

# Structural colours create a flashing cue for sexual recognition and male quality in a Neotropical giant damselfly

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## Summary

1. Structural coloration is common among animals that produce sexual displays involving motion or ultraviolet reflection. Different sources of colour may provide multicomponent signals that indicate the location, sex, and fitness of a potential mate or rival. We investigated the proximate basis and ultimate function of the wing coloration of the territorial damselfly *Megaloprepus caerulatus*, which produces a dynamic, high contrast display during flight. The wings of both sexes have blue and white bands, but the location of the white patches are sex specific.

2. Wax filaments produce diffuse, white areas through broadband scattering of wavelengths between 300 and 700 nm. Blue bands reflect wavelengths between 300 and 500 nm ( $\lambda_{\text{max}} = 398$  nm) and shift in hue with viewing angle, the result of thin layer interference produced by layers of cuticle and pigment within the wing membrane. Both wing bands strongly reflect UV wavelengths.

3. Both the iridescent UV-blue and white wing patches provide high contrast against the vegetation in forest light gaps where mating occurs. Moreover, the iridescent signal oscillates during flight. Angle-dependent UV-blue iridescence is periodically extinguished during each wing beat cycle, in contrast to the white areas, which remain bright.

4. Males distinguish potential mates from rivals by the presence of a female's white wing tip. Blackening the white wing bands of males and adding white wing tips to resemble a female elicits a sexual rather than aggressive response from males. Conversely, blackening the white wing tips of females reduces sexual responses.

5. The proportional area of the white wing bands of males is indicative of wing symmetry, correlated with body size, and in turn, territory residency suggesting that it may serve as a signal of male condition during intra- and intersexual interactions.

6. We propose that the flashing iridescent UV-blue wing bands provide a beacon to potential mates across forest light gaps, whereas the white patches serve in mate recognition and may indicate male quality or territorial status. Our study identifies a unique combination of interference and broadband reflectors that provide a dynamic multicomponent signal.

**Key-words:** damselfly, *Megaloprepus caerulatus*, structural colours, iridescence, ultraviolet, sexual signalling

## Introduction

The bright sexual signals of many animals include colours that arise from reflective nanostructures rather than absorptive pigments (Parker 1998; Kemp 2002). The unique

optical properties of these structural colours contribute to complex displays that involve viewing angle, motion, high contrast, or the reflection of ultraviolet (UV) light (Silberglied & Taylor 1978; Endler & Thery 1996; Marshall *et al.* 2003) as well as multicomponent signals in which different components convey different information about senders (Grether, Kolloru & Nerisissian 2004). Although most

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research on colours as indicators of fitness has focused on pigments, recent studies have shown that structural colours also may signal the condition of mates or competitors (Fitzstephens & Getty 2000; Doucet & Montgomerie 2003; Siefferman & Hill 2005; Loyau *et al.* 2007).

Among insects, studies of structural colours as sexual signals have focused primarily on butterflies (Lepidoptera) and shown that the brightness and spectral characteristics of wings, especially at ultraviolet wavelengths, serve to identify mates (Rutowski 1985, 1997) and in some species, influence mate choice (Robertson & Monteiro 2005; Kemp 2007a,b). This research has benefited from a thorough understanding of the reflective properties of photonic structures in wing scales that are the proximate source of iridescent and UV colours that play a role in mating behaviour (Stavenga *et al.* 2004; Kemp & Macedonia 2006; Morehouse, Vukusic & Rutowski 2007; Rutowski *et al.* 2007). Dragonflies and damselflies (Odonata) comprise another group of highly visual insects that exhibits sexual differences in iridescent and ultraviolet reflectance (Hilton 1986) in numerous taxa (i.e. Libellulidae; Calopterygidae, Pseudostigmatidae), however, we know of only two studies (Fitzstephens & Getty 2000; Vukusic, Wootton & Sambles 2004) that have investigated the source or function of these structural colours as sexual signals.

*Megaloprepus caerulatus*, the world's largest damselfly, inhabits primary forests in Central and South America. Males are typically larger than females and are sexually dimorphic in the placement of two types of wing markings (Fincke 1992a). Whereas both sexes have pigmented, metallic blue bands, in Panama and throughout most of their range (but see Fincke 2006), only males sport a proximal, matte-white band on the middle of all four wings (Fig. 1a). Females bear white wing tips that are lacking in males (Fig. 1b). Males defend water-filled tree holes in forest light gaps where females come to mate and lay their eggs (Fincke 1992b). Females mate only at defended sites and territory holders frequently chase and engage in face-offs with rival males. At very large territories, smaller males use a satellite tactic to obtain matings when the resident male is fighting with a rival or otherwise fails to detect a female that has entered the gap (Fincke 1992b). After detecting a female, a male typically hovers around her before she perches, permitting him to take her in tandem. Although not as well developed as courtship displays found in the territorial genus *Calopteryx* (Waage 1973), such behaviour could be used by females to assess the quality of a potential mate.

