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Structurally assisted blackness in butterfly scales

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Surfaces of low reflectance are ubiquitous in animate systems. They form essential components of the visual appearance of most living species and can explicitly influence other biological functions such as thermoregulation. The blackness associated with all opaque surfaces of low reflectivity has until now been attributed to strongly absorbing pigmentation alone. Our present study challenges this assumption, demonstrating that in addition to the requirement of absorbing pigmentation, complex nano-structures contribute to the low reflectance of certain natural surfaces. We describe preliminary findings of an investigation into the nature of the black regions observed on the dorsal wings of several Lepidoptera. Specifically, we quantify the optical absorption associated with black wing regions on the butterfly *Papilio ulysses* and find that the nano-structure of the wing scales of these regions contributes significantly to their black appearance.

Keywords: butterfly; blackness; structural colour; photonic structure

1. INTRODUCTION

Opaque synthetic surfaces that absorb 99.6% of incident light have recently been produced by chemical etching of electroless-deposited nickel–phosphorus (Brown *et al.* 2002). It was found that an optimum phosphorus content and etching regime were required to produce the appropriate surface morphology to minimize this reflectivity and thus enhance the absorption. While such ultra-blackness is technologically crucial in areas associated with the operation of optical instruments, it is equally important in natural systems. Controlled absorption of incident solar radiation is the principal method of temperature regulation in most insects (Nijhout 1991), for instance. As blackbody laws (Hecht 1974) of absorption control such thermoregulation, the quality of the blackness, or equally of the absorption, owing to an insect's surface, will have a bearing on its wing and body temperature. In addition to temperature control, however, visual appearance is also underpinned by absorption. The visual appearance of many brightly coloured creatures, especially insects, is influenced by both the quality of the dark frame that surrounds their colour regions as well as the underlying and absorbing medium beneath them. An indistinct border to a coloured region renders it significantly less conspicuous (Silberglied 1984). A strongly back-scattering substrate, rather than an absorbing one beneath a structurally coloured region, creates a saturated reflection that is relatively unremarkable (Vukusic *et al.* 2004). In terms of visual conspicuousness therefore, there is a need for effective broadband absorption to augment bright colour.

Lepidoptera form an ideal order from which to choose species that exhibit varying degrees of broadband absorption on their wings. The colours that signify this absorption exist as a range of light to dark browns and blacks. In all but a few Lepidoptera, the seat of both pigmentary and structural colour, for strong absorption or bright reflection, is associated with the scales, which imbricate both ventral and dorsal surfaces of each wing. Generally, there are two layers of scales on each surface; a superficial one often referred to as a cover scale (Ghiradella 1991) and a ground scale (Ghiradella 1991) that lies closer to the wing substrate. Very often a cover scale and its underlying ground scale may differ in pigment content, in structural design (Tada *et al.* 1998; Vukusic *et al.* 2000) and in biological function (Nijhout 1991).

A male *Papilio ulysses* butterfly was chosen for this preliminary study because it exhibits two regions of different blackness across parts of its dorsal wings; one region is deep matt black in appearance whereas the other is optically a much more lustrous black. These black regions completely frame the intense iridescent blue colour of the inner regions of both fore and hind dorsal wings.

2. MATERIAL AND METHODS

A dead male specimen of *P. ulysses jorea* was obtained from Worldwide Butterflies Ltd (Dorset, UK). Wing sections from the matt black and more lustrous black regions were used for scanning and transmission electron microscopy (SEM and TEM). A Hitachi S-3200N electron microscope was used for SEM, samples firstly cold sputtered with 4 nm of gold. TEM analysis 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 then followed by block staining in 2% aqueous uranyl acetate for 1 h, dehydration through an acetone series (ending with 100% acetone) and embedding in Spurr resin (Spurr 1969). Post microtomed sample sections were stained with lead citrate and examined using a JEOL 100S TEM instrument.

To determine the extent of the optical absorption associated with the black dorsal regions of *P. ulysses*, single scales were removed from the appropriate wing regions and mounted by their basal end onto ground-down tips of needles. Each needle-mounted scale was in turn positioned at the centre of an Euler Cradle, its centre coincident with the path of a laser beam. In this way, and using a scanning photodiode detector, optical reflection and transmission data could be taken on the single scale at any chosen angle (see Vukusic *et al.* (1999) for a full description of this method). Absolute values of reflection from, and transmission through, each scale were determined by successively illuminating each scale with a calibrated intensity of light from collimated lasers. An argon-ion laser and three helium–neon lasers provided six accessible wavelengths across the visible spectrum. These optical measurements were then repeated while each scale was immersed in liquid bromoform, a fluid with a refractive index closely matching that of the cuticle comprising the wing-scale structure.

