Mechanism of the wing colouration in the dragonfly *Zenithoptera lanei* (Odonata: Libellulidae) and its role in intraspecific communication

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ABSTRACT

Zenithoptera dragonflies are known for their remarkable bluish colouration on their wings and unique male behaviour of folding and unfolding their wings while perching. However, nothing is known about the optical properties of such colouration and its structural and functional background. In this paper, we aimed to study the relationship between the wing membrane ultrastructure, surface microstructure and colour spectra of male wings in *Zenithoptera lanei* and test the hypothesis that colouration functions as a signal in territorial fights between males. The results show that the specific wing colouration derives from interference in alternating layers of melanized and unmelanized cuticle in the wing membrane, combined with diffuse scattering in two different layers of wax crystals on the dorsal wing surface, one lower layer of long filaments, and one upper layer of leaf-shaped crystals. The results also show that the thicker wax coverage of the dorsal surface of the wings results in increased brightness and reduced chroma. In the field experiments, we have demonstrated that there is a reduction of aggressive reactions of rivals towards individuals with experimentally reduced amount of blue wing colouration.

1. Introduction

Colouration in insects is derived from several pigmentary and structural optical systems that create visual cues for intra and inter-specific communication (Stavenga, 2009). For instance, some dragonflies and damselflies have colourful wings that are used as cues for recognition of conspecifics (Anderson and Grether, 2010; Okamoto and Grether, 2013) and signals during territorial contests (Grether, 1996; Guillermo-Ferreira and Del-Claro, 2011a,b) and courtship displays (Siva-Jothy, 1999; Guillermo-Ferreira and Bispo, 2012).

Recent studies have shown the morphological mechanisms of some remarkable wing colouration in damselflies, such as the iridescence in *Neurobasis chinensis chinensis* (Linnaeus, 1758) (Vukusic et al., 2004), *Matronoides cyaneipennis* Foerster, 1897 (Nixon et al., 2013) *Calopteryx japonica* Selys, 1869 (Stavenga et al., 2012) and *Matrona basilaris basilaris* Selys, 1853 (Appel and Gorb, 2015). Colour pattern in odonates is usually based on

pigments embedded in the cuticle material (Stavenga et al., 2012) or situated just under transparent endocuticle, structural colouration derived from multi-layering structure of the cuticle (Vukusic et al., 2004; Schultz and Fincke, 2009; Stavenga et al., 2012; Nixon et al., 2013), nanospheres (Appel and Gorb, 2015), and wax crystals (Gorb, 1995; Gorb et al., 2000) on the epicuticle.

Dragonflies of the genus *Zenithoptera* Selys, 1869 exhibit an unusual striking blue colouration on the dorsal surface of the wings (Pujol-Luz, 1993) that resemble that of *Morpho* butterflies, granting them the fame of morpho-dragonflies. *Zenithoptera* males exhibit two distinct patterns of colouration on the wings: the dorsal surface is metallic blue, whereas the ventral surface is mostly black/metallic red. Another rare trait of these dragonflies is the ability to fold their wings dorsally, similar to representatives of Zygoptera (Ris, 1910; Jurzitza, 1982). This capability of folding the wings of this genus and *Cordulephya* Selys, 1870 (Anisoptera: Corduliidae) is considered unique among the Anisoptera (Paulson, 2004).

Pujol-Luz and Vieira (1998) suggested that this behaviour is involved in territorial displays, describing that males rest with their wings folded, but spread them when a rival male approaches. These displays show the striking bluish colouration of the dorsal wing surface to the rival (see Supplementary video). However,

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nothing is known about the optical properties of such colouration and its structural and functional background. In this paper, we aimed to study the relationship between the microstructure and colour spectra in wings of *Zenithoptera lanei* and to test the hypothesis that wing colouration functions as a signal in territorial fights between males.

2. Material and methods

2.1. Study species

Males of the dragonfly *Z. lanei* Santos, 1941 (Anisoptera, Libellulidae) were captured at a pond located in Assis, São Paulo state, Brazil (22°38′S, 50°27′W; altitude 522 m), killed by twisting their heads and taken to the laboratory where the wings were removed by scissors and kept in a plastic vial. The dorsal surface of the wings is metallic blue, while the ventral surface is mostly black/metallic red (Fig. 1). This species exhibit a sexual dimorphism in wing colouration, where females have no such blue colouration on the wings.

2.2. General methodology

2.2.1. Measurements of reflectance and transmittance spectra

Reflectance spectra were measured with a deuterium and tungsten halogen light source (DH-2000-BAL, Ocean Optics Inc, Dunedin, Florida, USA). The light source production ranged from 200 to 1100 nm (ultraviolet to near infrared), which was guided through a 200 μm glass fibre, positioned normal to the sample, 2 mm above it. The reflected light was collected by the same fibre and guided to a spectrometer (HR-4000, Ocean Optics). Spectral data were acquired and processed by the Spectral Suite software (Ocean Optics). Transmittance spectra were measured using the same set up. A glass fibre equipped with a collimator collected the light from beneath the sample and guided it to the spectrometer.