Here we investigated the proximate basis of wing coloration in *M. caerulatus* and its effect on damselfly behaviour to determine its function as a sexual signal. Using electron microscopy and spectral analysis we compared the structural and optical properties of the wing bands within and between the sexes. By manipulating the wing patterns of males and females in the field, we tested the hypothesis that the alternative location of the white patches served in mate recognition. Finally, we explored the potential of wing patterns to serve as indicators of male fitness by

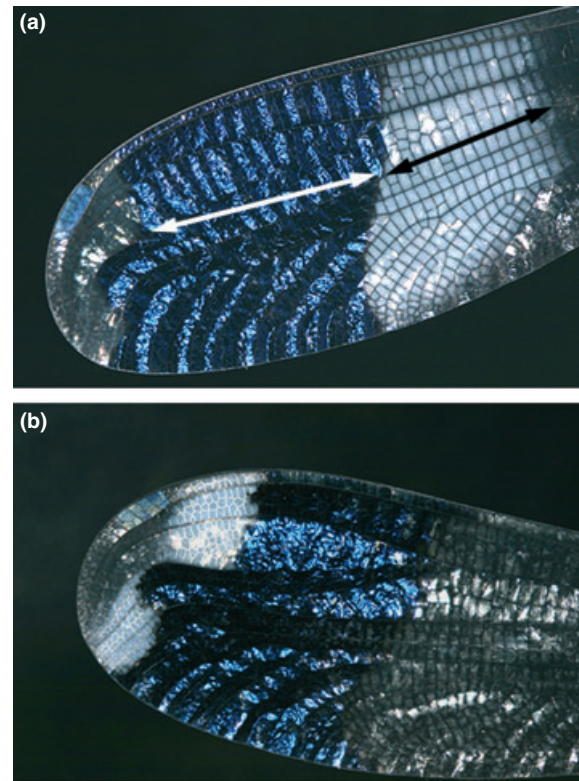


Fig. 1. Wing colour patterns of male (a) and female (b) *Megaloprepus caerulatus*. Arrows indicate width measurements of blue and white bands. White arrow = 13.1 mm.

comparing the wing signals of territorial and satellite males.

## Materials and methods

### STRUCTURAL AND SPECTRAL ANALYSES OF WING MARKINGS

All specimens of *Megaloprepus caerulatus* used in this study were obtained from Barro Colorado Island, Panama. Colour analyses were performed on individuals collected during the reproductive season in January 2004 and December 2008. The source of wing coloration was identified by immersing excised wing fragments in a medium (methyl salicylate) with a refractive index equal to that of cuticle (= 1.56; Vukusic *et al.* 1999), which extinguishes structural colours (Gilbert *et al.* 1988). To remove surface waxes that might contribute to colour (Gorb, Kesel & Berger 2000), other samples were scrubbed with a fine brush soaked in chloroform or by immersion in the lipid solvent for 24 h. Samples of wing membranes from two males and females were prepared for transmission electron microscopy (TEM) following the procedures of Vukusic, Wootton & Sambles (2004) and examined under an JEOL 2000-FX electron microscope, or sputter-coated with 20 nm of palladium-gold alloy and examined with a FEI NanoSEM scanning electron microscope. The thickness of cuticular layers was measured from ten randomly selected points in TEM sections from a forewing and hindwing of each sex. For SEM, we mounted and sputter-coated separate samples cut from clear, blue, and white areas of male and female wings and measured the length and width of 20 fully visible filaments from

each patch. The dimensions of nanostructures were measured from micrographs with ImageJ (NIH) and means were compared using parametric statistics and JMP 6.0.3 software (SAS Institute Inc., Cary, NC).

Spectral reflectances were obtained between 300 and 700 nm from the wings of freshly caught damselflies using an OceanOptics SD2000 spectrometer with a WS-1 diffuse white reflectance standard and a PS-2 xenon light source. We restrained live damselflies on their sides and focused the coaxial probe at a working distance of 2 mm and oriented 90° to the wing surface. The mean ( $\pm$  SE) peak reflectance ( $\lambda_{\text{max}}$ ) and brightness (% reflectance relative to standard) of blue and white wing bands were calculated and differences between sexes were analyzed with Student's *t*-tests.

To measure changes in reflectance with wing pitch during flight, we followed the methods of Rutowski *et al.* (2007) and obtained spectra from wing bands at angles from 10 to 40° between separate illuminating and collecting fibres, each fitted with a collimating lens. The illumination was focused on the blue forewing bands of five males and on the white wing tips of five females at a 90° elevation from the wing surface. Wing specimens were pinned on a universal stage and moved in the *x-y* horizontal plane and rotated around the *z*-axis until a maximum reflectance peak was obtained. The target area of the 10° scan was recorded with respect to wing venation and stage coordinates, and re-established for each subsequent scan after recalibration with the WS-1 reflectance standard. Spectra were recorded as the average of 50 scans at each angle.

