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# Light on the moth-eye corneal nipple array of butterflies

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The outer surface of the facet lenses in the compound eyes of moths consists of an array of excessive cuticular protuberances, termed corneal nipples. We have investigated the moth-eye corneal nipple array of the facet lenses of 19 diurnal butterfly species by scanning electron microscopy, transmission electron microscopy and atomic force microscope, as well as by optical modelling. The nipples appeared to be arranged in domains with almost crystalline, hexagonal packing. The nipple distances were found to vary only slightly, ranging from about 180 to 240 nm, but the nipple heights varied between 0 (papilionids) and 230 nm (a nymphalid), in good agreement with previous work. The nipples create an interface with a gradient refractive index between that of air and the facet lens material, because their distance is distinctly smaller than the wavelength of light. The gradient in the refractive index was deduced from effective medium theory. By dividing the height of the nipple layer into 100 thin slices, an optical multilayer model could be applied to calculate the reflectance of the facet lenses as a function of height, polarization and angle of incidence. The reflectance progressively diminished with increased nipple height. Nipples with a paraboloid shape and height 250 nm, touching each other at the base, virtually completely reduced the reflectance for normally incident light. The calculated dependence of the reflectance on polarization and angle of incidence agreed well with experimental data, underscoring the validity of the modelling. The corneal nipples presumably mainly function to reduce the eye glare of moths that are inactive during the day, so to make them less visible for predators. Moths are probably ancestral to the diurnal butterflies, suggesting that the reduced size of the nipples of most butterfly species indicates a vanishing trait. This effect is extreme in papilionids, which have virtually absent nipples, in line with their highly developed status. A similar evolutionary development can be noticed for the tapetum of the ommatidia of lepidopteran eyes. It is most elaborate in moth-eyes, but strongly reduced in most diurnal butterflies and absent in papilionids.

**Keywords:** eye reflectance; multilayer theory; refractive index gradient; butterfly evolution

## 1. INTRODUCTION

Insects have faceted, compound eyes, consisting of numerous anatomically identical units, the ommatidia. The eyes are classified according to the optical system that is used to efficiently focus light onto the light-sensitive parts of the photoreceptors. In apposition eyes, employed by butterflies, a facet lens together with its crystalline cone channels light into a fused rhabdom, a long, cylindrical structure, which contains the photoreceptors' visual pigment molecules. In optical superposition eyes, used by moths, light reaches the photoreceptive rhabdom via several facet lenses (Exner 1891, 1989; Nilsson 1989). Moths thus realize a much higher light sensitivity than butterflies, allowing a nocturnal instead of diurnal lifestyle (Warrant *et al.* 2003).

Well over four decades ago, Bernhard & Miller (1962) discovered that the outer surface of the facet lenses in moth-eyes consists of an array of cuticular protuberances termed corneal nipples (Bernhard & Miller 1962; Bernhard *et al.* 1965; Miller 1979). The optical action of the corneal nipple array is a severe reduction of the

reflectance of the facet lens surface. Accordingly, it increases the transmittance, and therefore the initial interpretation of the nipple array was that it helps to enhance the light sensitivity of the light-craving moths (Miller 1979). In other words, the corneal nipple array functions as an impedance matching device that improves vision. However, although the nipple array considerably reduces the reflectance of a smooth facet lens surface, from about 4 to less than 1%, this means only a very minor transmittance increase, from 96 to more than 99%. A more adequate consideration hence could be that moths are inactive in the daytime and therefore are vulnerable for predation. A moth with large, glittering eyes will be quite conspicuous, and therefore its visibility is reduced by the eye reflectance decreasing corneal nipple arrays (Miller 1979). This latter camouflage hypothesis seems to be plausible, but direct experimental proof has so far not been obtained.

Further research demonstrated that corneal nipple arrays are widespread among insects. In a comparative survey, Bernhard *et al.* (1970) inspected the corneal facet lenses of 361 insect species. They distinguished three classes of nipple arrays, depending on the height of the nipples. The corneas of class I have minor protrusions, less than 50 nm high, class II corneas have low-sized nipples,

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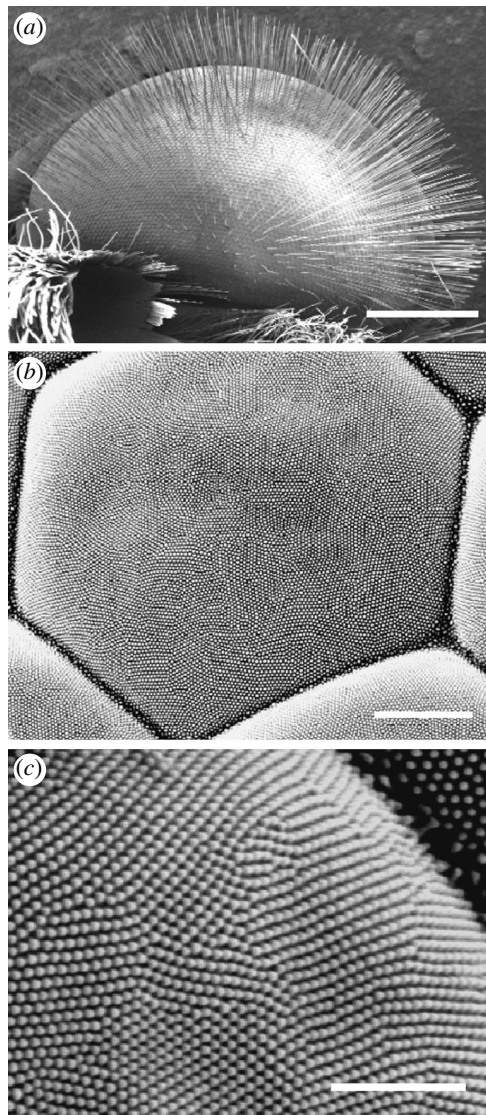


