Wrinkles enhance the diffuse reflection from the dragonfly *Rhyothemis resplendens*

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The dorsal surfaces of the hindwings of the dragonfly *Rhyothemis resplendens* (Odonata: Libellulidae) reflect a deep blue from the multilayer structure in its wing membrane. The layers within this structure are not flat, but distinctly ‘wrinkled’, with a thickness of several hundred nanometres and interwrinkle crest distances of 5 μm and greater. A comparison between the backscattered light from *R. resplendens* and a similar, but un-‘wrinkled’ multilayer in the damselfly *Matronoides cyaneipennis* (Odonata: Calopterygidae) shows that the angle over which incident light is backscattered is increased by the wrinkling in the *R. resplendens* structure. Whereas the reflection from the flat multilayer of *M. cyaneipennis* is effectively specular, the reflection from the wrinkled *R. resplendens* multilayer spans 1.47 steradians (equivalent to ±40° for all azimuthal angles). This property enhances the visibility of the static wing over a broader angle range than is normally associated with a smooth multilayer, thereby markedly increasing its conspicuousness.

1. Introduction

Brilliantly coloured, structurally based reflectance can be observed in a range of flora and fauna [1–4]. These colours are often iridescent, saturated and brighter than colours resulting from the more common colour-production method of wavelength-selective absorption by pigments [1]. Many insects exhibit sub-micrometre-sized photonic structures which produce a variety of interesting optical effects [5–7]. These are associated with, for example, signalling for courtship or camouflage [8–11].

In particular, several species of Odonata (dragonflies and damselflies) have been shown to exhibit structural colour [12–16]. This is predominantly achieved by multilayer interference from structures within their wing membrane, usually comprising alternating chitin and melanochitin layers. The damselfly *Neurobasis chinensis*, for example, exhibits a bright, saturated green reflected iridescence from its wing membranes [13]. Similarly, the damselfly *Calopteryx japonica* reflects green, gold and red colours from various regions on its thorax and abdomen, and metallic blue from the wings [14]. More recently, the damselfly *Matronoides cyaneipennis* was shown to produce brilliant blue and green iridescent reflectance from the dorsal and ventral sides of its hindwings, respectively [16]. This arises from two subtly different multilayer structures, one on either side of its hindwing membrane. A few examples of structural colour from non-multilayer structures in Odonata have also been reported. For instance, blue reflectance from the bodies of the damselfly *Enallagma civile* and dragonfly *Anax junius* arises from the coherent scatter of light from close-packed arrays of spheres in the endoplasmic reticulum of these regions’ epidermal pigment cells [17]. Where photonic structure is absent, reflected and transmitted intensity can often vary owing to scattering from the membranes’ two surfaces. This can manifest itself in the form of leaky guided modes, for instance, measured in the *Aeshna cyanea* dragonfly [18].

A study of the optical properties of the damselfly *C. japonica* has shown that the chitin and melanin components that comprise the multilayer structures in
2. Methods

2.1. Animals

Male specimens of *R. resplendens* were collected from a sun-dappled alluvial forest stream (shared with very large crocodiles) draining Eubenangee swamp in North Queensland, Australia (17° 24′30″ S, 145° 58′33″ E, 6 m.a.s.l., 1.iv.1999, leg. A.G. Orr; deposited in A.G. Orr collection). The wings were removed from the body to enable flat mounting on a microscope slide for imaging. Small sections were also removed and prepared for microscopy (TEM), atomic force microscopy (AFM), reflectance scanning electron microscopy (SEM), transmission electron microscopy and imaging epoxy resin flowed by sputter-coating the structure with approximately 8 nm of gold palladium. A Nova 600 NanoLab Dualbeam system was used with an electron beam voltage of 30 kV, a 0.13 nA beam current and a 5 mm working distance to image the sample.

2.2. Optical imaging

The wings of *R. resplendens* were imaged under bright-field epi-illumination using a Zeiss Axioscopic MRc5 connected to a Zeiss Axioscope 2 optical microscope, with lenses providing a range of magnifications.