#### BEHAVIOURAL EXPERIMENTS

The field component of this study was done over the reproductive season (i.e. between June and February) of 1995–1997. We manipulated the wing patterns of male and females using a black marker or Wite-Out® correction fluid (Bic Corp., Shelton, CT, USA) and assessed the responses of males to both sexes. The black marker removed the metallic blue colour and produced a flat reflectance of 2% between 300 and 700 nm. Artificial and natural white markings were equally bright at UV wavelengths, but the former were 5–10% brighter above 400 nm (Fig. 3). The wing markings of males were altered as follows (numbers refer to respective treatments): (1) a white tip was added (2) white band was blackened (3) white band was blackened and a white tip added. By using sham controls, we controlled for possible handling effects of treated individuals as well as the additional weight to the wing that resulted from the treatment manipulations. As a result, the signals of the sham controls were also somewhat altered. The controls for treatment 1 and 3 had Wite-Out® added to the white band (which increased reflectance by 20%) whereas controls for treatments 2 had the blue band blackened. Female manipulations involved: (4) white band added proximal to natural blue band, (5) the white spot or entire wing tip was blackened, (6) white band added and white wing tip was blackened. Female controls for treatment 4 and 6, had the white tips covered with Wite-Out® and controls for treatment 5 had the blue band blackened. The responses of males to the treatment individuals were compared with the responses made to the relevant control individuals, using two-tailed Fisher exact tests.

Manipulated individuals (i.e. 2–11 unique individuals used per treatment or control, mean of  $4.2 \pm 1.0$  SE) were tethered and presented one at a time to unique territorial resident males (range 5–14 unique males per treatment or control, mean of  $7.2 \pm 1.0$  SE). The manipulated individual was secured with a black thread around its thorax and tied to a meter-long dowel, about 3 cm from its end. A lead of 10 cm permitted the individual to flutter its wings and perch

naturally. Individuals were introduced to a territorial male near a defended tree hole, at a distance between 3–5 m from the male (i.e. the researcher slowly approached a perched male to a minimum distance of 3 m if the male failed to react initially). The time to react was measured to the nearest second. A male's reaction was broadly scored as neutral (male hovered and subsequently perched or otherwise ignored the individual), aggressive (male pursued the individual, faced-off with it, and/or hit it as in a fight), or sexual (male hovered and subsequently attempted or achieved tandem formation). In eight cases, a manipulated individual escaped the tether but the territorial male still reacted to it. The latter were also included in the Fisher Exact tests used to determine whether a male's reaction differed between manipulated and sham manipulated controls. To determine the constancy of a reaction to a given manipulated individual, 34 of the 43 total unique males used were presented with the same treatment individual three different times. In only one case did the male's reaction scores differ for a given treatment; then the more extreme reaction was used. Within a given treatment, the time required for a male to react varied only slightly within an individual (greatest range of a given individual measured multiple times = 0–20 s). Hence, analyses were based on a single reaction by unique resident males, and the longest time to react was used in the analysis.

#### FIELD MEASUREMENTS OF WING PATTERNS

Forewing and abdomen length of male and female *M. caerulatus* were measured over the reproductive season (i.e. between June and February) from 1995–1997. Asymmetry in wing length greater or equal to 1 mm was noted; inequalities in length of left and right forewing of less than 1 mm were conservatively considered symmetrical. In both sexes the width of the blue wing band was measured as the distance between the corner of the blue notch and the point at which the proximal edge of the blue patch intersected the second branch of the media vein ( $M_2$ , see Lencioni 2005 for wing vein designations). In males, the width of the white wing band was measured as the distance from the point at which the distal edge of the white band intersected the media vein, to where the proximal edge of the white patch intersected that same vein (Fig. 1a). The width of the blue or white band as measured above, divided by total wing length was used as a surrogate measure of the proportion of the total wing that was blue or white, respectively. To determine how well this surrogate measure corresponded to the actual proportion of the total wing area accounted for by the area of the blue and white spot of males, photos of a measured wing were taken for 40 individuals. Using ImageJ software and the wing length as the scaling measure, the total wing area, the area of the blue band and the white band of males, and white tip of females were calculated as a percent of total wing area. Both the surrogate and actual measures of areas are reported in terms of a proportion of the total wing (i.e. length and area, respectively), and hence control for differences in absolute wing size.

