Ommatidial adaptations for vision in nocturnal insects

Frederiksen, Rikard

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Rikard Frederiksen

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Cover image: *Onitis belial,* illustration by Pål Langöe

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Abstract

Nocturnal vision is a demanding task for insects with small eyes. As it gets dimmer the noise imposed by the stochastic nature of photon arrival makes vision unreliable. Despite this, there are quite a number of animal species that are active at night and apparently see well. In this thesis I show that the compound eyes of nocturnal insects are sufficiently flexible to adapt to the particular window of intensities in which the species is active. These ommatidial adaptations for vision in nocturnal insects are found in the optics and morphology of the eye (Chapters I - III) and in the physiology of the photoreceptors (Chapters IV & V). I have also discovered that the various visual adaptations that optimise sensitivity in a compound eye, and thus adapt the eye for a crepuscular or nocturnal lifestyle, do not contribute equally in different groups of insects. The crepuscular *Caligo memnon* has evolved large eyes with large facets but retained a reasonably high spatial and temporal resolution. In the nocturnal bee *Megalopta genalis*, the eyes and facets are enlarged, the rhabdoms are wide and the spatial and temporal acuity very poor compared to diurnal insects. In addition to this, *M. genalis* has a high gain of transduction present in its photoreceptors. The nocturnal dung beetle *Onitis aygulus* has refracting superposition eyes and increases optical sensitivity by possessing a wide superposition aperture. In addition, this beetle has slow photoreceptors with high gain. Thus, there are a variety of solutions to improve visual sensitivity and reliability in dim light. Exactly which solution evolved in which group most likely depends on constraints imposed by different phylogenetic histories, developmental constraints and different selection pressures due to different life styles.

Key words: Vision, Nocturnal insects, Superposition eye, Apposition eye, Optical sensitivity, Information rate, Signal-to-noise ratio, Gain of transduction

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"It has been suggested that zombies possess night vision, a fact that explains their skill at nocturnal hunting. This theory has been debunked by the fact that all zombies are expert night feeders, even those without eyes"

–Max Brooks: the Zombie Survival Guide

Pålitlig synförmåg hos nattaktiva insekter

I en värld full av konkurens och predation har många insekter antagit en nattlig livsstil. Ljuset som dessa djur upplever är i extrema fall endast en hundra milljontedel det som dagaktiva insekter utsätts för en solig sommardag på öppen mark. Trots detta använder många nattaktiva insekter synen som det primära sinnet för att till exempel navigera, söka föda eller finna en partner. Denna doktorsavhandling handlar om synsinnet hos dessa insekter som har antagit en nattlig livsstil och hur ögat är anpassat för att se i mycket svagt ljus.

I avhandlingens första kapitel har jag undersökt hur optiken och morfologin i ögat hos en centralamerikansk skymningsaktiv fjäril, *Caligo memnon,* är anpassad för ett liv i svagt ljus. Jag visar att hos denna fjäril så är det framförallt förstorade facettlinser som bidrar till ökad ljuskänslighet.

Kapitel två handlar om natt- och dagaktiva insekters förmåga att anpassa ögats ljuskänslighet och upplösningsförmåga till den ljusstyrka som de upplever för stunden (något som vi människor gör med hjälp av vår pupil). Jag har jämfört denna förmåga hos två arter av smalbin, det nattaktiva biet *Megalopta genalis* och det dagaktiva biet *Lasioglossum leucozonium*. Mina resultat visar att det nattaktiva biet har en mycket bättre förmåga att anpassa ögat till olika ljusnivåer än det dagaktiva biet. Detta beror troligen på att det nattaktiva biet normalt sett upplever en större variation av ljusintensiteter under sin aktivitetsperiod då ljusintensiteten under en natt med fullmåne är upp till 1000 gånger starkare än under en molnig natt utan måne. Det dagaktiva biet, å andra sidan, är endast aktivt mitt på dagen under soliga sommardagar.

I det tredje kapitlet har jag experimentellt testat en matematisk modell som länge har använts för att beräkna ett ögas optiska ljuskänslighet. Mina resultat visar att modellen stämmer väl överens med de värden jag har fått genom mina experiment.

Avhandlingens sista del, kapitel fyra och fem, handlar om hur fotoreceptorerna i ögat hos natt och dagaktiva insekter är anpassade för starkt eller svagt ljus. Jag har undersökt fotoreceptorernas signalförstärkning, tidsupplösning, samt deras förmåga att behandla information.

Fotoreceptorerna hos nattaktiva insekter har en stark signalförstärkning. Denna i kombination med en låg tidsupplösning innebär att enskilda fotoreceptorer kan behandla relativt lite information, men har en hög ljuskänslighet. Mina resultat visar dessutom skillnader mellan olika insekter med olika typer av ögon. Nattaktiva insekter med en ögontyp som har hög optisk ljuskänslighet har inte utvecklat lika högt signal-brusförhållande i fotoreceptorerna som de som har en ögontyp med lägre optisk känslighet.

Detta visar att det finns en rad olika lösningar för att öka ett ögas känslighet och anpassa detta för ett liv om natten. Exakt vilka lösningar som har utvecklats i olika insekter beror på vad synsystemet är anpassat för att se, samt insekternas evolutionära historia.

Main References:

I. Frederiksen, R. & Warrant, E. J. 2008a. Visual sensitivity in the crepuscular Owl butterfly *Caligo memnon* and the diurnal Blue Morpho *Morpho peleides*: a clue to explain the evolution of nocturnal apposition eyes? *Journal of Experimental Biology*, **211,** 844-851.

II. Frederiksen, R., Kreiss, E., Gislén, A., Wcislo, W. T., Smith, A. R. & Warrant, E. J. 2008a. Light and dark adaptation in diurnal and nocturnal halictid bees. *Manuscript submitted to Journal of Comparative Physiology A.*

III. Frederiksen, R. & Warrant, E. J. 2008b. The optical sensitivity of compound eyes – theory and experiment compared. *Manuscript submitted to Biology Letters.*

IV. Frederiksen, R., Wcislo, W. T. & Warrant, E. J. 2008b. Visual reliability and information rate in the retina of a nocturnal bee. *Current Biology*, **18,** 349-352.

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1. Introduction

Light environments on Earth are very diverse. The ambient light in the depths of the ocean is very different from that on an open meadow during the day or in a tropical rain forest at dusk or dawn. In fact, between the dimmest and the brightest habitat on Earth, daylight intensity varies over as much as 20 orders of magnitude (Warrant & McIntyre, 1992). No known single eye has evolved to be able to function over such an enormous span of intensities, but within any given light habitat, animals can be found that rely on vision for survival, often possessing amazing visual adaptations that match them to their own specific light-niche (Warrant, 2004). Some animals are strictly nocturnal and some are day active. There are even animals that have an activity period of just a few minutes during dusk and/or dawn. The range of intensities that a single animal is optimised to see well within is usually narrow and the animal's visual system is often found to be optimised to this particular intensity range.

No matter whether an eye is found in a deep-sea fish, a nocturnal cricket or a day-active bumblebee, it is still constrained by the physical nature of light, forming an image that can be interpreted by the animal's nervous system and which leads to a behavioural response. In order to do so the eye must first be able to collect enough light, secondly to distinguish the directions from which the incoming light has arrived and lastly to accurately code the changes in light intensity that result when the image moves over the retina. In a dim light habitat this becomes a major challenge for a visual system. Photons are rare and it is hard to capture enough light to achieve accurate contrast detection and acute vision. Despite this many animals have become nocturnal or crepuscular, because of the benefits that come with exploiting this new niche. These benefits could include a decrease in predation pressure or a decreased competition for limited resources such as food or territory.

In this thesis I will explore the visual systems of insects that have resorted to a crepuscular or nocturnal lifestyle and address the following questions. (1) What are the optical and morphological adaptations that crepuscular and nocturnal insects with apposition eyes possess, and what can these tell us about the evolution of nocturnal apposition eyes? This question will be addressed in Chapter I (Frederiksen & Warrant, 2008a), where I investigate how the visual system, in terms of eye morphology and optics, of two species of similar-sized and closely-related nymphalid butterflies (*Caligo memnon* and *Morpho peleides,* Fig. 1A,B) have been adapted to a crepuscular and a diurnal life style respectively. (2) In Chapter II (Frederiksen et al., 2008a), I address the question of how the different optics and morphologies of eyes in nocturnal and diurnal halictid bees (*Megalopta genalis* and *Lasioglossum leucozonium*, Fig 1C,D) affect their ability to light and dark adapt. (3) In Chapter III (Frederiksen & Warrant, 2008b), I use experimentally obtained data to test a commonly used theoretical model of optical sensitivity, the Land sensitivity equation, and ask how accurate this equation is as a tool for comparing optical sensitivities in insects with different activity periods and different eye types. In the last two chapters I explore how photoreceptor physiology is adapted for visual reliability and

eye design in nocturnal and diurnal insects. (4) Chapter IV (Frederiksen et al., 2008b) is a comparative study of photoreceptor physiology in nocturnal (*Megalopta genalis*) and diurnal (*Lasioglossum leiucozonium*) halictid bees. The main question here is how the physiology of the photoreceptors (in terms of frequency response bandwidth, gain of transduction, signal-to-noise ratio and information rate) in nocturnal and diurnal insects with apposition eyes is adapted to function in their particular light environment. (5) Finally, in Chapter V (Frederiksen et al., 2008c) I extend this question to nocturnal (*Onitis aygulus*, Fig. 1E) and diurnal (*Onitis belial*, Fig. 1F) insects with superposition eyes. Do the photoreceptors of superposition eyes (nocturnal and diurnal) share the same traits as apposition eyes (nocturnal and diurnal), or can they maintain a higher bandwidth and information rate because of their higher optical sensitivity?

To be able to address these questions and to understand how an animal can have a functional visual system in a very dim habitat we must first consider the fundamental physical principles that govern the function of the eye and also how evolution has shaped the vision of animals living in these types of habitats.

2. Light and its dual nature

If we wish to understand a sensory system, we must know something about the nature of the stimulus that it has evolved to detect, in this case light. Interestingly, light has a dual nature; it can be understood as stream of energy packages, or photons, each containing a defined amount of energy, or as a waveform with properties such as frequency and wavelength. Depending on the question, both descriptions are useful for understanding a visual system.

The wave nature of light imposes several constraints on vision. The wavelength, or ultimately the frequency, determines the colour of light. Refraction and reflection are important physiological phenomena that are due to the wave nature of light, and these allow an eye to focus an image.

Another important phenomenon arising from the wave properties of light is diffraction and this has the potential to limit visual acuity. When parallel plane wave fronts of light enter through a small aperture whose diameter is in the same order of magnitude as the light's wavelength, the wave crests and troughs will interfere with each other. The result of this interference is that light reaching the image plane has an intensity that is attenuated in some areas and reinforced in others.

The intensity distribution of a point source of light that has been subjected to diffraction is known as the Airy diffraction pattern. This consists of a central peak, the Airy disc, that has an approximately Gaussian distribution, and concentric rings of high and low intensity that surround it. The Airy disc and the brighter rings are the result of positive interference and the dark rings are the result of negative interference. The Airy diffraction pattern has a spatially broader distribution than what is expected from geometrical optics. Diffraction

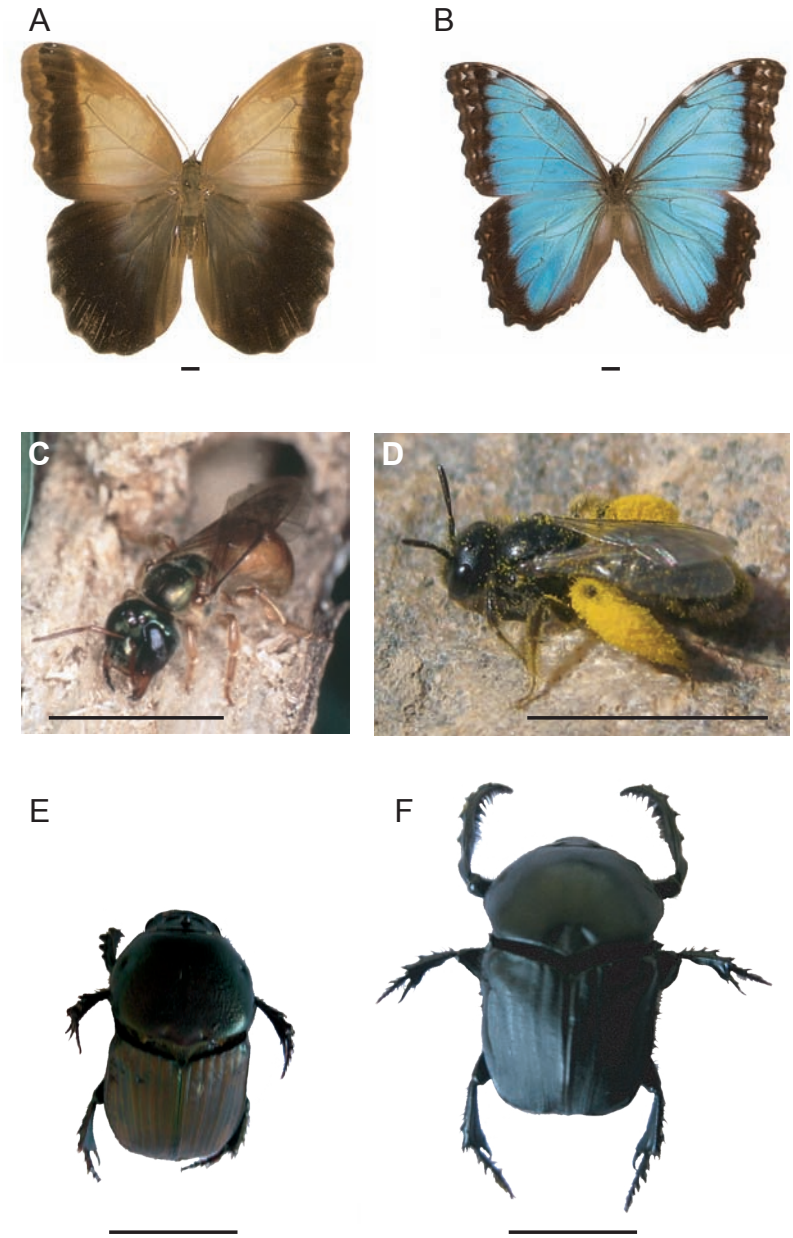

Fig. 1. The species investigated in this thesis. **A** The crepuscular butterfly *Caligo memnon*. Adapted from DeVries (1987) **B** The diurnal butterfly *Morpho peleides*. Adapted from DeVries (1987) **C** The nocturnal bee *Megalopta genalis*. Photograph by Michael Pfaff **D** The diurnal bee *Lasioglossum leucozonium*. **E** The nocturnal dung beetle *Onitis aygulus*. Adapted from Frederiksen et al. (2008c) **F** The diurnal dung beetle *Onitis belial*. Adapted from Frederiksen et al. (2008c). Scale bar all panels: 1 cm.

will therefore degrade spatial acuity in an eye. The half-width of the Airy disc, $\Delta\rho_p$ can be approximated by the ratio of the wavelength, λ, to the aperture diameter, D (Snyder, 1977):

$$\Delta\rho_l = \frac{\lambda}{D}. \quad (1)$$

From Eq. 1 it follows that the effect of diffraction is most prominent for small apertures and receptive structures and it is therefore an important constraint to consider when discussing the function of insect compound eyes.