2.3. Scanning electron microscopy

SEM imaging was undertaken after mounting a small region of the wing membrane onto an SEM stub with electrically conducting epoxy resin flowed by sputter-coating the structure with approximately 8 nm of gold palladium. A Nova 600 NanoLab Dualbeam system was used with an electron beam voltage of 30 kV, a 0.13 nA beam current and a 5 mm working distance to image the sample.

2.4. Transmission electron microscopy

TEM of wing sections was undertaken after fixing samples in 3% glutaraldehyde at 21°C for 2 h followed by rinsing in sodium cacodylate buffer. Subsequent fixing in 1% osmic acid in buffer for 1 h was followed by block staining in 2% aqueous uranyl acetate for 1 h, dehydration through an ethanol series ending with 100% ethanol, and embedding in Spurr resin. After ultramicrotoming, sample sections were stained with lead citrate and examined using a JEOL 1018 TEM instrument.

2.5. Atomic force microscopy

Small, flat regions of *R. resplendens* and *M. cyaneipennis* wings were cut and mounted on an AFM stub, then placed in a Bruker Innova AFM. The surface profiles from several 50 × 50 µm² regions within this sample were recorded using a semi-contact/tapping mode.

2.6. Reflectance spectrometry

A small region of the wing, with a diameter of 1.0 ± 0.2 mm, was fixed to the tip of a stretched glass pipette needle. This needle was positioned, so the sample was held at the exit hole of an integrating sphere. Light was incident from a 400 µm diameter optical fibre connected to an Ocean Optics HPX-2000 high-power xenon light source. After passing through a pinhole, this was focused onto the sample, the reflectance from which was collected by an Ocean Optics ISP-50-8-R-GT integrating sphere and measured using an Ocean Optics 2000+ high-resolution USB spectrometer.

2.7. Imaging scatterometry

The same samples that were used for reflectance spectrometry were also used for imaging scatterometry. These samples were positioned in an imaging scatterometer, allowing the backscattered light from a small region of this sample (beam spot size was approx. 0.5 mm²), under narrow-angle illumination, to be imaged. For specifics of this technique, see Stavenga et al. [20] and Vukusic et al. [21].

2.8. Finite-difference time-domain modelling

To simulate the electromagnetic response of theoretical models of the *R. resplendens* structure, finite-difference time-domain software was employed. Periodic boundary conditions were applied to the sides of many models’ unit cells, each of which represented two-dimensional cross sections of the physical structure. Each unit cell was between 50 and 71 µm wide (i.e. corresponding either to the length, width or diagonal of the AFM data field shown in figure 2b). This simulates a two-dimensional structure which is infinitely repeating in the x-direction (figure 4). An incident wave-port was assigned to deliver a broadband pulse of radiation, comprising wavelengths from 400 to 700 nm.

3. Results

Figure 1a,b shows optical images of a *R. resplendens* hindwing. The structural colour of *R. resplendens* is predominantly a deep blue/teal with the associated blue-shift of peak reflectance with increasing angle that is a characteristic of multilayer structures. This coloured reflectance is produced by coherent scattering from the structure shown in the TEM image of the wing-cell cross section (figure 1c). Here, a clear multilayer structure is present; however, unlike in most previously documented animal multilayers, the key interference layers in the top half of the membrane are not flat. Owing to the size and extent of the associated modulation in surface profile, we describe their appearance and physical configuration as ‘wrinkled’.
Figure 1. Images of R. resplendens and its photonic structure. (a) Photograph of R. resplendens (cropped version of original photograph by Bill Higham; adapted from Wikimedia Commons CC-BY-2.0). (b) Photograph of a region of the R. resplendens hindwing showing its blue/teal colour and its vein/cell structure. (c) TEM image of a cross section through a R. resplendens hindwing membrane. Scale bars are (b) 2 mm and (c) 1 μm.