The angular dependence of light reflectance was measured with the same fibre-optic collimator positioned at an angle of 45° relative to the sample at a distance of 2 mm from the illuminated area. Light from the light source illuminated the sample through a glass fibre equipped with a collimator. The glass fibre was placed at a fixed distance of 13.5 cm from the sample, with the possibility to vary illumination angle between 10° and 90°. Reflectance measurements were made with wings mounted on a bracket covered with black velvet. The measurements were made with wings oriented rostro-caudal relative to the light source. The angular dependence

was measured at only one location, with variation of the incident light angle in the range 10–90°. Spectral curves were smoothed using locally weighted scatterplot regression.

2.2.2. Scanning electron microscopy (SEM)

The right forewings of males were cut at their insertion and were mounted on aluminium stubs by using double-sided carbon conductive tape (Plano, Wetzlar, Germany). The samples were sputter-coated with gold–palladium (thickness 8 nm), and examined in a SEM (Hitachi S-4800; Hitachi Ltd., Tokyo, Japan) at an accelerating voltage of 3 kV.

2.2.3. Transmission electron microscopy (TEM)

For TEM, ultrathin sections of the wings with dimensions of around 0.5×0.5 cm were fixed in a solution of 2.5% glutaraldehyde in phosphate-buffered saline (PBS) (pH 7.4) for 8–12 h at 4 °C, washed three times in PBS for 20 min each, and once in double distilled water for 10 min at 4 °C. Then samples were fixed in 1% aqueous OsO_4 for one hour at 4 °C and washed three times in double distilled water for 20 min each. After this process, the samples were dehydrated in a series of 30–100% ethanol at 4 °C, infiltrated with Epon/ethanol and subsequently embedded in Epon 812 (Glycidether 100, Carl Roth GmbH, Karlsruhe, Germany). The samples were polymerised in Epon at 60 °C for 48 h and then cut using an ultra-microtome Leica EM UC7 (Leica Microsystems GmbH, Wetzlar, Germany) into ultra-thin sections of 50–80 nm thickness for TEM. The samples were then viewed and analysed in a TEM (Tecnai Bio TWIN, FEI, Eindhoven, The Netherlands).

2.3. Epicuticular wax

To visualise surface micro- and nanostructures, photographs were taken in the SEM at ten randomly selected positions of the dorsal and ventral parts of the forewings of three individuals ($N = 3$) at a magnification of 10,000×. Images of areas outside the striking blue colouration on the dorsal surface were also taken at same magnification. These images were then used to evidence the presence of wax crystals, and to identify their shape, coverage density, and damage level. Images of cross-sections of the wings were also taken at a magnification of 15,000×, in order to visualise cuticle structure and to measure the length and width of the wax crystals. The length and width of wax crystals were measured using software Adobe® Photoshop CS3©. The length and width of the crystals were compared between dorsal and ventral surfaces with Student *t* tests (software Statistica 10).