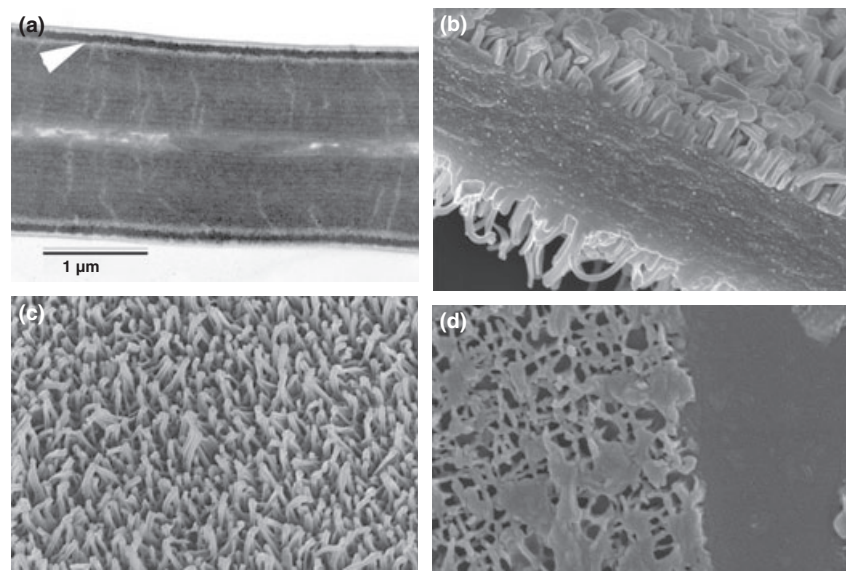
We distinguished between territorial males and satellite males to determine if they differed in the relative proportion of each signal component. Territorial males were defined as those known to successfully defend a territory for  $\geq 3$  days, whereas satellite males were those present at a defended site but which hid in the shadows and were only briefly chased if detected by the territory resident (see Fincke 1992a for behavioural details). Throughout, continuous variables were analyzed by Pearson correlations or General Linear Models (SAS version 9.1, SAS Institute), with day of marking as a covariate to control for seasonal effects (Fincke & Hadrys 2001). Bonferroni *post-hoc* tests were used to determine which of the independent variables contributed to a significant model effect.

## Results

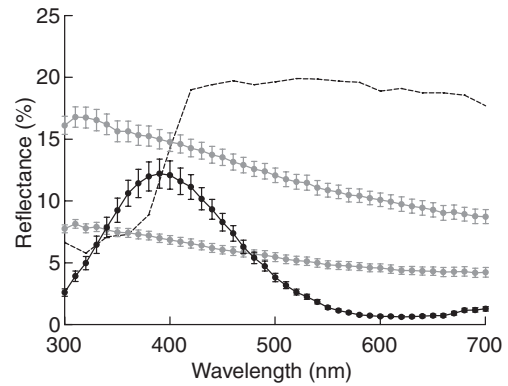
### STRUCTURAL AND SPECTRAL CHARACTERISTICS OF WING MARKINGS

The blue bands and white wing areas of *M. caerulatus* showed reversible colour change when immersed in a medium of similar refractive index confirming both colours as structural. The blue bands lost their hue and brightness in methyl salicylate, but remained dark brown in transmitted light, presumably due to the presence of the pigment melanin. Under TEM, cross-sections of wing from the blue bands showed three contrasting layers (Fig. 2a, arrow), c. 150 nm thick at both surfaces of the wing. The interior of the wing exhibited the helicoidal laminations and pore canals of exocuticle (Neville 1975) with lacunae in the centre. In micrographs, the apparent thicknesses of the white, black, and grey layers at the wing surface were  $48.06 \pm 5.46$ ,  $61.46 \pm 7.32$  and  $41.52 \pm 7.98$  nm ( $n = 40$ ), respectively. If the refractive indices of these layers are similar to those estimated by Vukusic, Wootton & Sambles (2004) (2003) for the iridescent wings of the damselfly *Neurobasis chinensis*, the layer thicknesses are within the range expected for a multilayer interference reflector (Vukusic & Stavenga 2009).

White areas of the wing became entirely transparent in methyl salicylate, demonstrating that the whiteness arises from broadband scattering by non-uniform unpigmented structures (Stavenga *et al.* 2004). Cross-sections of membranes from the white bands showed little internal ultrastructure, but SEM revealed a micropile of filaments (Fig. 2b,c) on the wing membrane similar to the 'rods' that Gorb, Kesel & Berger (2000) identified as a cuticular wax on the wings of other Odonata. Filaments from the white bands averaged  $539.74 \pm 19.97$  nm by  $113.52 \pm 6.76$  nm ( $n = 40$ ) and were significantly longer ( $F_{2,137} = 64.64$ ,  $P < 0.0001$ ) and thicker ( $F_{2,137} = 31.7$ ,  $P < 0.0001$ ) than filaments on the blue and clear portions of the wing. Chloroform removed the filaments



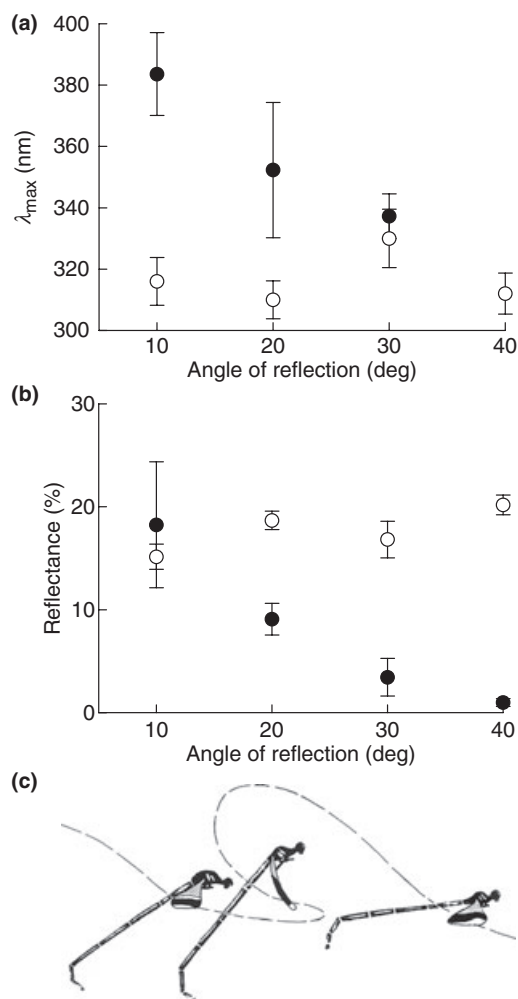
**Fig. 2.** (a) Transmission electron microscopy (TEM) image of a cross-section through the blue band of a male *M. caerulatus* hindwing showing thin layer reflectors (arrow) on both surfaces. (b, c, d) SEM images of the wing surface and wax covering from (b, c) an untreated white band and (d) a white band immersed in chloroform for 24 h.