The electron and atomic force microscopy images in figure 2a,b detail R. resplendens’ wing surface form. In both cases, the surface wrinkling of the wing-membrane structure can be discerned clearly. The wrinkling of the structure was quantified using AFM to obtain the profiles from several 50 × 50 μm² regions of the R. resplendens wing membrane. The surface profile of the M. cyaneipennis multilayer system was also measured in the same manner for comparison. This species was chosen for comparison as it is also an odonate system which reflects a similar colour, but from an unwrinkled multilayer structure. A two-dimensional surface-profile measurement of the wing membrane of R. resplendens, and one-dimensional line-profiles extracted from this, are presented in figure 2b,c. These show that the height variation of its membrane surface is approximately 500 nm. The analogous membrane and integument surfaces of other structurally coloured insects, such as many Coleoptera and Diptera, exhibit no such height variation [13,14,16,22]. For example, the surface height variation of M. cyaneipennis is much less than half that in R. resplendens (figure 2d,e). The height variation in M. cyaneipennis also occurs over a shorter length scale, namely it constitutes a surface roughness, rather than the surface wrinkling we describe for R. resplendens.

For a visual comparison between the wrinkling of R. resplendens and that of M. cyaneipennis, a 5 μm moving-average filter was applied to their measured height variations. This filter effectively removed the height variations that occur over shorter length scales which correspond to surface roughness. This leaves only the height variations that occur over longer length scales and which we describe as corresponding to surface wrinkling. To quantify the wrinkling spatially, fast Fourier transforms (FFTs) of the AFM-measured surface profiles of both R. resplendens and M. cyaneipennis were taken. A broad peak in the FFTs of R. resplendens’ AFM data can be seen below 0.2 μm⁻¹. This corresponds to effective wrinkle spacings of 5 μm and greater. This is absent from the FFTs of the M. cyaneipennis AFM data. While surface roughness is often present in biological structures as a result of both imperfections in structure and functional surface coatings [18,23,24], surface wrinkling is, however, far less common.

In order to obtain an estimate of the extent to which this type of wrinkling alters the reflectance and the optical properties of a multilayer structure, the R. resplendens and M. cyaneipennis samples were examined using an imaging scatterometer arrangement. A comparison between the two experimentally captured scattergrams is presented in figure 3.

While the measured reflectances of the two wings have a comparable peak wavelength (albeit arising from a more saturated colour in M. cyaneipennis), the scattergrams in figure 3 show that the solid angle over which incident light is scattered is much larger for R. resplendens than it is for M. cyaneipennis. As both these samples are blue-reflecting odonate multilayer structures, comprising the same constituent materials, this strongly suggests that the increased angle of scatter is due purely to the presence of the multilayer wrinkling in R. resplendens.

To confirm this, two contrasting theoretical models corresponding to representative two-dimensional cross sections of the R. resplendens’ photonic structure were created, one that included wrinkling (figure 4) and one that comprised equivalent multilayering but which was perfectly flat. The thicknesses of the modelled layers were determined from many measurements of individual layers using TEM images. The optical response of the flat multilayer model is shown in figure 5 (inset). To simulate the optical effect of the wrinkling, the multilayers were then convolved with a series of wrinkled profiles that matched those of many typical AFM-measured profiles of the sample’s surface (for instance the profile shown in figure 2c). These were taken from many different directions across the sample’s wing membrane. The
averaged (and angle-dependent) optical scatter from this ensemble of representative two-dimensional models is shown in figure 5. While this approach does not yield perfect three-dimensional model replicas of the insect’s original structure, it provides a viable way to accommodate the real variation of profile across the insect’s three-dimensional surface at a fraction of the computational cost. This approach has been successfully used before to model irregular structures on insect wing surfaces [25,26]. The far-field scatter patterns produced by these wrinkled models were averaged to give the wavelength- and the angle-dependent scatter plot shown in figure 5. This process was repeated for the flat models (figure 5, inset).

These modelling data show a peak reflection wavelength of approximately 475 nm at normal incidence; this fits well with the experimental reflectance measurement shown in

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**Figure 2.** Rhyothemis resplendens surface wrinkling analysis. (a) SEM image of the R. resplendens’ (wrinkled) wing membrane surface. (b) AFM image of the surface profile from a section of R. resplendens’ wing membrane. The colour axis represents the surface height at the corresponding X–Y location. (c) A line plot of the surface profile taken from the AFM image in panel (b). (d) Line plot from an AFM surface profile taken from M. cyaneipennis’ (flat) wing membrane (for (c,d), the black lines represent measured data, and the blue lines represent a 5 µm moving average applied to these data). (e) A comparison between the filtered profiles of R. resplendens (red line) and M. cyaneipennis (green line). (f) Fast Fourier transforms (FFTs) of the R. resplendens AFM line profile (red line) and of the M. cyaneipennis profile (green line). Scale bar in panel (a) is 2 µm.