Figure 1. Corneal nipple arrays in the peacock (*Inachis io*), a nymphalid butterfly, as revealed by SEM. (a) The complete eye. (b) The nipple array in one facet lens. (c) Detail, showing the local arrangement of domains with highly ordered nipple arrays. The scale bar is in (a) 500, (b) 5 and (c) 2  $\mu\text{m}$ .

with height between 50 and 200 nm, and class III corneas have full-sized nipples, with amplitude about 250 nm. Full-sized nipples were only found among the Trichoptera and Lepidoptera. The distribution over the three classes of the Trichoptera investigated was 5 : 5 : 5 (15 species in total). The distribution for the 170 lepidopteran species other than rhopalocerans (butterflies) was 42 : 26 : 102, and for the Hesperidae 7 : 2 : 1, Papilionidae 10 : 0 : 0, Pieridae 2 : 8 : 1, Lycaenidae 0 : 11 : 2 and Nymphalidae 1 : 9 : 20. The Papilionidae, where the corneal nipples are virtually non-existent, differed remarkably from the Nymphalidae, which have large or full-sized nipples. The latter feature is difficult to reconcile with the functional interpretations given for the moths, because the members of both Papilionidae and Nymphalidae are generally only active at bright light conditions and also advertise themselves with conspicuous colourations.

The optical properties of moth-eyes have received considerable biological as well as physical interest (Wilson & Hutley 1982; Parker *et al.* 1998). The operation of a moth-eye surface may be understood most easily in terms

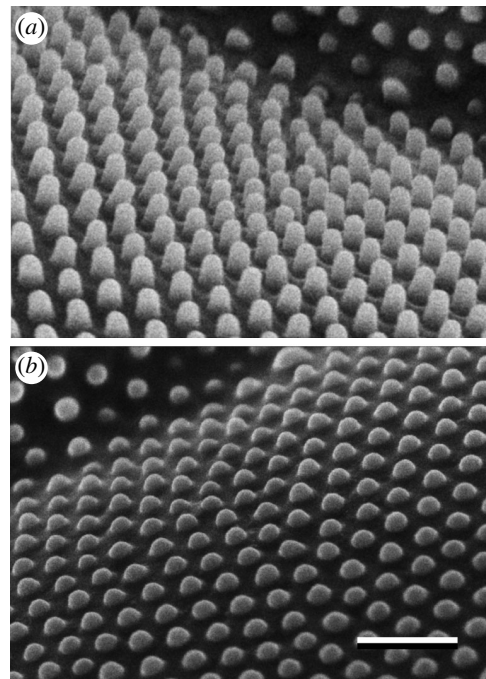


Figure 2. Corneal nipple arrays in the nymphalid *Polytonia c-aureum* (a) and the lycaenid *Pseudozizeeria maha* (b), showing differences in nipple height and shape. Bar, 500 nm.

of a surface layer in which the refractive index varies gradually from unity to that of the bulk material (Wilson & Hutley 1982). The insight that nipple arrays can strongly reduce surface reflectance has been widely technically applied, e.g. in window panes, cell phone displays and camera lenses (rev. Palasantzas *et al.* 2005; for further information and explanatory figures, see, for example [http://www.funktionale-oberflaechen.de/english/a1\\_ent\\_f.html](http://www.funktionale-oberflaechen.de/english/a1_ent_f.html), [http://www.ntt-at.com/products\\_e/motheye/](http://www.ntt-at.com/products_e/motheye/), <http://www.motheye.com/Index.swf>). In fact, some moth species (e.g. *Cephonodes hylas*) apply nipple arrays to reduce the reflectance of their scaleless and transparent wings (Yoshida *et al.* 1997).

In the course of our studies of butterfly vision, we have investigated the corneal nipple arrays of a number of butterfly species. We present novel data, calculate the reflectance for a number of nipple geometries using a simple multilayer modelling approach, and discuss the relevance of nipple arrays for vision and visibility.

