Visual noise

The major limitation for nocturnal vision in insects is consequently visual noise, the sources of which can be of three kinds within the photoreceptor: photon shot noise, dark noise and transducer noise (Warrant 2004). As photon arrival follows Poisson statistics, a photoreceptor absorbing a number of N photons experiences an uncertainty (or photon shot noise) of $\sqrt{N}$ photons (Land, 1981; Warrant and McIntyre, 1993). Decreasing photon catch in dim light results in an increasing noise level that degrades the ability of two photoreceptors to discriminate contrast. As two visual channels need to detect sufficient photons in order to reduce this noise level below the actual difference in intensity, the eye has to enhance its sensitivity in order to reliably detect the contrast. However, improved sensitivity only comes at the cost of losses in spatial and/or temporal resolution.

Dark noise also originates within the photoreceptor and consists of spontaneous thermal responses in the absence of photons, which are indistinguishable from membrane potentials (quantum bumps) produced by photons (Barlow, 1956). These fluctuations are more frequent at higher temperatures and introduce uncertainty at low light intensities. Nevertheless, compared to the high rates measured in vertebrates (Aho et al., 1988), dark noise is thought to have a low rate in invertebrates (Warrant, 1999).

Transducer noise is due to variations in the amplitude, latency and duration of quantum bumps produced upon photon absorption within the photoreceptors (Lillywhite and Laughlin, 1979). This uncertainty adds to the unreliability of vision, although is expected to be of lesser importance in dim light (Laughlin, 1990). Thus, as photon shot noise is the main source of uncertainty, nocturnal insects with apposition eyes have to maximise the photon catch or signal-to-noise ratio to enhance sensitivity, even if it comes at the cost of severe losses in resolution.

B. Resolution vs. sensitivity

An eye has to fulfil two basic tasks: first and foremost for dim-light vision, it has to be sufficiently sensitive, collecting enough photons to reduce the effects of photon noise. Secondly, it needs to possess spatial resolving power in order to determine the direction of the incident light (Warrant and McIntyre, 1993). These two prerequisites often stand in a cost-benefit relation, meaning that if an eye needs to function at low light intensities it has to trade resolution for sensitivity (Warrant and McIntyre, 1992; Warrant, 2004). A high signal-to-noise ratio is not only beneficial for vision at low light intensities, but also has advantages for various other aspects of vision, e.g. colour vision, motion vision, and contrast discrimination during the day. These advantages may have been selective for the transition to a diurnal lifestyle in insects with highly sensitive superposition eyes. The superposition eye of the diurnal hawkmoth *Macroglossum stellatarum* has excellent trichromatic colour vision (Kelber, 1999; Balkenius and Kelber, 2004) and remarkably, scotopic colour vision has even been shown to exists in the nocturnal hawkmoth *Deilephila elpenor* (Kelber et al., 2002). Despite the limitations for vision in dim light, various insects have managed the transition from a strictly diurnal to a nocturnal lifestyle. Hence, specific adaptations must have evolved in the apposition eyes of nocturnal insects to account for these shortfalls.

V. Adaptations for vision in dim light

Activity at light intensities up to 8 log units dimmer than during the day has led to the evolution of various specific adaptations in the visual systems of insects with apposition eyes. In general, sensitivity can be enhanced via optically collecting more photons within the eye, or by neurally summing signals in space or time (Lythgoe, 1979).

A. Optical adaptations

Independent of eye design, there are four major optical parameters that can affect light flux: facet size, changes of focal length, dimensional changes of the rhabdom, and pigment migrations within the retina (Nilsson, 1989). An ideal measure of quantifying optical sensitivity, which includes these factors, is to calculate the number of photons (*N*) a photoreceptor can capture per integration time (Δt) from a light intensity spectrum $I(\lambda)$ where λ is wavelength (Warrant and Nilsson, 1998; Warrant, 1999; Kelber et al., 2002; Warrant et al., 2004):

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

N depends on a number of optical and physiological parameters, namely on the size of the aperture (*D*), the length of the photoreceptor (*l*), the receptive field of the photoreceptor ($\Delta\rho$), the length of time a sample of photons is counted in the photoreceptor (integration time Δt), and on the absorption rate within the photoreceptor, which is based on the quantum efficiency of transduction (κ), the transmission of the optics (τ), the absorption coefficient of the rhabdom (*k*) and the spectral sensitivity of the photoreceptor $R(\lambda)$.

Thus, in apposition eyes specific optical and structural adaptations have evolved to enhance optical sensitivity, including changes in the facet diameter and the receptive field of the photoreceptors. This section describes these optical adaptations (i.e. facet size, focal length, pigment migrations and rhabdom diameter) and discusses their effect on sensitivity.

1. Facet size and corneal surface structure

Nocturnal bees and wasps typically possess prominent eyes and huge ocelli with an eye area 1.8 times larger than in their diurnal relatives (Kerfoot, 1967a; Jander and Jander, 2002; Greiner et al., 2004a; Greiner, 2005). In the case of bees, these large eyes show a regional increase of facet size, which directly affects the optical sensitivity of the eye (Greiner et al., 2004a). In contrast, the larger eyes of the nocturnal wasp *A. pallens* (Fig. 7a) contain 2000 more facets when compared to the eye of a diurnal relative (Fig. 7b), as facet size distribution is similar (Greiner, 2005). Optical measurements demonstrate that greater sensitivity in nocturnal wasps is instead achieved by a wide photoreceptor rhabdom diameter and a large visual acceptance angle (Greiner, 2005).

In mosquitoes, clear differences in eye structure are also related to light intensity (Land et al., 1999). Nocturnal species have significantly fewer facets of generally larger diameter compared to diurnal species (Land et al., 1999), indicating that nocturnal mosquitoes trade sensitivity against spatial resolution. Despite the obvious benefit of enlarging facets for nocturnal vision, nocturnal ants possess both smaller eyes and fewer facets of equal diameter compared to their diurnal relatives (Menzi, 1987). A pupil mechanism and extremely wide rhabdoms (see below) nevertheless support the importance of vision for these ants.

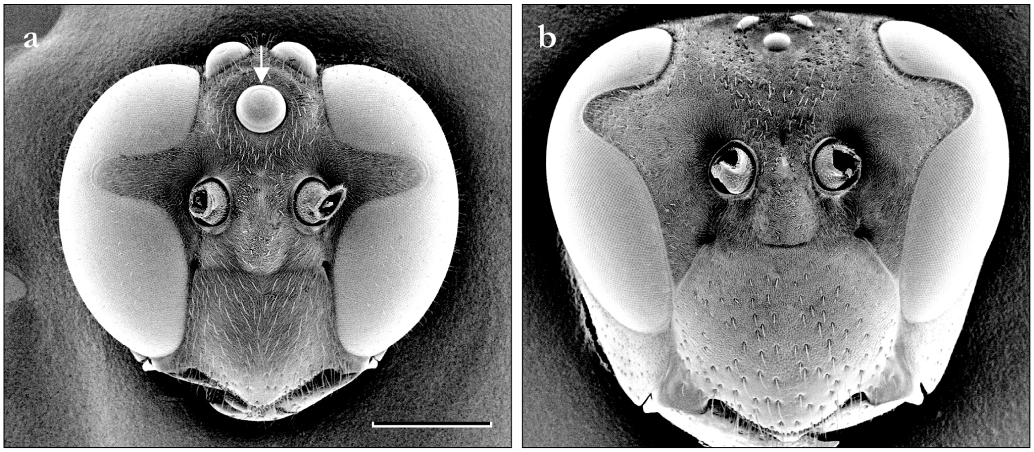


Fig. 7: Scanning electron microscopy images showing the large eyes and ocelli from (a) the nocturnal wasp *A. pallens* and (b) the diurnal wasp *Polistes occidentalis*. *Arrow* in (a) marks the medial ocellus, scale 1 mm for (a, b) (adapted from Greiner, 2005).

A detailed investigation of the corneal micro-surface reveals an array of nipple structures in some nocturnal species, which is thought to further enhance sensitivity (Miller, 1979; Stavenga et al., 2005). The anti-reflectance effect of this corneal nipple array facilitates the transition of light into the eye by reducing reflection. In nocturnal bees, the effect is calculated to be 4 % (Greiner et al., 2004a) and even such a small increase of sensitivity is likely to be important for a photon-starved eye. Despite these benefits for dim-light vision, corneal nipples are not present on the lens surface of the nocturnal wasp *A. pallens* (Greiner, 2005).

2. Dioptric apparatus and focal length

The dioptric apparatus of the compound eye, consisting of the corneal lens and the crystalline cone, focuses incoming light onto the distal rhabdom tip. Generally, the lens, either via refractive index gradients within the lens cylinder, or its convex curvature, forms the image. The crystalline cone represents a mere spacer and has no direct optical function (Nilsson, 1989). As most of the refractive power comes from the outer corneal surface, it is surprising that the corneal facets of nocturnal bees and wasps feature flattened outer and strongly convex inner curvatures instead (Fig. 8a,b) (Greiner et al., 2004a; Greiner, 2005). As this inner curvature is not present in their diurnal relatives (Fig. 8c,d), it may represent an optical adaptation for nocturnal vision. However, despite this inner corneal curvature, theoretical calculations using the thick lens formula (Land et al., 1999) predict a graded refractive index within the lens of the nocturnal bee *M. genalis*, in order to focus light onto the distal rhabdom (Greiner et al., 2004a).

In nocturnal mosquitoes, in contrast, focussing is achieved via an almost hemispherical curvature of the facet lens, which transmits a 65° cone of light onto the rhabdom (Land et al., 1997; Land et al., 1999). As the outermost rays of such lenses are highly over-focussed and cause severe spherical aberration, spatial resolution is sacrificed in order to enhance sensitivity. Despite this trade-off, the system minimises spherical aberration by allowing the position of the distal rhabdom to coincide with the smallest blur circle of the refracted light (Land et al., 1999).

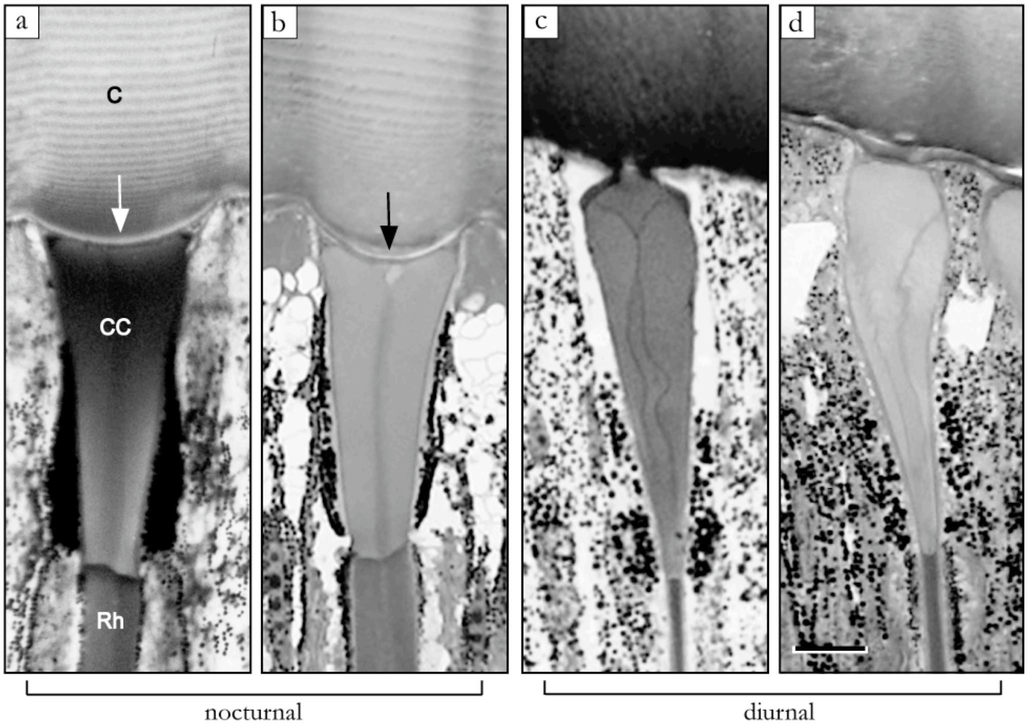


Fig. 8: The dioptric apparatus in the apposition eyes of (a) *M. genalis*, (b) *A. pallens*, (c) *Apis mellifera* and (d) *P. occidentalis*, showing the proximal cornea (C), crystalline cone (CC) and rhabdom (Rh) directly beneath. Note the strongly convex inner corneal curvature, its tight apposition to the CC and the extremely wide rhabdoms in the nocturnal bee (a) and wasp (b), which are not present in their diurnal relatives (c, d). Scale for (a-d) in (d) 10 μm (adapted from Greiner, 2005).

As for cameras, a large aperture (or facet diameter D in apposition eyes), and a relatively short focal length (f) lead to a system of high sensitivity defined by a low F-number:

$$F = f / D \tag{2}$$

Low F-numbers are adaptations commonly found in nocturnal animals, as large apertures and short focal lengths allow light to reach the rhabdom over a wide range of incident angles (Warrant and McIntyre, 1991). Diurnal insects with apposition eyes usually have eyes of high *F*-number, typically above 2.1 (Warrant and McIntyre, 1993). Eyes of low *F*-number, whilst sensitive, tend to have poorer spatial resolution. Due to the small difference in refractive index between the rhabdom and its surroundings, only rays with a maximum incident angle of about 10° can be trapped within a cylindrical rhabdom (Warrant and McIntyre, 1993). The minimum *F*-number for light to be totally reflected within the rhabdom is consequently 2.8 (Warrant and McIntyre, 1993). Most diurnal apposition eyes, and even many nocturnal insects, have *F*-numbers close to this value, ensuring that most of the light remains trapped within their rhabdoms (Greiner et al., 2004a; Greiner, 2005).