The particle nature of light imposes other constraints on vision. Photoreceptors effectively work as photon counters. One absorbed photon leads, via the transduction cascade, to a defined voltage response known as a photon 'bump' (Lillywhite, 1977). This will be discussed further in the second half of this thesis.

3. Eye designs

At first thought one might think that such a complex structure as an eye could have evolved once and be relatively conserved throughout time. However, it has been shown that an eye can evolve relatively quickly in geological time (Nilsson & Pelger, 1995). Eyes have evolved many times independently in the history of life (Land & Fernald, 1992; Land & Nilsson, 2002) and we should therefore expect to find a wide variety of 'eye designs'. Eight major types can be recognised (Nilsson, 1990). Three of these types are simple eyes such as the camera eye (found in for example vertebrates, arthropods, cnidarians, molluscs and annelids), the concave mirror eye (in the mollusc *Pecten* and in ostracod arthropods) and the pinhole eye (in the cephalopod mollusc *Nautilus* and in various invertebrates in less developed forms). The remaining five types are compound eyes: the apposition eye, the neural superposition eye, the refractive superposition eye, the reflecting superposition eye and the parabolic superposition eye. All of them can be found in arthropods (although not exclusively). I will not discuss all of them in detail, but will focus on the two main types that are found in insects: refractive superposition eyes (Fig. 2A) (which excludes the neural superposition eyes of dipterans) and the apposition eyes (Fig. 2B). Refractive superposition eyes will from here on be referred to simply as superposition eyes.

All compound eyes are composed of identical optical units called ommatidia. These consist of the corneal facet lens and the crystalline cone that together form the lens element (dioptric apparatus) of the eye. Light from a defined angle is focused by the lens element onto a rhabdom, which is composed of microvilli from the retinula cells (Fig. 2: Warrant & McIntyre, 1993).

The type of eye that is often found in a typical day-active insect is a focal apposition eye (Fig. 2B). In this design the crystalline cone adjoins the rhabdom, but only the cornea focuses light. The crystalline cone merely acts as a spacer between the cornea and the

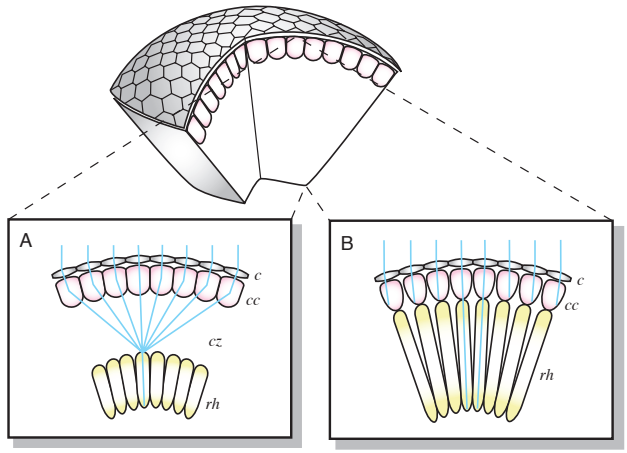

Fig. 2. Compound eyes of insects. **A** The superposition eye, the eye type often found in nocturnal and crepuscular insects (e.g. moths and beetles). The eye consists of ommatidia where the lens elements, corneal facet lenses (*c*) and crystalline cones (*cc*), focus light onto the rhabdom (*rh*). Between the dioptric apparatus and the rhabdom is a clear zone (*cz*) that allows light from multiple facet lenses to be focused onto one rhabdom. **B** The apposition eye, most often found in day-active insects (e.g. bees and wasps). This eye type lacks a clear zone and each rhabdom receives light from one facet lens only. Moreover, with the exception of the butterfly's afocal apposition eye (Nilsson et al., 1984), the crystalline cone have no refractive power and acts as a spacer between the corneal facet lens and the rhabdom. Illustration by Pål Langöe.

rhabdom. Since each ommatidium represents one optical unit and is shielded from other ommatidia by light-absorbing screening pigment this means that each rhabdom receives light from only one facet, and each ommatidium reconstructs one point, or pixel, in the visual field. Photon capture in an apposition eye is thus rather poor compared to that in superposition eyes (Warrant & McIntyre, 1993).

Many crepuscular and nocturnal insects, such as moths and many beetles, have superposition eyes (Fig. 2A) that are designed to capture as much of the available light as possible. In a superposition eye, a clear zone separates the optics from the rhabdoms. In order to form a superposition image, the images from the individual facet lenses need to be erect. In order to achieve this the crystalline cones of superposition eyes have powerful refractive index gradients that allow them to act as lens cylinders. This represents a remarkable adaptation for increased light capture since each rhabdom can receive light from hundreds of facets, but still from the same point in space (Land & Nilsson, 2002).

Moreover, it has also been shown that the superposition eye design can be quite flexible. For instance, in one species of hawkmoth, *Macroglossum stellatarum*, the retina has regions with up to four times as many rhabdoms as corneal facets, creating local acute zones (Warrant et al., 1999). In spite of the large benefits of having superposition eyes in a dim habitat, there are interesting examples of nocturnal and crepuscular insects in many groups that have retained their apposition eyes (e.g. the Apoidea: Warrant, et al., 2004; Greiner et al. 2004a; Somanathan et al., 2008, the Formicidae: Greiner et al., 2007, the Vespidae: Greiner, 2006, and the Nymphalidae: Järemo Jonson et al., 1998; Frederiksen & Warrant 2008a). There are also examples of diurnal insects in several taxa that possess superposition eyes (e.g. the Scarabaeidae: McIntyre & Caveney, 1985; Gokan & Meyer-

Rochow, 1990, the Lucanidae: Gokan et al., 1998, the Sphingidae: Exner, 1891; Warrant et al., 1999, the Hesperiidae: Swihart, 1969; Horridge et al., 1972, Land, 1984, the Noctuidae: Horridge et al., 1977; Horridge et al., 1983; Land, 1984, and the Neuroptera: Schneider et al., 1978; Eggenreich & Kral, 1990, Kral et al., 2000). The eyes of some of these examples will be discussed in more detail later in this thesis (Nympalidae: Chapter I, Apoidea: Chapters II-IV, Scarabaeidae: Chapters II and V).

4. Optical adaptations for nocturnal vision

What makes an eye sensitive to light? A number of physiological parameters are important for understanding the physical limitations that an eye has to cope with in order to produce an image in dim light. The F-number of the eye is the ratio of the focal length, *f*, and the pupil diameter, *D*:

$$F = \frac{f}{D}. \qquad (2)$$

The F-number is widely used among photographers to specify how bright an image a lens produces. A lower F-number will yield a brighter image because it implies wider angles of light incidence (i.e. a wider cone of rays from the lens to the retina). The F-number also has an impact on the spatial resolving power of the eye, as will be evident in the section below.

4.1. Spatial resolution

The spatial resolution of an eye defines the fineness of detail that the eye can resolve in space, easily understood if one makes a comparison with a digital camera. To evaluate the spatial resolution of the camera we must consider the number of pixels (the grain) that the CCD-chip can resolve, or even better, the angular spacing between the pixels. In a compound eye this is represented by the interommatidial angle, $\Delta\phi$. The interommatidial angle is defined as the angle between the optical axes of two neighbouring ommatidia (Land, 1981; Land, 1997).

The interommatidial angle is given by the ratio of the facet diameter, *D*, to the radius of curvature, *R*, of the eye (*D*/*R* radians), and this angle determines the packing density of ommatidia in a compound eye. The optical sampling frequency of an eye with a hexagonal sampling lattice (in cycles/degree) is $1/(\sqrt{3}\,\Delta\phi)$, a measurement of anatomical spatial acuity. Methods for the experimental determination of interommatidial angles can be found elsewhere (Land & Eckert, 1985).

The ultimate spatial resolution of the eye, however, cannot be defined by the interommatidial angle and the focused image quality alone. The light acceptance (i.e. optical) properties of the photoreceptors are also crucial. The angular sensitivity function accounts for all three of these ($\Delta\phi$, image quality and photoreceptor acceptance: Snyder, 1977). In the digital camera example used above, the angular sensitivity function can be likened to the angular size of a pixel.

Experimentally, the angular sensitivity function can be measured using electrophysiology in an intact eye. An electrode is inserted into a photoreceptor cell, and a point source of light is then flashed at known angular steps over the cell's receptive field and the cell's voltage response is recorded at each step. The angular sensitivity function is then calculated by converting the amplitudes of these responses to corresponding sensitivities through the cell's response-intensity curve.

The width of the angular sensitivity function at 50% maximum sensitivity is called the acceptance angle ($\Delta\rho$) of the cell. This angle is a good measure of the cell's spatial resolving power since it represents the size of one pixel in the visual field of the compound eye. Larger acceptance angles imply poorer resolution and *vice versa*. The acceptance angle can be simply approximated by several anatomical parameters in the eye. In an early model created by Snyder (1977), the angular sensitivity function is composed of a lens-pupil function due to diffraction (recognised from Eq. 1), and a rhabdom acceptance function defined by the ratio of rhabdom diameter, *d*, and focal length, *f*. From this model the acceptance angle can be calculated as (Fig. 3):

$$\Delta\rho = \sqrt{\left(\frac{\lambda}{D}\right)^2 + \left(\frac{d}{f}\right)^2}\,. \qquad (3)$$

A recent theoretical model provides a more rigorous description of the angular sensitivity

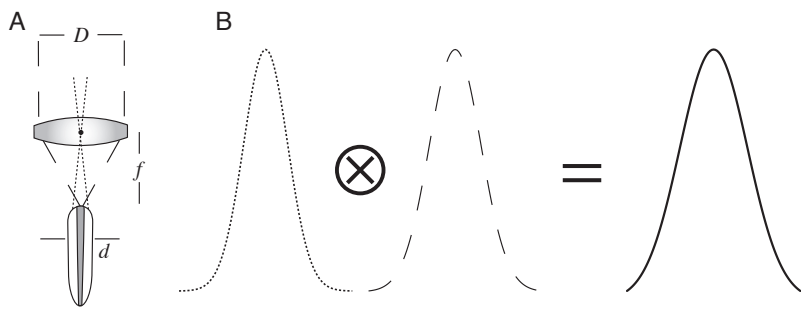

Fig. 3. A Cartoon of an ommatidium showing the optical parameters important for spatial spatial resolution: *D* - lens diameter, *f* - focal length, *d* - rhabdom diameter. **B** Snyder's model (Snyder, 1977) describes the angular-sensitivity function (*solid line*) as the convolution of the gaussian shaped rhabdom acceptance function (*dotted line*) and the lens spread function (*dashed line*), Eq. 3.

function (Stavenga, 2003a,b; Stavenga, 2004a). While the older model of Snyder approximates the angular sensitivity function from geometrical optics, this newer model also considers the waveguide properties (Smakman et al., 1984; van Hateren, 1984) of the rhabdom and thus the wavelength dependence. Snyder's model suggests that the only influence of the wavelength on the angular sensitivity is via facet lens diffraction, λ/D. Stavenga's more recent model, on the other hand, also considers the influence of wavelength on the waveguide properties of the rhabdom. A brief description is necessary.

When the rhabdom diameter is large in relation to the wavelength of the incoming light, the rhabdom behaves as a regular light guide and obeys the principles of geometrical optics (i.e. total internal reflection). If the rhabdom diameter approaches the wavelength of the incoming light it behaves as a waveguide and its optics must be described accordingly (Snyder, 1975). Many insect rhabdoms are less than 2 μm in width and thus subject to waveguide optics.

When light propagates in a waveguide it interferes and forms waveguide modes. The waveguide modes describe the intensity distribution of the wave in space. Which modes propagate depends on the waveguide parameter, V, and this in turn depends on the rhabdom diameter, d, the wavelength of the stimulating light, λ, and the refractive indices inside, n_r, and outside, n_o, the rhabdom:

$$V = \pi \frac{d}{\lambda} \sqrt{n_r^2 - n_o^2} \,. \tag{4}$$

For a review of waveguide modes see Snyder (1975) and Warrant & McIntyre (1993). A certain mode can only propagate if the waveguide parameter exceeds the cut-off value for that particular mode ($V > V_{CO}$). A wider rhabdom yields a larger V and allows more modes. As d grows larger in relation to λ, the superposition of all propagating modes will make the system approach one governed by geometrical optics (Snyder, 1975). The total power, P_{tot}, is then the sum of the powers (squared absolute amplitudes, a) of the individual waveguide modes (Stavenga, 2003a):

$$P_{tot} = \sum_p \left| a_p \right|^2 . \tag{5}$$

An important property of waveguide modes is that not all the energy travels inside the rhabdom – a proportion of the energy can be found on the outside, which has consequences for the angular sensitivity function. Light travelling outside the rhabdom can lead to optical crosstalk between the rhabdoms; this degrades the spatial acuity by blurring the image. This can be eliminated by screening pigments within the retinula cells which absorb this external light, although this also reduces light capture by the photopigments (Pask & Barrell, 1980). The wavelength dependence of the waveguide modes also has

consequences for angular sensitivity (Warrant & McIntyre, 1993; Stavenga, 2003a). In an eye with rhabdoms 2 μm wide two modes are permitted at 600 nm whereas at half that wavelength five modes are allowed (Stavenga, 2003a). The more modes that are present, the greater the intensity distribution found outside the rhabdom, for a given rhabdom diameter. The power of the absorbed light P_{abs} can be expressed as a function of the angle of incidence, θ, and wavelength, λ (Stavenga, 2003a,b):

$$P_{abs}(\theta,\lambda) = k(\lambda) l P_{tot}(\theta,\lambda), \tag{6}$$

where $k(\lambda)$ is the absorbance coefficient of the rhabdom and l is the rhabdom length. P_{tot} is the total effective power of the light (the sum of the power of all propagating waveguide modes). To obtain the angular sensitivity function at a specific wavelength from Eq. 6, we need to normalise either $P_{abs}(\theta, \lambda)$ or $P_{tot}(\theta, \lambda)$ (Stavenga, 2003b).