Fig. 1. *Zenithoptera lanei* male showing the black/metallic red ventral surface and the metallic blue dorsal surface of its wings. Left and right images show wings in folded and unfolded positions, respectively. Quick changes between these wing positions correspond to typical male territorial displays (see Supplementary video).
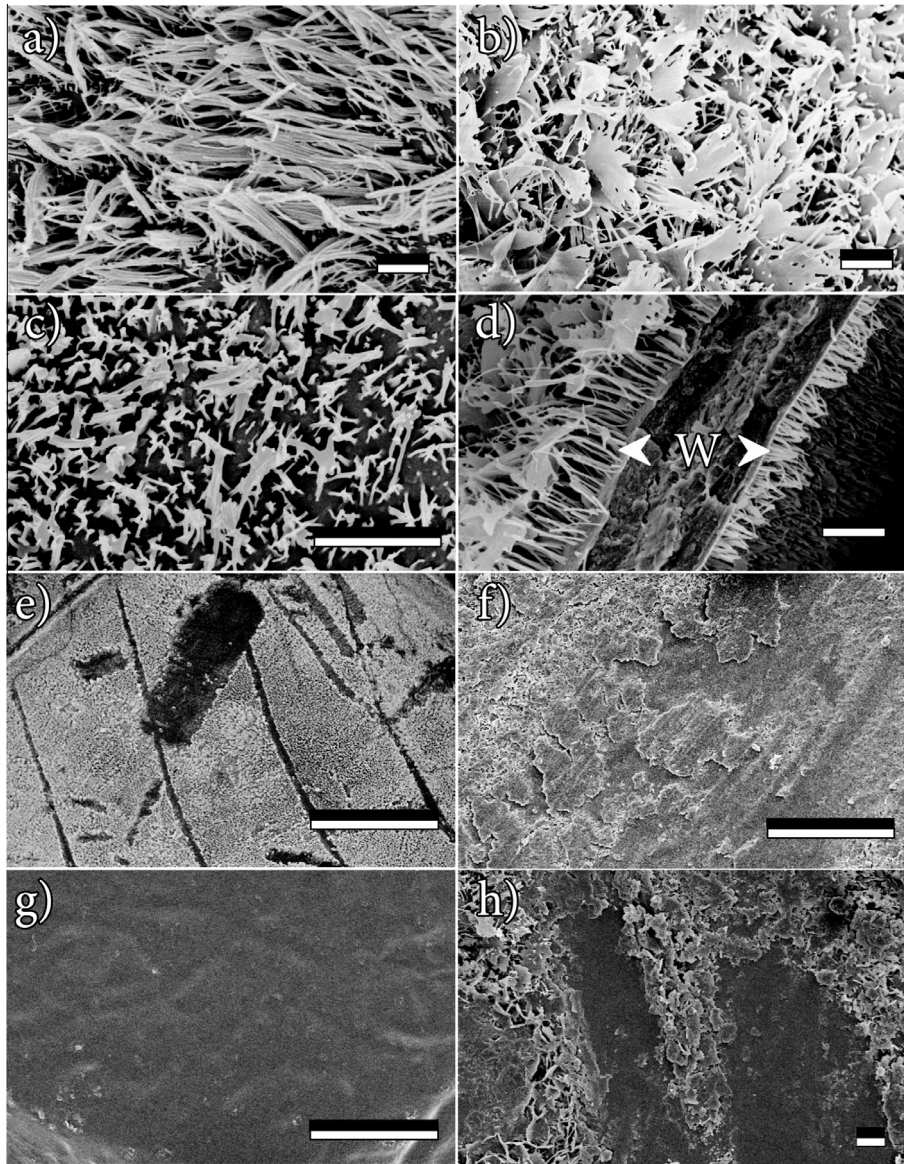
Fig. 2. Epicuticular wax crystals on the wings of males of *Zenithoptera lanei*. The crystals are long filaments (a) or leaf-shaped (b) on the dorsal surface, while they are short filaments, partially clustered on the ventral surface (c). The cross-section of the wing membrane shows the difference in wax layer thickness and crystals shape on dorsal (left) and ventral (right) surfaces (d). Wing wax surface with different levels of damage: (e) low damage, (f) severe damage, (g) removed wax, (h) crushed wax crystals. Scale bars = 2 μ in (a–d, h), 10 μ in (f) and 100 μ in (e) and (g). "W" and arrowheads indicate the wax crystals.

2.4. Wax removal

To demonstrate the influence of the wax crystals on the wing colouration and angle dependency, we measured the reflectance spectra of the dorsal surface of the wings of three males (N = 3). The wax was then removed from the wings by rinsing them in 20 ml of warm (60 °C) chloroform for 20 s (Kuitunen and Gorb, 2011). After wax removal, the measurements of optical spectra were repeated. After the spectral analyses, the samples were examined in the SEM to confirm wax removal.

2.5. Wax crystals morphology and its effect on colouration

We measured reflectance and transmission spectra of the dorsal surface of the right forewing of ten males (N = 10). These measurements were made by focusing the beam in five standard wing cells. Five SEM images were taken in each region of the wing where the spectra were measured. Then the relationship between various

parameters of colouration, the shape of wax crystals, and the degree of their damage was analysed. Crystal shape was categorised as (1) filaments (Fig. 2a) or (2) leaf-shaped (Fig. 2b). Wax coverage damage was categorised as (1) undamaged crystals (Fig 2a–d), (2) low damage and/or fused crystals (Fig. 2e), and (3) severe damage with removed (Fig. 2f and g) or crushed crystals (Fig. 2h) (Kuitunen and Gorb, 2011). We calculated values of brightness, hue, and chroma (Endler, 1990) according to the CIELAB pattern of colour measurements. Hue was measured as the hue angle according to the colour wheel; chroma as the saturation of the colour; and brightness as the intensity of light reflected from the sample. These variables were calculated as follows:

$$hue = \arctan\left(\frac{B_y - B_b}{B_r - B_g}\right),$$

$$chroma = \sqrt{(B_r - B_g)^2 + (B_y - B_b)^2},$$

$$brightness = R_{max},$$

where B_x is the relative reflectance in specific wavelengths intervals (r = 600–700 nm, y = 500–600 nm, g = 400–500 nm, and b = 300–400 nm), R_{max} is maximum reflectance. All the calculations were made using CLR v. 1.05 software (Montgomerie, 2008). The software also determines the values of UV and blue saturation that were also used in further data analysis. The relationship between colouration variables (i.e. brightness, chroma, hue, UV saturation, and blue saturation), the shape, and the damage degree of wax crystals was analysed with a factorial ANOVA test, using shape and damage as categorical factors, and colour variables as dependent variables.

