

## Optics of the butterfly eye

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**Summary.** The afocal apposition optics of butterfly eyes was examined from both a geometrical optics and a wave optics point of view. We used several different species of butterfly but put special emphasis on a common Australian nymphalid, *Heteronympha merope*. From the anatomy of the retina, the optics of isolated components of the eye and the ophthalmoscopy of the intact living eye we derived the following.

1. The proximal part of the crystalline cone behaves as a powerful lens which, according to our measurements of optical power, turns the complete optical system into an afocal telescope with an angular magnification of 6.4 (in *Heteronympha*). The rhabdom tip lies in the exit pupil of the telescope and is imaged into the cornea with a magnification of 9.1 (in the same species).

2. Using light reflected from the eye's tapetum, we studied the waveguide mode phenomena of the rhabdom. Different butterflies showed either one, two or three waveguide modes, depending on the rhabdom diameter. The mode patterns were observed at four different optical planes: at the cornea, at infinity, at the back focal plane of the corneal lens – which, for this measurement, was optically neutralised – and at the plane of the deep pseudopupil.

3. During light adaptation the closure of the pupil caused the modes to disappear in sequence, starting with the highest order. The behaviour of the fading modes indicates that the pupil acts by

absorption rather than by a change of refractive index around the rhabdom.

4. The modes were used to measure the waveguide parameter of the rhabdom, from which its refractive index was deduced to be 1.36.

5. The distinction between near-field and far-field versions of the mode patterns provided further evidence in favour of an afocal optical system.

Two different interpretations of the butterfly optical system are discussed and we present a hypothesis to explain how both afocal apposition and refracting superposition optical systems evolved in insect eyes.

### Introduction

Apposition compound eyes – in which the ommatidia are optically isolated from each other – have traditionally been considered to have a simple optical system consisting of a single lens focusing an inverted image on the tip of a waveguide which contains the photopigment. Such an optical system can be termed *focal*. In a short note, we recently reported the existence of an entirely different system in butterfly eyes (Nilsson et al. 1984), which uses *afocal*, or telescope-like, optics. At that time we felt uncertain about the relative merits of afocal apposition optics compared with conventional focal apposition optics, and about the reasons why butterflies use this system. However, following theoretical studies of the system (Dr. Colin Pask, pers. comm.; van Hateren and Nilsson, in press), we are now in the position to give a full account of the butterfly system and to compare its performance with the various alternatives.

It is only recently that a complete theory of conventional apposition optics has become avail-

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able. Snyder (1975, 1977) first combined the action of the lens with the waveguide properties of the rhabdom. A further improvement of this model was presented by Pask and Barrell (1980a, b). Finally, van Hateren (1984) and Smakman et al. (1984) demonstrated that a rigorous wave-optical approach accurately describes the angular sensitivity of fly photoreceptors. The agreement between theory and experiments is now so good that we can safely take the fly compound eye as being one of the best understood optical systems in the animal kingdom. It was generally believed that the same fundamental mechanism would apply to all apposition eyes in insects and crustaceans. However, the discovery of a second lens in the crystalline cone of butterfly eyes implies an alternative way of constructing an apposition optical system. In the butterfly eye the single lens of a traditional apposition ommatidium (focal optics) is exchanged for a two-lens telescope (afocal optics), with the fundamental consequence that the receptor visual field is determined not by the diameter of the rhabdom but by its angular acceptance properties (Nilsson et al. 1984).

In retrospect, it seems not surprising to find evidence for afocal apposition optics in butterflies because it has been known since Exner (1891) that most other lepidopterans possess an afocal system in their refracting superposition eyes. In this other major type of compound eye – the superposition eye – an erect superimposed image from many facets is formed on the retina, and this is most commonly realized by an afocal system in each facet. Our findings in butterflies thus provide a link between apposition and superposition eyes that otherwise seemed unrelated.