3. RESULTS

Minimal reflectivity (R, less than 2%) was measured from each scale in air. Transmission (T) through each scale in air was measured for each available incident wavelength. With these data, the optical absorption associated with each scale was calculated; it was between 90% and

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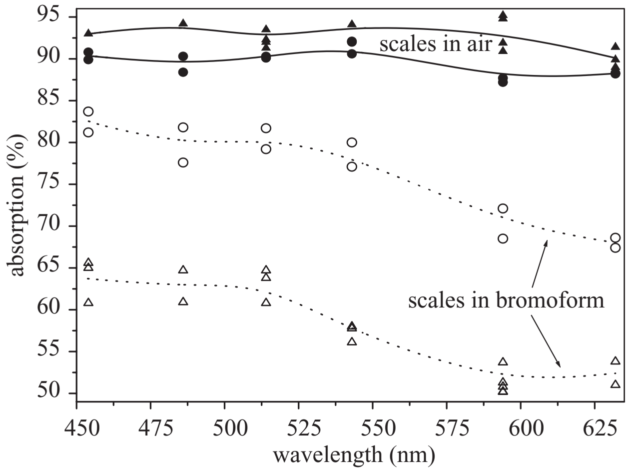

Figure 1. The wavelength-dependent absorption (at normal incidence), in air and immersed in bromoform, of single black scales from the lustrous black (filled and open circles) and the matt black region (filled and open triangles). The filled symbols represent data collected for the scales in air, while the open symbols represent data for the scales in bromoform. (The solid and dotted lines are included to allow for easier data tracking).

95% of all normally incident light at each wavelength (figure 1). To examine the extent that microstructure played in the optical absorption associated with each scale, the optical experiments were repeated while the scale on its needle was immersed in bromoform fluid. This process matches the refractive index of the scale material to that of its surroundings and effectively eliminates the optical functioning of the scales' nanostructure. Light incident on the scale will therefore only be subject to the absorption within it, and not to any interference, scattering or diffractive effects associated with the scale structure.

The data in figure 1 show the difference in absorption between a single scale from each black region before and after the structure has been index matched by bromoform. For single scales from the matt black region of wing, the immersion in bromoform causes a *ca*. 40% decrease in the optical absorption of the scale (this amounts to a four- or fivefold increase in transmission through the scale); for single scales from the lustrous region, there is a *ca*. 20% decrease in this absorption (derived from the two- or threefold increase in transmission through the scale).

4. DISCUSSION

The evidence from the index-matching experiments clearly shows that effective removal of the scale structure (by immersion in index-matching fluid) reduces the optical absorption of the scale. Without such a nanostructure, even with the same quantity of absorbing pigment, each scale would be a less efficient absorber of incident radiation; backscatter from the scales and wing substrate beneath these scales would create the appearance of a wing surface of inferior blackness.

Preliminary modelling confirms that one of the principal optical functions of the scale structure is to scatter incident radiation towards the ridging and about the scale interior. This has the effect of increasing the path length of the incident light through the absorbing pigmentation diffusely spread (Fox 1976; Nijhout 1991) throughout each scale. The structure of the black scales of *P. ulysses* (figure 2) is typical of dark brown and black scales found on many other Lepidoptera. Periodic ridging, of pitch *ca*. 2–3 μm runs the length of these scales. Between their ridging, scales from both regions exhibit an aperiodic latticework of struts and walls that extend from the surface toward the scale substrate beneath (figure 2*c*,*d*).

The change in optical absorption, on immersion in index-matching fluid, is different for both scale types. This is solely a consequence of their structural differences, which are particularly evident in images of the scale cross-sections (figure 2*c*,*d*). The scales from the matt black region (in figure 2*b*,*d*) clearly comprise a more intricate and densely distributed lattice of cuticle than the scales from the lustrous black region. Light that is incident on this structure, therefore, is more efficiently scattered toward the diffusely distributed pigmentation.