For those eyes with *F*-values less than 2.8, several solutions exist to avoid the severe spreading of unused stray light (Warrant and McIntyre, 1991). A shield of light-absorbing pigments would remove scattered light, but as this comes only at the cost of sensitivity it is not an ideal option for dim-light vision. In eyes where photoreceptors have large visual fields, non-cylindrical rhabdoms can trap light from an incident angle much greater than 10°, thus enhancing sensitivity, as demonstrated in nocturnal mosquitoes (Land et al., 1997). The most complete solution is to surround the rhabdom with a tapetal sheath (reflective pigment granules or tracheoles), however this has not yet been shown to exist in the apposition eyes of nocturnal insects.

Even though the trade-off between sensitivity and resolution represents a distinct limitation of eye design, most insect eyes also need to function over a wide range of light intensities. This is mediated by migrations of screening pigments.

3. Pigment migrations within the retina

Movements of screening pigments during light adaptation (LA) and dark adaptation (DA) constitute the most important pupillary mechanism found in compound eyes. These migrations can take place in primary and secondary pigment cells, as well as inside the retinula cells (Walcott, 1975; Autrum, 1981). Without optical mechanisms that adapt the eye to different light intensities, photoreceptors are unable to operate over a range of more than about 3 log units (Walcott, 1975). Animals active in a broader intensity range therefore need mechanisms to adjust the sensitivity of their eyes accordingly.

Just as humans use the iris as a pupil, screening pigments serve the same function in apposition eyes. Radial pigment movements, either in the retinula cells or in the primary pigment cells, are typical for apposition eyes, while longitudinal migrations are mostly found in superposition eyes (Warrant and McIntyre, 1996). The onset of pigment migration is often controlled directly by light intensity, but endogenous circadian rhythms can also influence migration (Menzi, 1987; Reisenman et al., 2002).

a. Radial pigment migration

In most apposition eyes, radial pigment migration is mediated by retinula or primary pigment cells (Walcott, 1975; Autrum, 1981). Within the retinula cells, pigments tightly surround the rhabdom in the LA state and migrate away from the rhabdom during the DA state (Ribi, 1978). Close to the rhabdom, these pigments absorb the light propagating outside the microvilli, which sharpens spatial acuity but decreases sensitivity (Land and Osorio, 1990). Many day-active insects use the benefits of this mechanism (Stavenga, 1979), but interestingly it is also present in some night-active insects, like cockroaches (Butler and Horridge, 1973) and nocturnal bees (Fig. 9) (Greiner et al., 2004a). As nocturnal bees are perfectly able to fly during daylight (although rarely doing so), this mechanism is important for extending their sensitivity range.

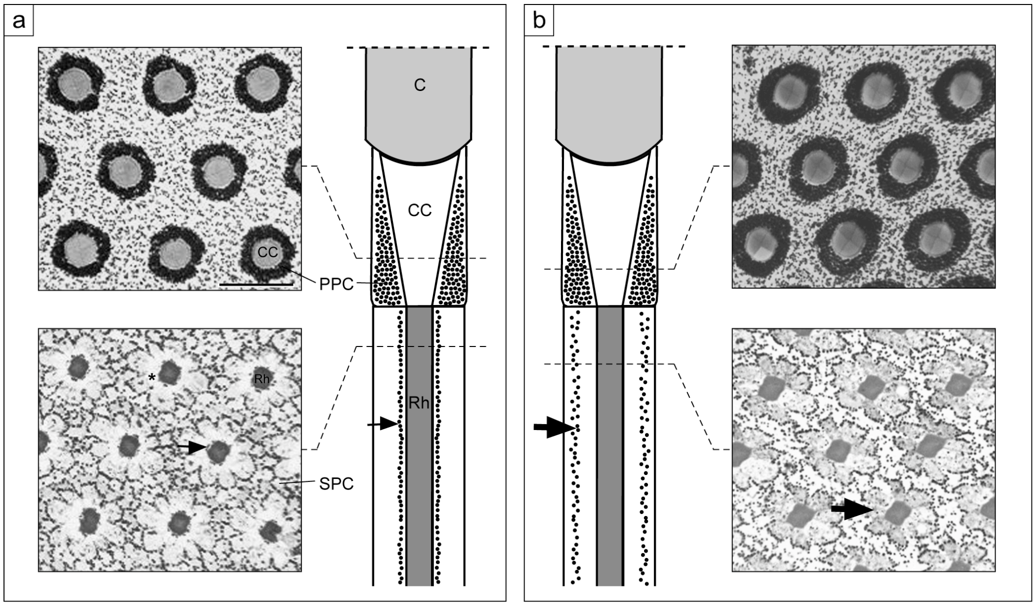


Fig. 9: Radial pigment migration in the retinula cells of the nocturnal bee *Megalopta genalis*. (a) Pigments tightly surround the rhabdom (Rh) during light adaptation (*small arrows*) and (b) move away from the rhabdom into the cytoplasm during dark adaptation (*large arrows*). Note that no pigment migration is present in the primary (PPC) and secondary (SPC) pigment cells. C – cornea, CC – crystalline cone, scale in (a) for (a, b) 25 µm (from Greiner et al., 2004a).

By effectively reducing the intensity during bright light, radial migrations of retinula cell pigments have an analogous effect to dynamic pupils located distally to the rhabdom (Autrum, 1981). Such pupils often cause strong retinomotor movements affecting not only the primary pigment cells, but also the shape of the crystalline cone and the position of the distal rhabdom (Lüdtke, 1953; Eckert, 1968). During LA, when the primary pigment cells form a closed pupil, a long narrow crystalline cone tract appears and the rhabdom distance towards the lens increases. During DA, in turn, this adaptive mechanism shortens the focal length and enhances the eye's sensitivity by widening the visual field of the photoreceptor. Little is known, however, regarding the mechanisms driving these retinomotor movements, as microtubuli can be found in the visual cells as well as in the primary pigment cells and the crystalline cone (Autrum, 1981).

Primary pigment pupils are typically found in apposition eyes with open rhabdoms that lack neural superposition (Lüdtke, 1953; Walcott, 1971; Ioannides and Horridge, 1975; Williams, 1980; Nilsson and Ro, 1994; Reisenman et al., 2002). The pupil mechanism mediates high resolution/low sensitivity via the central rhabdomeres when the pupil is narrow (during LA) and low resolution/high sensitivity when the pupil is wide open and exposes the outer rhabdomeres to light (during DA) (Nilsson, 1989). The significant gain in sensitivity, when the eye is dark-adapted, is of great benefit for crepuscular and nocturnal species. Primary pigment pupils also occur in some eyes with fused rhabdoms, where the pupil instead regulates the visual angle of the entire rhabdom during LA and DA (Menzi, 1987; Land et al., 1999).

b. Longitudinal pigment migration

Longitudinal pigment migrations are generally only present in superposition eyes, where secondary pigments may migrate into the clear zone to optically isolate the ommatidia in the light-adapted state (Autrum, 1981; Nilsson, 1989). However, in the apposition eye of the nocturnal bug *Triatoma infestans* and the common European earwig *Forficula auricularia*, movement of retinula cell pigments along the longitudinal axis of the rhabdomeres adds to the effect of the primary pigment pupil during LA (Nilsson and Ro, 1994; Reisenman et al., 2002). Longitudinal retractions of secondary and retinula cell pigments during DA could greatly improve the sensitivity of an apposition eye, but without specific superposition optics this would result in severely blurred images as light crosses between the ommatidia.

Not all insects use the dynamic changes of pigment migrations. Instead, some have large cisternae (or palisades) surrounding the rhabdom in the DA state (Horridge and Barnard, 1965; Butler and Horridge, 1973), which could function as spacers between the rhabdom and the light absorbing pigment granules (Snyder and Horridge, 1972). As the optical density of these palisades is lower than the cytoplasm, they are also thought to improve the light-guiding properties of the rhabdom by retaining the light energy within the rhabdom (Horridge and Barnard, 1965; Ioannides and Horridge, 1975).

4. Changes of rhabdom size and shape

Rhabdom size plays a crucial role in the apposition eyes of nocturnal insects as enlarging its effective area directly enhances sensitivity. Most insects that are active at low light intensities have wide rhabdoms where the diameter either remains constant or changes according to a circadian rhythm. Comparative studies between diurnal and nocturnal hymenopterans show that a constant rhabdom diameter of 8 µm is present within the nocturnal apposition eyes of the halictid bee *M. genalis* (Greiner et al., 2004a), the wasp *A. pallens* (Greiner, 2005) and the ant *Camponotus ligniperda* (Menzi, 1987).

A circadian rhythm, where rhabdom diameter widens significantly during DA, is present in the apposition eyes of locusts and mantids (Rossel, 1979; Horridge et al., 1981; Williams, 1982). As locusts perform migration flights at low light intensities (Chapman, 1980), this circadian change in rhabdom diameter widens the receptive field of the photoreceptors to enhance sensitivity at night and subsequent narrowing optimises spatial resolution during the day (Tunstall and Horridge, 1967).

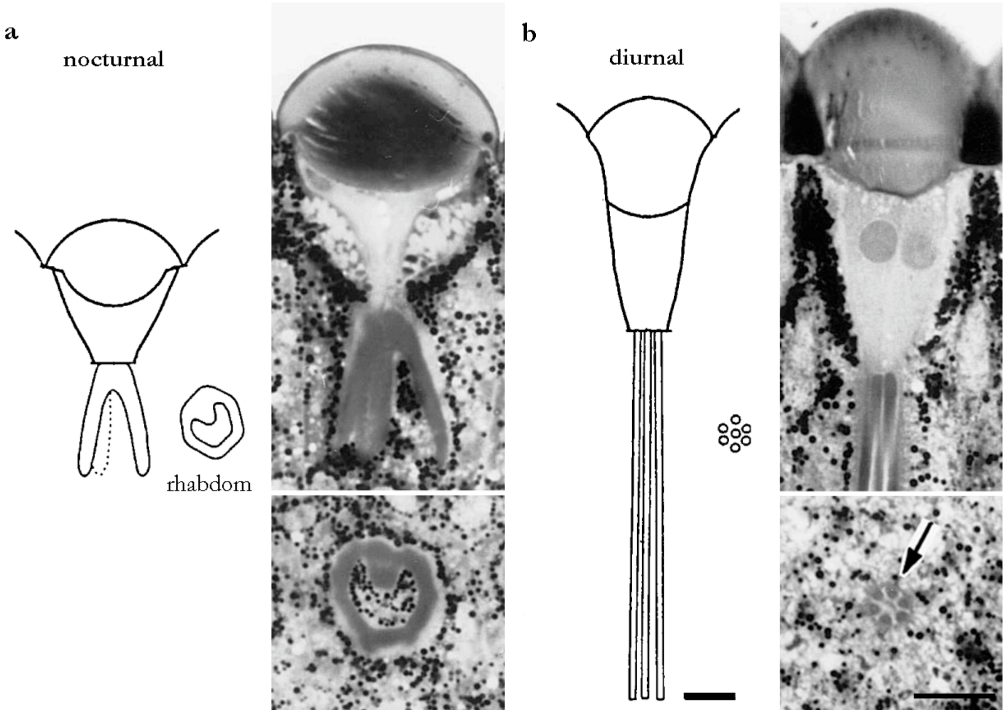


Fig. 10: The ommatidial structure of (a) nocturnal and (b) diurnal mosquitoes, shown as a schematic drawing and as histological sections. (a) The rhabdom of nocturnal mosquitoes has a unique hollow cone shape with a blunt apex and traps all the light reaching the eye through the large, almost hemispherical lenses. (b) In contrast, diurnal mosquitoes have narrow, open rhabdomeres and much smaller apertures. Scale for (a,b) in (b) 10 µm, *arrow* in (b) points at the open rhabdomeres in the eyes of the diurnal mosquito (modified after Land et al., 1999).

The nearly hemispherical lenses of crepuscular and nocturnal mosquitoes focus a 65° wide cone of light onto the rhabdom during DA. In order to capture this wide cone, a unique rhabdomeric shape, consisting of a wide fused apex and proximal hollow cone, has evolved in these species (Fig. 10a) (Mazokhin-Porshnyakov and Kazyakina, 1978; Land et al., 1997; Land et al., 1999). Only with such a conical rhabdom, is all the light effectively trapped within the rhabdom (Land et al., 1997). As diurnal species have open rhabdomeres with a narrow cylindrical shape, conical rhabdoms represent a purely nocturnal adaptation (Fig. 11b) (Land et al., 1999). Another extreme case is the apposition eye of the deep-sea crustacean *Cirolana borealis*, where the rhabdom width and length are equal and remarkably large (90 × 90 μm) (Nilsson and Nilsson, 1981). However, the overall cost of widening the visual field to such extents is the almost complete loss of spatial resolution.

5. Resolution, visual field and optical sensitivity

The anatomical resolution of an apposition eye is determined by the ommatidial packing density, which is given by the interommatidial angle ($\Delta\phi$). In conventional apposition eyes, $\Delta\phi$ is typically narrow and together with a small acceptance angle ($\Delta\rho$, half-width of the photoreceptor's angular sensitivity curve) this leads to good spatial resolution. Surprisingly, in the nocturnal bees *M. genalis*, the minimal $\Delta\phi$ of 1.4° in the frontal eye region is extremely narrow for an insect that needs to maximise sensitivity (Fig. 11a) (Warrant et al., 2004).