**Figure 3.** A comparison between the optical properties of R. resplendens and M. cyaneipennis. (a) Integrating-sphere-measured reflectance from R. resplendens (red line) and M. cyaneipennis (green line). (b) Experimentally imaged scattergram of R. resplendens. (c) Experimentally imaged scattergram from a similar, but unwrinkled, multilayer structure (M. cyaneipennis) for comparison. The red lines correspond to scattered angles of 5°, 30°, 60° and 90°. (Note the sample in panel (c) was rotated to an angle of approximately 12°, as at normal incidence the sample’s scatter is blocked by the sample shadow.)
Figure 4. A comparison between natural and modelled R. resplendens structures. (a) TEM image of a cross section through a region of the wrinkled structure. (b) The binary image generated from the AFM measurement in figure 2 to be used for theoretical modelling. Scale bar in panel (a) is 2 μm.

Figure 5. Modelled wavelength-dependent and angle-dependent scatter from the wrinkled multilayer structure arising from broadband, normal-incidence illumination. Inset shows scatter results for the equivalent planar (unwrinkled) multilayer.

Figure 6. A comparison between theoretically calculated and experimentally captured scatter from R. resplendens. (a) Experimentally measured scatter from an R. resplendens wing-cell region and (b) the theoretical scattergram showing the far-field scatter from the modelled R. resplendens wrinkled multilayer structure for comparison.

4. Discussion

The surface (and underlying multilayer) profile of R. resplendens’ wing membranes is strikingly different from that of M. cyaneipennis and of other previously documented odonate wing membranes [13,16]. Although the surfaces of biological photonic structures are rarely completely flat, their profile variations are largely owing to inherent surface roughness, the common result of what we have called biological noise, owing to their complex growth dynamics. The type of wrinkling in R. resplendens, however, which extends below the surface, fundamentally changing the photonic structure, is certainly uncommon. The effect of the structural wrinkling is evidenced by comparing the light scattered both by wrinkled (R. resplendens) and by unwrinkled (M. cyaneipennis) multilayer wing membranes. The scattergrams in figure 3 show that light incident on a region of R. resplendens' wrinkled wing membrane is scattered over a much broader angle range than it is by the unwrinkled analogue wing membrane of M. cyaneipennis.

This striking increase in the angle over which incident light is scattered enables coloured reflectance from multilayer interference to be visible over a much wider range of angles than is normally possible for flat multilayer structures, thereby markedly increasing the solid angle over which light from the R. resplendens system is broadcast. The colour scattered from conventional, flat multilayer structures is visible only at specular angles. For a species with a macroscopic wing structure comprising different orientations of individual wing cells, this often leads to the reflected colour being visible only from certain cells, creating a fractured, sparkling colour appearance over the entire wing. This is described elsewhere for M. cyaneipennis [16]. For the wrinkled structure of R. resplendens, however, this is not the case, as the increased angular visibility of the wing membrane’s reflectance allows for viewing over a greatly increased range of viewing angles.

Similar strategies in other biological photonic systems allow colour from a multilayer-type structure to be reflected to non-specular angles. For instance, the ‘boomerang’-shaped breast feather barbules of the Pharotis innessi bird of paradise (Aves: Paradisaeidae) reflect different wavelengths of light in different directions; shorter (blue) wavelengths of light are predominantly reflected by the thin-film barbule envelope to approximately 60°, whereas longer (yellow) wavelengths are reflected back by the multilayer located within the barbule [27,28]. Morpho butterflies (Lepidoptera: Nymphalidae: Morphinae) also exhibit diffuse multilayer reflectance; this is
achieved by the additional diffraction and scattering conferred by the spacing between each individual discrete ‘Christmas-tree’-shaped photonic structure [5,29]. In some *Morpho* species, there is an additional layer of ‘glass’ scales that increases angle-broadening scatter of incident light [5,30].