## 2. MATERIAL AND METHODS

### (a) *Experimental animals*

Butterflies of the families Papilionidae, Pieridae, Lycaenidae and Nymphalidae were captured in the Netherlands, Taiwan, Japan and Uganda. Two nymphalid species (*Bicyclus anynana* and *Heliconius melpomene*) were obtained from a laboratory culture maintained by Prof. P. Brakefield (Leiden University). The investigated eyes of dead butterflies were often slightly deteriorated, but the nipple structures appeared to be unaffected (see Bernhard *et al.* 1970).

### (b) *Electron microscopy*

The corneal nipple arrays were studied by standard scanning electron microscopy (SEM, Philips XL30 ESEM), using palladium sputtering of heads severed from dead specimens (figures 1 and 2). For transmission electron microscopy (TEM), isolated eyes were prefixed overnight at 4 °C in 2%

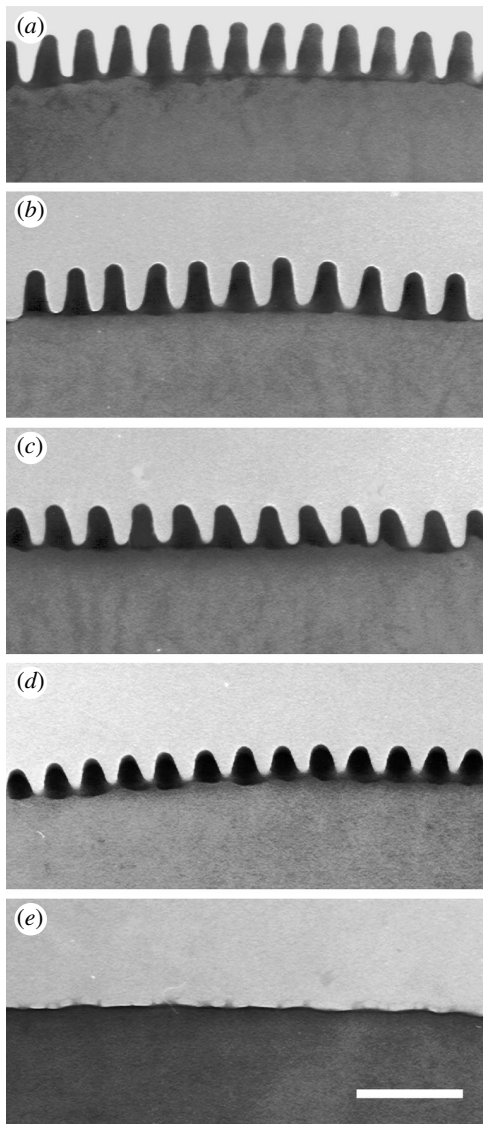


Figure 3. Corneal nipple arrays in the nymphalids *Bicyclus anynana* and *Polygonia c-aureum* (a,b), the pierid *Pieris rapae* (c), the lycaenid *Pseudozizeeria maha* (d) and the papilionid *Papilio xuthus* (e). Bar, 500 nm.

glutaraldehyde and 2% paraformaldehyde in 0.1 M sodium cacodylate buffer (CB, pH=7.4). After being washed with CB briefly, the tissues were postfixed in 2% osmium tetroxide in CB for 2 h at room temperature. The tissues were then dehydrated with a graded series of acetone and embedded in Epon. Ultrathin sections cut with a diamond knife were observed with a transmission electron microscope (JEM 1200EX, JEOL Tokyo Japan) without staining (figure 3).

#### (c) Atomic force microscopy

An atomic force microscope (AFM, Dimension 3100) was used in tapping mode, to avoid sample damage, on a few butterfly species. Sputtered as well as non-sputtered corneas yielded reliable results, confirming the estimates obtained by SEM, but only when the nipples were low- or medium-sized. AFM on full-sized nipple arrays appeared to be problematic, presumably due to the high aspect ratio of the nipple arrays.

#### (d) Optical modelling

The reflectances of three types of nipple arrays, with cone, paraboloid and Gaussian-shaped nipples, were calculated with a multilayer model. A coordinate system was used with

