Supplemental Data: The cause of colouration in the ctenophore *Beroë cucumis*

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Supplemental Experimental Procedures

Sample Preparation and Transmission Electron Microscopy

The lengths of comb row were fixed immediately after dissection in a 2.5% glutaraldehyde solution and left overnight. The following day, they were washed with sodium cacodylate buffer solution, before being osmicated in 1% osmium tetroxide for one hour in the dark. The osmicated samples were washed with distilled water and then dehydrated in an alcohol series, before being passed to propylene oxide, then araldite. The araldite-impregnated samples were embedded in araldite and dried in an oven at 60° for 48 hours. The resulting araldite blocks were trimmed with a razor blade and cut into thin sections with a Reichurt Jung "Ultracut", to give sections 1µm thick, which were examined by light microscope, to find a suitable part from which to cut ultra-thin sections 60-90nm in thickness. The ultra-thin sections were cut and the grids stained with saturated uranyl acetate solution and 1% lead citrate solution. They were examined with a Philips FEI Techni 12 Biotwin transmission electron microscope.

Optical Modelling of Reflection Properties

The animal's observed appearance *in* vivo changes, according to the location, orientation and state of deformation of the animal body, relative to the observer. In our model, these changes were accounted for by changes in the emergence angle: defined as that between the exiting light ray and the normal to the surface structure. This emergence angle is related to the incident light direction and, in an optimal situation, any light beam emergent to an observer below the animal arise from some point at the water surface, which can be considered as a spatially extended source of white light. For an animal that is seen from below, aiming vertically towards the surface, this gives an incidence-emergence plane along the axes of the cilia. As the animal changed location or its body deformed as it moved, this would lead to a change of orientation of the cilial structure

and produce variations on the emergence and incidence angles, which would change the dominant wavelength reflected to the observer.

Algebraic Derivation of Optical Model for Reflection

In order to predict mathematically the optical properties of the photonic crystal structure, we used an optical model based on that outlined below. In the first instance, we will treat the structure as if it were divided into planes parallel to the surface and averaged the refractive indices within each of these planes.

The whole structure has a three-dimensional average refractive index $\overline{n}$. The dispersion equation for light waves propagating normal to the multilayer structure in this average homogeneous material is

$$\omega = k_z \frac{c}{\overline{n}}$$

where k_z is the normal wave-number, c is the speed of light and ω is the angular frequency. This equation is modified slightly by the distribution of the microtubules, because they have refractive index that is higher than the surrounding solution and higher than the average, $\overline{n}$, so they introduce a periodic contrast in refractive index.

When (light) waves travel in media with regular (periodic) refractive index contrasts, such as in this structure, only waves whose wave-numbers differ by an integer amount of $2\pi/a$ (where a is the one-dimensional lattice parameter) can interact and produce a band gap. The only values of angular frequency (ω) for which this interaction occurs are those located at the edges of what is called the "Brillouin zone" of the Bravais lattice, which extends between k_z = - n/a and k_z=π/a and at integer multiples of these wave-numbers. That is to say that band gaps are only produced at wave-numbers given by

$$k_z = m\frac{\pi}{a}$$

(m being an integer: $m = 1, 2, 3...$). The normal wave-number k_z is related to the wave frequency and to the conserved parallel component k_y of the wave travelling through the structure

$$k_z = \sqrt{\left(\bar{n}\frac{\omega}{c}\right)^2 - k_y^2}$$

If the one-dimensional photonic crystal ends at a surface, we can make a connection between the wave travelling in the structure and an incident wave, because the lateral wave number k_y does not change when the medium is homogeneous in a lateral plane. In water (refractive index n_0), with an incidence angle θ, this is

$$k_y = n_0 \frac{\omega}{c}\sin\theta$$

Finally, using these equations, we can calculate the wavelengths where a "multilayer" directional gap occurs. These can be expressed as

$$\lambda = \frac{2a}{m}\sqrt{\bar{n}^2 - n_0^2 \sin^2\theta}$$

Since we are interested in visible light wavelengths (rather than, for example, microwave radiation), we are only concerned with the integer values of m which lead to visible light wavelengths. When only one integer value, m, leads to a visible wavelength, λ, the coloration of the multilayer is readily determined from this formula.