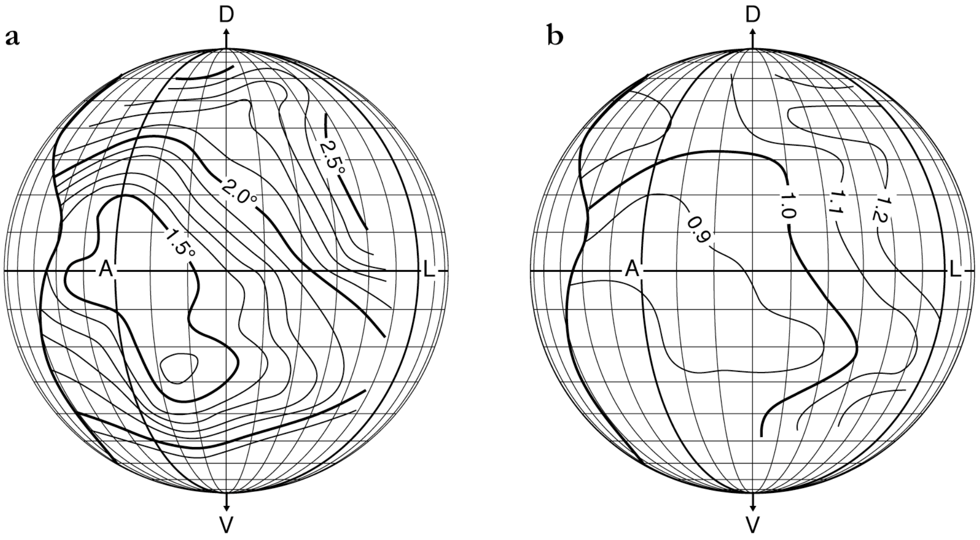


Fig. 11 (a) Interommatidial angles $\Delta\phi$ and (b) the eye parameter p of *Megalopta*'s left eye. Data are plotted onto a sphere representing the three-dimensional space around the bee. Latitude and longitude are shown in intervals of 10°. D – dorsal, V – ventral, A – anterior, L – lateral (adapted from Warrant et al., 2004).

However, as insects active in dim light have in general larger facets, we can examine the trade-off between sensitivity and resolution using the eye parameter (p), which relates $\Delta\phi$ to the facet diameter D (Snyder, 1979):

$$p = D\Delta\phi \tag{3}$$

Diurnal insects active in bright light intensities typically optimise resolution by having smaller eye parameters. In *M. genalis*, the values for p reach a minimum of 0.9 μm·rad in the frontal eye region (Fig. 11b) (Warrant et al., 2004). This is high compared to most diurnal insects but still lower than generally predicted for nocturnal insects, which is greater than 2 μm·rad (Snyder, 1979). Paradoxically, in nocturnal bees the anatomical resolution of the eye seems more suited for activity in bright light, which brings us to the question of whether the visual fields of the photoreceptors also reflect this.

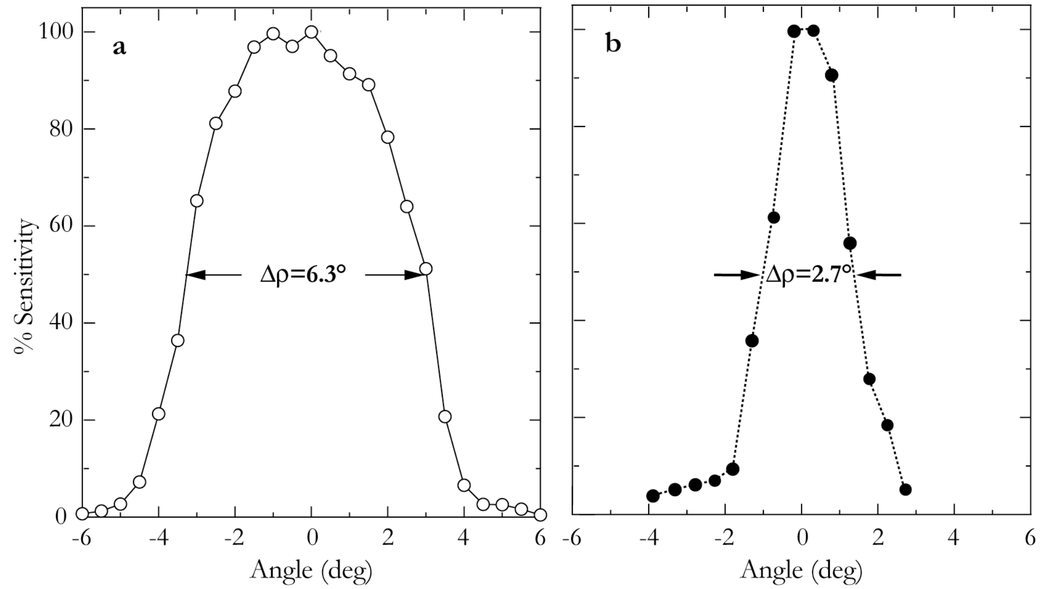


Fig. 12. The angular sensitivity function of dark-adapted photoreceptors in (a) the nocturnal bee *Megalopta genalis* and (b) the worker honeybee *Apis mellifera*. The half-width $\Delta\rho$ of *M. genalis* is more than twice as wide as in *A. mellifera*. (a, adapted from Warrant et al., 2004; b, redrawn from Laughlin and Horridge, 1971).

Despite its detrimental effects on resolution, a useful and common mechanism to enhance sensitivity in dim light is to increase the angle in space from which the photoreceptor receives light (Snyder, 1977; Land, 1981). Essentially all the optical mechanisms discussed earlier in this section, affect the size of the photoreceptor's visual field: large apertures, short focal lengths, wide rhabdom diameters and large dark-adapted pupils all effectively widen $\Delta\rho$ (Snyder, 1979). Typically, day-active insects like bees, butterflies and dragonflies, have a narrow $\Delta\rho$ even during DA. However, insects active in dim light are able to effectively use these optical mechanisms to widen their dark-adapted $\Delta\rho$. The wide rhabdom diameter in the nocturnal bee *M. genalis*, for example, leads to a $\Delta\rho$ of around 6° (Fig. 12a) (Warrant et al., 2004), which is more than twice as wide as in the worker honeybee ($\Delta\rho_{DA} = 2.6°$, Fig. 12b) (Laughlin and Horridge, 1971). These values compare to a dark adapted $\Delta\rho$ of almost 6° in locusts (Tunstall and Horridge, 1967), and about 7° in cockroaches (Butler and Horridge, 1973), both also caused by wider rhabdom diameters at night. Pupil mechanisms usually have a much stronger effect on the dark-adapted $\Delta\rho$: in the backswimmer *Notonecta glauca* and the cranefly *Tipula pruinosa*, $\Delta\rho$ increases almost 3 and 4-fold during dark adaptation, respectively (Nilsson and Ro, 1994).

At bright light intensities, a visual overlap $\Delta\rho/\Delta\phi$ of less than 2 is optimal for resolution (Snyder, 1979). For example, in the worker honeybee, $\Delta\rho$ matches the anatomical resolution of the eye ($\Delta\phi = 1.9°$: van Hateren et al., 1990), leading to a visual overlap of only 1.4. However, in dim-light conditions a larger $\Delta\rho$ improves the signal-to-noise ratio of the photoreceptors for low spatial frequencies. This is when a large visual overlap is beneficial, because without enhanced sensitivity, photon noise will disable the detection of all spatial frequencies (Snyder, 1979; Land, 1981; Nilsson and Ro, 1994). Nocturnal mosquitoes have a visual overlap of almost 5 during dark-adaptation (Land et al., 1999), while in the tenebrionid beetle *Zophobas morio*, the earwig *Forficula auricularia*, the backswimmer *N. glauca*, and the cranefly *T. pruinosa* ratios of about 6 are present (Nilsson and Ro, 1994). In *M. genalis*, with its large $\Delta\rho$ and small $\Delta\phi$, the visual overlap is about 4, clearly showing that these nocturnal bees have prioritised sensitivity over spatial acuity (Warrant et al., 2004). Moreover, in eyes where $\Delta\rho$ is larger than $2{\cdot}\Delta\phi$, additional neural pooling of signals from neighbouring ommatidia to an angle of $\Delta\rho$, will result in an increase of sensitivity without further loss in resolution (Fig. 21).

For eyes where only anatomical and optical parameters are available, an alternative measure of sensitivity to (compared to Eq. 1) is the optical sensitivity (*S*). This value defines the amount of light energy that is absorbed by a photoreceptor when it views an extended source of white light (Kirschfeld, 1974; Land, 1981; Warrant and Nilsson, 1998):

$$S = (\pi/4)^2 D^2 (d/f)^2 [kl/(2.3 + kl)] \tag{4}$$

Similar to Eq. 1, the parameters in Eq. 4 include facet diameter and the receptive field, where the term $[(\pi/4)D^2]$ is the facet area, $[(\pi/4)(d/f)^2]$ is the solid angular visual field of the rhabdom, l the rhabdom length and k the absorption coefficient of the photoreceptors. Wider facets, larger visual fields and longer, as well as more absorptive, photoreceptors all increase sensitivity.

In eyes with *F*-numbers less than 2, Eq. 4 has limitations (Stavenga, 2003). However, in those nocturnal apposition eyes with *F*-numbers larger than 2, it can be applied with safety. Recent work on nocturnal bees and wasps has shown that despite differences in facet size, *S* is very similar: 2.7 $\mu m^2 sr$ in *Megalopta* and 3.0 $\mu m^2 sr$ in *Apoica*. Compared to *S*-values of 0.1 $\mu m^2 sr$ in their diurnal relatives, this represents a 25 to 30-fold enhancement of sensitivity (Greiner et al., 2004a; Greiner, 2005). Superposition eyes are in contrast much more efficient in gathering light as shown by an optical sensitivity of 69 $\mu m^2 sr$ in the nocturnal elephant hawmoth *Deilephila elpenor* and 59 $\mu m^2 sr$ in the nocturnal dung beetle *Onitis aygulus* (Warrant, 2004).

In *M. genalis*, the physiological parameters are known and the number of photons *N* a single photoreceptor absorbs within its integration time (Δt), and at a nocturnal light intensity (*I*), was calculated to be 0.15 photons (Warrant et al., 2004). As the photoreceptors of *M. genalis* absorb 28 times more photons per integration time than those of *A. mellifera* at the same light intensity, the results calculated with either Eq. 1 or Eq. 4 are highly comparable.

A 30-fold improvement in photon capture is remarkable for an apposition eye, however, this improvement alone is not sufficient to account for a light intensity difference of up to 8 log units. As the photoreceptors of *M. genalis* would need to absorb about 100 times more photons per integration time to detect the contrast at its nest entrance (Warrant et al., 2004), additional mechanisms need to be involved.

B. Neural adaptations

Due to their small facet apertures, optical improvements in sensitivity are particularly limited in apposition eyes. An increase in the response gain of the photoreceptors with decreasing light intensity can further enhance sensitivity but does not improve photon capture itself (Laughlin, 1981). Instead, the ultimate solution to optimise sensitivity at low light intensities is to process the incoming visual signal using a strategy of neural summation in space and time (Pirenne, 1967; Lythgoe, 1979; Snyder, 1979; Laughlin, 1981; Warrant et al., 1996; Warrant, 1999).

1. Temporal summation

As is well known to photographers, a longer camera exposure time leads to a brighter image. This improvement in sensitivity, however, only comes at a cost - fast moving objects appear 'smeared' or could even completely disappear from the photograph. In an analogous manner, visual systems can also improve image reliability at night by slowing vision down. By lengthening the eye's visual integration time at night, the signal-to-noise ratio of lower temporal frequencies is improved at the expense of noisier and less reliable higher temporal frequencies.

This temporal summation results in a slower but more reliable visual world. The duration of the visual integration time can be altered via the transduction cascade in the photoreceptor membrane, or by higher circuits that neurally integrate temporal information (Warrant, 2004). Extremely long photoreceptor integration times of about 1.5 s in nocturnal toads (Donner, 1989; Aho et al., 1993), and 160 ms in a deep-sea crustacean (Moeller and Case, 1995), indicate the presence of temporal summation in the photoreceptors. For sit-and-wait predators and slowly moving animals, temporal summation is certainly a good strategy; however, the challenge to perceive flow-field information during nocturnal flight may instead be better met via neural photon summation in space.

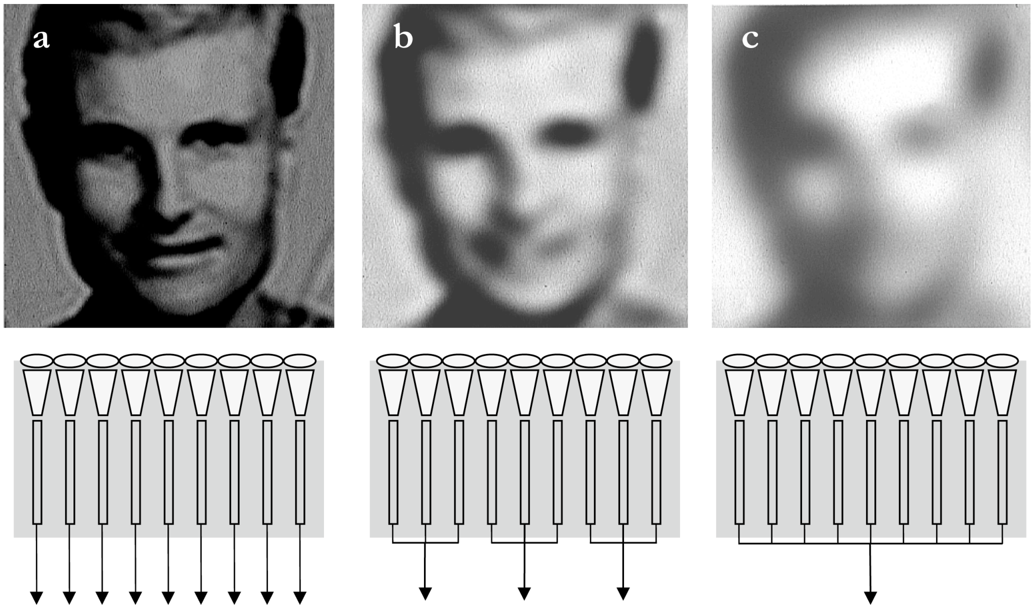


Fig. 13: Theory of spatial summation. (a) Without summation each visual channel within an array of ommatidia remains isolated from all others creating a sharp but dark image. (b, c) Increasing the amount of spatial summation (pooling of ommatidia) enhances the brightness but degrades the resolution of the image.

2. Spatial summation

Another strategy photographers use to improve image quality at night is to choose films of coarser grain (higher ASA value), which makes the image brighter at the expense of larger pixels (Fig. 13). In apposition eyes, such spatial summation can be achieved by neurally summing the output of neighbouring visual channels, or ommatidia, which would dramatically increase photon capture and thus image brightness. Hence, with an increasing extent of neural pooling, the brighter but also the blurrier the image becomes (Fig. 13). This is due to the fact that photons are integrated over wider visual fields, which is similar to a widening of the angular sensitivity function, and therefore has the same effect: reduced spatial acuity. Only when neural summation is matched to the extent of the visual overlap present in the eye, can sensitivity be maximised without further degrading resolution.