**Fig. 3.** Reflectance spectra (mean  $\pm$  SE) of blue wing bands (black symbols) of *M. caerulatus* and white wing bands (grey symbols) of females (upper) and males (lower). Spectrum of a painted wing is indicated by the dashed line. We measured the spectra of wings oriented  $90^\circ$  relative to the coaxial light source and probe.

from the wing membrane (Fig. 2d) and simultaneously rendered white areas transparent.

The reflectance spectra of blue bands had a unimodal peak with an average  $\lambda_{\max}$  of  $397.75 \pm 11.86$ ,  $n = 15$  of each sex (Fig. 3). Males and females did not differ in the  $\lambda_{\max}$  ( $t = -0.703$ ,  $P = 0.49$ ) or relative brightness ( $t = 0.51$ ,  $P = 0.61$ ) of their blue bands. However, the white wing tips of females were significantly brighter than the mid-wing bands of males ( $t = -9.39$ ,  $P < 0.0001$ ). Both produced a flat spectrum that decreased gradually from 300 nm to 700 nm. The  $\lambda_{\max}$  and brightness of the UV-blue bands decreased linearly as the angle between the illumination source and collection probe was increased from 10 to  $40^\circ$  (Fig. 4a,b), the definitive characteristic of iridescence (Fox 1976), whereas the white bands remained unchanged.



**Fig. 4.** Change in mean ( $\pm$  SE) peak wavelength (a) and relative reflectance (b) with the angle between illumination and collection for the blue ( $\bullet$ ) and white ( $\circ$ ) wing bands. (c) Change in wing pitch during the wing beat of *M. caeruleatus* (modified from R uppell & Fincke 1989).

#### MALE RESPONSES TO ALTERED WING PATTERNS

Territorial males reacted sexually to all of the sham control females, whereas they reacted aggressively to all but one of the sham control males (Table 1). This suggests that our manipulations of these individuals to control for handling and additional wing weight did not significantly interfere with the sexual cues used by males to distinguish between the sexes. Territorial males reacted as aggressively to treatment males whose white wing band had been blackened as they did to the controls whose blue wing patches were blackened (Table 1). In contrast, territorial males reacted neutrally to a male with a white wing tip added, and reacted sexually when both a white wing tip was added and a male's white band was blackened. Territorial males reacted sexually to both sham control females and to females that had a white band added to their wing. The male reaction to a female with a blackened white spot did not differ from their reaction to females whose entire

wing tip was blackened ( $\chi^2_2 = 0.43$ ,  $P = 0.8$ ), so these results were pooled. These male reactions were the most variable. Adding a white band and blackening the white wing tips to make a female most resemble a male resulted in a neutral response by the territorial males.

Most (73%) of the males reacted to presented individuals within 1 s (mean =  $2.9 \pm 0.8$  s, range = 1–58 s,  $n = 78$ ), and male reaction times did not differ between sham controls and the experimental treatments ( $F_{1,63} = 0.09$ ,  $P = 0.76$ ). However, there was a significant effect of treatment on reaction times ( $F_{10,53} = 2.72$ ,  $P = 0.009$ ), caused by the relatively slower reaction of males to females to which a white wing patch had been added (Bonferroni test,  $P < 0.05$ ). The slowest male took 58 s to react, even though he still reacted sexually to the female.

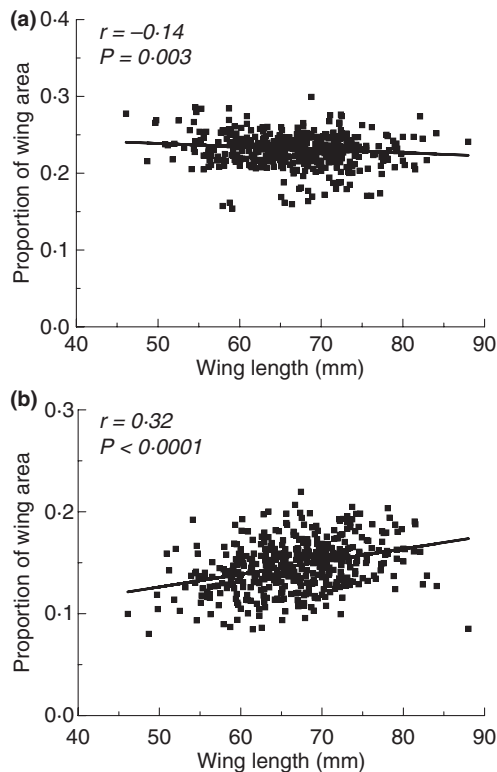
#### WING PATTERN, BODY SIZE, AND TERRITORIAL STATUS