Z-axis perpendicular to the corneal surface, so that the nipple array troughs were at  $z=0$  and the nipple peaks at  $z=h$ . The  $z$ -coordinate relative to the peak value,  $h$ , is  $z^*=z/h$ , and the distance  $r$  to the nipple axis relative to the distance of two adjacent nipples,  $d$ , is  $r^*=r/d$ . The three nipple types then are described by  $z^*=1-r^*/p$  (cone),  $z^*=1-(r^*/p)^2$  (paraboloid), and  $z^*=\exp(-4 \ln 2 (r^*/p)^2)$  (Gaussian), with the condition that  $z^*\geq 0$  for all  $r^*$ ; the parameter  $p$  determines the width of the nipple. The nipple lattice is assumed to be hexagonal (figures 1 and 2), and thus the area taken up by a nipple equals  $A_n=\sqrt{3}d^2/2$ . The area of the cone and paraboloid at their base, where  $z^*=0$  (or  $r^*=p$ ) is  $\pi p^2$ , and this area equals  $A_n$  when the width parameter  $p$  equals  $p_0=\sqrt{(\sqrt{3}/2\pi)}=0.53$ . A plane at level  $z^*$  contains a fraction  $f(z^*)=\pi r^2/A_n=2\pi r^{*2}/\sqrt{3}$  of corneal material, with refractive index  $n_c$ , and the remaining fraction,  $1-f(z^*)$ , then is air, with refractive index 1. Because the distance of the nipples is small with respect to the wavelength of light, light propagation is governed by the effective refractive index of the nipple array, which can be calculated from effective medium theory (Bruggeman 1935). At height  $z^*$ , the effective refractive index,  $n_e(z^*)$ , then is  $n_e=[g+(g^2+8n_c)^{1/2}]^{1/2}/2$ , with  $g=(3f-1)n_c^2-3f+2$ . We note here that for  $n_c=1.52$  (Vogt 1974),  $n_e(f)$  is well approximated by  $n_e=[fn_c^q+(1-f)]^{1/q}$ , with  $q=2/3$ , and that this function yields values that only slightly deviate from values given by the simple weighting formula  $n_e=fn_c+(1-f)$ . In the case of paraboloid nipples, the volume fraction is therefore very approximately a linear function of  $z^*$ , and consequently the refractive index profile of the nipple array is then very approximately a linear function of  $z^*$ . The corneal reflectance was calculated from the refractive index gradient by first dividing the transition layer of the nipples, between  $z=0$  and  $h$ , in 100 layers with thickness  $h/100$ , and calculating the effective refractive index value for each layer. The stack of 100 layers then can be treated as a multilayer system where the layers have different refractive indices. The reflectance of such a system can be calculated with a matrix multiplication procedure for a stack of thin layers (Macleod 1986). The calculations were performed for five nipple heights: 50, 100, 150, 200 and 250 nm.

### 3. RESULTS

The set of facet lenses of a butterfly eye, the cornea, is approximately a hemisphere (figure 1a). The convex outer surface of the facet lenses of a peacock (*Inachis io*) consists of protuberances, the corneal nipples, which locally are arranged in a highly regular, hexagonal lattice (figure 1b,c). The nearest-neighbour distance of the nipples,  $d$ , is about 210 nm, and their height,  $h$ , is ca 200 nm.

The dimensions of the nipples, estimated by SEM, TEM as well as AFM, appeared to vary among the butterfly species (figures 2–4; table 1). The five investigated papilionid species, having facet lenses with an average diameter of  $29\pm 3\ \mu\text{m}$ , had very minor nipples, with height less than or equal to 30 nm. When visible, the nipples were arranged in an irregular pattern with distance  $d=235\pm 10\ \text{nm}$ . The non-papilionid species had clear nipples arranged regularly in a hexagonal pattern, in domains with a diameter of roughly  $2\ \mu\text{m}$  (about 10 nipple distances; figure 1). The nipple distance was  $200\pm 20\ \text{nm}$  in the (small-sized) lycaenids, with facet lens diameter  $19\pm 2\ \mu\text{m}$  (figure 2b, 3d and 4), and  $210\pm 10\ \text{nm}$  in the

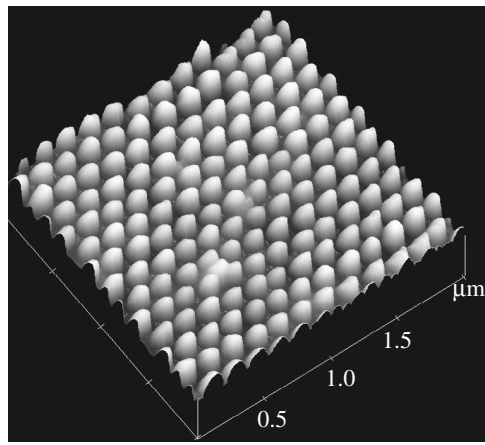


Figure 4. AFM image of the nipple array in a facet lens of the lycaenid *Pseudozizeeria maha*. The nipple distance is  $d=170\pm 10$  nm and the height is  $h=130\pm 15$  nm.

Table 1. Dimensions of the corneal nipple array of butterflies. (Average values of measurements by SEM, TEM and AFM.  $D$ , facet diameter;  $d$ , nipple distance;  $h$ , nipple height; n.d., not determined. Errors:  $\Delta D=3$   $\mu\text{m}$ ,  $\Delta d=10$  nm,  $\Delta h=10$  nm.)