2.6. Multilayer interference

The theoretical reflectance spectrum of the wing multilayer system in *Z. lanei* was directly calculated based on the dimensions of layers and the values of their complex refractive indices (RI), where the imaginary part of RI corresponds to the light absorption. The thickness of layers was measured from TEM micrographs using Adobe Photoshop's measuring tools. The thickness of each layer was adjusted within minimum and maximum values to obtain a theoretical spectrum, which resembles the experimentally measured spectrum (Vukusic and Stavenga, 2009; Nixon et al., 2013). The RIs of cuticular layers used in this study were based on the non-dispersive real (n) and imaginary (k) refractive index values used in the literature (Vukusic and Stavenga, 2009; Nixon et al., 2013), considering the contrast between layers as predictors of melanised and non-melanised layers. Pale layers were considered to have chitin as its main component, hence, $n = 1.56 + i0.03$. Dark layers were considered to be melanised, thus, $n = 1.70 + i0.17$. The outermost layer was assumed to have a value of $n = 1.38 + i0.00$ (Vukusic et al., 1999; Nixon et al., 2013). The alternation between pigmented and non-pigmented cuticular layers usually results in iridescence in odonate wings (Vukusic et al., 1999; Nixon et al., 2013). The theoretical spectra were built using the OpenFilters software (Larouche and Martinu, 2008).

2.7. The role of the blue colouration in territorial behaviour

Males were tethered to a cotton line and to a wood stick (Guillermo-Ferreira and Bispo, 2012; Guillermo-Ferreira et al., 2014), and presented to territorial males. Tethered males were divided in two groups: (i) sham control, with wing colouration manipulated by painting the tips (~10% of wing area) of the wings on the dorsal surface with a black marker (Faber-Castell) that did not reflect UV light; (ii) treatment, the wings were painted from the tip to the nodus (~55% of wing area), leaving the basal part of the wings with the original blue colouration. Three males were used in each category, presenting each male to five unique intact territorial males at a distance of 20 cm. The behavioural responses of the territorial males were recorded for the presence/absence of physical attacks (N = 30 territorial males). As each male model was presented to five territorial males, we consider these males as repetitions for the same model. Therefore, we used a Binary Logistic Generalized Estimating Equations (GEE) model to control for repeated measures. The proportion of non-aggressive/aggressive displays was compared between the sham control and treatment groups, using male response (aggression or not) as a binary dependent variable. The analysis was built with model males as subjects and territorial males as repeated measures. Aggressive displays consisted of physical attacks, grabbing and biting, while non-aggressive displays included neutral behaviours such as approach-retreat and ward-off displays, when males spread their wings as a warning signal (e.g. Guillermo-Ferreira et al., 2014).

3. Results

3.1. Epicuticular wax

The wax crystals cover almost the entire surface of the wings. Nevertheless, the crystals exhibit different shapes on dorsal and ventral surfaces. This difference can be seen both in upper view (Fig. 2b and c) and cross-section (Fig. 2d). On the dorsal surface, the crystals form a hierarchically composed structure, with a filamentous basal/lower layer (aspect ratio 1:14) and a leaf-shaped secondary/upper layer. In some cells, the secondary layer is not present (Fig. 2a). On the ventral surface, the crystals are filament–like with much lower aspect ratio (1:12). The upper layer is not present on the ventral surface. In cross section, the crystals are significantly longer on the dorsal surface, when compared to the ventral surface (0.91 ± 0.38 μm vs 0.32 ± 0.10 μm, respectively) (Student t-test, t = 8.083, N = 30, p < 0.00001). The wax crystals were also thicker on the dorsal surface (0.055 ± 0.017 μm), when compared to the thickness of crystals on the ventral surface (0.045 ± 0.012 μm) (Student t-test, t = 2.518, N = 30, p = 0.01).

3.2. Reflection and transmission spectra with/without wax layer

The results show that the ventral surface of male wings reflect more in the red (600–700 nm) with two smaller peaks of reflectance in 300–350 and 450–500 nm ranges (Fig. 3a), while the dorsal surface reflects mainly ultraviolet (300–400 nm) (Fig. 3b). The transmission spectra are similar for both surfaces, with enhanced transmission in red (Fig. 3c and d). After complete removal of the wax layer from the wing with chloroform (Fig. 2g), the transmission in red was increased by illumination from the ventral surface. The reflection intensity from the ventral wing surface has significantly dropped down after wax removal. The removal of the thick wax layer from the dorsal surface resulted in the reduction of reflection intensity in 380–600 nm wavelength range, and an increased intensity in the range 650–700 nm. Wax removal did not influence the transmission spectra by illumination from the dorsal surface.