Wavelength-dependent diffraction effects in the angular-sensitivity function are compensated by the wavelength-dependence of the waveguide mode propagation (Stavenga, 2003a). At longer wavelengths diffraction becomes a considerable problem for an eye with a small aperture (Eq. 1), but because there are fewer waveguide modes propagating in the rhabdom (Eq. 4), most of the light intensity distribution is nevertheless inside the rhabdom. Even though the system will also suffer less diffraction at shorter wavelengths, more modes will propagate and a larger proportion of the light intensity distribution will be outside the rhabdom. Stavenga thoroughly modelled this and concluded that the acceptance angle approximates the geometrical acceptance function of the rhabdom $\Delta\rho_r$ (Stavenga, 2004a,b):

$$\Delta\rho \approx \Delta\rho_r = \frac{d}{f}. \tag{7}$$

The F-number (Eq. 2) affects both the waveguide properties and the geometrical optics of an ommatidium. It have been shown that a low F-number broadens the angular sensitivity function due to a less efficient waveguide mode excitation in the rhabdom (Stavenga, 2003a).

The acceptance angle may be a convenient measurement of spatial resolution, but it lacks important information present in the shape of the angular sensitivity function, particularly in the function's flanks. The relevance of the shape of the angular sensitivity function and its flanks becomes evident in its frequency domain representation: the spatial modulation transfer function, or MTF, of the eye. The MTF is simply the Fourier transform of the angular sensitivity function and reveals the spatial frequencies (the fineness of a black and white grating in cycles per degree) that an eye can resolve (Dubs, 1982). It typically has high values and a flat shape for low spatial frequencies and falls off rapidly close to the

highest resolvable spatial frequency. The frequency where the MTF falls to zero is the cut-off frequency of the system and this is the highest spatial frequency that can be passed (Snyder, 1977). Two angular sensitivity functions with the same acceptance angles but different shapes could potentially have different representations in the frequency domain. Wider flanks are typically equated with poorer spatial resolution and this can be seen in the MTF cutting off at a lower frequency (Warrant & McIntyre, 1992).

The spatial resolution of the eye has implications for vision in dim light; normally a trade-off exists between the ability to capture light and spatial resolution. That is, a wider light capturing angle (i.e. a wider receptive field) that captures more light invariably means poorer spatial resolution and *vice versa* (Warrant & McIntyre, 1992).

4.2. Temporal resolution

The eye must be able to accurately follow movements of the image across the retina due to the animal's own movement or because objects in the scene move. That is, the eye must have temporal resolution. The importance of temporal resolution can be understood in terms of motion detection. If an animal or its surroundings moves with a particular angular velocity, the neural image will be blurred if the eye does not have a sufficient temporal resolution (Snyder, 1977). Fast-moving insects thus tend to have a higher temporal resolution than slow-moving insects (Howard et al., 1984).

The temporal resolution of a photoreceptor can be determined by measuring the cell's 'impulse response' using intracellular electrophysiology. In this case the stimulus is a brief and dim pulse of light. Plotted as a function of time, the impulse response is the graded electrical response of the cell to this stimulus. The impulse response contains two good indicators of the speed of vision. The time-to-peak, τ_p, is the time from the stimulus onset to the maximal response of the cell. The integration time, Δt, is the half-width of the impulse response. A simplified but convenient analogy for understanding the integration time is the exposure time of a camera. A longer integration time allows the cell to improve signal reliability, but slows down vision. At the same light intensity a short integration time

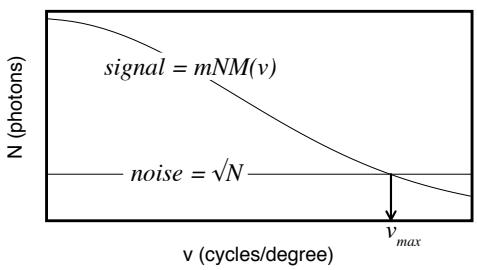

Fig. 4. Signal (Eq. 13) and noise (Eq. 14) as a function of spatial frequency. The stimulus is a sinusoidal grating with a contrast of *m*. The signal has a shape similar to that of the MTF and falls with increasing spatial frequencies. The noise, on the other hand, is independent of spatial frequency. The spatial frequency, ν_{max}, where the signal is equal to the noise is the highest spatial frequency resolvable by the eye. Redrawn from (Warrant & McIntyre, 1992).

allows a high temporal resolution but less reliable vision (Warrant, 2004). A longer impulse response time course improves the reliability of vision by suppressing noise at higher temporal frequencies (van Hateren 1993; Laughlin, 1996).

Just like the angular sensitivity function, the impulse response contains a lot of information that is not revealed by its half-width alone. Its shape is also important since one impulse response could have a broad base but a narrow Δt. The Fourier transform of the impulse response is thus more informative about temporal vision than the impulse response itself. The resulting power spectrum reveals those temporal frequencies that are perceivable and important to the animal. The important details of this topic will be discussed further below in the section dealing with photoreceptors.

4.3. Sensitivity inferred from the static image

What are the adaptations that make an eye sensitive to light? As mentioned previously, the spatial and temporal resolution of an eye both affect the amount of light that it can capture. Moreover, the F-number (Eq. 2) has consequences for both spatial resolution and the brightness of an image produced by the optics. However, other parameters are also important. The Kirschfeld-Land sensitivity equation (Kirschfeld, 1974; Land, 1981), modified for white light (Warrant & Nilsson, 1998), describes the optical and anatomical parameters that determine the eye's sensitivity to a broad extended source of light:

$$S = \left(\frac{\pi}{4}\right)^2 D^2 \left(\frac{d}{f}\right)^2 \left(1 - e^{-kl}\right), \qquad (\textit{monochromatic}) \qquad (8a)$$

$$S_W = \left(\frac{\pi}{4}\right)^2 D^2 \left(\frac{d}{f}\right)^2 \frac{kl}{(2.3 + kl)}. \qquad (\textit{broad spectrum}) \qquad (8b)$$

The model reveals that the lens diameter, D (or more precisely the lens area, $\pi D^2/4$), is one important factor. A larger aperture lets through more light and thereby improves sensitivity. If the focal length, f, is smaller, the sensitivity also increases. We recognise these parameters from the F-number introduced above (Eq. 2). Lenses with low F-numbers form the brightest images because the aperture is large in relation to the focal length. But if the F-number is low, other optical problems will occur, namely spherical and chromatic aberration. These will blur the image. One way to increase both sensitivity and resolution is to make the whole eye larger (Land, 1981; Frederiksen & Warrant, 2008a), but then of course the animal may face other survival problems that have nothing to do with vision. In addition, larger eyes are energetically more expensive (Laughlin & de Ruyter van Steveninck, 1998).

We can also see the influence of the size of the receptive structure, the rhabdom. A rhabdom with a longer length, l, has the potential to capture more light. Another recognisable feature in Eq. 8 is the rhabdom acceptance function, d/f, or more correctly the solid angle of visual space (in sr) viewed by the photoreceptor, ($\pi d^2/4f^2$). This illustrates well the trade-off between sensitivity and spatial resolution. A large ($\pi d^2/4f^2$) implies poor spatial resolution but yields a high sensitivity value. Even though the model is a robust comparative tool (Chapter III: Frederiksen & Warrant, 2008b) it is an approximation from geometrical optics, and quite large errors can occur for narrow rhabdoms, in particular if the F-number is also low (Stavenga, 2003a,b). To obtain a better approximation of sensitivity in narrow rhabdoms we must consider waveguide optics. This has been done by (Stavenga, 2003b). The light power absorbed from a monochromatic point source of 1 Watt is:

$$P_{abs} = \sum P_{exc}(\lambda)\left[1 - e^{-\bar{\eta}(\lambda)k(\lambda)l}\right], \tag{9}$$

where $P_{exc}(\lambda)$ is the power excited into a particular waveguide mode, $\bar{\eta}$ is the fraction of the mode propagating inside the rhabdom (the fraction that can be absorbed by the visual pigments), $k(\lambda)$ is the wavelength dependent absorption coefficient, and l the rhabdom length.

Even though we get a better approximation of sensitivity if we use waveguide optics instead of geometrical optics to model the angular sensitivity, it is even better to use experimentally obtained data. A recent model (Warrant, 1999; Kelber et al. 2002; Warrant et al., 2004) does this to calculate the number of photons, N, that a photoreceptor cell, with spectral sensitivity $R(\lambda)$, absorbs from an illumination spectrum of quantal intensity $I(\lambda)$ during one integration time, Δt:

$$N = 1.13n\frac{\pi}{4}\Delta\rho^2 D^2 \Delta t \int \kappa\tau\left[1 - e^{-kR(\lambda)l}\right] I(\lambda)d\lambda \tag{10}$$

The parameters in Eq. 10, have a lot in common with those in Eqs. 8 and 9. The integral term is analogous to the term $[kl/(2.3+kl)]$ of the Kirschfeld-Land equation (Eq. 8b) and to the term $[1-e^{-\bar{\eta}(\lambda)k(\lambda)l}]$ of Stavenga's equation (Eq. 9). We also recognise the facet diameter, D, the absorption coefficient of the rhabdom, k, and the rhabdom length, l, from above. The equation also includes, n, the number of facet lenses that contribute light to one rhabdom, which makes it suitable to use for superposition eyes as well (Kelber et al., 2002). κ is the quantum efficiency of transduction and τ is the transmission fraction of the optics. The remaining parameters concern the spatial and temporal resolution of the photoreceptor; the acceptance angle, $\Delta\rho$, and the integration time, Δt.

4.4. Spatial information capacity

To really assess the optical performance of an eye in dim light we need a single-number quantity that accounts for both resolution and sensitivity and the given trade-off between them. One possible method of quantification is to consider the spatial information capaciity, H_S (in sr^{-1}), of the eye (Snyder et al., 1977a,b). The spatial information capacity is a measure of the number of 'pictures' that can be reconstructed by an array of photoreceptors, that is, the amount of information an eye can extract from a visual scene. The simplest possible way is to consider an array of photoreceptors with a density of p photoreceptors per steradian of visual space. If each photoreceptor can discriminate i intensity levels, then the eye can maximally reconstruct i^p pictures. The spatial information capacity can be calculated by taking the natural logarithm of this:

$$H_S = \ln i^p = p \ln i. \tag{11}$$

4.4.1. The packing density of photoreceptors p

The simplicity of Eq. 11 is very appealing. A high density of photoreceptors means that much information can potentially be coded (a highly resolved image). But we must not forget the limitations imposed by high spatial resolution: one cannot continue to increase the density of photoreceptors without seriously compromising the sensitivity of each. Moreover, the photoreceptor density cannot be increased infinitely because of the constraints imposed by the physical nature of light discussed above: diffraction and waveguide effects will eventually limit the acceptance function and thus the photoreceptor density.

A convenient way to express the uncertainty caused by the rhabdom acceptance function and imperfect optics is to consider the MTF introduced above. For simplicity we assume an angular sensitivity function with a Gaussian intensity distribution. The frequency domain representation of the angular sensitivity, or the MTF, can the be expressed as (Snyder, 1977):

$$M(v) = e^{-3.65(v\Delta\rho)^2}, \tag{12}$$

where M is the normalised amplitude modulation, v is the spatial frequency and $\Delta\rho$ is the acceptance angle (assuming a Gaussian angular sensitivity function). The MTF alone is not very informative about the eye's ability to code images. It is, however, a basis for quantifying the spatial signal that the eye has to code (Snyder et al., 1977a,b):

$$signal = mNM(v). \tag{13}$$

The parameters *m* and *N* account for properties of the stimulus, that is, the image that has to be coded. *N* is the average light intensity (the mean number of photons absorbed by each photoreceptor in the retinal matrix during one integration time from a infinite uniform source: Eq. 10) and *m* is the mean contrast of the scene (in this case the contrast modulation of a sinusoidal grating). What is apparent from Eqs. 12 and 13 is that the signal is highly dependent on both the light intensity and the spatial resolution of the eye and that these trade-off against each other. As with the MTF we can find the cut-off frequency of the system where the signal approaches zero. Since the system is invariably subject to noise, this cut-off frequency is an over-estimate. A more realistic, and convenient, estimate of the cut-off frequency is the spatial frequency at which the signal falls to the level of the noise (i.e. the spatial frequency where the signal-to-noise ratio is one: Fig. 4).

4.4.2. The number of discriminable intensity levels i

The discussion so far has mostly concerned the packing density of the photoreceptors, *p*, in Eq. 11. The other parameter from Eq. 11 – the number of intensity levels that each photoreceptor is able to code *i* – is highly dependent on the noise level in the system. This, as will be evident, has a major impact on the reliability of vision when the scene gets dimmer.