The width of the iridescent UV-blue band was correlated positively with wing length for both males ( $r = 0.69$ ,  $n = 447$ ,  $P < 0.0001$ ) and females ( $r = 0.52$ ,  $n = 228$ ,  $P < 0.001$ ). For both sexes, our surrogate measure for relative wing area occupied by the UV-blue band (i.e. the width of the UV-blue band/total wing length) was correlated positively with the actual relative area of the band (i.e. area of blue band/total wing area,  $r = 0.81$ ,  $P < 0.0001$ ,  $n = 41$  males;  $r = 0.83$ ,  $P = 0.006$ ,  $n = 9$  females). Similarly for males, the width of the white band/total wing length was correlated positively with the proportion of the total wing area that was white (i.e. white band area/wing area,  $r = 0.70$ ,  $P < 0.0001$ ,  $n = 40$  males). For females, the area of white wing tip was positively correlated with wing area ( $r = 0.71$ ,  $P = 0.03$ ,  $n = 9$  females).

Across males, the proportion of the wing that was white was a positive indicator of male size as measured by forewing length ( $r = 0.32$ ,  $P = 0.0001$ ,  $n = 447$ , Fig. 5b), and abdomen length ( $r = 0.35$ ,  $P < 0.001$ ). In contrast, the proportion of the wing that was iridescent UV-blue decreased slightly as wing length increased ( $r = -0.14$ ,  $P = 0.003$ ,  $n = 446$ , Fig. 5a). Symmetrical males had a higher proportion of white on their wings than asymmetrical males ( $F_{1,443} = 8.28$ ,  $P = 0.004$ ). In contrast, the proportion of UV-blue on the wing did not differ between symmetrical and asymmetrical males ( $F_{1,443} = 1.45$ ,  $P = 0.23$ ). Satellite males were smaller than territorial males ( $F_{1,41} = 15.25$ ,  $P < 0.001$ ). Territory residents had a greater proportion of their wing covered by the white band than did satellites ( $F_{1,41} = 6.19$ ,  $P = 0.02$ ), whereas the proportion of the wing covered by the blue band did not differ ( $F_{1,41} = 1.68$ ,  $P = 0.20$ ). In contrast to males, the proportion of a female's wing that was blue was not correlated with wing length ( $r = -0.10$ ,  $P = 0.14$ ,  $n = 228$ ) or abdomen length ( $r = -0.05$ ,  $P = 0.48$ ,  $n = 228$ ). Nor did symmetrical females differ from asymmetrical ones in the proportion of UV-blue on the wing ( $F_{1,220} = 0.06$ ,  $P < 0.80$ ).

**Table 1.** Responses by 78 unique male *Megaloprepus caerulatus* to males and females with modified wing patterns ( $n = 42$ ).  $P$  values are given for 2-tailed Fisher exact tests used to compare the results of each treatment with the relevant sham control (i.e. 1 vs. a, 2 vs. b, 3 vs. 1, 4 vs. c, 5 vs. d, 6 vs. c)

| Male responses to treated males             |        |            |         |       |
|---|--------|------------|---------|-------|
| Treatment                                   | Sexual | Aggressive | Neutral | $P$   |
| Sham control:                               |        |            |         |       |
| (a) White –out on white band                | 0      | 4          | 1       |       |
| (b) Blue band blackened                     | 0      | 7          | 0       |       |
| (1) White tip added                         | 0      | 0          | 5       | 0.048 |
| (2) White band blackened                    | 0      | 6          | 1       | 1.0   |
| (3) White tip added/white band blackened    | 8      | 0          | 0       | 0.001 |
| Male responses to treated females           |        |            |         |       |
| Treatment                                   | Sexual | Aggressive | Neutral | $P$   |
| Sham control:                               |        |            |         |       |
| (c) White tips whitened                     | 5      | 0          | 0       |       |
| (d) Blue band blackened                     | 5      | 0          | 0       |       |
| (4) White band added                        | 6      | 0          | 0       | 1.0   |
| (5) White spot or entire wing tip blackened | 6      | 13         | 6       | 0.035 |
| (6) White tip blackened & white band added  | 0      | 0          | 5       | 0.008 |



**Fig. 5.** Correlation between wing length and the estimated proportion of the wing surface covered by (a) blue or (b) white bands of 447 males of *M. caerulatus*.