3.3. Wax crystal morphology and its effect on wing colouration

The results show that there is a relationship between the wax crystal morphology and wing colouration (Table 1). According to the ANOVA analysis the Brightness and Chroma depend on the shape of the wax crystals (leaf-like or filaments) and the other colouration parameters such as hue, UV and blue saturations do not depend on the shape of the wax crystals. The colouration parameters do not depend on the damage degree or its' combination with the shape of the wax crystals within the spectra measurement precision. Native wing membrane cells with leaf-shaped wax crystals had higher brightness (0.25 ± 0.08) and lower chroma (0.31 ± 0.06), when compared to the brightness (0.20 ± 0.06) and chroma (0.10 ± 0.04) of cells with only filamentous crystals. This result indicates a possible relationship between phenotypic variation and the concentration of leaf-shaped crystals that combines with the multi-layer interference to create the optical effect observed on the dorsal surface of the wings.

3.4. Multi-layer interference

The exocuticle exhibits a set of pigmented (dark) and non-pigmented (pale) layers, which may result in the red ventral (Fig. 3a) and UV-blue-red dorsal (Fig. 3b) metallic colouration. The theoretical reflection models were based on both the thickness and RI of these layers (Table 2). The thickness of layers in the
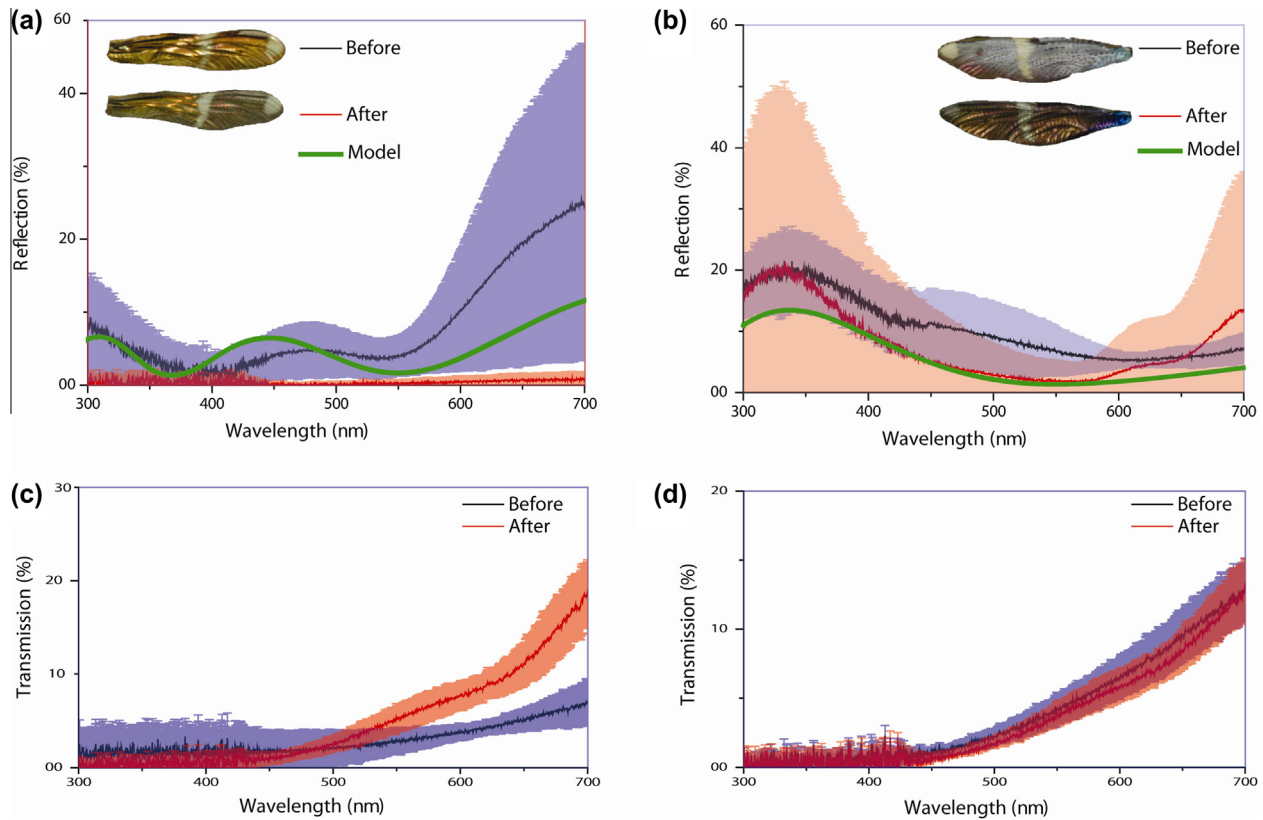
Fig. 3. Real and theoretically modelled reflection (a, b) and transmission (c, d) spectra of the ventral (a, c) and dorsal (b, d) surfaces of the wings of *Zenithoptera lanei* males, before (blue curves) and after (red curves) wax removal. Bars denote standard deviations (SD) calculated for 15 spectra taken at three individual wings. Green lines indicate the theoretical spectra. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Results of factorial ANOVA showing relationships between colour variables (i.e. brightness, chroma, hue, UV saturation, and blue saturation) and the shape and degree of damage of wax crystals on the male wings of the dragonfly *Zenithoptera lanei*. Significant results are highlighted in bold.