Recently, a mechanism for producing structural whiteness via multilayer-type interference has been elucidated. The ‘pyjama squid’ *Sepioloides lineolata* (*Sepiida: Sepiidiidae*) contains clusters of iridophores comprising iridose plate stacks; although small regions of this structure produce coloured reflectance, it has a diffuse white macroscopic appearance [31]. Diffuse structural whiteness can also be produced by disordered structures such as those seen in the *Cyphochilus* sp., *Lepidia stigma* (*Coleoptera: Scarabidae*) and *Calothyris margaritifera* (*Coleoptera: Cerambycidae*) beetles [25,32]. The random sizes and arrangement of filaments in their scale cross sections lead to their bright-white angle-tles [25,32]. Diffuse, but coloured, reflectance in their scale cross sections lead to their bright-white angle-independent appearance. Diffuse, but coloured, reflectance can also be created with quasi-ordered structures possessing short range, but not long range, order. This can be seen, for instance, in the diffuse colour reflected from blue scales of the *Eupholus magnificus* (*Coleoptera: Curculionidae*) weevils [33]. Ordered photonic crystals (PCs) in weevils can also produce diffuse reflectance, especially where the structure is polycrystalline, namely where the structure comprises many individual PC domains, each with a different orientation, thereby projecting the colour they reflect over a range of angles [10,33]. This strategy of PC domaination, to increase the angular range over which colour is scattered or suppress iridescence, is also employed by the butterflies *Callophrys rubi* (*Lepidoptera: Lycenidae*) [34] and *Parides sesostris* (*Lepidoptera: Papilionidae*) [35].

The wrinkling of the *R. resplendens* structure represents a different approach for obtaining diffuse reflectance from a multilayer-type system, and as such, is likely to be of interest to behavioural biologists and optical physicists/engineers alike. Wing coloration in odonates (and many other animals) typically has an intraspecific semiotic function in courtship and territorial display, hence it is in the evolutionary interests of the individual that these visual signals are communicated to conspecifics with maximal efficiency and intensity. In odonates, exhibiting structural wing colour thus far studied, a significant element of their visibility and conspicuousness derives from the movement of the wings as they are presented in flight [11,36].

In members of the genus *Rhyothemis* generally the wings are boldly patterned, but not necessarily iridescent. The pattern occurs in both sexes and hence it is probably more important as a species recognition signal with no special function in courtship. It is, however, likely to signal territory occupancy, of either breeding or feeding territories. *R. resplendens* is a rather sedentary and solitary insect which perches for long periods in sunny patches over water in alluvial forest or forested swamp, generally in dappled sunlight with a dark background (A.G. Orr 1999, unpublished data). Males defend sections of habitat, attacking intruding conspecific males. They perch always with their wings open, either depressed as in figure 1a, or held in several other static postures so that the brilliant uppersides are exposed to direct sunlight and are highly visible. The broad angle over which the brilliant blue signal is broadcast would serve to indicate to conspecifics from afar that a territory was occupied, with minimal expenditure of energy on the part of the perching insect. In the case of a territorial male, this signal might be expected to deter invading males, thus avoiding physical conflict, or attract females seeking a mate. For whatever reason, by a subtle adaptation in design, this insect has evolved a highly efficient mechanism of advertising its presence to conspecifics.

5. Conclusion

*Rhyothemis resplendens* is a dragonfly which reflects a deep blue structurally based colour from the dorsal side of its fore- and hindwings. Unlike conventional flat multilayer structures, *R. resplendens*’ structure is distinctly wrinkled; the dorsal multilayer follows a profile which varies in height by about half a micrometre owing to this wrinkling. Experimentally measured scatter from this structure and that from a similar, but unwrinkled odonate multilayer, shows that the angle over which incident light is scattered is greatly increased by the presence of the wrinkling. This is confirmed by theoretical models that compare the far-field scatter produced by wrinkled and unwrinkled versions of the *R. resplendens* multilayer structure.

Owing to the habit of *R. resplendens* of perching for long periods in dappled forest sunlight with wings held open and stationary this would appear to be a system by which a species identification signal can be broadcast over a wide area with a minimal expenditure of energy.

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References


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