Optical modelling and phylogenetic analysis provide clues to the likely function of corneal nipple arrays in butterflies and moths

Authors

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Abstract

The lenses in compound eyes of butterflies and moths contain an array of nipple-shaped protuberances, or corneal nipples. Previous work has suggested that these nipples increase light transmittance and reduce the eye glare of moths that are inactive during the day. This work builds on but goes further than the analysis of Stavenga and co-workers [1] suggesting a functional role for these structures including, for the first time, an explanation of why moths are attracted to UV light. Using a phylogenetic approach and 3D optical modelling, we show empirically that these arrays have been independently lost from different groups of moths and butterflies and vary within families. We find differences in the shape of nipples between nocturnal and diurnal species, and that anti-glow reflectance levels are different at different wave-lengths, a result thereby contradicting the currently accepted theory of eye glow for predator avoidance. We find that there is reduced reflectance, and hence greater photon absorption, at UV light, which is probably a reason why moths are attracted to UV. We note that the effective refractive index at the end of the nipples is very close to the refractive index of water, allowing almost all the species with nipples to see without distortion when the eye is partially or completely wet and providing the potential to keep eyes dry. These observations provide a functional explanation for these arrays. Of special interest is the finding that their repeated and independent loss across lepidopteran phylogeny is inconsistent with the explanation that they are being lost in the ‘higher’, more active butterflies.

First, I apologize that my comments may include some irrelevant ones, since I am neither an entomologist nor a (bio-)physician. I hope some will be useful to improve the manuscript. This study
examined presence or absence and size ranges of the corneal nipple arrays in various lepidopteran species and estimate the light reflection on the nipple arrays based on the optical modeling of several types of arrays. Combination with phylogenetic analysis showed that the nipples have appeared and disappeared several times in the lepidopteran lineages and refuted the hypothesis that nipples are a vanishing trait associated with evolution. The authors discussed that that reduction in reflectance would be an incidental by-product of increased nipple height. They also suggest that one of the functions of the nipples is the reduction of distortion of images when water droplets sit on the nipple surface as, estimating the refractive index at the tops of the nipples is close to those of water. This article provides some new ideas on the functions of the corneal nipple array, and I recommend the publication after appropriate revisions.

To estimate the gradient of the refractive index on the structure, the refractive index of the material (chitin?) that form nipples should be necessary. Are there any measurements of the material? Please show the value, method, and representative reference.

Yes, Chitin on its own has been measured in the literature to be 1.61 [1] but from other references [2]–[5] the nipple material is 1.52 refractive index due to chitosan [1]. This was the value used in calculations and modelling, this has been included in the text along with more detail on the modelling method used.

I could not well understand the parameters in the estimation of the reflectance. Consider to add a schematic drawing of the section of the nipple array used in the simulation for 100, 200, 300 nm in height that correspond to Fig 5. (See Fig. 2c, f in [16] for reference.) The author should explain the incident angle of the light (º), interval of the nipples (nm)and arrangement of the nipples (grid or hexagonal), as well as the height (already shown). These parameters affect the reflectance. Although I do not know the examples in the insect research, but those in marine animals are found in Hirose et al. (J. Mar. Biol. Assoc. UK95:1025–1031, 2015) and Sakai et al. (Zool. Lett. 4:7, 2018).

Yes the modelling method could be clearer so we have created a new diagram which is hopefully clear without being misleading in terms of the optical theory behind it. This has been explained in detail in the text also. The simulation was carried out only for normal incidence light (0 degrees incidence) with spacing of 200nm between nipples. The array pattern was assumed hexagonal with equal spacing between all nipple axes. Although this was all input for calculating the effective refractive index,, the actual reflectance was simulated (following fundamental Fresnel equations) using ray tracing software where the effective medium layers (figure below) represented the layers of the nipple pattern going from top to bottom. Each nipple height has a different gradient refractive index and hence a different reflectance dependent on incident wavelength. The simulation software also allows us to include the refractive dispersion relationship, this allowed each layer’s refractive index (which changes depending on incident wavelength) to be adjusted according to the refractive dispersion of chitosan (included in new figure 5). This has been explained in the text as clearly and as concisely as we could without taking up too much of the papers focus.
UV-lights shorter than 280 nm are absorbed by the ozone layer and do not reach the ground, although Figure 5 ranges from 0 to 3000 nm. The author should mention this elsewhere.

Good point, this has been included in the text but also that man made light sources may still produce wavelengths in this region (e.g. UV lamps at 200-300nm). The modelling also does not hold for wavelengths lower than 200nm so these have been cut out of the graph in case misleading also.

New figure 5:

Line 146: Are there any reasonable explanations for the grouping based on nipple height, i.e., group 1–3?
We followed previous studies (e.g. Bernhard et al. 1970 and Stavenga et al. 2005.) and the nipples seemed to fall into these groups. We are certain about the need to have a group for the no nipples or small ridges group (group 1). There should be a group for the ~100nm height nipples (group 2), as these achieve the water matching and are mostly made up of domed top (see figure 1). Then we have group 3 for longer nipples.

Figure 2: Because scale bar is shown in each figure, magnification need not shown in the figure legends. Brightness and contrast should be adjusted for E and G, appropriately. We think the scale bar is still useful but we have adjusted brightness and contrast for E and G.

Figure 3: Consider to add phylogenetic tree based on [31] and order the species according to phylogenetic relatedness. There is no detailed species-level phylogeny for Lepidoptera – as we write “Even so, higher-level relationships within the Lepidoptera, and particularly within the species-rich subclade Ditrysia, are still generally far from understood.” The main problem is that the Erebidae have been split fairly recently and they are not all Noctuidae – some are Arctiidae, and these are constantly being moved around. With limited information available on the phylogeny of our species, and no reliable phylogeny that includes the Eribidae we decided not to muddy the waters by including a phylogenetic tree which could well be wrong. We have considered it and as we only have family-level data and no separate phylogeny for the Erebidae we decided not to add it.

Indicate diel/diurnal/nocturnal types. We have done this.

Line 204: I cannot understand what ‘UV end’ means. Show it as wavelength. Yes, this has now been included as UV end (<380nm as shown in figure 5)

Figure 5. Consider to add the curve of 0 nm height (flat surface) as a control. Clarify this is a real measurement or a simulation based on an optical model. This has been done, assuming the material is chitosan with refractive index 1.52 as discussed previously in the responses and explained in the text as to why that value.

Line 256–263: Consider to summarize this part in a table. We don’t think this would work very well as a table.

Line 305–307: Flat surface (0 nm height) should be compared with the 100-nm nipple array in Figure 5. Done.

Minor comments and corrections were directly added in the manuscript pdf.

Line 27: We think absorption is fine

Line 88: we are not sure How were the specimens dried.

Line 129: our results over the visible range match particularly well to Stavenga’s figure 6c – not a paraboloid model