Laterally spreading neurons that sum photons from a large number of ommatidia may provide the neural basis of spatial summation. Such neurons, which in insects are likely to be found within the first optic ganglion (or lamina), could create a wide receptive field or 'neural pupil'. Prime neural candidates, due to their regular arrangement and extensive lateral spreading in nocturnal insects (Greiner et al., 2004b), are the retinula cell axons of the photoreceptors (Fig. 14a), and the first-order interneurons, also known as L-fibres or lamina monopolar cells (Fig. 14b).

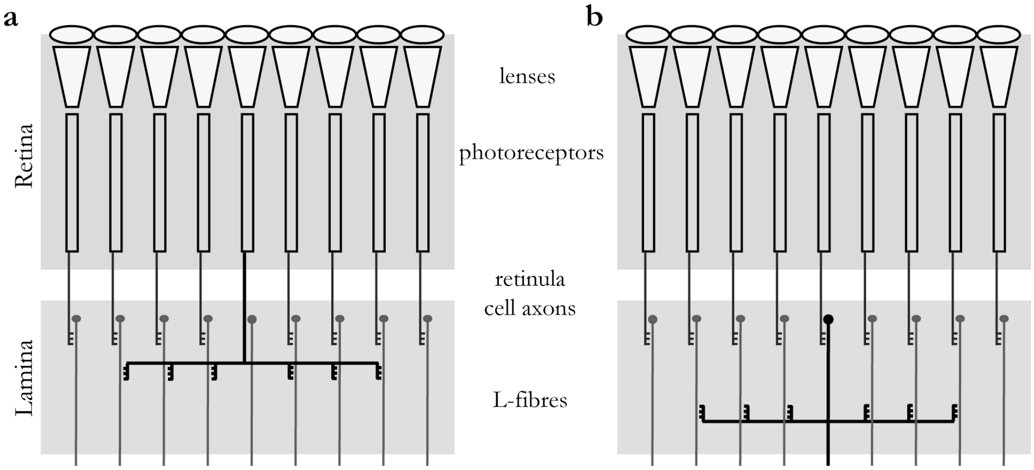


Fig. 14. Possible neural basis of spatial summation in the lamina. Visual information is pooled from several ommatidia via (a) lateral branching of retinula cell axons from the photoreceptors or (b) wide dendritic fields of L-fibres (adapted from Warrant et al., 2004).

3. Organisation of the lamina and spatial summation

Remarkably, the neural circuitry of the lamina (i.e. cell identity and position), has remained highly conserved over more than 200 million years (Shaw and Moore, 1989). Instead of the evolution of novel neurons, changes in the branching pattern and synaptic connectivity of existing neurons appear to mediate the high adaptability of the visual system. The optic lobes of insects contain an array of repeated neural units known as cartridges (Strausfeld, 1976; Meinertzhagen, 1991). In apposition eyes, all the retinula cells from one ommatidium directly project, via an axon bundle, to one specific lamina cartridge (Horridge and Meinertzhagen, 1970; Ribi, 1974). Thus, there are as many cartridges in the lamina as there are ommatidia in the eye and neighbouring ommatidia project to neighbouring laminar cartridges. This retinotopic representation of the visual environment is maintained across the three optic neuropils (lamina, medulla and lobula) via an outer chiasm between the lamina and medulla and an inner chiasm between the medulla and the lobula (Fig. 15) (Meinertzhagen, 1976; Strausfeld, 1976).

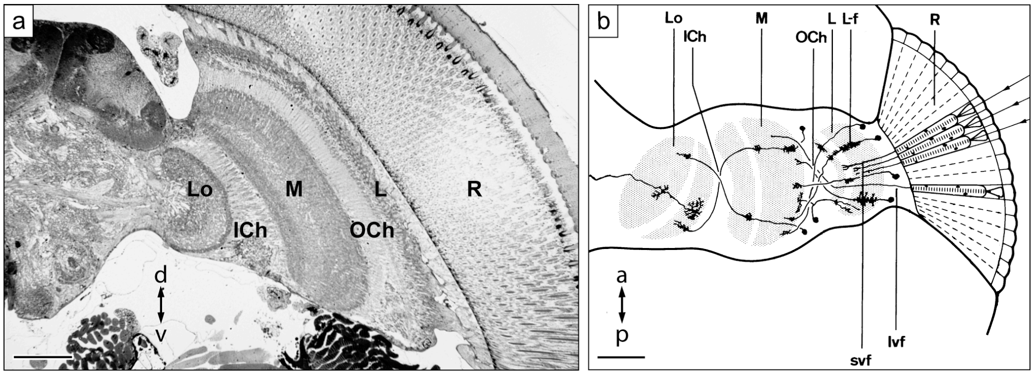


Fig. 15: The hymenopteran visual system features the retina (R) and three optic ganglia - the lamina (L), the medulla (M) and the lobula (Lo). Retinula cell axons project as short visual fibres (svf) to the lamina and as long visual fibres (lvf) to the medulla. In the lamina, a set of first-order interneurons, or L-fibres (L-f), joins the receptor cell axons forming neural units known as cartridges. ICh – inner chiasm, OCh – outer chiasm. d – dorsal, v – ventral, a – anterior, p – posterior. Scales in a, b 200 μm (b, adapted from Ribi 1987b).

There are two types of retinula cell axons: short visual fibres, which terminate within the lamina, and long visual fibres, which pass through the lamina and end in the medulla (Fig. 15b). A set of first-order interneurons (L-fibres) joins each cartridge in the distal lamina and a dense network of tangential glial cells tightly surrounds the cartridge bundles. In addition, the lamina contains various accessory cells, including amacrine cells, tangential fibres and centrifugal cells (Strausfeld, 1976). However, due to our limited understanding of their functions, these cells are discussed no further.

a. Lateral branching of retinula cell axons

In apposition eyes, the exact projection of the retinula cell axons from neighbouring ommatidia to the lamina cartridges directly beneath serves the purpose to conserve the image within the optic lobes. However, in eyes where a significant visual overlap is present, pooling of signals from neighbouring ommatidia which matches this overlap will enhance sensitivity without further decreasing resolution (Snyder, 1979). Such large visual overlaps exist in many dark-adapted apposition eyes of the open-rhabdom type, such as aquatic hemipterans and primitive dipterans (Nilsson and Ro, 1994). As predicted, lateral branching can be found in the short visual fibres of these insects and interestingly, most dendritic collaterals diverge in an asymmetrical manner into several cartridges (Fig. 16a,b).

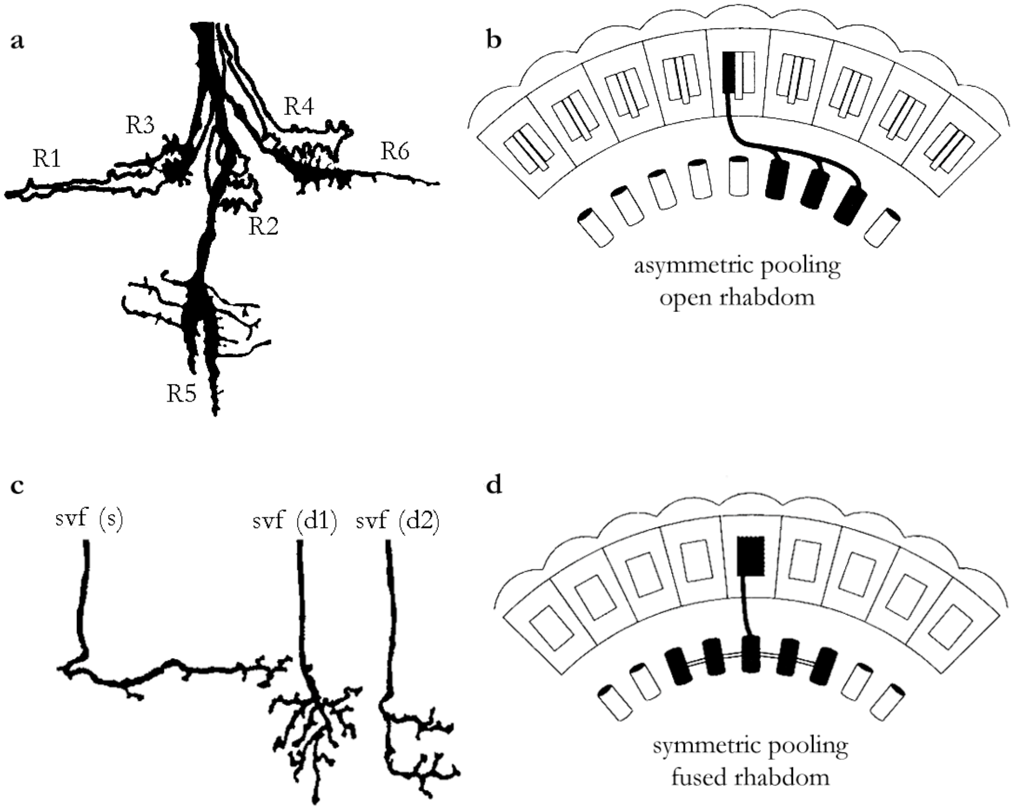


Fig. 16: Lateral branching of short visual fibres (svf) in the lamina of (a) the backswimmer *Notonecta glauca* and (c) the cockroach *Periplaneta americana*. Asymmetric branching is clearly visible in retinula cell axons R1 and R6 of the backswimmer, as well as svf (s) and svf (d2) of the coackroach, while symmetric branching is present in R5 of the backswimmer and svf (d1) in the cockroach. These short visual fibres might therefore be involved in either (b) asymmetric or (d) symmetric pooling to enhance sensitivity. (a, adapted from Wolburg-Buchholz, 1979; c, adapted from Ribi, 1977; b, d adapted from Nilsson and Ro, 1944).

Lateral branching over at least 3-5 rows of neighbouring cartridges is present in all of the species studied: the backswimmer *N. glauca*, the water strider *Gerris lacustris*, the water boatman *Corixa punctata*, the waterbug *Benatus griseus*, the phantom midge *Chaoborus crystallinus* and the cranefly *T. rufina* (Meinertzhagen, 1976; Strausfeld, 1976; Wolburg-Buchholz, 1979; Melzer et al., 1997). From these, the backswimmer (Fig. 16a) and the cranefly possess the longest collaterals (Wolburg-Buchholz, 1979; Melzer et al., 1997). The pupil mechanisms of both species, and the spectral sensitivity of *Notonecta*'s outer rhabdomeres, seem to be optimized for vision at low light intensities (discussed in Ro and Nilsson, 1995).

The asymmetrical lateral branching pattern of the primitive dipterans has been intensely discussed regarding the evolution of neural superposition (Nilsson and Ro, 1994; Melzer et al., 1997). The hypothesis is that asymmetrical pooling originally evolved to enhance sensitivity (explaining its presence in nocturnal insects), and subsequently led to the neural superposition eyes of advanced flies, by narrowing the pooling fields in favour of resolution. All of these insects show strong dynamic pupils, which narrow to the approximate width of the central rhabdomere during light adaptation (Nilsson and Ro, 1994). These central rhabdomeres remain within their own cartridge and terminate as long visual fibres in the medulla and may therefore mediate high spatial resolution. Consequently, the proposed neural pooling would only occur in the dark-adapted eye, when the pupil is open and the outer six rhabdomeres (R1-6) are exposed to light. In animals that are active at twilight and experience large variations in light intensity, several parallel spatial summation channels may be an additional benefit. In *Notonecta*, for example, this might be mediated by different extents of lateral spreading in the six short visual fibres (Fig. 16a).

In most apposition eyes with fused rhabdoms the retinula cell axons from one ommatidium project to a single cartridge and their dendritic branches remain within that same cartridge (Ribi, 1975; Meinertzhagen, 1976; Meinertzhagen et al., 1980; Ribi, 1987a). As apposition eyes are typically present in diurnal insects, neural summation to enhance sensitivity should not be necessary. Accordingly, one might expect lateral branching in nocturnal insects. Indeed, in the lamina of the cockroach, all short visual fibre types show wide asymmetric and symmetric lateral branching (Fig. 16c) (Ribi, 1977). The dendritic fields of these retinula cell axons (as well as those of their L-fibres) could thus enhance sensitivity through neural pooling. The reason for the presence of asymmetric projections in fused rhabdoms is however unclear, as all photoreceptors receive light from the same visual angle in space. In nocturnal bees, lateral branching was found in one of the three short visual fibre types. This is the first time that lateral branching of retinula cell axons has been demonstrated in hymenopterans (Greiner et al., 2004b; Greiner et al., 2005) and in order to relate this lateral spreading to nocturnal vision, more comparative studies of other nocturnal and diurnal hymenopterans are needed. In nocturnal mosquitoes, lateral branching into adjacent cartridges may also be present (Land and Horwood, 2005), and due to large visual overlap (Land et al., 1997), spatial summation of adjacent cartridges would be beneficial.

Neural pooling in fused rhadoms does not allow for separation of spatial channels, like those proposed for open rhabdom eyes. Thus, to retain good spatial resolution during the day and use spatial summation at night, a dynamic control in the neurons involved in spatial summation would be of great benefit (Warrant and McIntyre, 1993). Circadian changes of synapses in the fly lamina indicate that a neural basis for such dynamics may in fact exist (Pyza and Meinertzhagen, 1993).