Colour variable (continuous)	Shape/damage (categorical)	F	p
Brightness	Shape	6.24	**0.01**
	Damage	0.10	0.89
	Shape × damage	1.45	0.24
Chroma	Shape	7.60	**0.008**
	Damage	0.12	0.87
	Shape × damage	1.51	0.23
Hue	Shape	1.72	0.19
	Damage	0.67	0.51
	Shape × damage	1.02	0.36
UV saturation	Shape	0.60	0.44
	Damage	0.35	0.70
	Shape × damage	0.62	0.54
Blue saturation	Shape	1.56	0.21
	Damage	0.69	0.50
	Shape × damage	0.96	0.38

models was estimated based on mean values obtained for each layer in TEM micrographs (Fig. 4). The models show qualitatively similar profiles for both dorsal and ventral surfaces real reflectance spectra. The calculated spectrum of the dorsal surface fitted better the real spectra after wax removal, indicating non-trivial influence of the wax coverage on the shape of the measured spectra.

3.5. The angle dependency of the dorsal colouration and the scattering effect of epicuticular wax coverage

The measurements demonstrated that the wing colouration exhibits angle dependency (Fig. 5a), which is enhanced after wax removal (Fig. 5b). This suggests that there is a scattering enhancement and modulation by the wax crystals.

3.6. The role of the blue colouration in territorial behaviour

The results of the behavioural experiment (Table 3) show that the wing colouration influences aggressive responses by males towards rival intruders. Males were more aggressive to males with
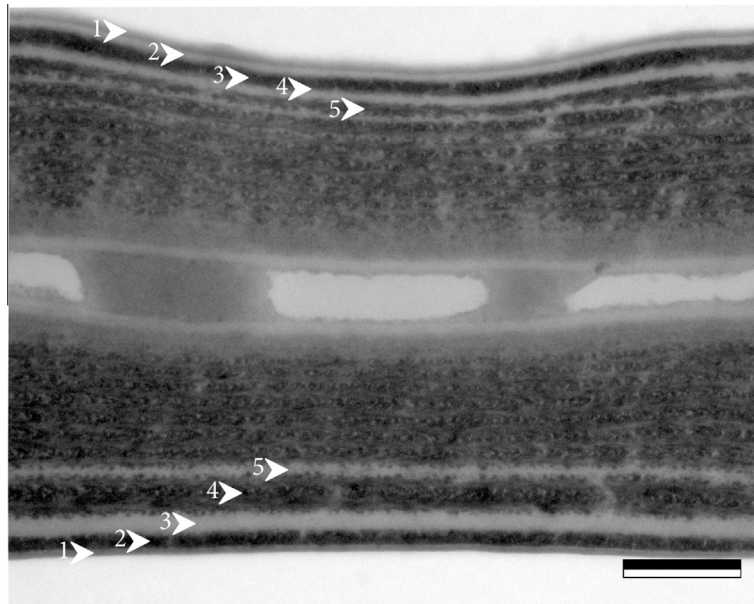
Table 2
Thickness and refractive indices of exocuticle layers in the wings of males of *Zenithoptera lanei*.

Layer	Dorsal			Ventral		
	Modelled thickness (nm)	Real thickness (nm)	Refractive index	Modelled thickness (nm)	Real thickness (nm)	Refractive index
1	30	31 ± 3.1	1.38 + i0.00	30	32 ± 2.3	1.38 + i0.00
2	40	40 ± 3.66	1.56 + i0.03	60	64 ± 5.41	1.70 + i0.17
3	80	81.6 ± 3.3	1.70 + i0.17	140	133 ± 9.8	1.56 + i0.03
4	30	33 ± 4.72	1.56 + i0.03	100	110 ± 11.33	1.70 + i0.17
5	30	33 ± 3.52	1.70 + i0.17	30	25 ± 5.95	1.56 + i0.03

Fig. 4. TEM micrograph of the cross section of the *Zenithoptera lanei* wing showing the contrasting layers in the exocuticle of ventral (bottom) and dorsal (top) surfaces. The layers are numbered and indicated by arrowheads. Scale bar = 0.5 μ.

a larger area of the wings covered by the metallic blue colouration (GEE, Wald Chi-square = 61.498, g.l. = 1, *p* < 0.0001).