|                                 | $D$ ( $\mu\text{m}$ ) | $d$ (nm) | $h$ (nm) |
|---------------------------------|-----------------------|----------|----------|
| Papilionidae                    |                       |          |          |
| <i>Graphium sarpedon</i>        | 28                    | 230      | 30       |
| <i>Papilio memnon</i>           | 31                    | n.d.     | (10)     |
| <i>Papilio protenor</i>         | 33                    | 240      | 20       |
| <i>Papilio xuthus</i>           | 25                    | 230      | 20       |
| <i>Pachliopta aristolochiae</i> | 26                    | 235      | 20       |
| Pieridae                        |                       |          |          |
| <i>Pieris rapae</i>             | 22                    | 210      | 210      |
| <i>Anthocharis cardamines</i>   | 24                    | 215      | 170      |
| Lycaenidae                      |                       |          |          |
| <i>Everes argiades</i>          | 17                    | 215      | 140      |
| <i>Pseudozizeeria maha</i>      | 21                    | 180      | 120      |
| <i>Narathura japonica</i>       | 17                    | 200      | 90       |
| Nymphalidae                     |                       |          |          |
| <i>Inachis io</i>               | 23                    | 210      | 200      |
| <i>Heliconius melpomene</i>     | 27                    | 205      | 180      |
| <i>Bicyclus anynana</i>         | 23                    | 205      | 210      |
| <i>Mycaliesia francisca</i>     | 28                    | 205      | 130      |
| <i>Polygonia c-aureum</i>       | 29                    | 200      | 190      |
| <i>Polygonia c-album</i>        | 24                    | 215      | 165      |
| <i>Euphaedra</i> sp.            | 35                    | 215      | 160      |
| <i>Euxanthe wakefieldii</i>     | 28                    | 220      | 230      |
| <i>Charaxes fulvescens</i>      | 30                    | 205      | 40       |

(larger) nymphalids, where the facet lens diameter was  $26\pm 3$   $\mu\text{m}$  (figure 1, 2a and 3a,b). The nipple height,  $h$ , was in the pierid species  $185\pm 20$  nm (figure 3c), in the lycaenids  $120\pm 20$  nm (figure 2b, 3d and 4), and in the nymphalids  $180\pm 30$  nm (figure 1, 2a and 3a,b), except for one species with  $h\approx 40$  nm (see table 1).

The shape of the nipples appeared to be somewhat variable and, therefore, we performed reflectance calculations for a few model shapes, a cone, paraboloid and Gaussian bell, respectively (figure 5), assuming a hexagonal nipple lattice. The height was increased from 50 to 250 nm in steps of 50 nm. Two nipple widths, given by the parameter  $p$ , were taken:  $p=0.40$  (figure 5) and 0.53; for the latter value the cone and paraboloid nipples have a base area equal to that of the lattice unit cell (see §2). Incident light faces a gradually increasing effective

refractive index, from  $n_e=1$  at  $z^*=1$  to  $n_e=1.52$  at  $z^*=0$  (Vogt 1974). The cone and paraboloid-shaped nipples have a base area smaller than that of the lattice unit cell when  $p=0.40$  (figure 5a), and hence the effective refractive index value suddenly jumps to 1.52 at  $z^*=0$  (figure 5b).

A thin-film multilayer model was used to calculate the reflectance of the three types of nipple array for normally incident light. The data of the effective refractive index profiles for the three nipple shapes, the two widths and the five heights yielded the reflectance spectra of figure 6. When the nipples are small, with height 50 nm, the refractive index gradient is steep, and accordingly the reflectance approximates the value of 0.043, predicted by the Fresnel equations for light in air normally incident on a medium with refractive index 1.52. The reflectance decreases with increasing nipple height, becoming minimal when the height is about 250 nm. The height-induced changes in the reflectance somewhat depend on the wavelength, especially for the non-touching nipples (figure 6a,c,e). The strongest reduction in reflectance occurs for paraboloid nipples with  $p=0.53$  that is for nipples that approximately touch each other in the troughs (figure 6d).

At normal incidence the degree of polarization is irrelevant. The reflectance, however, depends on the polarization when the angle of incidence is non-zero. Figure 7a,b show how the reflectance for 500 nm light depends on the angle of incidence for different nipple heights, that is for TE (s-) polarized and TM (p-) polarized light, respectively. The nipples were taken here to be touching paraboloids (cf. figure 6d). Again, for low nipples the angle dependence of the reflectance approximates that predicted by the Fresnel equations for a smooth surface. The reflectance for TE waves decreases monotonically with nipple height at all angles of incidence. A similar reduction occurs for TM waves when the angle of incidence is smaller than *ca* 50°, but the reflectance for TM waves hardly changes at angles above 50°. Qualitatively very similar angle and polarization dependences of the reflectance follow from calculations for the other nipple shapes. No striking differences occurred for wavelengths within the visible range.

#### 4. DISCUSSION

We investigated the corneal nipple arrays on the facet lenses of 19 species of butterflies with SEM, TEM and AFM (table 1). The nipple distance is, generally, about 210 nm. Slightly lower values occur in small facets, with diameter around 20  $\mu\text{m}$ , and larger distances correlate with large facets, around 30  $\mu\text{m}$ . The nipples are created during growth by secretions from regularly spaced microvilli in the corneagenous cells (Gemne 1971). Possibly the number of microvilli per ommatidium is about constant, resulting in a larger separation of the nipples in the bigger facet lenses.