Photons strike the surface of the eye at random intervals and the intensity can be thought of as the average photon arrival per unit time. In dim light, photons enter the eye at a low rate. This makes it difficult for the eye to accurately determine the stimulus intensity, or more importantly the contrast. The randomness of photon arrival is best described by Poisson statistics (Rose, 1942; De Vries, 1943). The noise in a sample of a Poisson-distributed pool is the square root of the sample size:

$$noise = \sqrt{N} \tag{14}$$

If we approximate the reliability of contrast discrimination with the signal-to-noise ratio it follows that vision improves with increasing intensity as $N/\sqrt{N} = \sqrt{N}$. The signal-to-noise ratio, SNR, is obtained by taking the ratio of Eq. 13 and Eq. 14 (Fig. 4: Snyder et al., 1977a,b):

$$\mathrm{SNR} = m\sqrt{N}M(v) \tag{15}$$

The essence of Eq. 15 is that when the scene becomes dimmer, the reliability of contrast discrimination decreases. This means that the number of light levels, *i*, that a photoreceptor can code decreases. As is evident from the previous section on spatial acuity, one solution to the problem is to trade $M(v)$ in favour of a higher sensitivity. The change in sensitivity as a response to a change in ambient light intensities is known as visual adaptation.

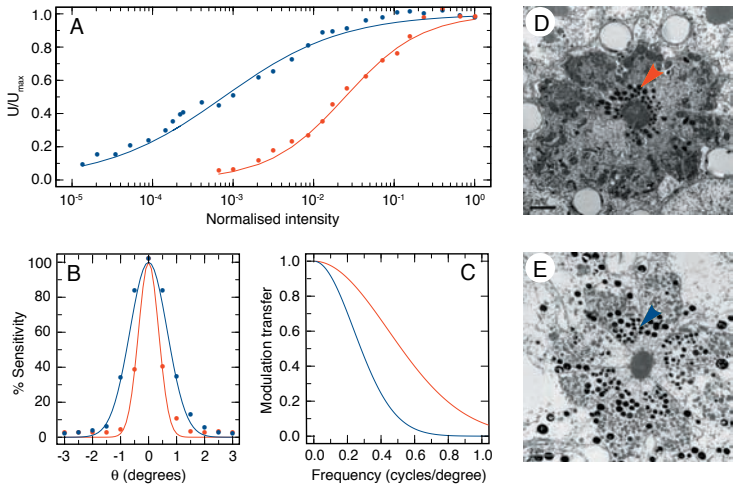

Fig. 5. The effects of pigment migration during light adaptation. *Blue* – dark adapted, *red* – light adapted. **A** VlogI curves recorded from a photoreceptor of the crepuscular butterfly *Caligo memnon* (Frederiksen & Warrant, 2008b). The response-intensity relationship shows a shift that signifies light adaptation. **B, D, E** During light adaptation, pigments (indicated by *arrowheads* in **D** and **E**) migrate towards the rhabdom, which cuts off high-order waveguide modes and results in a narrower angular sensitivity function **B**. Gaussians are fitted to the data, a good approximation since the rhabdoms are wide enough to obey geometrical optics. **C** Modulation transfer functions, frequency domain representations of **B**.

4.4.3. Adaptation

There are a number of mechanisms described that are involved in visual adaptation (reviews: Walcott, 1975; Autrum, 1981). First, there are structural adaptational mechanisms that change the morphology and optical properties of the ommatidium. These structural changes include alterations in rhabdom size and position (Walcott, 1971b; Walcott, 1971a; Williams, 1982; Menzi, 1987), and migration of pupillary screening pigment granules (Stavenga & Kuiper, 1977). In insect apposition eyes the main pupil mechanism consists of light-dependent radial migrations of pigment granules in the retinula cells (Kirschfeld & Franceschini, 1969; Kolb & Autrum, 1972; Butler, 1973; Stavenga & Kuiper, 1977; Frederiksen et al., 2008a). During light adaptation, the pigment granules migrate close to the rhabdom (Fig. 5). This narrows the photoreceptor's acceptance function (its angular receptive field) and reduces the light-flux to the rhabdom (Stavenga & Kuiper, 1977; Land & Osorio, 1990). The action of this pupil prevents photoreceptor saturation and maintains a high SNR at a wide range of light intensities (Howard et al., 1987). During dark adaptation, the opposite happens – the retinula cell pigment granules migrate away from the rhabdoms and disperse within the retinula cell matrix (Fig. 5), widening the angular width of the receptive field and increasing the light flux (Land & Osorio, 1990). Thus, visual adaptation also has a strong impact on the spatial resolution of the photoreceptors (Fig. 5).

In addition to these morphological mechanisms, there are also adaptation mechanisms within the photoreceptors that affect the biochemical process of phototransduction, and the nontransductive photoreceptor membrane (Juusola & Hardie, 2001a), altering the response properties in the photoreceptors. During light adaptation, these mechanisms

decrease the photoreceptor's gain of transduction and speed up its light-response (i.e. increase the temporal resolution: Howard et al., 1984), thereby utilising more of the light to increase the visual signal-to-noise ratio (Snyder, 1977; Juusola & Hardie, 2001a). Upon dark adaptation, on the other hand, gain is increased and temporal resolution decreases (Howard et al., 1984), with the photoreceptor response becoming low-pass-filtered in order to decrease high frequency noise (van Hateren, 1993; Laughlin, 1996). Adaptation is thus an effective way of maximising signal-to-noise ratio and acuity over a wide range of intensities without saturating the photoreceptors (Howard et al., 1987; Järemo Jonson et al., 1998). This means that the eye can, in a particular state of adaptation, retain a fine discrimination of different intensity levels and thereby maintain high contrast reliability.

4.4.4. The moving image and temporal resolution

As mentioned above, eyes must possess sufficient temporal resolution to perceive an accurate neural image as the optical image moves over the retina. Since the SNR in Eq. 15 is expressed as a function of spatial frequency we need to incorporate temporal resolution as a change in spatial frequency, that is, as motion blur. Consider the angular velocity, υ (in degrees per second), of the moving image. A good approximation of the motion blur function (degrees) can be obtained simply by multiplying the angular velocity by the integration time of the photoreceptor; $\upsilon\Delta t$. The motion blur function can be incorporated into Eq. 12 by convolution with the angular sensitivity function (Snyder, 1977), a simple operation if one assumes that both functions are Gaussians. As the animal moves, motion blur degrades the spatial acuity and decreases the reliability of the signal, thus indicating that there is a close relationship between spatial and temporal resolution. High spatial acuity is usually associated with high temporal resolution whereas low spatial resolution usually implies low temporal resolution. This has indeed been confirmed in the blowfly *Calliphora vicina* (Burton et al., 2001), where areas in the visual field with narrow angular sensitivity functions (acute zones) also have high temporal resolution.

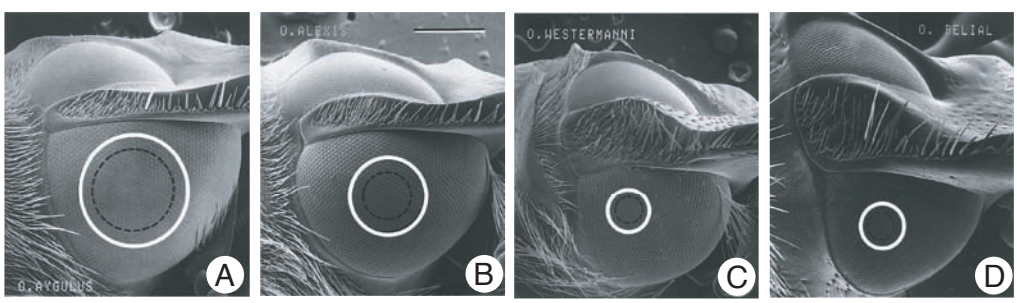

Fig. 6. Superposition apertures in four species of dung beetles of the genus *Onitis*. **A** *Onitis aygulus*, nocturnal; **B** *Onitis alexis*, crepuscular; **C** *Onitis westermanni*, crepuscular; **D** *Onitis belial*, diurnal. The size of the superposition aperture (*white rings*) increases with decreasing habitat intensity, in which the species is active. Adapted from (McIntyre & Caveney, 1998) with kind permission from the authors. Scale bar: 0.5 mm

Table 1. Sensitivity of nine species of insects with different activity periods and eye types. The sensitivity is represented by N (Eq. 10), the number of photons captured by one green-sensitive photoreceptor (λ_{max}=540 nm) from a green foliage spectrum with a quantal intensity of 0.1 $h\nu\ \mu m^{-2}\ sr^{-1}\ s^{-1}$ at 540 nm during one integration time. S_w is the sensitivity to white light (Eq. 8).

Species	Activity[A]	Eye type[B]	N (photons)	S_w ($\mu m^2 sr$)	Ref.[C]
Deilephila elpenor	N	RSU	95	441	1
Macroglossum stellatarum	D	RSU	5.4	31	2,3
Onitis aygulus	N	RSU	141	31	3,4
Onitis alexis	C	RSU	105	31	3,4,5
Onitis belial	D	RSU	0.88	0.6	3,4
Megalopta genalis	N	AP	1.5	2.7	6,7
Lasioglossum leucozonium	D	AP	0.078	0.1	7,8
Apis melifera	D	AP	0.052	0.1	7,9
Caligo memnon	C	AA	0.17	1.3	10
Morpho peleides	D	AA	0.049	0.4	10

[A]N – nocturnal, D – diurnal, C – Crepuscular. [B]RSU – refracting superposition eye, AP – focal apposition eye, AA – afocal apposition eye. [C]The data used to calculate N and S_w was obtained from the following references: [1] (Kelber et al., 2002); [2] (Warrant et al., 1999); [3] (Frederiksen unpublished data); [4] (McIntyre & Caveney, 1998); [5] (Warrant & McIntyre, 1990); [6] (Warrant et al., 2004); [7] (Greiner et al., 2004a); [8] (Frederiksen et al., 2008a); [9] (Laughlin & Horridge, 1971); [10] (Frederiksen & Warrant, 2008b).

4.5. Optical adaptations found in nocturnal insects

The discussions above predict that nocturnal insects should have certain adaptations to see in dim light - is this the case? Indeed, adaptations have been found that increase photon capture, improve the signal-to-noise-ratio and thus increase the spatial information rate of vision in nocturnal insects. Perhaps the most obvious adaptation found in insects is the superposition eye design, that permits light from many corneal facet lenses to reach a single rhabdom. McIntyre & Caveney (1998) concluded that the size of this superposition aperture in four closely related species of dung beetles correlates with the time of activity of the species. They investigated the nocturnal *Onitis aygulus*, the crepuscular *O. alexis* and *O. westermanni*, and the day-active *O. belial* and found the largest superposition aperture in the nocturnal species, a somewhat smaller aperture in the crepuscular species and the smallest aperture in the day-active species (Fig. 6).

The superiority of the superposition eye in dim light becomes evident if we calculate the number of photons a photoreceptor in a superposition eye can capture during one integration time compared to an apposition eye viewing the same light source. Calculations of the photon catch of a photoreceptor in *Deilephila elpenor* (superposition eyes) and the nocturnal sweat bee *Megalopta genalis* (apposition eyes) reveals a sensitivity ratio of 63 to 1

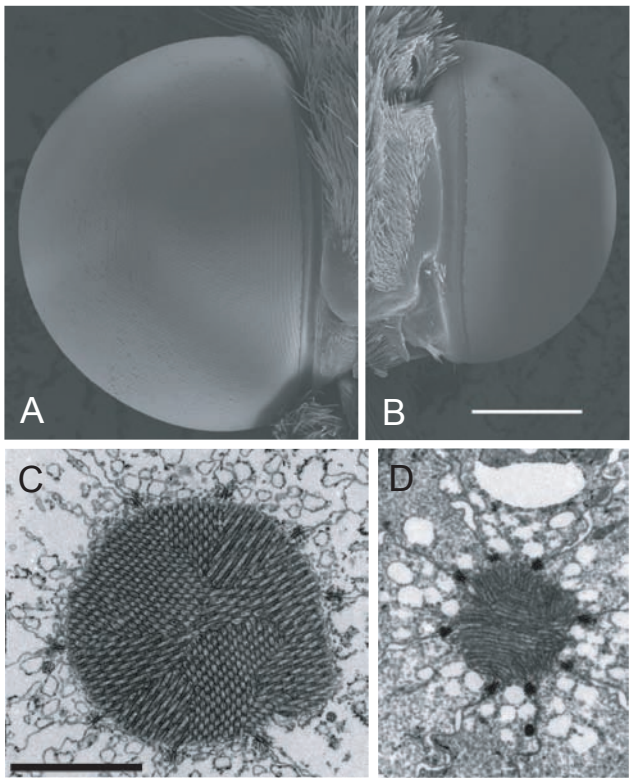

Fig. 7. A, B Scanning electron micrographs of the eyes of *Caligo memnon* (**A**) and *Morpho peleides* (**B**). The local eye radius of *C. memnon* is almost twice as large as that of *M. peleides*. **C, D** Transmission electron micrographs showing distal transverse sections through the rhabdoms of *Caligo memnon* (**C**) and *Morpho peleides* (**D**). Scale bar for A and B: 1 mm, scale bar for **C** and **D**: 2 μm.

(Table 1) which shows the superiority of the superposition design for vision in dim light.

In both the superposition and apposition eyes of nocturnal animals, an increased sensitivity due to sacrificed spatial and temporal resolution is common. For instance, the superposition eyes of the nocturnal elephant hawkmoth, *Deilephila elpenor*, have acceptance angles of about 3 degrees and integration times of around 36 ms in the dark-adapted state (Kelber et al., 2002). In contrast, the day-active superposition eye of the hummingbird hawkmoth, *Macroglossum stellatarum*, has acceptance angles of about 1 degree and integration times of around 23 ms (Rikard Frederiksen, unpublished data). All in all, the eye of *D. elpenor* is 19 times more sensitive to light than that of *M. stellatarum* (Table 1).