Surprisingly, the lamina of the diurnal skipper butterfly *Parnara guttata* features lateral branching of both short and long visual fibres (Shimohigashi and Tominaga, 1999). Skipper butterflies, despite living in bright light, are highly unusual in that the secondary pigment granules of their superposition eyes always remain in a position typical of dark-adapted nocturnal moth eyes (Horridge et al., 1972). However, as light intensity represents no limitation to their eyes, these collaterals may be involved in visual tasks other than enhancing sensitivity.

b. Lateral branching of L-fibres

Lateral spreading of L-fibres is commonly found in insects and shows an interesting correlation, where the amount of spreading is greater in the lamina of crepuscular and nocturnal insects than in diurnal species (Greiner et al., 2004b). This hypothesis – that a nocturnal lifestyle should affect the amount of lateral branching in L-fibres – was recently investigated in nocturnal and diurnal bees (Fig. 17) (Greiner et al., 2004b; Greiner et al., 2005). In bees, the structure of the lamina as well as the number and identity of the four different L-fibres is highly conserved. The lamina contains three morphologically distinct layers, A, B and C, and the four L-fibre types L1-L4 are categorised by their branching pattern within these three layers. L-fibres with branches in all three layers are categorized as the L1-type, L-fibres with processes in layers A and B are of the L2-type, L-fibres arborising in layer B (in the honeybee) or A and C (in the nocturnal bee) are of the L3-type, and L-fibres branching exclusively in layer C are of the L4-type (Fig. 17) (Ribi, 1976; Greiner et al., 2004b).

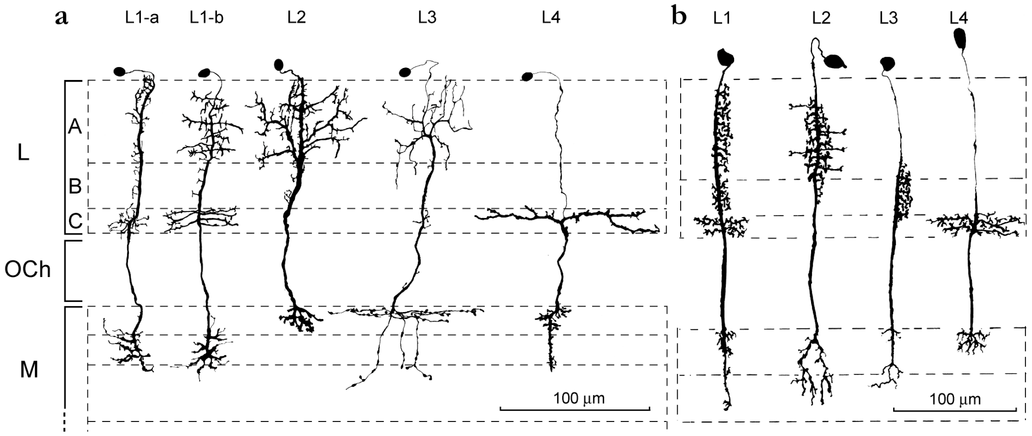


Fig. 17: The L-fibre types L1-L4 of (a) the nocturnal bee and (b) the worker honeybee shown in frontal orientation. All L-fibre types feature extensive lateral branching in the nocturnal bee, which is significantly wider than in the honeybee. Only the distal part of the medulla containing the L-fibre terminals is shown. Note the wide lateral branching of L3 in the medulla. L – lamina, OCh – outer chiasm, M – medulla, A, B, C – layers of the lamina (a, adapted from Greiner et al., 2004b; b, adapted from Ribi, 1975).

Extensive lateral branching is present in the lamina of nocturnal bees, and this branching is significantly wider than in diurnal bees (Fig. 17) (Greiner et al., 2004b). The largest dendritic fields of the L-fibres, L2 and L3, extend to 12 and 13 cartridges respectively (Fig. 23). L4 targets a remarkable 18 cartridges (Fig. 18), and this is considerably larger than the largest dendritic fields of L-fibres found in the honeybee (L2, which visits 9 cartridges: Ribi, 1981).

Behavioural data show that the honeybee *A. mellifera* is also able to see at moonlight intensities, even though the optical structure of their eyes should render them blind already at mid dusk (Warrant et al., 1996). Theoretical modelling predicts that neural summation should also occur in the honeybee to enhance their vision at low light intensities (Warrant et al., 1996). The lateral branching of L2 and L4 may well serve this purpose and might also explain why the African race of the honeybee *A. mellifera adansonii* and the Asian giant honeybee *A. dorsata* are able to continue to forage on nights when at least a half-full moon is present in the sky (Fletcher, 1978; Dyer, 1985). Subsequent widening of the dendritic fields in L1, L2 and L4, together with the modifications of the branching pattern in L3, may all have evolved in bees as a response to the benefits of a nocturnal lifestyle. Whether other nocturnal hymenopterans have followed a similar evolutionary path, or whether they have evolved an alternative solution to enhance vision at night, is a fascinating open question that needs further attention.

How do these findings in bees compare to the L-fibre branching present in other insects? Is there a correlation with their preferred light intensity? In most strictly diurnal species, like the cabbage butterfly *Pieris* (Strausfeld and Blest, 1970), the dragonfly *Sympetrum* (Meinertzhagen and Armett-Kibel, 1982) and the housefly *Musca* (Boschek, 1971; Strausfeld, 1971), the dendrites of all L-fibres are confined to their own parental cartridge. However, there are interesting exceptions, like the orchard butterfly *Papillio*, which shows branching pattern similar to those of the diurnal honeybee (Ribi, 1981; Ribi, 1987a). Again, these branches may be involved in other visual tasks (e.g. colour vision), as diurnal insects with apposition eyes have no need for spatial summation.

An interesting correlation with intensity and L-fibre branching can be found within the previously discussed aquatic hemipterans, the backswimmer *N. glauca*, the water boatman *C. punctata* and the waterstrider *G. lacustris* (Wolburg-Buchholz, 1979). In all three species, four L-fibre types L1-L4 are present. In *Notonecta* three of them contact neighbouring cartridges, with L3 having the largest dendritic field with contact over eight cartridges. In the water boatman, only L3 spreads laterally, in this case over five cartridges, and in the water strider all four L-fibres remain in their own cartridge. Their behaviour matches the hypothesis: *Notonecta* visually navigates during crepuscular periods (Schwind, 1983), *Corixa* spends time on the pond floor (Chinery, 1973), where a sensitive visual system might be beneficial, and the water strider hunts on the water surface in bright light.

The cockroach *Periplaneta americana* is the only insect with apposition eyes where all L-fibres are wide-field neurons with large dendritic fields (Ribi, 1977). As *Periplaneta* is purely nocturnal, these laterally branching neurons may effectively sum the visual input reaching the lamina from several ommatidia in order to enhance sensitivity.

Similar wide-field neurons are present in the lamina of nocturnal insects with superposition eyes, like the male firefly *Phausis splendidula* (Ohly, 1975) and nocturnal hawkmoths (Strausfeld and Blest, 1970). Interestingly, when comparing nocturnal and diurnal hawkmoths, nocturnal species have significantly wider lateral branching L-fibres (A. Balkenius, unpublished data). This correlation, similar to the one found in bees, strongly indicates that neural summation may be a general strategy to enhance sensitivity in insects.

As pointed out by Ribi (1977), differences in the lamina organisation found across insect groups may depend on their visual requirements. A more differentiated lamina is characterised by a multilayered appearance and a defined location of the neurons within the lamina (Kral, 1987). Cockroaches have a less organised lamina, where a cartridge may contain anything from 6 to 20 fibres (Ribi, 1977). The greater visual demands of day-flying insects might have led to the evolution of highly organised laminas, such as the laminas of neural superposition eyes, whose exact wiring arose from the unorganised asymmetric cell branching found in primitive dipterans. In bees, however, nocturnal activity has most likely evolved from an originally diurnal lifestyle, and the extent of lateral L-fibre branching simply widened in response to the low light intensities.

How does the receptive field size of photoreceptors compare to that of L-fibres? In dragonflies, dark-adapted L-fibres receive their major input from retinula cell axons and therefore have the same field of view (Laughlin, 1973). As no lateral connections into neighbouring cartridges exist in the lamina of dragonflies (Meinertzhagen and Armett-Kibel, 1982), it makes sense that the receptive field is not increased. Such recordings have not yet been done in the L-fibres of nocturnal insects and an interesting question is whether the receptive fields of wide-field L-fibres are actually larger than the receptive fields of the photoreceptors. In addition, one might expect the receptive field size to be controlled by a circadian rhythm and/or changes in light intensity. Future investigations, especially with respect to these possible dynamic regulations during day and night, both physiologically and anatomically, will also advance our knowledge of neuronal plasticity.

Spectral sensitivity recordings of the L-fibres in the honeybee lamina revealed a highly complex response pattern (de Souza et al., 1992). Whether responses in the nocturnal bee *M. genalis* are equally complex remains to be seen as no attempts have so far been made to physiologically characterise the functions and receptive fields of the four L-fibre types. The possible roles of the different L-fibres in the lamina of *M. genalis* can therefore only be discussed on the basis of their anatomy and by direct comparison to the thoroughly studied synaptic connectivity in the lamina of the worker honeybee (Ribi, 1981; Ribi, 1987b; Greiner et al., 2005). For example, in *M. genalis*, L4 shows the largest dendritic field, extending over 18 cartridges in an extreme dorso-ventral direction (Fig. 18). As L4 is restricted to layer C, it does not receive direct input from retinula cell axons, but probably does so via L1 instead. In addition, L2 and L3 have large dendritic fields of 12 and 13 cartridges and are therefore potential candidates for spatial summation (Fig. 23).

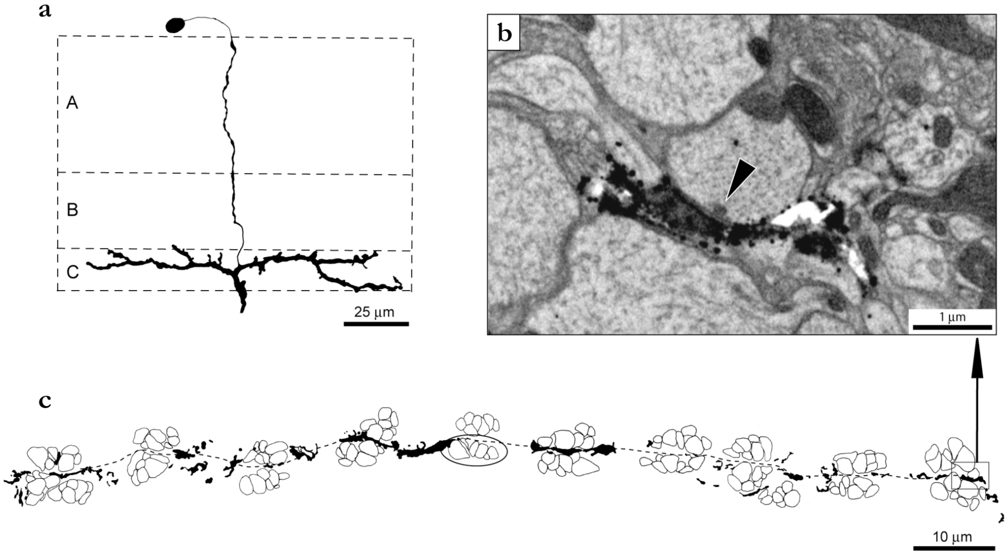


Fig. 18 (a) The dendritic field of the L-fibre L4 showing wide lateral branching exclusive to lamina layer C and oriented in an extreme dorso-ventral orientation. (b) Example of an EM section where the membrane of L4 is silver-stained (Golgi) and therefore appears black. *Arrowhead* marks a synapse in an adjacent axonal stem of another L-fibre. (c) L4's dendritic field of 18 cartridges in a reconstructed serial-cross sectioned layer C. The L4's own cartridge is circled in the centre. Dendritic branching pattern was reconstructed from alternating Golgi-electron microscopy (EM) ultra-thin sections (drawn in black) and light microscopy semi-thin sections (*dotted lines*). A, B, C – lamina layers A, B, C (adapted from Greiner et al., 2005).

Only a combination of single cell recordings (receptive fields) and intracellular staining can reveal whether wide-field L-fibres actually mediate spatial summation in the lamina. Nevertheless, theoretical modelling predicts a great deal about the optimal amount of spatial and temporal summation that is needed in order to allow nocturnal vision in bees (Theobald et al., 2005). Whether these theoretical predictions match the anatomical data, is the topic of the final section.

C. A model of neural summation

As discussed earlier, spatial and temporal summation enhances sensitivity at the cost of spatial and temporal resolution. Using the analytical model developed by Warrant (1999) one can now test this trade-off theoretically and determine whether neural summation can improve vision in dim light or whether the sacrifices in resolution actually outweigh the sensitivity gain and make vision worse. By calculating the number of photons N sampled by a visual channel (Eq. 4, explained in section III-A) and the total visual noise (photon shot noise $\sqrt{N}$ plus the effect of dark noise σ^2_D), at a particular light intensity I and image velocity v, the model predicts the maximum detectable spatial frequency ν_{max} that an animal can see with and without optimal spatial and temporal summation. A criterion used in the model is that ν_{max} occurs when signal and noise become equal: all spatial frequencies below ν_{max} can be reliably distinguished, whereas those higher than ν_{max} are lost in the noise (Fig. 19).

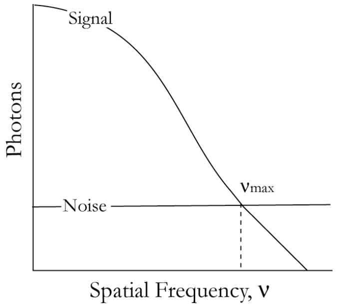


Fig. 19: Signal decreases with increasing spatial frequency ν (cycles deg^{-1}), while noise remains constant. At the maximum detectable spatial frequency ν_{max}, signal and noise become equal and are indistinguishable from each other (adapted from Warrant, 1999).

The Gaussian angular sensitivity function describes visual performance in the eye. Similarly, the summation model defines a Gaussian output channel receptive field (with a half-width $\Delta\rho_T$) that predicts visual performance after spatiotemporal summation (Fig. 20d). This summation function is defined by the convolution of three Gaussians: the receptive field of each input channel of half-width $\Delta\rho$ (Fig. 20a), the spatial summation function of half-width $\Delta\rho_P$ (Fig. 20b) and a motion blurring function $v\Delta t$ (temporal summation) (Fig. 20c) (Warrant, 1999).