4. Discussion

Several mechanisms of wing colouration have been shown for Odonata. Most of them have pigments embedded in the cuticle (Stavenga et al., 2012), some exhibit metallic visual effects derived from multi-layering structure of the cuticle (Vukusic et al., 2004; Gorb et al., 2009; Nixon et al., 2013), and others have specialised structures such as nanospheres and cuticular wax coverage that also contribute to colouration (Gorb, 1995; Gorb et al., 2000; Prum et al., 2004; Gorb et al., 2009; Kuitunen et al., 2014). Here, we have shown that wing colouration in the dragonfly *Z. lanei* presents three different components, the bluish colouration on dorsal surface is due to a double layer of wax crystals (filamentous basal layer and leaf-shaped upper layer), the dark colouration derived from pigments, and the iridescent colour (reddish in ventral and UV-bluish in dorsal surface) derived from multi-layered interference. The low accuracy of the modelled reflectance of the ventral surface of the wings may be a result of the great variation in thickness and number of cuticular layers (see Supplementary Fig. S1).

Many hypotheses have been proposed for the role of wing colouration in odonates. Melanin based wing pigmentation (Stavenga et al. 2012) is considered to be a sexual ornament that indicates male quality (Contreras-Garduño et al., 2006, 2008) and influences territorial contests (Guillermo-Ferreira and Del-Claro, 2011a; Guillermo-Ferreira et al., 2015), mate-choice (Siva-Jothy, 1999) and mate recognition (Frantsevich and Mokrushov, 1984; Anderson and Grether, 2010; Guillermo-Ferreira et al., 2014). For instance, the structural colouration in *Megaloprepus caerulatus* (Drury, 1782) (Zygoptera: Pseudostigmatidae) was suggested to act as a cue for sexual recognition and male quality (Schultz and Fincke, 2009). Wing UV reflection was suggested to play a role in sexual and age recognition in *Mnesarete pudica* (Hagen in Selys, 1853) (Guillermo-Ferreira et al., 2014).

The reflection in the UV-blue spectral range is well described by Tyndall/Rayleigh scattering. The intense wax derived UV-bluish colouration observed in several odonates and on the wings of *Z. lanei*, is often called pruinosity, which is known as a type of structural colouration that reflects ultraviolet light (Robertson, 1982; Hilton, 1986). It derives from the epicuticular wax filaments and plate-like crystals covering wings and body (Gorb, 1995; Gorb et al., 2000, 2009). Such structures, besides producing colouration, exhibit self-cleaning and hydrophobic properties that reduce wing wettability (Wagner et al., 1996; Hasan et al., 2012), crucial for an aquatic insect that is often in contact with water or even submerged to oviposit (Guillermo-Ferreira and Del-Claro, 2011b; Guillermo-Ferreira and Del-Claro, 2012).

The wax layer on the dorsal surface of the wings of *Z. lanei* exhibits two different layers, the lower one with long filaments and the upper one composed of leaf-shaped crystals. The results suggest that these structures are partially responsible for an enhanced brightness and chroma (but not hue) of the striking blue colouration of the *Morpho*-dragonfly *Z. lanei*. The visual effect of this composite wax layer resembles the Tyndall scattering and the bluish colouration generated by wax crystals in the body of some dragonflies (Gorb, 1995; Parker, 2000). Nevertheless, our finding of the two layered wax system presented here is the first record of such complex hierarchical wax structures on the wings of an insect. To the best of our knowledge, similar complex wax structure is only described for the slippery zone of the trapping organs of the pitcher plants from the genus *Nepenthes* (Gorb et al., 2013).

This thick and complex wax layer of *Z. lanei* may impose a physiological burden for males; hence, not every male can probably afford to cover the wings with the complex arrangement of leaf-shaped wax crystals. Recently, it has been shown that odonate wax derived UV colouration can affect territorial contests, suggesting a possible condition dependence of this trait (Xu and Fincke, 2015). Whether the production of a more pronounced and complex wax layer imposes a higher physiological burden for males and, thus, cannot be afforded by every male, remains unclear. Nevertheless, since there is evidence on the possible trade-off between cuticular hydrocarbons and fitness in insects (Thomas and Simmons, 2009; Kelstrup et al., 2014), future studies should address the energetic costs of complex wax production.