Such adaptations are perhaps more prominent in nocturnal apposition eyes. The lack of a clear zone in apposition eyes constrains light capture, and increased sensitivity is achieved by a much greater sacrifice in spatial and temporal acuity. A good example of a nocturnal insect with apposition eyes is the halictid bee *Megalopta genalis*, an insect capable of navigating through a neotropical rainforest at dusk and dawn in light intensities dimmer than starlight (Warrant et al., 2004; Kelber et al., 2006). The eyes of *M. genalis* have a 27 times higher optical sensitivity to white light than those of the honeybee worker, *Apis mellifera* (Table 1), and the diurnal halictid bee *Lasioglossum leucozonium* (Greiner et al., 2004a). This is due to a lower temporal resolution (Δt = 32 ms), wider rhabdoms and larger facet

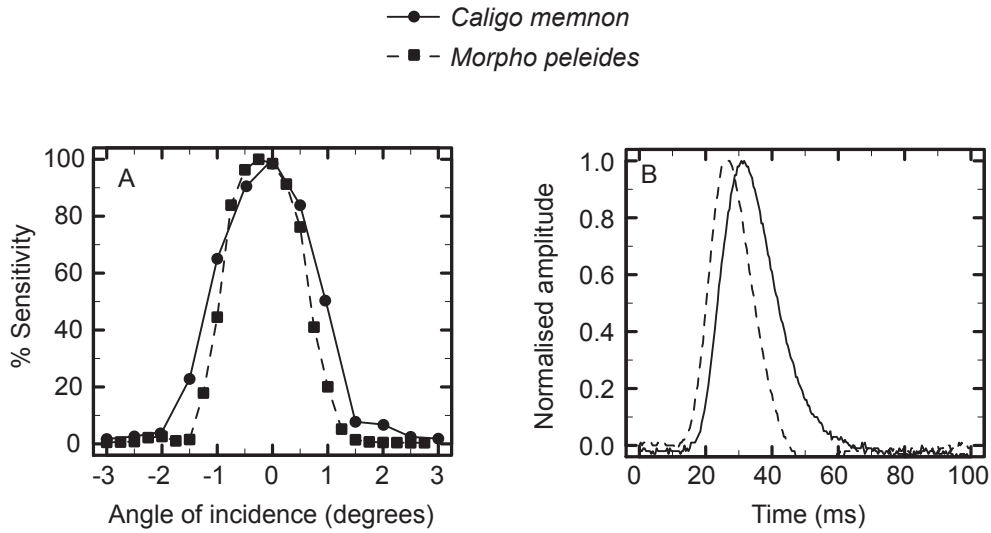

Fig. 8. A Typical angular-sensitivity functions recorded from one dark-adapted photoreceptor cell in the crepuscular *Caligo memnon* and the diurnal *Morpho peleides*. The acceptance angle of the angular-sensitivity functions for these cells is slightly larger in *C. memnon* ($\Delta\rho$ = 2.1°) than in *M. peleides* ($\Delta\rho$ = 1.7°). **B** Impuse responses recorded from the same cells as in **A**. The integration times and times-to-peak are *C. memnon*: Δt = 18 ms, τ_p = 31 ms; *M. peleides*: Δt = 15 ms, τ_p = 26 ms.

lenses, producing a wider acceptance angle (theoretical: $\Delta\rho$ = 4.7 degrees: Greiner et al., 2004a, measured: $\Delta\rho$ = 5.6 degrees: Warrant et al., 2004).

These physiological, anatomical and optical adaptations possessed by *Megalopta genalis* to increase its sensitivity are not sufficient to explain the bee's behaviour in the dim rainforest at night. Recent evidence (Greiner et al., 2004b; Greiner et al., 2005; Theobald et al., 2006) suggests that *M. genalis* uses a strategy of optimal spatial and temporal summation in the first optic lobe of the brain, the *lamina ganglionaris*, in order to achieve sufficient sensitivity for its nocturnal lifestyle, a strategy probably also used by the Africanised honeybee worker to extend its foraging period on moonlit nights (Warrant et al., 1996).

Another example reveals that optical, anatomical and physiological adaptations can be found in apposition eyes on a smaller scale when the differences in intensity between the habitats of the compared species are not as great as in the example of *Megalopta genalis*. The crepuscular nymphalid butterfly *Caligo memnon*, for instance, has vision that is four times more sensitive than that of its similarly-sized day-active relative *Morpho peleides* (Table 1, Chapter I: Frederiksen & Warrant, 2008a). This is mainly due to larger corneal facet lenses and a larger eye (Fig. 7), but somewhat broader angular sensitivity functions and slower impulse responses also contribute (Fig. 8).

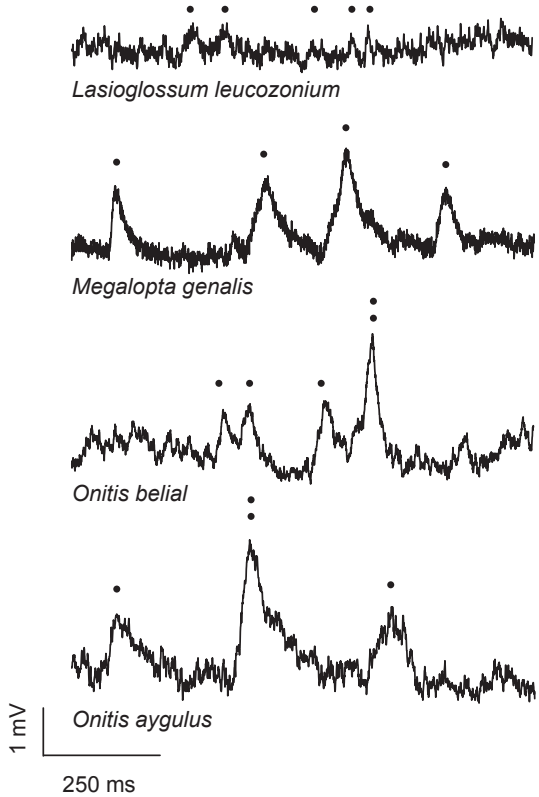

Fig. 9. Photon bumps recorded from four different species of insects (*Lasioglossum leucozonium*, *Megalopta genalis*, *Onitis belial* and *Onitis aygulus*) as indicated. Dots mark bumps. Adapted from Chapter III: (Frederiksen & Warrant, 2008a).

5. Photoreceptor adaptations for nocturnal vision

The discussion so far has mostly concerned the properties of the optics of the eye and the photoreceptor acceptance and how it can be adapted to increase sensitivity and information capacity. To get a more complete picture of how the eyes are adapted to function in their visual environment it is necessary to consider the physiology of the receptor cells as well. If we can understand the different steps that occur between the absorption of light and the production of an electrical response in the receptor cell we can pose proper questions concerning how this system may be adapted, together with the optics of the eye, for a life in dim light. The biochemical phototransduction cascade works together with the electrical properties of the cell membrane, to produce the graded response that is ultimately transmitted, via synapses, to the optic lobes. Amplification and filtering of the graded response already occurs at the photoreceptor level.

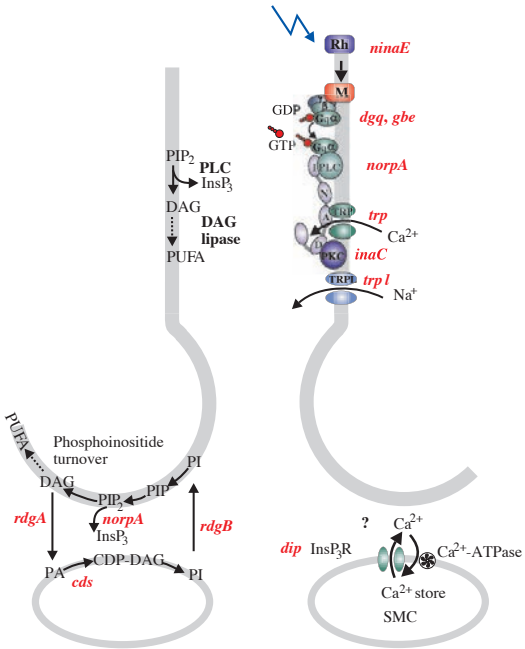

Fig. 10. The biochemical phototransduction cascade in *Drosophila melanogaster* photoreceptors. The cascade starts with the absorption of one photon and the photoisomerisation of rhodopsin, Rh, to metarhodopsin, M. Metarhodopsin activates G-protein G_q that releases the $G_{q\alpha}$ subunit via GTP-GDP conversion. $G_{q\alpha}$ activates phospholipase C, PLC, that converts phophatidyl inositol 4,5-bisphosphate, PIP_2, into inositol 1,4,5-trisphosphate, $InsP_3$, and diacyl glycerol, DAG. TRP and TRPL channels are activated via a so far unknown mechanism. It is known, however, that protein kinase C, PKC and the scaffolding protein INAD are involved in the process. At the base of the microvilli Ca^{2+} is believed to be released from submicrovillar cisternae, SMC. The most important function of these, however, is probably the turnover of phosphoinositide as indicated in the lower left corner. The red text indicates the genes coding the protiens involved in the different steps of phototransduction. Modified from Hardie (2001) with kind permission from the author.

5.1. Signal transduction

The complete detailed mechanisms of the biochemical phototransduction cascade in insect photoreceptors are not yet known, and what is known is mostly based on research from a single species, *Drosophila melanogaster*. It is definitely questionable how "general" these mechanisms are between insect taxa considering the variety of morphological and optical solutions within this group, and that substantial differences to other groups of arthropods have been found (e.g. *Limulus*: Grzywacz et al., 1992; Hecker et al., 1992; Kirkwood & Lisman, 1994; Fein & Cavar, 2000). However, in order to put the following sections into context, I find it necessary to begin with a short summary.

The absorption of one photon of light by rhodopsin leads to the formation of rhodopsin's activated state – metarhodopsin (Vogt & Kirschfeld, 1984; Hardie, 2001). The photoisomerisation activates G-proteins (G_q) via exchange of GTP-GDP. The $G_{q\alpha}$ subunit is released and activates phospholipase C (PLC) that generates inositol 1,4,5-trisphosphate ($InsP_3$) and diacyl glycerol (DAG) from phosphatidyl inositol 4,5-bisphosphate (PIP_2). The biochemical cascade results in the opening of at least two types of ion channels (Hardie & Minke, 1992; Reuss et al., 1997) – TRP (transient receptor potential) and TRPL (TRP-like) – resulting in a voltage response (Hardie, 2001). A single absorbed photon leads to a discrete

voltage response of a few millivolts amplitude (Lillywhite, 1977) called a photon "bump" (Fig. 9). The ability to respond to a single absorbed quantum of light is not restricted to the rhabdomeric photoreceptors of insects, but can also be found in vertebrate cilliary photoreceptors, for instance in human rods (Hecht et al., 1942). The exact mechanism of activation of the ion channels is not yet completely known and is beyond the scope of this report. A more detailed description of the biochemical phototransduction cascade can be found elswhere (Ranganathan et al., 1995; Hardie, 2001; Hardie & Raghu, 2001).

The opening of TRP and TRPL channels causes light-induced currents of ions to flow across the cell membrane. The main light-induced ion current is from the TRP channel, and this constitutes about 95% of the total light induced current (Reuss et al., 1997). Interestingly, the TRP channel also has a high selectivity for Ca^{2+} ions while the TRPL channel is more non-selective (Hardie, 2001), indicating the importance of Ca^{2+} in the generation of photon bumps. Light-induced increments of intracellular Ca^{2+} concentration are partly due to release from intracellular stores (Baumann & Walz, 1989; Zeigler & Walz, 1990) and partly due to influx from the extracellular space (Fig. 10, Zeigler & Walz, 1989).

The photopigments are situated in the microvilli of the retinula cells and the whole transduction mechanism is believed to take place in the same. It has been suggested that the response of the photoreceptor to one photon of light is restricted to a single microvillus (Howard et al., 1987; Hochstrate & Hamdorf, 1990). Opening of the first TRP channel raises the Ca^{2+} concentration in the microvillus and this sensitises more TRP channels throughout the whole microvillus. A positive feedback loop forms due to the presence of (sub-threshold) DAG from the PLC cascade (Hardie et al., 2002; Hardie, 2003). This results in an explosive opening of virtually all TRP channels in the microvillus, resulting in a rise in Ca^{2+} concentration to about 200 μM (Oberwinkler & Stavenga, 2000) and the generation of a bump, that is then terminated by the negative feedback from the raised Ca^{2+} concentration (Baumann & Walz, 1989). The whole procedure has a finite but variable latency period before the current starts to flow through the cell membrane and depolarise the cell.

Perhaps the most important feature of the biochemical cascade from a dim light perspective is the early amplification that results from recruitment of several ion channels by a single photon of light. Amplification in the phototransduction cascade was previously believed to occur downstream of the PLC (Pak et al., 1976; Laughlin, 1990; Scott et al., 1995). However, it has lately been suggested that the amplification is dependent on the recruitment of several G-proteins and molecules of PLC. Therefore an earlier amplification step is more likely (Hardie et al., 2002). This is in accordance with information theory that states that amplification should take place as early as possible in a system in order to minimise the noise (Laughlin, 1990; Pelli, 1993). In *Drosophila melanogaster* the amplification results in the opening of approximately fifteen ion channels per absorbed photon, generating a current of approximately 10 pA (Henderson et al., 2000). This figure may vary somewhat between species of insects, depending on the size of their microvilli and the density of

ion channels within them. Amplification can also occur due to summation of bumps from different receptors. Observations of two distinct classes of bump amplitudes in locust photoreceptors led Lillywhite (1978) to suggest that locust photoreceptors are electrically coupled. Electrical coupling has also been observed in blowflies (van Hateren, 1986) and in vertebrate cones (DeVries et al., 2002) where it has been suggested to improve visual reliability (DeVries et al., 2002; Laughlin, 2002).

The light-induced current initiated by the phototransduction cascade generates a voltage response that depends on the impedance of the photoreceptor cell membrane (Vallet et al., 1992). Light-induced currents are accompanied by voltage-induced currents that shape the response (Weckström & Laughlin, 1995). The filtering properties of the photoreceptor membrane and the transduction cascade are not static but are influenced, for example, by the state of adaptation (Howard et al., 1984; Laughlin & Weckström, 1993; Weckström & Laughlin, 1995; Burton, 2002) and the temperature (Tatler et al., 2000; Juusola & Hardie, 2001b; Faivre & Juusola, 2008).