The nipple height is much more variable. Bernhard *et al.* (1970) classified the nipples in classes I–III, with heights  $h<50$  nm,  $50\text{ nm}<h<200$  nm, and  $h>200$  nm, respectively. According to that classification, the distribution of the five investigated papilionid species was 5 : 0 : 0, of the two pierids 0 : 1 : 1, of the three lycaenids 0 : 3 : 0 and of the nine nymphalids 1 : 5 : 3. The corresponding values obtained by Bernhard *et al.* (1970)

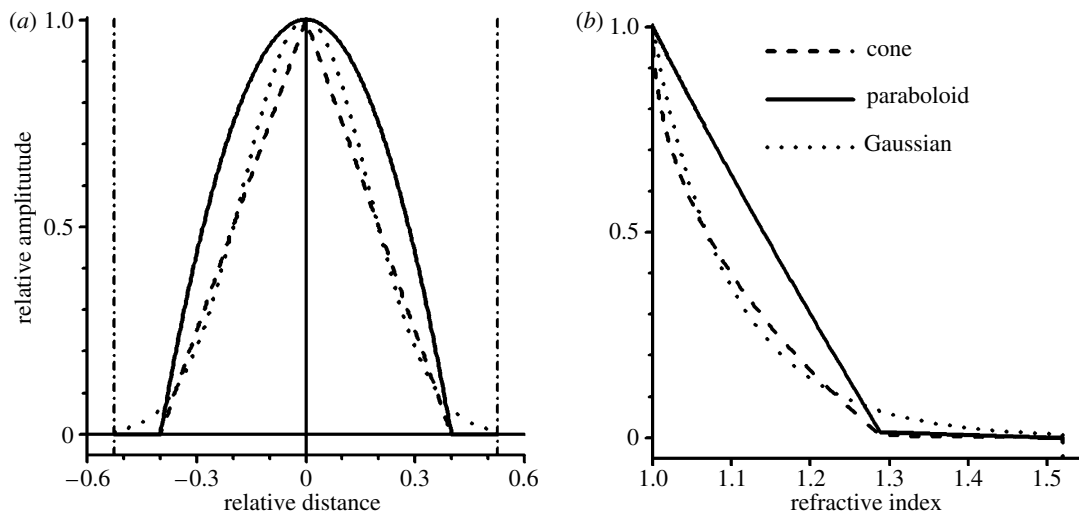


Figure 5. Three model nipple types with a cone, paraboloid and Gaussian-bell shape, and the resulting effective refractive index. (a) The amplitude of the three types of nipples relative to the peak value,  $z^*$ , shown as a function of the distance relative to the distance of two adjacent nipples,  $r^*$ . The boundary value for the width parameter,  $p_0=0.53$  (see §2), is given by vertical, dot-dashed lines. For the nipples shown in (a),  $p=0.40$ . (b) Effective refractive index values at level  $z^*$  for arrays of the three nipples of (a); note that the relative amplitude,  $z^*$ , is the independent variable here; the refractive index is the dependent variable. When  $z^* < 0$ , the refractive index is that of the facet lens medium,  $n_c=1.52$ , and when  $z^* > 1$  the refractive index is 1, that of air. The refractive index for  $0 < z^* < 1$  follows from effective medium theory (see §2). Paraboloid nipples yield a nearly linear refractive index gradient. Cone and paraboloid nipple arrays with  $p=0.40$  yield an effective refractive index jump at  $z^*=0$  from 1.29 to 1.52.

are 10 : 0 : 0 (papilionids), 2 : 8 : 1 (pierids), 0 : 11 : 2 (lycaenids), and 1 : 9 : 20 (nymphalids). The distribution that we obtained for the nymphalids was close to the boundary of 200 nm, which according to Bernhard *et al.* (1970) should not be taken as very sharp. We, therefore, conclude that our data are in good agreement with those of the earlier workers.

Using microwave models, Bernhard *et al.* (1965) experimentally demonstrated the strong reflectance reduction by a nipple array with cone-shaped nipples. The optical properties of moth-eye antireflection surfaces in the visible wavelength range have been firstly investigated on nipple arrays produced in photoresist by Wilson & Hutley (1982). The early work has induced many technical applications, known as ‘moth-eye’ arrays, which are widely applied for glare reduction as well as transmittance enhancement (review Palasantzas *et al.* 2005). Recently, Yoshida *et al.* (1997) investigated the effect of the nipple array discovered on the scaleless wings of a hawkmoth. The reflectance of the native wing was *ca* 1.5%, but removing the nipples by scraping resulted in a distinct reflectance increase to 4%, showing that the nipple array on the wings indeed functions as an impedance matching system. A similar prominent nipple array exists in cicada wings (SEM, Wagner *et al.* 1996; AFM, Watson & Watson 2004).