Iridescent metallic colouration discussed here may also be a trait used in animal communication. For instance, UV-green iridescence in jumping spiders is used as a signal of male quality during
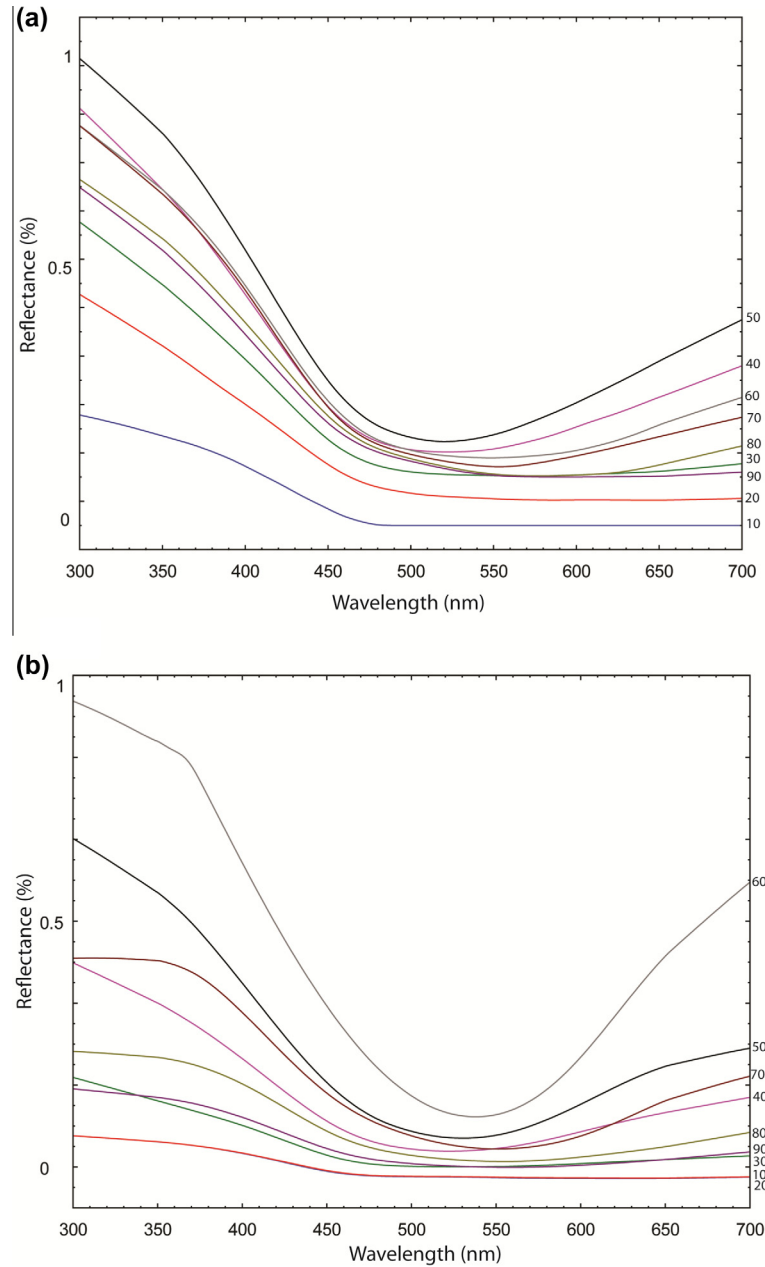
Fig. 5. The angle dependency of light reflection on the dorsal surface of the wing of *Zenithoptera lanei* before (a) and after (b) wax removal.

Table 3
Results of Chi-square tests comparing behavioural responses of territorial males of *Zenithoptera lanei* to rival males with manipulated wing colouration. Sham control males had only the tips of the wings painted black, while males of treatment group had more than 50% of the wings painted black.

Male type	Non-aggressive	Aggressive	p
Sham control	3	12	**<0.0001**
Treatment	9	6	

male agonistic interactions (Lim and Li, 2013). Such structural colouration, derived from the multi-layered structure of the cuticle, may also play an important role in species recognition, sexual recognition and age identification (Doucet and Meadows, 2009). In *Z. lanei*, the thickness and pigment concentration of layers may result in variations in their refractive index and different optical properties (Land et al., 2007), which may finally have an influence on male-male fights and mate attraction.

Altogether, the combined visual effects of light scattering by the epicuticular wax coverage and iridescence by multi-layer interference probably constitute a multi-component signal for intra-specific communication. Indeed, our results of the experimental manipulation suggest that males *Z. lanei* use the UV-bluish colouration of the dorsal surface of the wings as a signal for rival recognition. These results corroborate the predictions of Pujol-Luz and Vieira (1998), suggesting that the blue colouration of the wings of *Zenithoptera* may be a signal used by males during territorial contests.

5. Conclusions

The results allow us to conclude that the striking UV-blue colouration of wings in the *Morpho*-dragonfly *Z. lanei* is derived from a combination of at least two optical effects (light scattering due to complex wax structures and multilayer interference of pigmented layers). Through behavioural manipulations we found

evidence that this wing colouration elicits aggressive responses by other males, suggesting a role in male-male competition. Our findings demonstrate that specific bright blue wing colouration, mainly a characteristic found in butterflies, may be achieved by another structural means in dragonflies. Hierarchical organisation of crystalline wax layers of such complexity has been not previously demonstrated for odonate wings.

Acknowledgements

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jinsphys.2015.07.010.

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