A variety of ion channels in the photoreceptor membrane have been identified to be involved in signal filtering and amplification. Apart from the TRP and TRPL channels that mediate the light-induced currents, the voltage-gated delayed-rectifying K^+ channel (Weckström et al., 1991; Laughlin & Weckström 1993; Weckström & Laughlin, 1995; Vähäsöyrinki et al., 2006), the *Shaker* K^+ channel (Niven et al., 2003; Juusola et al., 2003, Niven et al., 2004; Vähäsöyrinki et al., 2006) and voltage-gated Na^+ channels (Coles & Schneider-Picard, 1989; Vallet et al., 1992) are examples of channels that mediate important conductances and which influence the kinetics of the photoreceptor cell membrane and reduce the deleterious effects of noise that degrades the visual reliabillity.

5.2. Three types of noise

Like all signalling systems, photoreceptors are subject to noise. Noise arises from several sources and depending on its origin it has different properties and will affect the signalling differently. Perhaps the most obvious source of noise is the above mentioned Poisson distributed photon shot noise (Rose, 1942; De Vries, 1943; Scholes, 1964), arising from the random nature of photon arrival. Because of this, the photon shot noise is directly dependent on the light intensity (Eq. 14) and clearly a limiting factor for nocturnal vision. There is in addition, intrinsic noise in the photoreceptors that does not directly depend on the light intensity. Two distinct types can be identified: dark noise, or dark light (Barlow, 1956; Lillywhite, 1977), and transducer noise (Lillywhite & Laughlin, 1979).

Spontaneous depolarisations of the photoreceptor membrane in the absence of light are known as dark noise. The dark noise can originate from different steps in the phototransduction chain and if its origin is early, the spontaneous depolarisations are virtually indistinguishable from responses due to single photons. Spontaneous activation of the phototransduction chain can also occur downstream of the early amplification, and

this results in smaller voltage responses that can be distinguished from photon bumps by their smaller amplitudes (Hardie et al., 2002). Dark noise is important in vertebrates that have relatively noisy cilliary photoreceptors. For instance, it sets a limit to the visual threshold in toads (Aho et al., 1988). This is, however, not true for invertebrate rhabdomeric photoreceptors, because these are far less noisy: the rate of spontaneous bumps in the locust photoreceptor is less than ten per hour (Lillywhite, 1977).

The second type of intrinsic noise, transducer noise, has its origin in the shape of the waveform of the voltage response. When the photoreceptor responds to a single photon there is a finite but variable time lag from the absorption of the photon until the voltage response. The variability in the time course and in amplitude constitutes transducer noise (Lillywhite & Laughlin, 1979). It has been suggested that part of the variability is due to different parts of the photoreceptor responding with different time courses (Pece & French, 1989). The distal part of the locust photoreceptor, for example, responds with a longer time-to-peak compared to the proximal part.

The relevance of the different types of noise varies between systems and what is studied. For instance, Lillywhite & Laughlin (1979) showed that for insect photoreceptors in very

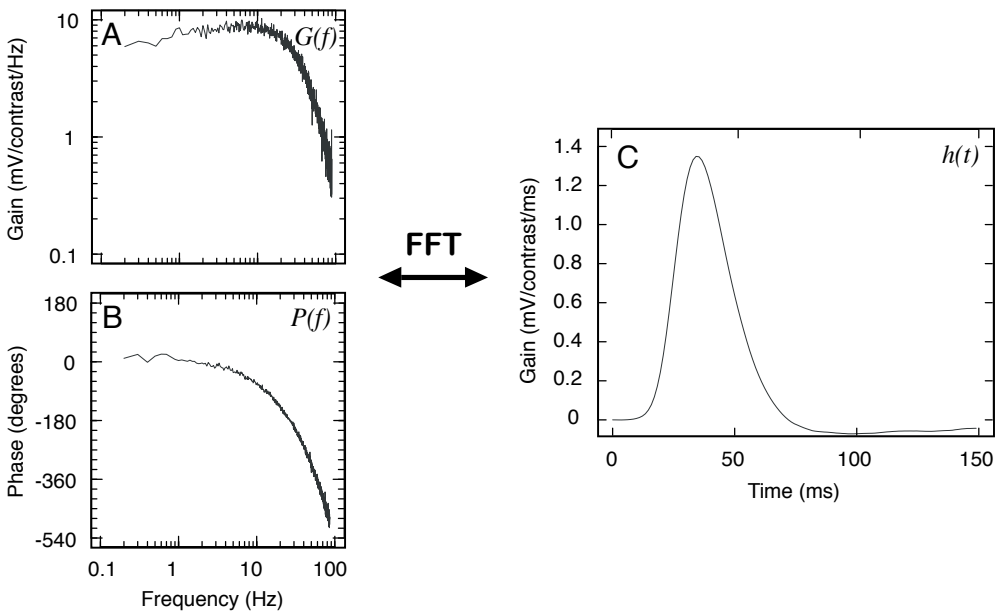

Fig. 11. Transfer function of a photoreceptor in *Megalopta genalis*. **A** The contrast gain function, *G*(*f*), shows the bandwidth and the contrast gain of the cell. **B** The phase function, P(*f*), shows the lag of the response as a function of frequency. **C** The impulse response, *h*(*t*) calculated as the first order Wiener kernel (French & Butz, 1973), is the linear part of the time-domain representation of the transfer function.

dim light, the contribution by transducer noise and photon shot noise is approximately equal. In motion-sensitive neurons in flies, on the other hand, researchers have suggested that the main source of noise limiting neural computation is intrinsic to the nervous system, and is not photon shot noise (Grewe et al., 2003).

Noise has a major impact on the amount of information that can be coded in a channel. In colour vision, for instance, the colour thresholds are noise limited (Vorobyev & Osorio, 1998; Vorobyev et al., 2001) and animals with scotopic colour vision, such as the elephant hawkmoth (Kelber et al., 2002) or the nocturnal helmet gecko (Roth & Kelber, 2004) must have a very low level of intrinsic photoreceptor noise since they cannot avoid photon shot noise arising from the low light intensities in which they live.

5.3. An information theoretic approach

A convenient way to dissect the performance of the photoreceptor is to use an information theoretic approach (e.g. Watson, 1990; Kouvalainen et al., 1994; de Ruyter van Steveninck & Laughlin, 1996; Abshire & Andreou, 2001; Niven et al., 2003). The signalling properties of the photoreceptor are described by its transfer function and this characterises its receptive field. The response $r(t)$ of a photoreceptor with a spatiotemporal receptive field $l(x,t)$, to a stimulus $f(x,t)$ can be modelled if we assume a linear behaviour of the photoreceptor (Watson, 1990):

$$r(t) = \iint f(x,t)l(x,\tau - t)dxd\tau, \tag{16}$$

where x represents a spatial coordinate and t time. Since the spatial receptive field of a photoreceptor has already been discussed above, we can simplify Eq. 16 by only considering the temporal receptive field:

$$r(t) = \int f(t)l(\tau - t)d\tau. \tag{17}$$

The impulse response of the system $h(t)$ can now be derived if the stimulus is set as a Dirac delta function, $\delta(t)$, an impulse of infinite positive amplitude and a temporal width that approaches zero. The integral of $\delta(t)$ is a constant equal to one and the impulse response is therefore:

$$h(t) = l(-t). \tag{18}$$

Since the delta function by definition has a white spectrum (equal amplitude at all frequencies), the impulse response is very useful for characterising the time course of the

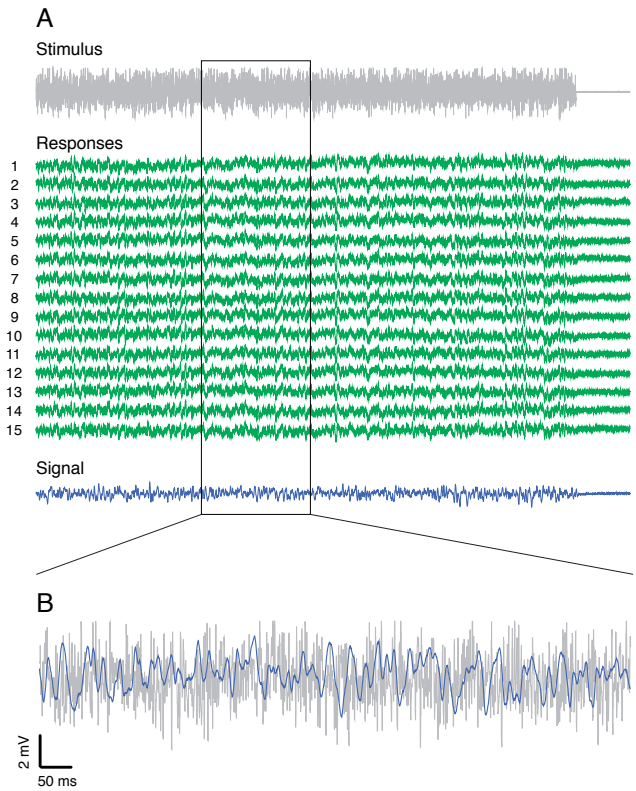

Fig. 12. A 15 response traces (*green*) to a white noise light stimulus (*grey*). The signal (*blue*) is calculated as the average of the response traces. **B** Magnified image of the siganal and stimulus traces in **A**.

receptor and is thus informative about its temporal resolution. Moreover, the response of the cell is the convolution of the stimulus and the impulse response:

$$r(t) = h(t) \otimes f(t). \tag{19}$$

The frequency domain representation of the impulse response is known as the transfer function, $H(f)$, or the frequency response of the cell:

$$H(f) \leftrightarrow h(t), \tag{20}$$

where $H(f)$ and $h(t)$ are Fourier transform pairs. The transfer function shows the input-output relation of the cell and thus informs us of transfer characteristics such as gain, bandwidth and lag. These characteristics are best shown by decomposing the transfer function (Eq. 20) into gain (Eq. 21, Fig. 11A) and phase (Eq. 22, Fig. 11B) functions. The gain function, $G(f)$, provides information about the amplification of the stimulus per unit bandwidth. If the stimulus has a white amplitude spectrum it reveals the bandwidth of the cell. The phase function, $P(f)$, on the other hand, shows the absolute lag of the response

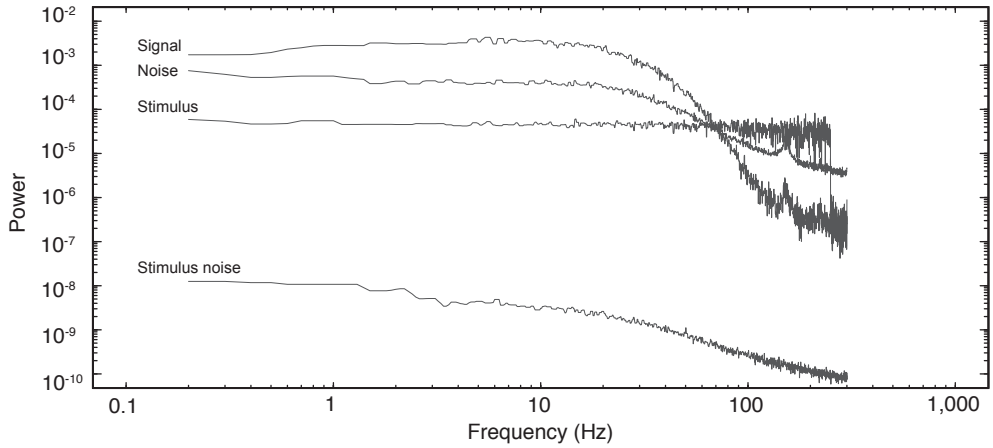

Fig. 13. Power spectra of signal, noise, stimulus and stimulus noise from one photoreceptor recording in *Megalopta genalis*.

to the stimulus (Fig. 11):

$$G(f) = \left|H(f)\right|, \tag{21}$$

$$P(f) = \tan^{-1}\frac{\Re H(f)}{\Im H(f)}. \tag{22}$$

From the previous discussion we have seen that it is possible to measure the impulse response of a photoreceptor and thus its temporal resolution. To get a fair approximation of the contrast transfer function, the stimulus has to have a flat spectrum at all relevant frequencies, as is the case for the delta function. It is however impossible to deliver an infinitely short light flash. The best approximation possible is to deliver a flash that is short and dim enough to contain a single effective photon, resulting in a photon bump. In spite of this, the recorded impulse response has the advantage of being an easy and quick way to obtain the temporal resolution experimentally and is widely used (e.g. Howard et al., 1984; Tatler et al., 2000; Rutowski & Warrant, 2002; Frederiksen & Warrant, 2008a).

There is another approach for measuring the contrast transfer function that is more informative than the impulse response. By using a stimulus of Gaussian distributed white noise (Fig. 12, Weckström et al., 1988; Kouvalainen et al., 1994; Juusola et al., 1994) – also with a flat spectrum for a defined band of frequencies (Fig. 13) – the contrast transfer function can be measured. Moreover, from the analysis of the response (Fig. 12) to a white noise stimulus it is possible to accurately calculate the signal-to-noise ratio of the

photoreceptor as a function of frequency (Kouvalainen et al., 1994) and to calculate the amount of information, *R*, that can be processed by the photoreceptor in bits/s (Shannon, 1948a; Shannon, 1948b, Kouvalainen et al., 1994; Juusola et al., 1994, Eq. 23). The signal is calculated as the assemble average of multiple responses to a single pseudorandom Gaussian white noise stimulus. The noise is obtained by subtraction of the signal from the individual responses. The final step is to divide the power spectrum of the signal with the power spectrum of the noise. The exact experimental protocol used in these methods can be found elsewhere (Kouvalainen et al., 1994; Burton, 2002).

If the signal-to-noise ratio as a function of temporal frequency, *SNR*(*f*), has been measured using Gaussian distributed white noise as a stimulus, the information rate, *R*, can be calculated (Shannon, 1948a):

$$R = \int \log_2\left[SNR(f) + 1\right] df \,. \qquad (23)$$

The use of white noise in obtaining the signal-to-noise ratio tends to somewhat overestimate the information rate since natural scenes generally have a higher low-frequency content than high frequency content (Laughlin & de Ruyter van Steveninck, 1998; Juusola & de Polavieja, 2003) (a better approximation of a natural stimulus would in fact be 1/*f*-noise). This, however, is of minor importance if the white noise analysis is used as a comparative tool and a small systematic error in the approximation would have only a minor impact on the outcome of the actual comparison.