Although several theoretical treatises have been given for the effect of specific nipple profiles on the reflectance for light at normal incidence (e.g. Southwell 1991), quantitative data can be easily obtained by treating the nipple array as an interface with a gradient effective refractive index. The reflectance of such a medium can be straightforwardly calculated with matrix multiplication procedures for thin-film multilayers. It thus appeared that the precise shape of the nipples is rather unimportant for the reduction of the reflectance, that the nipple width plays a secondary role, and that the height of the nipples is the crucial factor (figure 6). An extreme reduction to nearly zero is realized by tall paraboloids, touching each other at

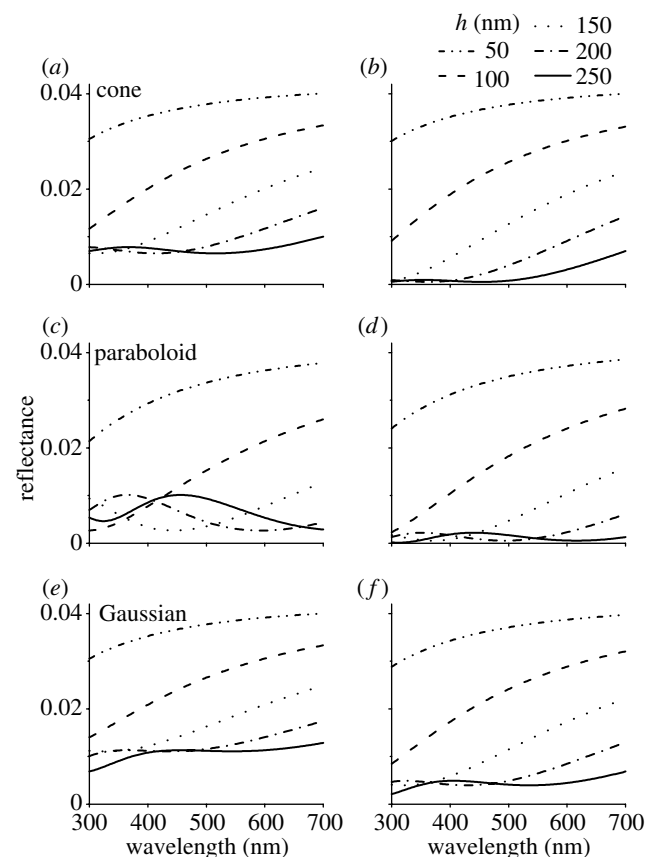


Figure 6. Reflectance of nipple arrays with the three types of nipples for normally incident light. The spectra were calculated with a model multilayer, consisting of 100 layers with thickness  $h/100$ , where  $h$  is the height of the cone (a,b), paraboloid (c,d) or Gaussian-shaped (e,f) nipples. The height was varied from 50 to 250 nm in steps of 50 nm. The width parameter  $p$  was taken to be 0.40 (a,c,e) or 0.53 (b,d,f). The reflectance for 50 nm high nipples approximates the value 0.043, predicted by the Fresnel equations, at the longer wavelengths. The reflectance is strongly reduced at nipple heights of *ca* 250 nm, notably when the nipples are paraboloids.

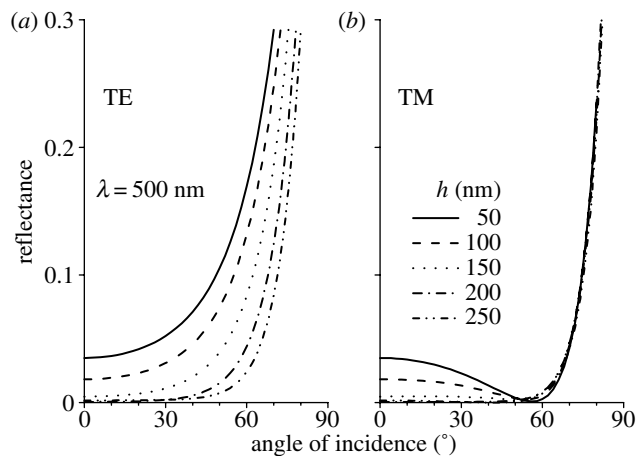


Figure 7. Dependence of the reflectance on polarization and angle of incidence. The corneal nipples were assumed to be paraboloids that touch each other at their base ( $p=0.53$ ; see figure 6*d*), and the nipple height was varied from 50 to 250 nm. The light wavelength was 500 nm. (a) The reflectance of TE (s-) polarized light is strongly reduced with increasing nipple height. (b) With TM (p-) polarized light, the strong reflectance reduction only occurs at angles of incidence below 50°.

the base (figure 6*d*). This situation is well approximated by the classical moth cases (Bernhard & Miller 1962; Bernhard *et al.* 1965).