The tapering of the ridging in both scale types is also a significant feature. Optically, reflection from surfaces (not including interference effects from multilayering) is brought about by abrupt changes in optical impedance between the material and the medium surrounding it. Therefore a gradual transition of optical impedance from one medium to the next reduces the magnitude of this reflection. Normally in insect systems this is done using nanostructure of subwavelength dimensions (Bernhard 1965). However, the ridge tapering in this species, although larger than conventional anti-reflective nanostructure, also serves an impedance-matching purpose. In this way it reduces the extent of back-reflection and scatter when incident light first encounters the scale. Closer inspection of a single ridge cross-section reveals that the tapered sides of each ridge comprise the more typical subwavelength impedance-matching elements (figure 2 insert); optically, these reduce backscatter and reflection from the ridge surfaces at non-normal angles of incidence.

The lustrous patches on the dorsal forewings are only found in male *P. ulysses* specimens (D. Vane-Wright, personal communication). The lustre is not created by the highly absorbing black ground scales examined here and shown in figure 2*a*,*c*. It is instead created by low-intensity scatter from microstructures that comprise very long hair-like androconial scales that overlie the ground scales. Their principal function is presumed to be associated with pheromone storage or transfer (D. Vane-Wright, personal communication). In *P. ulysses*, these scales have minimal absorbing pigment; multi-wavelength scatter from their surfaces, although relatively inefficient, is sufficient to produce the optical effect of surface lustre. Incident light that is not scattered by the androconial scales in these regions, is transmitted through them to be efficiently absorbed (*ca*. 90%) by the underlying ground scales.

Until this preliminary study, nanostructure was not regarded as an intrinsic component of highly absorbing natural surfaces. The experiment described in this paper shows that such a nanostructure as is found in many Lepidoptera, assists in creating strong optical absorption and significantly enhances the appearance of black on their wings.

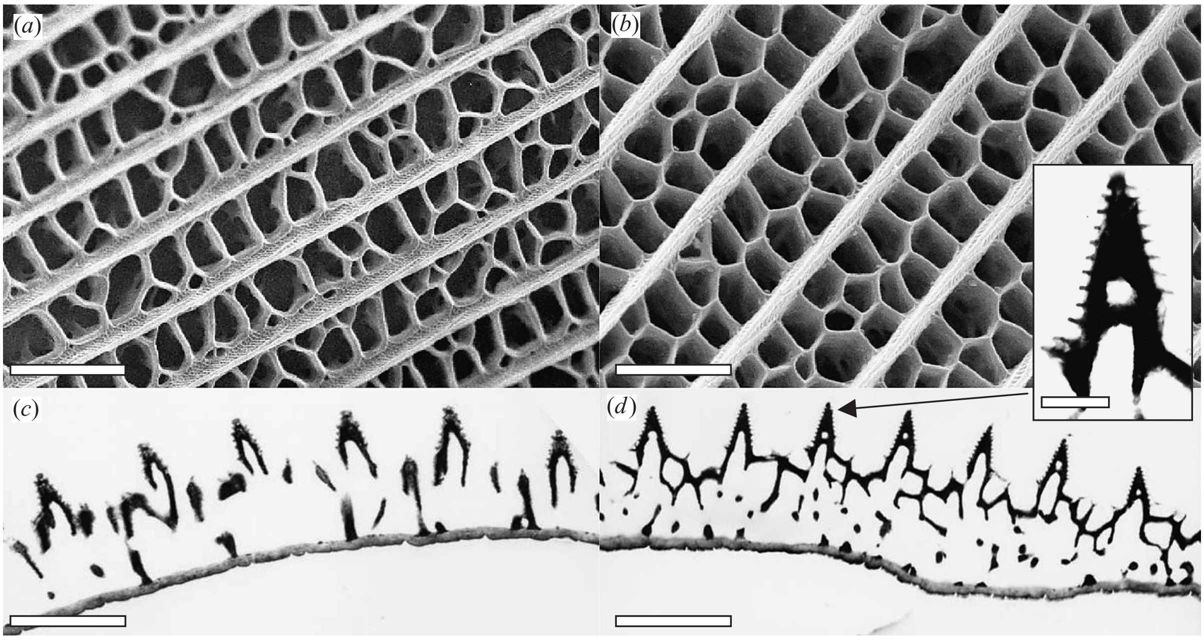

Figure 2. The nanostructure of a single black ground scale from two regions of wing of *Papilio ulysses*. (*a*,*b*) SEM images of the surface of a single scale from the lustrous and matt black regions, respectively. (*c*,*d*) TEM images of the cross-section through a single scale from the lustrous and matt black regions, respectively. The inset image shows the cross-section through a solitary ridge of a scale from the matt black region. Scale bars: (*a*) 3 μm; (*b*) 2 μm; (*c*,*d*) 2 μm; inset, 300 nm.

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