As evident from above, there thus exist powerful mathematical tools that can help us

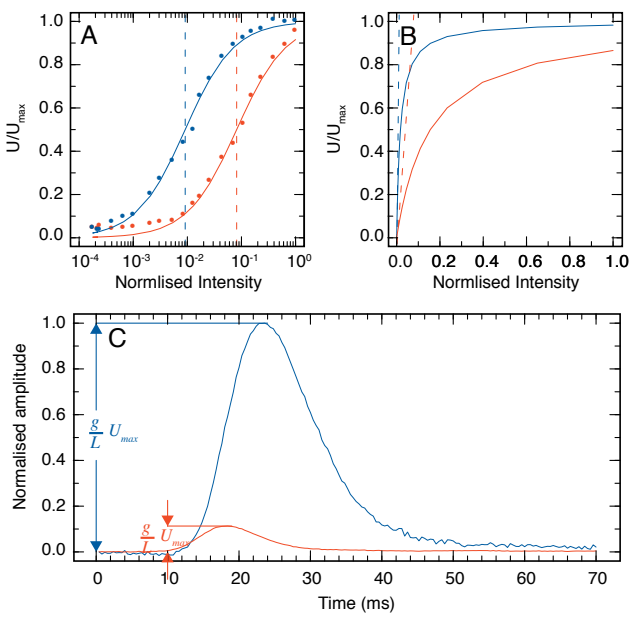

Fig. 14. Voltage-intensity relationships. *Blue* represents dark-adapted and *red* represents light-adapted. **A** VlogI curves of a photoreceptor in the crepuscular butterfly *Caligo memnon*. Eq. 24 is fitted to the data. The dashed lines represents the reciprocals of the range sensitivities, S_R. **B** Voltage intensity relationship of Eq. 25 on linear scales. The dashed lines have slopes of $U_{max}\, g/L$ which are also the amplitudes of single photon responses, represented by impulse responses in **C**.

characterise the performance of the photoreceptors. Although it is easy to be fascinated and thrilled about the methods *per se*, it is more important to use these methods to ask what information theory can tell us about visual ecology. At this point we must reflect once more on Eq. 11 and the term *i*, the number of intensity levels that the photoreceptor can code, since it is precisely this parameter we are trying to characterise here. The number of intensity levels is ultimately limited by the noise level that the photoreceptor experiences and the transfer characteristics of the receptor itself. To assess this, it is crucial to understand how the light intensity is mapped to voltage depolarisation in the photoreceptor. The detailed physiological mechanisms will be covered below; here we discuss the physical mapping.

At dim light levels the photoreceptor sums the electrical responses to single photons linearly (Howard, 1981; Laughlin, 1989). As more photons are transduced the summation becomes non-linear: the voltage gain decreases. The non-linearity stems from two causes (Laughlin, 1981). The first cause is a physiological constraint in the photoreceptor. The driving force of the depolarisation due to light is ultimately the difference in electrical potential between the cell and its surround. As the cell depolarises in response to light this driving force decreases and so does the voltage gain. This mechanism is known as self-shunting of the cell membrane and is inherent in the neuron's design. The second cause of the non-linear graded response is a reduction in conductance gain, or the number of activated light gated ion channels per photon absorbed. It can also be due to hyperpolarising voltage-gated conductances in the cell membrane. Plotted as a function of intensity, I, the normalised voltage response, U/U_{max}, can be approximated by a hyperbolic function (Laughlin, 1981):

$$\frac{U}{U_{\max}} = \frac{(S_R I)^{\psi}}{(S_R I)^{\psi} + 1}, \tag{24}$$

where S_R is the range sensitivity, the reciprocal of the intensity that will produce a voltage response that is equal to 1/2 *Umax*. The exponent, ψ, is a constant that is species-specific and can be empirically derived. The constant ψ always has a value of less than or equal to one. If, for instance, $\psi = 1$, all non-linearities in the photoreceptor are due to self-shunting.

A useful measure, related to S_R, is PAQ_{50}. PAQ_{50} is defined as the peak axial quantal intensity (in photons/cm^2/s) at which a photoreceptor responds with an amplitude of 1/2 U_{max} (Laughlin, 1976; Laughlin & Hardie, 1978). The lowest PAQ_{50} (i.e. highest sensitivity) is found in the photoreceptors of nocturnal animals. Typically, PAQ_{50} ranges from about 10^5 photons/cm^2/s in nocturnal arthropods (5×10^5 photons/cm^2/s in the postero-medial camera eyes of the nocturnal spider *Dinopsis*: Laughlin et al., 1980) to 10^{11} photons/cm^2/s in day-active arthropods (6.7×10^{11} photons/cm^2/s in the diurnal dragonfly *Hemicordulia*: Laughlin & Hardie, 1978). PAQ_{50} is also related to κ, the quantum

efficiency of transduction, which is the fraction of the incident photons that is transduced (results in a voltage response).

The non-linear summation of photons in the photoreceptor response starts at relatively dim intensities (three to five effective photons or a few mV of depolarisation: Lillywhite, 1977; Howard, 1981, Fig. 14). The reason for this is to extend the range of intensities that the photoreceptors can transduce and prevent saturation of the voltage response at bright light levels (Fig. 14A). In order to obtain a better understanding of the causes and consequences of the non-linear intensity-to-voltage mapping in the insect photoreceptor we need a better expression than Eq. 24, that contains physiological terms instead of empirically derived constants (Fig. 14, Laughlin, 1989):

$$\frac{U}{U_{\max}} = \frac{\kappa g I}{\kappa g I + L}. \tag{25}$$

The term κgI is the light activated conductance that generates the voltage response, where g is the conductance activated by a single photon, κ the quantum capture efficiency and I the intensity. L is related to S_R in Eq. 24. It is the value of the conductance, κgI, that generates a half maximal voltage response and equals the load conductance of the cell. At very dim intensities κgI is much smaller than L and we can approximate the cell's voltage-to-intensity relationship with a linear expression:

$$U = U_{\max} \frac{\kappa g I}{L}. \tag{26}$$

The amplitude of a single photon response ($\kappa I = 1$) is then $U_{max}\, g/L$ (Fig. 14C). Since the relationship is linear, this is also the slope of the curve (in mV/photon) (Fig. 14B) and therefore represents the sensitivity of the cell. Supporting this, research has shown that photoreceptors in nocturnal arthropods produce larger bumps compared to those of day-active animals (Lillywhite, 1978; Hardie, 1979; Laughlin, 1981; Pirhofer-Walzl et al., 2007).

These powerful tools of information theory can allow us to understand how information is processed by the photoreceptors, and how the light response is shaped both in time course and amplitude.

5.4. Photoreceptor adaptations found in nocturnal insects

With all these powerful tools of information theory available we can address questions about how the performance of a photoreceptor is adapted to different visual inputs (e.g. contrasts, temporal frequencies and mean intensity levels) that follow from different lifestyles. For instance, studies have been made on sexual aspects of visual ecology (Coles & Schneider-Picard, 1989; Vallet et al., 1992; Vallet & Coles, 1993; Hornstein et al., 2000). A good example is the 'love spots' of male house flies. The love spot is an area in the frontal visual field of male flies that has increased spatial and temporal resolution, adapted for detection of female flies (Land & Collett, 1974). The love spot photoreceptors of the male housefly, *Musca domestica*, have a gain function with a corner frequency of 75.9 Hz while the female's corner frequencies in corresponding regions of the visual field only reach 46.7 Hz (Hornstein et al., 2000). The photoreceptors located in the love spot of male houseflies have an input resistance that is about 40% lower than that of female photoreceptors.

From the discussion above one can infer that there should be a correlation between the physical and biological constraints imposed by dim light vision and a nocturnal animal's way of life. If we fully want to understand the visual ecology of nocturnal animals, it is of great importance to investigate all levels of the visual system, from physiology, optics and anatomy to behaviour and ecology. I have in the first half of this thesis attempted to cover the optical aspects of ommatidial adaptations for vision in nocturnal insects. I will now discuss the physiology of their photoreceptors.

5.4.1. Gain control, SNR and information rate in nocturnal photoreceptors

How can a photoreceptor be adapted to suit the lifestyle of a nocturnal insect? In Chapter IV (Frederiksen et al., 2008b) we show that the photoreceptors of the nocturnal Halictid bee *Megalopta genalis* has an increased contrast gain, and a narrow low-passed-filtered signalling bandwidth compared to those of its close diurnal relative *Lasioglossum leucozonium*. A similar trend is found among dung beetles of the genus *Onitis* (Chapter V: Frederiksen et al., 2008c). The nocturnal *Onitis aygulus* has a higher contrast gain and a narrower bandwidth in dim light than the diurnal *Onitis belial*, although the difference in gain between these two species is not as substantial as between the Halictids (Fig. 15: Frederiksen et al., 2008c). This is in agreement with previous studies that have shown that nocturnal arthropod photoreceptors are characterised by a high gain of transduction (Laughlin & Weckström, 1993; Laughlin, 1996; Heimonen et al., 2006; Pirhofer-Walzl et al., 2007) and a narrow response bandwidth (Howard et al., 1984; Laughlin & Weckström, 1993; Laughlin, 1996; Heimonen et al., 2006).

Another interesting observation is that in both Halictid bees (Chapter IV) and Onitine dung beetles (Chapter V), the nocturnal species of each group (Fig. 15A,C) have highest contrast gain at both lower intensities and lower temporal frequencies. Moreover, the nocturnal species show low-pass-filter properties throughout the intensity range used (Fig.

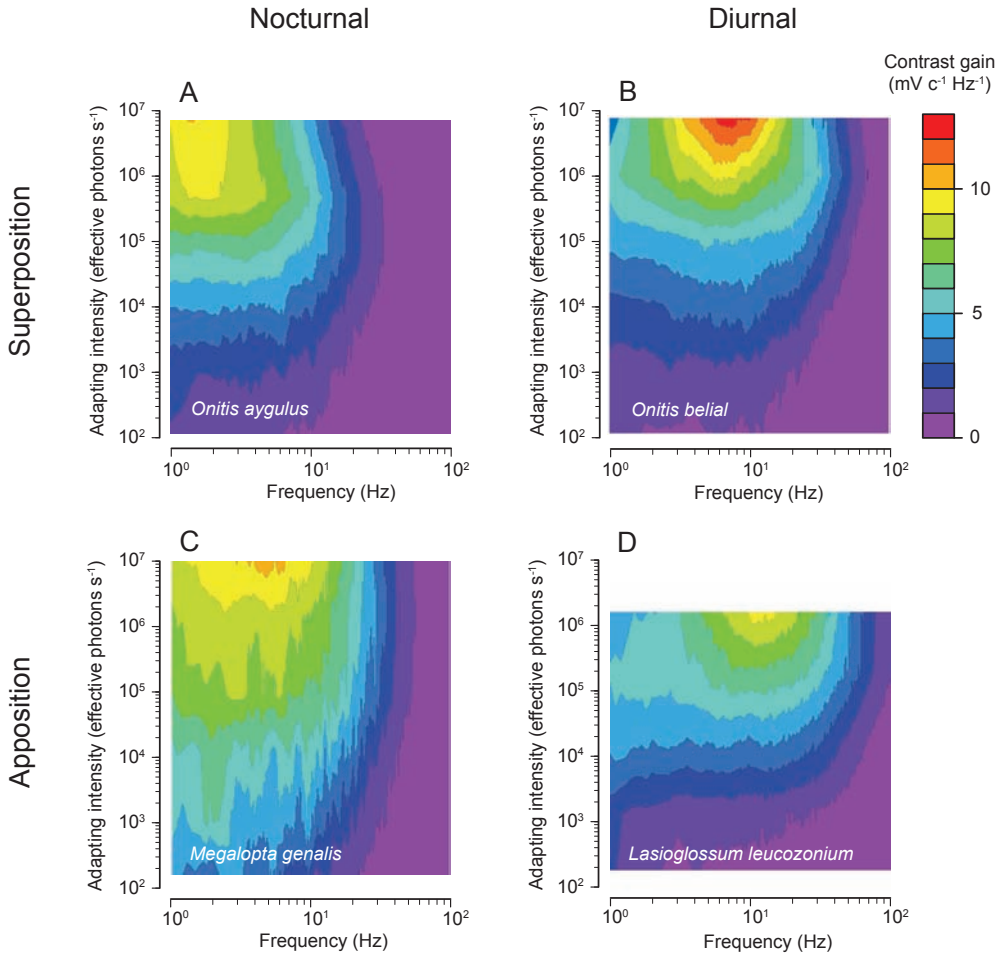

Fig. 15. Contrast gain as a function of temporal frequency and adapting intensity in the dung beetles *Onitis aygulus* (**A**) and *Onitis belial* (**B**), and the halictid bees *Megalopta genalis* (**C**) and *Lasioglossum leucozonium* (**D**). The nocturnal *O. aygulus* (**A**) and *M. genalis* (**C**) have their highest contrast gains shifted towards lower intensities and lower temporal frequencies compared to *O. belial* (**B**) and to *L. leucozonium* (**D**) respectively. The dung beetles (**A** and **B**) have a narrower temporal bandwidth than the halictid bee from the same light habitat (**C** and **D** respectively). Note that *M. genalis* (**C**) maintains a much higher contrast gain at dimmer intensities compared to all other species. Note also that the plots differ in size and position along the intensity axis. This is because the calibration to equal intensities in effective photons per second does not correspond to identical neutral density filters in the experimental apparatus.