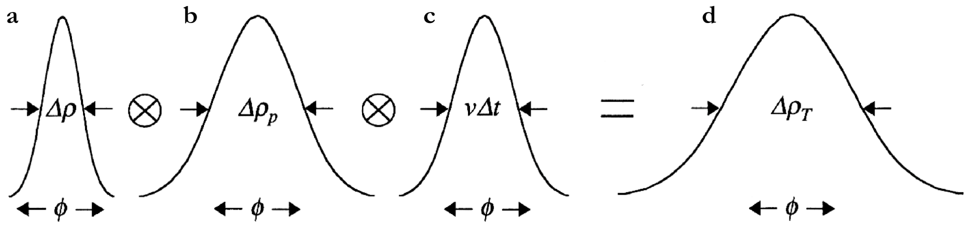


Fig. 20: In the spatiotemporal summation model, the output channel receptive field $\Delta\rho_T$ (d) depends on (a) the receptive fields of the input channel $\Delta\rho$, (b) the extent of spatial summation (spatial summation function $\Delta\rho_P$) and (c) the extent of temporal summation (motion blurring function $v\Delta t$). The circles with crosses signify convolution, the ϕ-axis defines angles in degrees and the vertical amplitudes of the function relates to sensitivity (adapted from Warrant, 1999).

The spatial summation function (of half-width $\Delta\rho_P$) also describes the strength of coupling within the field of channels. This Gaussian assumes that nearest-neighbour channels contribute most to the summed signal, which gradually decreases with increasing distance. In dim light, this function is generally much wider than the angular sensitivity function, or input receptive field (half-width $\Delta\rho$), of the photoreceptor. Thus, a much greater visual overlap is present between the channels. This leads to an increased photon capture in each visual channel, but only at the cost of spatial resolution (Fig. 21a). Despite a drastic decrease in resolution, spatial summation nevertheless proves to be beneficial in dim light due to the enhanced signal-to-noise ratio at low spatial frequencies (Warrant, 1999).

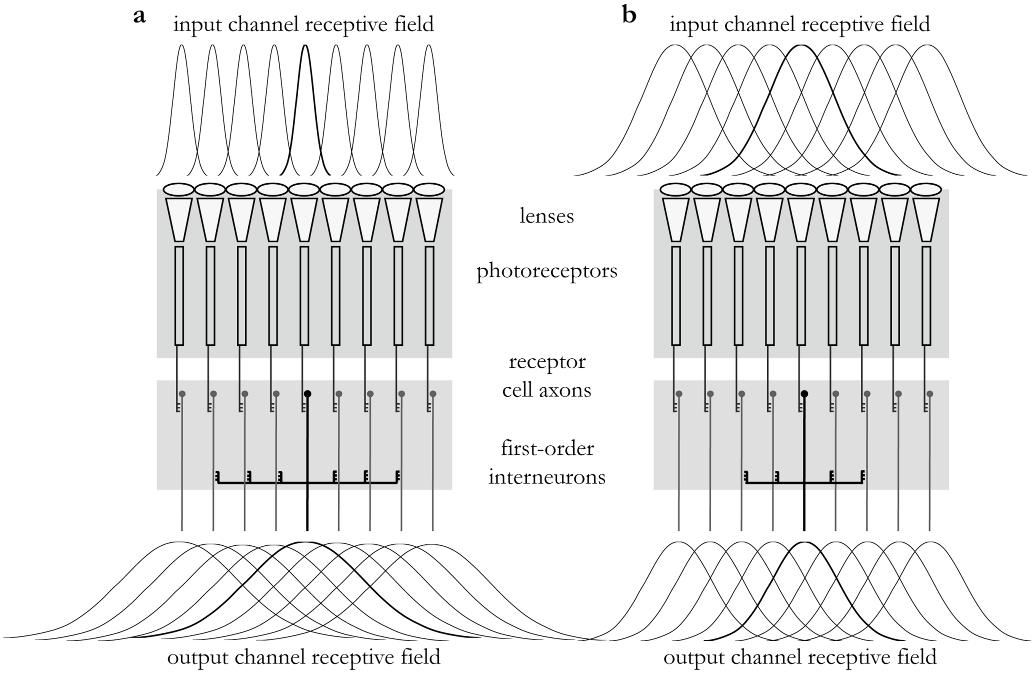


Fig. 21: Theory of spatial summation mediated by the receptive field of a wide-field first-order interneuron, which couples the input channels from the photoreceptors. (a) In a conventional apposition eye this leads to a large visual overlap and poor spatial resolution. (b) In some nocturnal apposition eyes large visual overlap is already present in the retina. In these eyes, a laterally branching neuron with a matched summation field would enhance sensitivity without further losses in resolution.

Irrespective of whether the visual system increases sensitivity by optically widening the receptive field of the input channels ($\Delta\rho$) or neurally widening the receptive field of the output channels ($\Delta\rho_P$), resolution will always be limited by the larger of the two. Consequently, to optimise both resolution and sensitivity, the size of $\Delta\rho$ and $\Delta\rho_P$ should be matched. In eyes where the input receptive field

$\Delta\rho$ is much larger than the anatomical resolution of the eye, spatial summation from the number of ommatidia representing $\Delta\rho$, will enhance sensitivity without further decreasing resolution (Fig. 21b) (Nilsson and Ro, 1994). In the case of dynamic or circadian changes in $\Delta\rho$ (e.g. pupil mechanisms or changes in rhabdom diameter), $\Delta\rho_P$ could also be under dynamic control (possibly via synaptic changes within neurons). Accordingly, the model assumes intensity-related dynamics, where pooling is turned off at bright light intensities and gradually 'switched on' as the intensity level decreases. Subsequent Fourier transformation of the output channel receptive field yields the modulation transfer function (MTF), which is used to calculate the signal. MTFs show the potential range of spatial frequencies seen by the eye, where wider receptive fields lead to a narrower range of frequencies. In practice, inherent visual noise (Fig. 19) reduces this range significantly (for more details on the model see Warrant 1999).

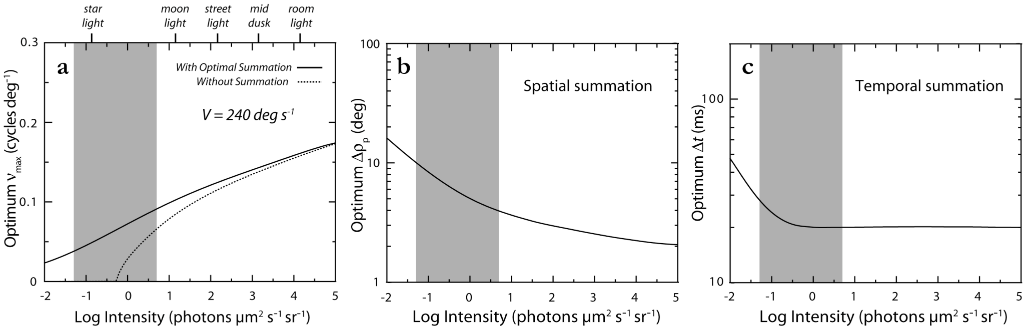


Fig. 22: Spatial and temporal summation modelled for *Megalopta genalis* at different light intensities for a constant image velocity of 240 deg s^{-1}. *Grey areas* mark the light intensity window at which *M. genalis* in normally active. (a) The maximum detectable spatial frequency $\nu_{\max}$ with (*solid line*) and without (*dashed line*) optimal summation. (b) The spatial summation function half-width $\Delta\rho_P$ increases over the entire activity range, while integration time Δt (c) is only predicted to contribute to performance at dimmest intensities (adapted from Theobald et al., 2005).

As apposition eyes have small apertures and isolated visual channels, the model predicts that spatial and temporal summation will be of great benefit for this eye design in dim light. Indeed, in locusts and bees, the model predicts that neural summation can dramatically improve visual performance as measured by the maximum detectable spatial frequency $\nu_{\max}$ (Warrant et al., 1996; Warrant, 1999; Theobald et al., 2005). Using spatiotemporal summation, locusts can extend their vision from mid-dusk into starlight, a remarkable difference of 5 log units (Warrant, 1999). In the honeybee, the optical sensitivity of their apposition eyes alone should render them blind by mid dusk. However, behavioural data shows that visual performance in dim light is much greater than expected from the optics, and the model predicts that this performance can be achieved by optimal spatiotemporal summation (Warrant et al., 1996). In the interesting case of the nocturnal bee *M. genalis*, the limited optical sensitivity of its eyes does not allow reliable vision at the low light intensities in which they are active. Thus, neural summation is crucial for the visual performance of nocturnal bees (Fig. 22) (Theobald et al., 2005).

The model also predicts the optimum balance between spatial and temporal summation for different light intensities and image velocities. For an animal experiencing low image velocities, visual performance is better enhanced via temporal summation, whereas at high image velocities temporal resolution needs to be retained. In the latter situation, enhanced visual performance in dim light is best achieved by spatial summation, as seen in insects capable of fast flight even at low light intensities, like locusts and nocturnal bees (Warrant et al., 1996; Warrant, 1999; Theobald et al., 2005). At constant image velocity and in dim light, spatial summation contributes most to the enhancement of visual performance (Fig. 22).

How does the spatial summation function relate to the branching pattern of wide-field L-fibres? Lateral branching is present in one of the six L-fibres of the locust lamina (Nowel and Shelton, 1981), however, it is unknown whether the dendritic field of its branching pattern matches this amount of spatial summation. In nocturnal bees, however, the L-fibre's dendritic fields are known (Greiner et al., 2005), and after transforming the receptive field of the summation function into numbers of cartridges, the results from the theoretical model can be directly compared with the anatomical data (Fig. 23) (Theobald et al., 2005).

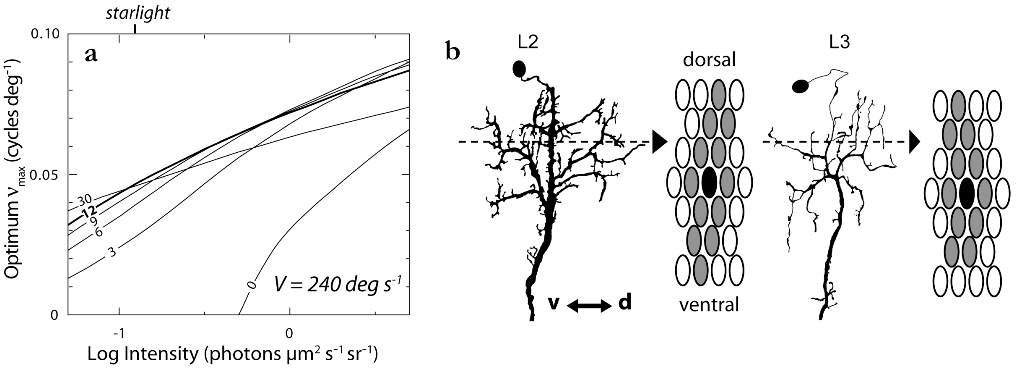


Fig. 23 (a) Optimum summation in *M. genalis* expressed as various extent of spatial summation: curves for no summation (0), and for 3, 6, 9, 12, and 30 summing cartridges. v_{max} was measured at a constant velocity (240 deg s^{-1}) and over the range of light intensities experienced by *M. genalis* (-1.3 to 0.7 log units). The *heavy black line* marks the summation of 12 cartridges, which overall proves optimal with respect to the entire range of light intensities (adapted from Theobald et al., 2005) (b) Equivalent dendritic field sizes can be found in L-fibres L2 and L3 of *M. genalis* reconstructed from frontal sections. The schematic drawings represent the cross-sectioned layer marked by the *dashed arrows*. The ovals illustrate the schematic arrangement the L-fibre's own cartridge (*black*) and its dendritic field (*grey*). v-ventral, d-dorsal (adapted from Greiner et al., 2005).

Over the entire intensity range of *Megalopta*'s activity period, the model predicts a summation of about 12 cartridges to be optimal (Fig. 23a). Even though summation from 30 cartridges would be better at the lowest intensities it reduces visual performance at brighter intensities. The data generated by the model match the anatomical results remarkably well, where the dendritic fields of L2 and L3 visit 13 and 12 cartridges, respectively (Fig 23b, c), and L4 as many as 18 (Greiner et al., 2005). Furthermore, a simulation of the spatial resolution confirms that the optical resolution of the bee's eye is already blurred at the level of the retina, so that spatial summation would hardly worsen the image (Theobald et al., 2005).

Thus, neural summation is extremely useful for insects active in dim light and is able to convert an apposition eye into a reasonably well functioning nocturnal eye. With optimal spatiotemporal summation, vision can be extended into significantly dimmer light intensities, and one can expect that this clearly beneficial strategy is widely used by nocturnal insects with apposition eyes (Warrant, 1993; Warrant et al., 1996; Warrant, 1999; Theobald et al., 2005).

VI. Concluding Remarks

Despite our own preference for bright light, several groups of insects seek the cover of night in search of food and to reduce the risk of predation and competition. The fact that some insects were able to achieve this transition to a nocturnal lifestyle while retaining their apposition eyes is remarkable and intriguing, as this eye design is highly unsuitable for vision at low light intensities. How is it possible that small tropical bees can navigate in an entangled rainforest using landmarks at light intensities where it is not possible to see your own hand in front of your face? This review presents numerous optical and neural adaptations that nocturnal insects have evolved to significantly enhance the sensitivity of their apposition eyes for dim-light vision. One remarkable example is certainly the apposition eye of nocturnal mosquitoes, where unique structural changes of the photoreceptors match the wide cone of light reaching the eye through almost hemispherical lenses. Additional neural adaptations are crucial in other insects, like nocturnal bees, and a hypothesis of spatiotemporal summation for further enhancing sensitivity is proposed. Although a large amount is still unknown, recent anatomical evidence of laterally spreading neurons in the first optic ganglion, and convincing neural modelling, strongly suggest that spatial summation is present in the eyes of these nocturnal bees. Further knowledge of the neural networks within the first optic ganglion of nocturnal insects, and the mechanisms of neural plasticity modulating their early visual processing, will reveal the principles responsible that regulate vision at low light. Such discoveries may be instrumental in the development of new technologies, including night-vision devices.