## Discussion

The contrasting wing pattern of *M. caerulatus* is comprised of two structural colours produced by different means. The UV-blue iridescence of the pigmented wing bands results from the interference of light reflected by thin cuticular layers that include melanin as an absorptive component. Similar reflectors have been identified in the iridescent bodies and hindwings of calopterygid damselflies (Fitzstephens & Getty 2000;

Vukusic, Wootton & Sambles 2004), but they contain more birefringent layers and reflect wavelengths greater than 400 nm. The white wing tips and bands of *M. caerulatus* are produced through broadband scattering by cuticular wax filaments on the wing membrane. Pruinescence and UV reflectance from the body of several Odonata species have been attributed to surface waxes (Hilton 1986) and Gorb, Kesel & Berger (2000) suggested that one function of cuticular waxes on odonate wings may be to produce UV signals. We confirmed both hypotheses and found a correspondence between the size of wax filaments and the reflectance of damselfly wings between 300 and 700 nm; the efficiency of broadband scattering and intensity of UV reflectance depends on the shape and sub-micron size of wax structures (Grant *et al.* 2003).

*Megaloprepus caerulatus* display in open treefall gaps where the ambient light is white and in adjacent woodland shade, which is relatively rich in short wavelength light (Endler 1993). The reflectance of tropical forest vegetation is deficient at wavelengths below 500 nm (Doucet, Mennill & Hill 2007). The few studies of odonate colour vision (Lavoie-Dornik *et al.* 1988; Yang & Osorio 1991) suggest that damselfly vision is at least trichromatic with UV (366 nm), blue (410 nm), and green (525 nm) photoreceptors (Schultz, Anderson & Symes 2008). Given these conditions, the UV reflective white wing bands would be conspicuous to *M. caerulatus* either in gaps or woodland shade, while the UV-blue iridescence is brightest in the direct sunlight of treefall gaps. Iridescent-blue and white colour patterns are also exhibited by tropical birds that display near light gaps (Endler & Thery 1996), butterflies (e.g. *Morpho achillaena*), and even antlions (e.g. *Glenurus peculiaris*, Hogue 1993). The wing patterns of the butterfly *Heliconius cydno chioneus* are sufficiently similar to *M. caerulatus* as to sometimes confuse the males of both species (Fincke 1984).

When flying or hovering, the pitch of the *M. caerulatus* wing surface changes 90° during each wing stroke (Fig. 4c;

Rüppell & Fincke 1989). The reflectance of the iridescent bands is maximized when the incident light is normal to the wing surface but is extinguished as the wing rotates and the angle of incidence increases relative to normal (Fig. 4b). Consequently, the iridescent bands of a flying *M. caerulatus* flash on and off with each wing beat providing a dynamic, conspicuous signal between 350 and 450 nm. Silberglied (1984) and Vukusic *et al.* (1999) have suggested that the flickering iridescent and UV-reflecting wings of *Morpho* butterflies are especially effective as long-range conspecific signals. We propose that the iridescent wing bands shared by both male and female *M. caerulatus* facilitate detection and identification of conspecifics across the tropical forest light gaps where individuals meet.

The white wing markings of *M. caerulatus* change slightly in brightness with wing orientation (Fig. 4b), but remain conspicuous throughout the slow and synchronous wing beats. Despite our inadvertent modification of the signal of our sham controls, territorial males reacted to our sham control females in a sexual fashion, and to the sham control males in an aggressive fashion, similar to their behaviour towards free flying individuals (see Rüppell & Fincke 1989). Manipulations using a zinc-based white paint with a UV signal that realistically mimicked the natural white band produced preliminary results similar to the current study (M. Xu, O. Fincke and T. Schultz, unpublished data). Our manipulations of wing patterns of the treatment individuals suggested that a female's white wing tip functions to cue sexual recognition. Males reacted sexually to, and even took in tandem, males that were given a white wing tip and whose white wing band had been darkened (Table 1). Conversely, experimental males lacking a white wing tip elicited aggressive reactions even if the white band were absent suggesting that a male's white band is not used as a cue to his sex. In addition to wing markings, an individual's behaviour should act as an additional cue to sex. Although we occasionally found males that we had painted as females subsequently defending a territory, we have yet to tease apart the relative strength of behavioural and wing signal cues. Nevertheless, our results from female manipulations strengthened the conclusion that the white wing tip is a prominent sexual cue whereas the white wing band is not. Males reacted sexually to females with white wing tips even when a white wing band was added (Table 1). Blackening the wingtips reduced the sexual responses of males to altered females, similar to the results obtained by Ballou (1984) when she blackened the white stigma of female *Calopteryx maculata*.

Our results failed to indicate a clear role for the white wing patch of males in sexual recognition. This may not be surprising in light of the fact that in the Pacific coast populations at Corcovado, Costa Rica, and the Atlantic coast Mexican population at Los Tuxlas, the white band is absent in males altogether (Fincke 2006). The white bands may be used in species recognition by providing additional contrast with the visual background under cloudy or shaded conditions when iridescent colours are less reflective. In addition to that possible

function, our data suggest that the white wing bands of males could serve as an honest indicator of male size and condition, either during male-male contests for territories, or in female choice of a mate. However, once in tandem, females do not seem to discriminate against satellite males (see Fincke 1992b). In *M. caerulatus*, male size is the best predictor of winners in territorial fights. Because females mate only at territories defended by males, body size is positively correlated with mating success (Fincke 1992a). The proportion of the total wing that was occupied by the white band increased as a function of male size (Fig. 4), was greater for territorial males than for satellites, and was greater in symmetrical males than asymmetrical ones. Hence, males might use this non-pulsating UV signal above the flashing signal as an indicator of the size of a competitor, whereas females might use it to assess the quality of a potential mate. Preliminary manipulation of the UV component of the male's white patch suggested it affects the outcome of male-male encounters (M. Xu, O. Fincke and T. Schultz, unpublished data).