In this paper we give a detailed description of the butterfly optical system and interpretations of it. Much of the paper is, however, concerned with the waveguide mode phenomena of the rhabdom which are both visible and important (Nilsson et al. 1984), just as they are in fly eyes (van Hateren 1984). Due to an unusual set of anatomical circumstances, butterflies are almost ideal for studying waveguide modes in photoreceptors. The principle reason is the presence of a reflecting tapetum at the bottom of each rhabdom (Miller 1979; Miller and Bernard 1968). The reflected light emerging from the rhabdom can be studied using ophthalmoscopic techniques, and the mode patterns observed have proved to be useful tools in working out the optics of butterfly eyes and would indeed be so for apposition eyes in general.

The fundamentals of waveguide optics and Fourier optics may be unfamiliar to many biolo-

gists and with the introduction of afocal systems we considered it important to include a comprehensive description of how wave optics can be applied to both focal and afocal systems.

Originally, we considered focal and afocal systems to be discrete and even opposite solutions to apposition eye design, and no intermediates appeared to make sense. However, when we considered wave and geometrical optics together we came to realize that under some circumstances alternative interpretations of the same optical system could legitimately co-exist. Some of these problems are considered in the Discussion.

## Materials and methods

For this study we have mainly used an Australian woodland butterfly, *Heteronympha merope*, which was caught wild and kept for no more than two days before use. In some experiments and for comparison, we also used other Australian butterflies: *Zizina labradus*, *Vanessa itea*, *Junonia villida*, *Xoia arctoa*, *Melanitis leda*, *Pieris rapae* and *Papilio aegaeus*. Finally, we made ophthalmoscopic and histological checks on a few European species to confirm the generality of our conclusions. These were *Argynnis paphia*, *Lycaena phlaeas* and *Gonepteryx rhamni*.

Histological work was performed on eyes excised into a solution consisting of 2.5% glutaraldehyde, 2% paraformaldehyde, 2% glucose and 5 mM EGTA in 100 mM Na-cacodylate buffer. After 2 h fixation, the specimens were rinsed in buffer solution and part of the material was treated with 1% OsO<sub>4</sub> for 2 h. Dehydration was performed in an alcohol series and embedding in Epon. Sections for light microscopy were cut 2 µm thick and stained with toluidine blue. For electron microscopy, ultrathin sections were cut on a diamond knife and post-stained with lead citrate and uranyl acetate.

Nomarski microscopy, which was used to study the shape of intact isolated crystalline cones, was performed on excised eyes immersed for 1 h in the histological fixative and then torn apart with fine needles. In optical experiments requiring a Ringer solution, we used the following composition (in mM): 119 NaCl, 8.5 KCl, 2.4 CaCl<sub>2</sub> and 1.6 MgSO<sub>4</sub> in 10 HEPES buffer. Refractive indices were measured on fresh components of the eye immersed in Ringer solution using a Jamin-Lebedeff type interference microscope. The optical properties of the crystalline cones were investigated on 4–8 µm cryosections cut from eyes prepared for 30 min in Ringer solution with 1% glutaraldehyde. Focal length measurements were performed on cross-sectioned cones in the cryosections and on hanging drop preparations of isolated pieces of cornea. The technique was to present an object of specified size far from the preparation and measure the image magnification with a microscope.

Observations of rhabdom mode patterns were all made on live, intact animals immobilized with wax. We used a Zeiss Universal microscope with epi-illumination through the objective lens. For the different experiments we used a selection of objective lenses; 6.3 × /0.16, 16 × /0.32 and 40 × /1.30 oil. The illumination area and angle were controlled by iris diaphragms. Light sources were either a 100 W halogen bulb or a 200 W mercury arc lamp. Some experiments (infinity projections of ommatidia) were made with an ophthalmoscope, described by Land (1984), fitted with an image intensifier. All photography of mode patterns was done with Kodak Tri-X film developed in Acufine. Further details of the various ophthalmoscopic techniques are given in the relevant sections of the results.