References

Aho, A. C., K. Donner, C. Hyden, L. O. Larsen, and T. Reuter. 1988. Low retinal noise in animals with low body temperature allows high visual sensitivity. *Nature*, 334:348-350.

Aho, A. C., K. Donner, S. Helenius, L. O. Larsen, and T. Reuter. 1993. Visual performance of the toad (*Bufo bufo*) at low light levels: retinal ganglion cell responses and prey-catching accuracy. *J Comp Physiol A*, 172:671-682.

Arneson, L., and W. T. Wcislo. 2003. Dominant-subordinate relationships in a facultatively social, nocturnal bee, *Megalopta genalis* (Hymenoptera: Halictidae). *J Kansas Entomol Soc*, 76:183-193.

Autrum, H. 1981. Light and dark adaptation in invertebrates. In H. Autrum (ed.), *Handbook of sensory physiology, vol VII/6C*, pp. 1-91. Springer, Berlin Heidelberg New York.

Baker, H. G. 1961. The adaptation of flowering plants to nocturnal and crepuscular pollinators. *Q Rev Biol*, 36:64-73.

Baldridge, R. S., C. W. Rettenmeyer, and J. F. Watkins, II. 1980. Seasonal nocturnal and diurnal flight periodicities of nearctic army ant males (Hymenoptera: Formicidae). *J Kansas Entomol Soc*, 53:189-204.

Balkenius, A., and A. Kelber. 2004. Colour constancy in diurnal and nocturnal hawkmoths. *J Exp Biol*, 207:3307-3316.

Barlow, H. B. 1956. Retinal noise and absolute threshold. *J Opt Soc Am*, 46:634-639.

Bohart, G. E., and N. N. Youssef. 1976. The biology and behaviour of Evylaeus galpinsiae Cockerell (Hymenoptera: Halictidae). *Wasmann J Biol*, 34:185-234.

Boschek, C. B. 1971. On the fine structure of the peripheral retina and lamina ganglionaris of the fly, *Musca domestica*. *Z Zellforsch Mikrosk Anat*, 118:369-409.

Braitenberg, V. 1967. Patterns of projection in the visual system of the fly. I. Retina-lamina projections. *Exp Brain Res*, 3:271-298.

Brünnert, U., A. Kelber, and J. Zeil. 1994. Ground-nesting bees determine the location of their nest relative to a landmark by other than angular size cues. *J Comp Physiol A*, 175:363-369.

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

Butler, R., and G. A. Horridge. 1973. The electrophysiology of the retina of *Periplaneta americana*. I. Changes in receptor acuity upon light/dark adaptation. *J Comp Physiol*, 83:263-278.

Caveney, S., and P. Mcintyre. 1981. Design of graded-index lenses in the superposition eyes of scarab beetles. *Philos Trans R Soc B-Biol Sci*, 294:589-632.

Chandler, L. 1961. Light trap collections of the nocturnal bee, *Sphecodogastra texana* (Cresson) (Hymenoptera, Halictidae). *Proc Indiana Acad Sci*, 71:124-129.

Chapman, R. F. 1980. *The insects. Structure and function.* Hodder and Stoughton, London.

Chinery, M. 1973. *A field guide to the insects of Britain and northern Europe*. Collins Publishers, London.

Clements, A. N. 1963. *The physiology of mosquitoes.* Pergamon, Oxford.

Cockerell, T. D. A. 1923. Two nocturnal bees and a minute *Perdita*. *Am Mus Novit*, 66:1-4.

Collett, T. S., and M. Lehrer. 1993. Looking and learning - a spatial pattern in the orientation flight of the wasp *Vespula vulgaris*. *Proc R Soc Lon B Biol Sci*, 252:129-134.

Collett, T. S., and M. Collett. 2002. Memory use in insect visual navigation. *Nat Rev Neurosci*, 3:542-52.

de Souza, J., H. Hertel, D. F. Ventura, and R. Menzel. 1992. Response properties of stained monopolar cells in the honeybee lamina. *J Comp Physiol A*, 170:267-274.

Debaisieux, P. 1944. Les yeux des Crustacés: structure, développement, réactions à l'éclairement. *Cellule*, 50:9-122.

Donner, K. 1989. Visual latency and brightness - an interpretation based on the responses of rods and ganglion-cells in the frog retina. *Vis Neurosci*, 3:39-51.

Dyer, F. C. 1985. Nocturnal orientation by the Asian honey bee, *Apis dorsata*. *Anim Behav*, 33:769-774.

Eckert, H. 1968. Hell-Dunkel-Adaptation in aconen Appositionsaugen der Insekten. *Zool Jb Physiol*, 74:102-120.

Exner, S. 1891. *Die Physiologie der facettirten Augen von Krebsen und Insecten*. Deuticke, Leipzig.

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

Gemperlein, R., and U. Smola. 1972. Transfer characteristics of visual cell of *Calliphora erythrocephala*. III. Improvement of signal-to-noise ratio by presynaptic summation in lamina ganglionaris. *J Comp Physiol*, 79:393-409.

Gibson, G. 1995. A behavioural test of the sensitivity of a nocturnal mosquito, *Anopheles gambiae*, to dim white, red and infra-red light. *Physiol Entomol*, 20:224-228.

Greiner, B. 2005. Visual adaptations in the night active wasp *Apoica pallens*. *J Comp Neurol*, accepted.

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

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

Greiner, B., W. A. Ribi, and E. J. Warrant. 2005. A neural network to improve dim-light vision? Dendritic fields of first-order interneurons in the nocturnal bee *Megalopta genalis*. *Cell Tissue Res*, in press.

Horridge, G. A., and P. B. T. Barnard. 1965. Movement of palisade in locust retinula cells when illuminated. *Quart J Micr Sci*, 106:131-135.

Horridge, G. A., and I. A. Meinertzhagen. 1970. The exact projection of the visual fields upon the first and second ganglia of the insect eye. *Z Vergl Physiol*, 66:369-378.

Horridge, G. A., C. Giddings, and G. Stange. 1972. The superposition eyes of skipper butterflies. *Proc R Soc Lon B Biol Sci*, 182:457-495.

Horridge, G. A., J. Duniec, and L. Marcelja. 1981. A 24-hour cycle in single locust and mantis photoreceptors. *J Exp Biol*, 91:307-322.

Howard, K. J., A. R. Smith, S. O'Donnell, and R. L. Jeanne. 2002. Novel method of swarm emigration by the epiponine wasp, *Apoica pallens* (Hymenoptera, Vespidae). *Ethol Ecol Evol*, 14:365-371.

Hunt, J. H., R. L. Jeanne, and M. G. Keeping. 1995. Observations on *Apoica pallens*, a nocturnal neotropical social wasp (Hymenoptera: Vespidae, Polistinae, Epiponini). *Insect Soc*, 42:223-236.

Ioannides, A. C., and G. A. Horridge. 1975. The organization of visual fields in the hemipteran acone eye. *Proc R Soc Lon B Biol Sci*, 190:373-391.

Jander, U., and R. Jander. 2002. Allometry and resolution of bee eyes (Apoidea). *Arthropod Struct Dev*, 30:179-193.

Janzen, D. H. 1964. Notes on the behavior of four subspecies of the Carpenter bee, *Xylocopa* (*Notoxylocopa*) *tabaniformis*, in Mexico. *Ann Entomol Soc Am*, 57:296-301.

Janzen, D. H. 1968. Notes on nesting and foraging behavior of *Megalopta* (Hymenoptera: Halictidae) in Costa Rica. *J Kansas Entomol Soc*, 41:342-350.

Kelber, A. 1999. Trichromatic colour vision in the hummingbird hawkmoth, *Macroglossum stellatarum* L. *J Comp Physiol A*, 184:535-541.

Kelber, A., A. Balkenius, and E. J. Warrant. 2002. Scotopic colour vision in nocturnal hawkmoths. *Nature*, 419:922-925.

Kelber, A., E. J. Warrant, M. Pfaff, R. Wallen, J. C. Theobald, W. T. Wcislo, and R. Raguso. 2006. Light intensity limits the foraging activity in nocturnal and crepuscular bees. *Behav Ecol*, in press.

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

Kerfoot, W. B. 1967b. The lunar periodicity of *Sphecodogastra texana*, a nocturnal bee (Hymenoptera: Halictidae). *Anim Behav*, 15:479-486.

Kirschfeld, K. 1967. Die Projektion der optischen Umwelt auf das Raster der Rhabdomere im Komplexauge von *Musca*. *Exp Brain Res*, 3:248-270.

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

Kral, K. 1987. Organization of the first optic neuropil (or lamina) in different insect species. In A. P. Gupta (ed.), *Arthropod Brain: Its Evolution, Development, Structure, and Functions*, pp. 181-201. John Wiley & Sons, New York.

Land, M. F. 1981. Optics and vision in invertebrates. In H. Autrum (ed.), *Handbook of sensory physiology, vol VII/6B*, pp. 471-592. Springer, Berlin Heidelberg New York.

Land, M. F. 1984. The resolving power of diurnal superposition eyes measured with an ophthalmoscope. *J Comp Physiol A*, 154:515-533.

Land, M. F., and D. C. Osorio. 1990. Waveguide modes and pupil action in the eyes of butterflies. *Proc R Soc Lon B Biol Sci*, 241:93-100.

Land, M. F., G. Gibson, and J. Horwood. 1997. Mosquito eye design: conical rhabdoms are matched to wide aperture lenses. *Proc R Soc Lon B Biol Sci*, 264:1183-1187.

Land, M. F., G. Gibson, J. Horwood, and J. Zeil. 1999. Fundamental differences in the optical structure of the eyes of nocturnal and diurnal mosquitoes. *J Comp Physiol A*, 185:91-103.

Land, M. F., and J. Horwood. 2005. Different retina-lamina projections in mosquitoes with fused and open rhabdoms. *J Comp Physiol A*, 191:639-47.

Laughlin, S. B. 1973. Neural integration in the first optic neuropile of dragonflies. I. Signal amplification in dark-adapted second-order neurons. *J Comp Physiol*, 84:335-355.

Laughlin, S. B. 1981. Neural principles in the peripheral visual system of invertebrates. In H. Autrum (ed.), *Handbook of sensory physiology, vol VII/6B*, pp. 133-280. Springer, Berlin Heidelberg New York.

Laughlin, S. B. 1990. Invertebrate vision at low luminances. In R. F. Hess, L. T. Sharpe and K. Nordby (eds.), *Night vision*, pp. 223-250. Cambridge University Press, Cambridge New York Melbourne Sydney.

Laughlin, S. B., and G. A. Horridge. 1971. Angular sensitivity of the retinula cells of dark-adapted worker bee. *Z Vergl Physiol*, 74:329-335.

Lazzari, C. R. 1992. Circadian organisation of locomotion activity in the haematophagous bug *Triatoma infestans*. *J Insect Physiol*, 38:895-903.

Lehrer, M. 1993. Why do bees turn back and look. *J Comp Physiol A*, 172:549-563.

Leigh, E. G. J. 1999. *Tropical forest ecology: A view from Barro Colorado Island*. Oxford University Press, Oxford.

Lillywhite, P. G., and S. B. Laughlin. 1979. Transducer noise in a photoreceptor. *Nature*, 277:569-572.

Linsley, E. G. 1962. The colletid *Ptiloglossa arizonensis* Timberlake, a matinal pollinator of *Solanum* (Hymenoptera). *Pan-Pacific Entomol*, 38:75-82.

Linsley, E. G., J. W. MacSwain, and R. F. Smith. 1955. Biological observations on *Xenoglossa fulva* (Smith) with some generalizations of biological characters of other Eucerine bees. *Bull South Californian Acad Sci*, 54:128-141.

Linsley, E. G., and M. A. Cazier. 1970. Some competitive relationships among matinal and late afternoon foraging activities of caupolicanine bees in southerneastern Arizona (Hymenoptera, Colletidae). *J Kansas Entomol Soc*, 43:251-261.

Lythgoe, N. J. 1979. *The ecology of vision.* Oxford University Press, New York.

Lüdtke, H. 1953. Retinomotorik und Adaptationsvorgänge im Auge des Rückenschwimmers (*Notonecta glauca* L.). *Z Vergl Physiol*, 35:129-152.

Machado, I. C., and S. Vogel. 2004. The north-east-Brazilian Liana, *Adenocalymna dichilum* (Bignoniaceae) pollinated by bats. *Ann Bot*, 93:609-613.

Marshall, A. G. 1983. Bats, flowers and fruit - evolutionary relationships in the old world. *Biol J Linnean Soc*, 20:115-135.

Matsuura, M. 1999. Size and composition of swarming colonies in *Provespa anomala* (Hymenoptera, Vespidae), a nocturnal social wasp. *Insect Soc*, 46:219-223.

Mazokhin-Porshnyakov, G. A., and V. I. Kazyakina. 1978. The morphology of the compound eye of bloodsucking mosquitoes (Diptera, Culicidae). *Nauchnye Dokl Vysshei Shkoly, Biol Nauki*, 7:59-63.

McIntyre, P., and S. Caveney. 1985. Graded-index optics are matched to optical geometry in the superposition eyes of scarab beetles. *Philos Trans R Soc B-Biol Sci*, 311:237-269.

Meinertzhagen, I. A. 1976. The organization of perpendicular fibre pathways in the insect-optic lobe. *Philos Trans R Soc B-Biol Sci*, 274:556-594.

Meinertzhagen, I. A. 1991. Evolution of the cellular organisation of the arthropod compound eye and the optic lobe. In J. R. Cronly-Dillon and R. L. Gregory (eds.), *Vision and visual dysfunction, vol. 2, Evolution of the eye and the visual system.*, Vol. 2, pp. 341-363. Macmillan Press, London.

Meinertzhagen, I. A., C. J. Armett-Kibel, and K. L. Frizzell. 1980. The number and arrangement of elements in the lamina cartridge of the dragonfly *Sympetrum rubicundulum*. *Cell Tissue Res*, 206:395-401.