15A,C) while the diurnal species gradually change from low-pass filtering in dim light to band-pass filtering in bright light (Fig. 15B,D). For nocturnal insects, low-pass filtering and amplification of the visual signal in dim light is beneficial because it improves receptor

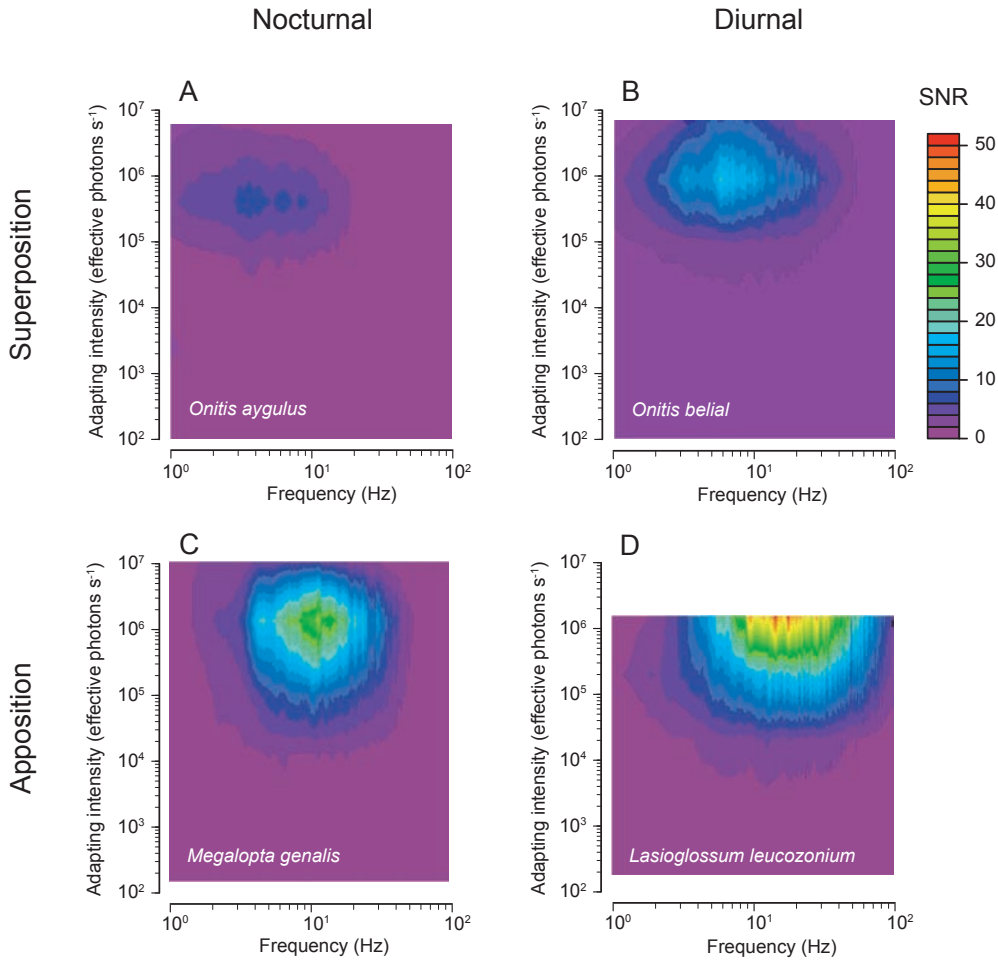

Fig. 16. Signal-to-noise ratio (SNR) as a function of temporal frequency and adapting intensity in the dung beetles *Onitis aygulus* (**A**) and *Onitis belial* (**B**), and the halictid bees *Megalopta genalis* (**C**) and *Lasioglossum leucozonium* (**D**). The dung beetles (**A** and **B**) have much lower maximum SNRs than the bees (**C** and **D**) and peak SNRs shifted towards lower frequencies. The nocturnal species (**A** and **C**) in each taxon have lower SNR than the corresponding diurnal species (**B** and **D**). Note that the plots differ in size and position along the intensity axis. This is because the calibration to equal intensities in effective photons per second does not correspond to identical neutral density filters in the experimental apparatus.

sensitivity and visual reliability at low temporal frequencies (van Hateren, 1993; Laughlin, 1996). This pattern is also clear from the amplitudes of the photon bumps. Numerous studies have shown that nocturnal arthropods produce large bumps in response to single photons (Lillywhite, 1977; Laughlin et al., 1980; Heimonen et al., 2006; Pirhofer-Walzl et al., 2007). In *O. aygulus* bump amplitudes are slightly higher than in *O. belial* (Chapter V:

Frederiksen et al., 2008c). This is also the case in *M. genalis*, which has larger bumps than in *L. leucozonium* (Chapter IV: Frederiksen et al., 2008b), although the difference between the bees is larger than between the dung beetles (Fig. 9).

If we instead plot SNR as a function of frequency and adapting intensity (Fig. 16), further features of nocturnal photoreceptors are revealed. First, the filtering properties discussed above are also evident here. Compared respectively to the diurnal *O. belial* (Fig. 16B) and *L. leucozonium* (Fig. 16D), the maximal peaks of SNR in the nocturnal *O. aygulus* (Fig. 16A) and *M. genalis* (Fig. 16C) are shifted towards the low frequency end of the spectrum. Secondly, the peak SNR amplitude is much higher in the two diurnal species (Fig. 16). Intuitively one might imagine that in dim light a higher SNR should be found in the photoreceptors of nocturnal insects. However, the data show a different pattern – in both nocturnal halictid bees (Chapter IV: Frederiksen et al., 2008b) and in nocturnal dung beetles (Chapter V: Frederiksen et al., 2008c), the SNR is much poorer relative to that found in diurnal species of the same taxon. The poor SNR and low temporal bandwidth of nocturnal photoreceptors have consequences for their information rates (Fig. 17) since the information rate in a photoreceptor depends on both of these parameters. The information rate of *O. aygulus* falls well below that of *O. belial* at all adapting intensities, and

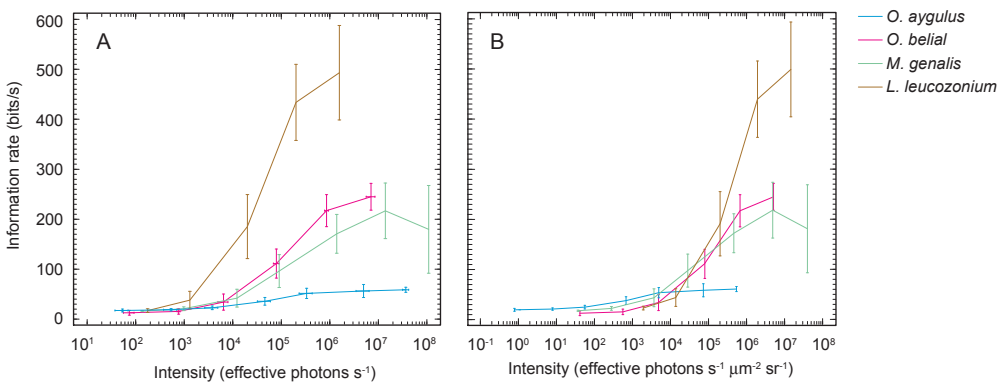

Fig. 17. A Information rates of photoreceptors in *O. aygulus* (nocturnal superposition eye, *blue*, n=8), *O. belial* (diurnal superposition eye, *red*, n=8), *Megalopta genalis* (nocturnal apposition eye, *green*, n=8) and *Lasioglossum leucozonium* (diurnal apposition eye, *brown*, n=8) plotted as a function of intensity (in effective photons per second). The information rate of the diurnal species in each group is higher than that of the nocturnal species in each group at all adapting intensities. Moreover, the nocturnal bee has a higher information rate than the nocturnal dung beetle and the diurnal bee has a higher information rate than the diurnal dung beetle. **B** Information rates of photoreceptors of the same species as in **A** but plotted as a function of intensity adjusted for differences in sensitivity (in effective photons s^{-1} μm^{-2} sr^{-1}). The information rate in the photoreceptors of the nocturnal *O. aygulus* is highest at the dimmest intensities. However, when calibrated in this manner, the largest differences in information rate are due to the much greater optical sensitivity in the nocturnal species. The error bars represent standard deviations.

the information rate of *M. genalis* is much lower than that of *L. leucozonium* (Fig. 17A).

The poor SNR and information rate of nocturnal photoreceptors is not likely an adaptation to nocturnal vision, but a necessary compromise. It has been suggested that nocturnal vision is energetically very costly. The large photoreceptors of nocturnal insects most likely pay a higher energetic price to maintain a similar SNR and bandwidth to those found in the smaller photoreceptors of diurnal insects (Niven et al., 2007). However, larger photoreceptors, with larger collecting areas (wide rhabdoms), are also much more sensitive (Land, 1981). Thus, it is likely that SNR and information rate is traded for increased sensitivity in nocturnal insects.

5.4.2. The influence of optical sensitivity and eye design

In the previous section we have seen that there are certain properties that characterise the physiology of photoreceptors in nocturnal insects. Are there also properties that are influenced by differences in eye design and optical sensitivity among different nocturnal insects? In previous sections we have seen that the superposition eyes of dung beetles are inherently more sensitive than the apposition eyes of bees (Table 1, Chapter III: Frederiksen & Warrant, 2008b) and I will argue that this has also influenced the evolution of photoreceptor physiology in these eyes. The contrast gain in *O. aygulus* and *O. belial* falls approximately equally with falling adapting intensity (Fig. 15A,B) while the high photoreceptor contrast gain evident in the less sensitive apposition eye of *M. genalis* is maintained at much dimmer adapting intensities (Fig. 15C). This suggests that the lower contrast gains found at dimmer intensities in the two refracting superposition eyes are compensated by their inherently greater optical sensitivities.

The effect of the greater optical sensitivity of the superposition design is even more apparent when one considers SNR (Fig. 16): compared to the apposition eyes, the more sensitive refracting superposition eyes of the dung beetles have photoreceptors with much poorer SNR. This is also reflected in their much lower information rates (Fig. 17A). As argued above, it is energetically very expensive for a neuron to maintain a high information rate (Laughlin & de Ruyter van Steveninck, 1998; Laughlin, 2001; Niven et al., 2007). Since there is a high cost associated with maintaining a high signal-to-noise ratio and a broad signalling bandwidth there are likely to be evolutionary forces that work to reduce these costs. On the other hand, there are opposing evolutionary forces that drive nocturnal insects to maintain reliable vision in dim light. Since the refracting superposition eye design is inherently much more sensitive, this has probably driven the balance between these two forces towards the former. Thus, more sensitive optics allows the energetic cost to be minimised.

In order to see the benefits of the evolutionary trade-off above we must consider the whole system, with the photoreceptors and optics working together. We can do this by calibrating the recordings of information rate according to the calculated sensitivities for each species (Fig. 17B). It is clear, that due to much more sensitive optics, both nocturnal species perform much better in dim light than either of the corresponding diurnal species.

O. aygulus, with the most sensitive eyes, also has the highest information rate at the dimmest light levels.

Thus, even if bump amplitude is higher in superposition eyes than in apposition eyes (from the same light habitat), contrast gain and signal-to-noise ratio are lower. This implies that in superposition eyes photoreceptor adaptations to improve signal reliability are not as pronounced as in apposition eyes. The greater optical sensitivity of superposition eyes may be almost sufficient on its own to improve reliability, a conclusion supported by the fact that, when adjusted for their higher optical sensitivity, their information rate is nonetheless higher than in apposition eyes in dim light (Fig. 17B).

Do the above conclusions hold if we instead compare the two diurnal species, *L. leucozonium* and *O. belial*? Both of these insect species are active in bright sunshine and have the possibility to collect sufficient light to maximise the signal-to-noise ratio, bandwidth and information rate. This is especially the case for *O. belial* due to its sensitive superposition eyes. Nonetheless, our data show that the photoreceptor SNR (Fig. 16), bandwidth (Fig. 15) and information rate (Fig. 17) in *O. belial* are much lower than in *L. leucozonium*. However, several factors (and not solely the light intensities at which the different species are active) have driven the evolution of photoreceptor signalling properties and may confound comparisons between taxa as widely unrelated as the Onitini and Halictidae. Bees and dung beetles use their eyes for considerably different tasks and this, as much as light level, has most likely influenced their photoreceptor physiology. While halictid bees make advanced visually guided flights that involve extensive turning, dung beetles of the genus *Onitis* probably use their eyes only for obstacle avoidance while flying from dung pile to dung pile (which are initially detected using olfaction). This means that the photoreceptors of the two groups are used for tasks that are not equally demanding, experiencing different contrasts, spatial frequencies and temporal frequencies. The narrower signal bandwidths found in dung beetles (Fig. 15) probably reflect this since the speed of vision is likely to be influenced by life history traits such as flight speed (Howard et al., 1984; Laughlin & Weckström, 1993). The dung beetles in this study are slower are less manoeuvrable flyers than the bees and probably experience lower angular velocities. This has almost certainly diminished the demand for dung beetle photoreceptors to maintain a high temporal bandwidth.

Moreover, diurnal bees are nectar-feeding insects that rely on well-developed trichromatic colour vision for flower detection. The size of the colour space that can be perceived by an animal depends ultimately on the photoreceptor SNR (Vorobyev & Osorio, 1998; Vorobyev et al., 2001) – colour discrimination is likely to have been a strong selective force that improved SNR in diurnal bees such as *L. leucozonium*. In *Onitis*, however, colour is probably of less importance, since only two spectral classes of photoreceptors have been reported in these beetles (Warrant & McIntyre, 1990).

6. Final remarks

In this thesis I have presented data that show that the compound eyes of insects are sufficiently flexible to adapt to the particular window of intensities in which the species is active. These ommatidial adaptations for vision in nocturnal insects can concern the optics and morphology of the eye (Chapters I - III) or the physiology of the photoreceptors (Chapters IV & V). We can observe that that all visual properties that contribute to sensitivity in a compound eye, and thus adapt it for a crepuscular or even a nocturnal lifestyle, have apparently not changed equally in different groups. The crepuscular *Caligo memnon* has evolved large eyes with large facets but has retained a reasonably high spatial and temporal resolution. In the nocturnal bee *Megalopta genalis* the eyes and facets are enlarged, the rhabdoms are wide and the spatial and temporal acuity are very poor compared to diurnal insects. In addition to this, *M. genalis* has a high gain of photoreceptor transduction. The nocturnal wasp *Apoica pallens* has retained small facet lenses but evolved wide rhabdoms (Greiner, 2006). The nocturnal dung beetle *Onitis aygulus* has refracting superposition eyes and thus gains optical sensitivity via a wide superposition aperture that results from the clear zone and the graded-index lens system that is present in its crystalline cones. This beetle has, in addition, slow photoreceptors with high gain. Thus, there are a variety of solutions to improve visual sensitivity and reliability in dim light. Exactly which solutions evolved in which group most likely depended on the constraints imposed by different phylogenetic histories, on developmental constraints, and on different selection pressures arising from different life styles.

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