A system of regular, radial ridges was reported to exist in the corneal surface of the tiny moth *Leucoptera coffeella* by Meyer-Rochow & Stringer (1993). They also found the same arrangement of microridges in the strongly curved facets of 'other species of tiny flying insects', with spatial dimensions similar to those of the nipple arrays of the larger insects. Parker *et al.* (1998) provided further data for extant flies as well as for an Eocene dolichopodid fly. The latter authors reproduced the ridge structures in photoresist and thus demonstrated a severe reflectance reduction of light incident over a large range of angles of incidence, to about 60°, especially for TE waves. The reported results correspond well with the calculations of figure 7. Many extant dolichopodid flies have facet lenses with minor nipples, however, and in fact have in the distal region of the facet lens alternating layers of high and low refractive index material (Bernard & Miller 1968). The multilayer structure acts as a spectrally selective reflector, which possibly functions to improve colour discrimination (Trujillo-Cenóz 1972; Stavenga 2002*a*). Extant brachyceran flies have facet lenses with front surface curvature slightly smaller than the lens diameter (Stavenga *et al.* 1990). The maximal angle of incidence is then at most 40°. The reflectance for light incident at this extreme angle does not severely deviate from that for normal incidence (figure 7), causing some doubt about the effect of the corneal ridges of flies. Furthermore, the corneal facets of the tiny insects investigated by Meyer-Rochow & Stringer (1993) and Parker *et al.* (1998) are remarkably flat in the centre and, therefore, reflectance reduction will there be minimal. Nevertheless, in some cases the facets appear to be very strongly curved at the lens periphery, so that the ridge structures could serve as an effective impedance matching device there. This may indeed be an important factor in mosquitoes, which have about 200 nm high, hexagonally packed nipples (Brammer 1970) in a virtually

hemispherical facet surface (Land *et al.* 1997). All the same, the resulting light sensitivity increase due to the corneal corrugations in dipterans will presumably be no more than a few per cent. This could still be useful, of course, as several mechanisms are known that enhance the sensitivity of insect eyes by only a small amount, e.g. the afocal optics of butterfly eyes compared to the conventional focal optics (van Hateren & Nilsson 1987), the tapetum basal to the butterfly rhabdom (Stavenga, unpublished work), or the sensitizing pigment in fly eyes (Stavenga 2004).

Nipple-like structures have been encountered in several insects that are evolutionary ancestral to moths and butterflies; for instance, Thysanura (Parker *et al.* 1998), Collembola (Bernhard *et al.* 1970; Barra 1971) and Trichoptera (Bernhard *et al.* 1970), and their presence hence must be considered a potential property of all insect facet lenses. We temporarily conclude that the most likely biological function of the nipple arrays is glare reduction, especially in the scaleless, transparent wings. An additional consequence of the nipple arrays in insect corneal facet lenses will be a slight improvement of the transmittance, which cannot be disadvantageous (Miller 1979). Neither of both functions seems to be crucial for butterfly eyes, however, as numerous species have low nipples or even have completely discarded them, as for example all known papilionids. This raises again the question of which eye type is ancestral in the Lepidoptera, and inextricably linked to this is the question whether the first moths were diurnal or crepuscular/nocturnal (Warrant *et al.* 2003).

The most likely evolutionary scenario for the corneal nipple arrays of butterflies is that the diurnal butterflies descended from nocturnal moths (Yack & Fullard 2000; Grimaldi & Engel 2005; Wahlberg *et al.* 2005). Most nymphalids, considered to be the least evolved butterflies, thus have retained the full-grown nipples of the moths, but the highly developed papilionids have completely lost the nipple trait.

A similar reasoning can be erected for the lepidopteran tapetum. Moth-eyes have extremely well developed tapeta, created by tracheoles that surround the fat rhabdoms. They form efficient reflectors that enhance light sensitivity as well as visual acuity (Warrant *et al.* 2003). Most diurnal butterflies have an intricate tapetal reflector proximally to each ommatidial rhabdom, which is formed by tracheoles, as in moths. The function of the tapetum is that light which travelled through the length of the rhabdom and reached the proximal end without having been absorbed is reflected back into the rhabdom, so having another chance of absorption. The diurnal butterflies thus feature a unique remnant of the extensive moth tapetum. The tapetal reflector is fully absent in papilionids, however, presumably because the gain in sensitivity is very slight. We recently found that this loss of tapetum also has occurred in certain pierids. The orange tip, *Anthocharis cardamines*, as well as the yellow tip, *Anthocharis scolymus*, appear to lack the tracheolar tapetum (Stavenga & Arikawa, unpublished work).

The hypothesis that butterflies developed from nocturnal moths runs somewhat counter to the view that the optical superposition eyes of nocturnal moths gradually developed from the afocal apposition eyes of diurnal butterflies (Nilsson *et al.* 1988). It may be too early yet to decide (Warrant *et al.* 2003), but we note that recently

studied nocturnal bees have not developed optical superposition eyes. The only major modification is a huge increase of the rhabdom diameter, whereas the apposition optics is essentially unchanged (Greiner *et al.* 2004).

As a final remark, we note that the corneal nipples of butterflies have a favourable consequence for optical studies on butterfly eyes. Epi-illumination of butterfly eyes with tracheolar tapeta reveals beautiful eye shines, which can be studied with large aperture optics when using an adequate set-up (Stavenga 2002b). Background light due to the reflecting facet lens surfaces is in many species appreciably suppressed by the corneal nipple arrays.

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