Meinertzhagen, I. A., and C. Armett-Kibel. 1982. The lamina monopolar cells in the optic lobe of the dragonfly *Sympetrum*. *Philos Trans R Soc B-Biol Sci*, 297:27-49.

Melzer, R. R., T. Zimmermann, and U. Smola. 1997. Modification of dispersal patterns of branched photoreceptor axons and the evolution of neural superposition. *Cell Mol Life Sci*, 53:242-247.

Menzel, R. 1981. Achromatic vision in the honeybee at low light intensities. *J Comp Physiol A*, 141:389-393.

Menzel, R., U. Greggers, A. Smith, S. Berger, R. Brandt, S. Brunke, G. Bundrock, S. Hulse, T. Plumpe, F. Schaupp, E. Schuttler, S. Stach, J. Stindt, N. Stollhoff, and S. Watzl. 2005. Honey bees navigate according to a map-like spatial memory. *Proc Natl Acad Sci USA*, 102:3040-3045.

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

Meyer-Rochow, V. B., and J. Gál. 2004. Dimensional limits for arthropod eyes with superposition optics. *Vision Res*, 44:2213-23.

Michener, C. D. 2000. *The bees of the world.* Johns Hopkins University Press, USA.

Miller, W. H. 1979. Ocular optical filtering. In H. Autrum (ed.), *Handbook of sensory physiology, vol VII/6A*, pp. 69-143. Springer, Berlin Heidelberg New York.

Moeller, J. F., and J. F. Case. 1995. Temporal adaptations in visual systems of deep-sea crustaceans. *Mar Biol*, 123:47-54.

Nilsson, D.-E. 1989. Optics and evolution of the compound eye. In D. G. Stavenga and R. C. Hardie (eds.), *Facets of vision*, pp. 30-73. Springer, Berlin Heidelberg New York.

Nilsson, D.-E., and H. L. Nilsson. 1981. A crustacean compound eye adapted for low light intensities (Isopoda). *J Comp Physiol A*, 143:503-510.

Nilsson, D.-E., M. F. Land, and J. Howard. 1984. Afocal apposition optics in butterfly eyes. *Nature*, 312:561-563.

Nilsson, D.-E., M. F. Land, and J. Howard. 1988. Optics of the butterfly eye. *J Comp Physiol A*, 162:341-366.

Nilsson, D.-E., and A.-I. Ro. 1994. Did neural pooling for night vision lead to the evolution of neural superposition eyes? *J Comp Physiol A*, 175:289-302.

Nowel, M. S., and P. M. J. Shelton. 1981. A Golgi-electron microscopical study of the structure and development of the lamina ganglionaris of the locust optic lobe. *Cell Tissue Res*, 216:377-401.

Ohly, K. P. 1975. The neurons of the first synaptic region of the optic neuropil of the firefly, *Phausis splendidula* L. Coleoptera. *Cell Tissue Res*, 158:89-109.

Park, O. 1940. Nocturnalism - the development of a problem. *Ecol Monogr*, 10:486-536.

Pirenne, M. H. L. 1967. *Vision and the eye.* Chapman & Hall, London.

Pyza, E., and I. A. Meinertzhagen. 1993. Daily and circadian rhythms of synaptic frequency in the first visual neuropile of the housefly's (*Musca domestica* L.) optic lobe. *Proc R Soc Lond B Biol Sci*, 254:97-105.

Rau, P. 1933. *The jungle bees and wasps of Barro Colorado Island.* Von Hoffman Press, St. Louis.

Reisenman, C. E., C. R. Lazzari, and M. Giurfa. 1998. Circadian control of photonegative sensitivity in the haematophagous bug *Triatoma infestans*. *J Comp Physiol A*, 183:533-541.

Reisenman, C. E., T. C. Insausti, and C. R. Lazzari. 2002. Light-induced and circadian changes in the compound eye of the haematophagous bug *Triatoma infestans* (Hemiptera: Reduviidae). *J Exp Biol*, 205:201-210.

Ribi, W. A. 1974. Neurons in the first synaptic region of the bee, *Apis mellifera*. *Cell Tissue Res*, 148:277-286.

Ribi, W. A. 1975. The organization of the lamina ganglionaris of the bee. *Z Naturforsch (C)*, 30:851-852.

Ribi, W. A. 1976. The first optic ganglion of the bee. II. Topographical relationships of the monopolar cells within and between cartridges. *Cell Tissue Res*, 171:359-373.

Ribi, W. A. 1977. Fine structure of the first optic ganglion (lamina) of the cockroach, *Periplaneta americana*. *Tissue Cell*, 9:57-72.

Ribi, W. A. 1978. Ultrastructure and migration of screening pigments in the retina of *Pieris rapae* L. (Lepidoptera, Pieridae). *Cell Tissue Res*, 191:57-73.

Ribi, W. A. 1980. The phenomenon of eye glow. *Endeavour*, 5:2-8.

Ribi, W. A. 1981. The first optic ganglion of the bee. IV. Synaptic fine structure and connectivity patterns of receptor cell axons and first order interneurones. *Cell Tissue Res*, 215:443-464.

Ribi, W. A. 1987a. Anatomical identification of spectral receptor types in the retina and lamina of the Australian orchard butterfly, *Papilio aegeus aegeus* D. *Cell Tissue Res*, 247:393-407.

Ribi, W. A. 1987b. The structural basis of information processing in the visual system of the bee. In R. Menzel and A. Mercer (eds.), *Neurobiology and Behavior of Honeybees*, pp. 130-140. Springer, Berlin Heidelberg New York.

Roberts, R. B. 1971. Biology of the crepuscular bee *Ptiloglossa guinnae* n. sp. with notes on associated bees, mites and yeasts. *J Kansas Entomol Soc*, 44:283-294.

Rose, R., and R. Menzel. 1981. Luminance dependence of pigment color discrimination in bees. *J Comp Physiol A*, 141:379-388.

Rossel, S. 1979. Regional differences in photoreceptor performance in the eye of the praying mantis. *J Comp Physiol A*, 131:95-112.

Roulston, T. H. 1997. Hourly capture of two species of *Megalopta* (Hymenoptera: Apoidae; Halictidae) at black lights in Panama with notes on nocturnal foraging by bees. *J Kansas Entomol Soc*, 70:189-196.

Sakagami, S. F. 1964. Wiederentdeckung des Nestes einer Nachtfurchenbiene, *Megalopta* sp. am Amazonas (Hymenoptera, Halictidae). *Kontyû*, 32:457-463.

Schremmer, F. 1972. Observations on biology of *Apoica pallida* (Olivier, 1791), neotropic social wasps (Hymenoptera, Vespidae). *Insect Soc*, 19:343-357.

Schwind, R. 1983. A polarisation-sensitive response of the flying waterbug *Notonecta glauca* to UV light. *J Comp Physiol A*, 150:87-91.

Shaw, S. R. 1989. The retina-lamina pathway in insects, particularly diptera, viewed from an evolutionary perspective. In D. G. Stavenga and R. C. Hardie (eds.), *Facets of vision*, pp. 186-212. Springer, Berlin Heidelberg New York.

Shaw, S. R. 1990. The photoreceptor axon projection and its evolution in the neural superposition eyes of some primitive brachyceran Diptera. *Brain Behav Evol*, 35:107-125.

Shaw, S. R., and I. A. Meinertzhagen. 1986. Evolutionary progression at synaptic connections made by identified homologous neurones. *Proc Natl Acad Sci USA*, 83:7961-7965.

Shaw, S. R., and D. Moore. 1989. Evolutionary remodeling in a visual system through extensive changes in the synaptic connectivity of homologous neurons. *Vis Neurosci*, 3:405-410.

Shimohigashi, M., and Y. Tominaga. 1999. Synaptic organization in the lamina of the superposition eye of a skipper butterfly, *Parnara guttata*. *J Comp Neurol*, 408:107-124.

Smith, A. R., W. T. Wcislo, and S. O'Donnel. 2003. Assured fitness returns favor sociality in a mass-provisioning sweat bee *Megalopta genalis*. *Behav Ecol Sociobiol*, 54:14-21.

Snyder, A. W. 1977. Acuity of compound eyes: physical limitations and design. *J Comp Physiol*, 116:161-182.

Snyder, A. W. 1979. The physics of compound eyes. In H. Autrum (ed.), *Handbook of sensory physiology, vol VII/6A*, pp. 225-313. Springer, Berlin Heidelberg New York.

Snyder, A. W., and A. Horridge. 1972. The optical function of changes in the medium surrounding the cockroach rhabdom. *J Comp Physiol*, 81:1-8.

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

Stavenga, D. G. 1979. Pseudopupils of compound eyes. In H. Autrum (ed.), *Handbook of sensory physiology, vol VII/6A*, pp. 357-440. Springer, Berlin Heidelberg New York.

Stavenga, D. G. 2003. Angular and spectral sensitivity of fly photoreceptors. II. Dependence on facet lens F-number and rhabdomere type in Drosophila. *J Comp Physiol A*, 189:189-202.

Stavenga, D. G., S. Foletti, G. Palasantzas, and K. Arikawa. 2005. Light on the moth-eye corneal nipple array of butterflies. *Proc R Soc Lon B Biol Sci*, in press.

Strausfeld, N. J. 1971. Organization of insect visual system (light microscopy) II. Projection of fibres across first optic chiasma. *Z Zellforsch Mikrosk Anat*, 121:442-454.

Strausfeld, N. J. 1976. *Atlas of an insect brain.* Springer, Berlin.

Strausfeld, N. J., and A. D. Blest. 1970. Golgi studies on insects. Part I. The optic lobes of Lepidoptera. *Philos Trans R Soc B-Biol Sci*, 258:81-134.

Strausfeld, N. J., and D. R. Nässel. 1981. Neuroarchitecture of brain regions that subserve the compound eyes of crustaceans and insects. In H. Autrum (ed.), *Handbook of sensory physiology, vol VII/6B*, pp. 1-132. Springer, Berlin Heidelberg New York.

Takken, W., and B. G. Knols. 1999. Odor-mediated behavior of Afrotropical malaria mosquitoes. *Annu Rev Entomol*, 44:131-157.

Theobald, J. C., B. Greiner, W. T. Wcislo, and E. J. Warrant. 2005. Visual summation in night-flying sweat bees: a theoretical study. *Vision Research*, submitted.

Trujillo-Cenóz, O. 1965. Some aspects of the structural organisation of the intermediate retina of Dipterans. *J Ultrastruct Res*, 13:1-33.

Tunstall, J., and G. A. Horridge. 1967. Electrophysiological investigation of the optics of the locust retina. *Z Vergl Physiol*, 55:167-182.

van Hateren, J. H., M. V. Srinivasan, and P. B. Wait. 1990. Pattern recognition in bees: orientation discrimination. *J Comp Physiol A*, 167:649-654.

Vesey-FitzGerald, D. 1938. Social wasps (Hym. Vespidae) from Trinidad, with a note on the genus *Trypoxylon* Latreille. *Trans R Entomol Soc Lond*, 87:181-191.

Vigier, P. 1909. Mecanisme de la synthèse des impressions lumineuses par les yeux composés des Diptères. *C R Acad Sci Paris*, 148:1221-1223.

Walcott, B. 1971. Cell movement on light adaptation in the retina of *Lethocerus* (Belastomatidae, Hemiptera). *Z Vergl Physiol*, 74:17-25.

Walcott, B. 1975. Anatomical changes during light-adaptation in insect compound eyes. In G. A. Horridge (ed.), *The compound eye and vision of insects*, pp. 20-33. Clarendon Press, Oxford.

Warrant, E. J. 1993. Can neural pooling help insects see at night? *Proc IEEE Int Conf Syst Man Cybern*, 3:150-155.

Warrant, E. J. 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. J. 2001. The design of compound eyes and the illumination of natural habitats. In F. G. Barth and A. Schmid (eds.), *Ecology of sensing*, pp. 187-213. Springer, Berlin Heidelberg New York.

Warrant, E. J. 2004. Vision in the dimmest habitats on earth. *J Comp Physiol A*, 190:765-789.

Warrant, E. J., and P. D. McIntyre. 1991. Strategies for retinal design in arthropod eyes of low F-number. *J Comp Physiol A*, 168:499-512.

Warrant, E. J., and P. D. McIntyre. 1992. The trade-off between resolution and sensitivity in compound eyes. In R. B. Pinter and B. Nabet (eds.), *Nonlinear vision*, pp. 391-421. CRC Press, Boca Raton Ann Arbor London Tokyo.

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

Warrant, E. J., and P. D. McIntyre. 1996. The visual ecology of pupillary action in superposition eyes. *J Comp Physiol A*, 178:75-90.

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

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

Warrant, E. J., K. Bartsch, and C. Günther. 1999. Physiological optics in the hummingbird hawkmoth: a compound eye without ommatidia. *J Exp Biol*, 202:497-511.

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

Wcislo, W. T., L. Arneson, K. Roesch, V. Gonzalez, A. Smith, and H. Fernandez. 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*, 83:377-387.

Williams, D. S. 1980. Organization of the compound eye of a tipulid fly during the day and night. *Zoomorphology*, 95:85-104.

Williams, D. S. 1982. Ommatidial structure in relation to turnover of photoreceptor membrane in the locust. *Cell Tissue Res*, 225:595-617.

Wolburg-Buchholz, K. 1979. The organization of the lamina ganglionaris of the hemipteran insects, *Notonecta glauca*, *Corixa punctata* and *Gerris lacustris*. *Cell Tissue Res*, 197:39-59.

Wolda, H., and D. W. Roubik. 1986. Nocturnal bee abundance and seasonal bee activity in a Panamanian forest. *Ecology*, 67:426-433.

Zeil, J. 1979. A new kind of neural superposition eye: the compound eye of male Bibionidae. *Nature*, 278:249-250.

Zeil, J. 1983. Sexual dimorphism in the visual system of flies: the divided brain of male Bibionidae (Diptera). *Cell Tissue Res*, 229:591-610.

Zeil, J. 1993. Orientation flights of solitary wasps (Cerceris, Sphecidae, Hymenoptera). 1. Description of Flight. *J Comp Physiol A*, 172:189-205.

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