With this particular structure the period, a, is the length of the unit cell, in the direction normal to the structure surface, after the refractive index has been laterally averaged and was calculated as $a = (215\text{ nm}) \times \sin(77°) = 210\text{ nm}$. The mean refractive index can be estimated to be 1.47 (somewhat larger than water, but smaller that the cilial index). (see below for choice of cilial index).

The model we used is described elsewhere [S1]; but is based upon a more complex version of the calculations described above, in which the refractive index has not been averaged in any dimension, but, instead, the periodicities in refractive index contrast have been retained in both dimensions.

Choice of Cilial Refractive Index

The refractive index of cilia, or, more accurately, of their microtubules is not known and could not be measured experimentally in this instance. The refractive indices of their chemical components - tubulin, dynein and nexin, have also not been reported, so it was necessary to make an estimate. The basis of this estimate was as follows- the refractive index of sea water (and therefore cytosol) is 1.34, so the lowest refractive index a biological material can have above this (to 2 decimal places) is 1.35. The highest refractive index found in a biological colour-producing structure is 1.83, corresponding to guanine. So we assumed the refractive index of the microtubules to lie within the range 1.35 to 1.83 and chose the mid-point 1.57 for the purposes of our calculations. Many biological materials found in colour-producing structures have refractive indices within the range 1.47 to about 1.57 (e.g. chitin=1.52, keratin=1.53, collagen= 1.47), so 1.57 seems a reasonable value. If the true refractive index is higher, the reflected wavelength will experience a red-shift which, at most, will not exceed 7%, whereas, if the true refractive index is lower, the reflection band will be displaced to shorter wavelengths, but this shift will be well under 6%.

Optical Model for Light Transmission

The transmission properties of the *B.cucumis* photonic crystal structure were calculated as a function of the angle of incidence for light of a wavelength of 489nm, using the same model as we used for calculating the structure's reflection, above. In such calculations, the refractive indices of the structure's component materials are often adjusted to account for the fact that all materials absorb some of the light travelling within them. In this case, the distances of propagation of the light waves through the structure are too short for significant absorption to occur so adjusting the refractive indices to allow for absorption is not justified.

Supplemental Figure Captions

Figure 2 (main article)

(A) The two dimensional photonic structure is the result of the arrangement of parallel cilia with reinforced refractive index. In the plane normal to the common cilia axis

direction, the invariance is limited to translations which correspond to integer multiples of the vectors $\vec{a}_1$ and $\vec{a}_2$. The sides of the square section of the highly-refractive cilia (with refractive index $n_r = 1.57$) is h=40nm in each direction. These cilia are inserted in a transparent tissue with refractive power very similar to that of water (n_0=1.34). Each parallelogrammatic unit cell defined by the vectors $\vec{a}_1$ and $\vec{a}_2$ contains 10 identical microtubules: one of them is the axoneme; the others are evenly distributed on a circle of diameter d=146nm about the axoneme axis. None of these microtubules overlap in the final crystalline structure.

(C) The incidence plane contains the surface normal and this translation vector $\vec{a}_1$.

The polarization is TM, which means that the spectra relate to light rays where the magnetic component of the incident radiation is normal to the incidence plane. This is the polarization of most of the light crossing the sea surface into the sea. The reflectance is weak everywhere in the visible range, except for a single narrow band, which shifts rapidly to the blue when the angle of incidence is increased. (D) Transmission predicted through the structure shown in Figure 2, assuming it to be 512 cilia thick for light of wavelength of exactly 489 nm.

Supplemental References

S1. Pendry, J.B., and MacKinnon, A. (1992). Calculation of photon dispersion relations. Phys. Rev. Lett. 69, 2772–2775.