Although we found that the white wing tips of females were about twice as bright as the white wing bands of the brightest males that were measured, we did not measure signal brightness of the males measured over the 3-year field portion of our study. Nevertheless, because the white wing markings result from waxes secreted after eclosion, their brightness could represent the status of fat reserves and age. Through the abrasion of nanostructures, structural colours are more prone to fade than pigmentary colours and provide an honest signal of age (Kemp 2006). In several species of calopterygid damselflies, the extent of male wing pigmentation is positively correlated with fat reserves, parasite resistance, territory holding ability, and mating success (Grether 1996; Siva-Jothy 1999, 2000; Cordoba-Aguilar 2002; Cordoba-Aguilar, Leshner-Trevino & Anderson 2007; Contreras-Garduno *et al.* 2008).

Sexual signals evolve under selection to enhance both perception and informative content (Doucet, Mennill & Hill 2007). Different elements in a complex colour pattern may be under selection to enhance contrast and conspicuousness (Endler 1990) or signify different information (Grether, Kolloru & Nerisissian 2004; Meyers *et al.* 2006). Through a combination of structural colours, the wing patterns of *M. caerulatus* are well adapted for the efficient transmission of sexual identity in forest light gaps. Whether these multi-component signals, which also indicate relative size and symmetry, are actually used to assess the quality of a potential mate or rival male, or species identity is currently being investigated.

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## References

- Ballou, J. (1984) Visual recognition of females by male *Calopteryx maculata* (Odonata: Calopterygidae). *Great Lakes Entomologist*, **17**, 201–204.
- Contreras-Garduno, J., Buzatto, B.A., Serrano-Meneses, M.A., Najero-Cordero, K. & Cordoba-Aguilar, A. (2008) The size of the red wing spot of the American rubyspot as a heightened condition-dependent ornament. *Behavior Ecology*, **19**, 724–732.
- Cordoba-Aguilar, A. (2002) Wing pigmentation in territorial male damselflies, *Calopteryx haemorrhoidalis*: a possible relation to sexual selection. *Animal Behaviour*, **63**, 759–766.
- Cordoba-Aguilar, A., Leshner-Trevino, A.C. & Anderson, C.N. (2007) Sexual selection in *Hetaerina titia*: a possible key species to understand the evolution of pigmentation in calopterygid damselflies (Odonata: Zygoptera). *Behaviour*, **144**, 931–952.
- Doucet, S.M., Mennill, D.J. & Hill, G.E. (2007) The evolution of signal design in manakin plumage ornaments. *The American Naturalist*, **169**, S62–S80.
- Doucet, S.D. & Montgomerie, R. (2003) Multiple sexual ornaments in satin bowerbirds: ultraviolet plumage and bowers signal different aspects of male quality. *Behavioral Ecology*, **14**, 503–509.
- Endler, J.A. (1990) On the measurement and classification of color in studies of animal color patterns. *Biological Journal of the Linnean Society*, **41**, 315–352.
- Endler, J.A. (1993) The color of light in forests and its implications. *Ecological Monographs*, **63**, 1–27.
- Endler, J.A. & Thery, M. (1996) Interacting effects of lek placement, display behavior, ambient light, and color patterns in three Neotropical forest-dwelling birds. *The American Naturalist*, **148**, 421–452.
- Fincke, O.M. (1984) Giant damselflies in a tropical forest: reproductive biology of *Megaloprepus coerulatus* with notes on *Mecistogaster* (Zygoptera: Pseudostigmatidae). *Advances in Odonatology*, **2**, 13–27.
- Fincke, O.M. (1992a) Behavioral ecology of the giant damselflies of Barro Colorado Island, Panama (Odonata: Zygoptera: Pseudostigmatidae). *Insects of Panama and Mesoamerica: Selected Studies* (eds D. Quintero & A. Aiello), pp. 102–113, Oxford University Press, Oxford.
- Fincke, O.M. (1992b) Consequences of larval ecology for territoriality and reproductive success of a Neotropical damselfly. *Ecology*, **73**, 449–462.
- Fincke, O.M. (2006) Use of forest and tree species, and dispersal by giant damselflies (Pseudostigmatidae): their prospects in fragmented forests. *Forests and Dragonflies* (ed A. Cordero Rivera), pp. 103–125, Pensoft Publishers, Sofia.
- Fincke, O.M. & Hadrys, H. (2001) Unpredictable offspring survivorship in the damselfly *Megaloprepus coerulatus*, shapes parental behaviour, constrains sexual selection, and challenges traditional fitness estimates. *Evolution*, **55**, 762–772.
- Fitzstephens, D.M. & Getty, T. (2000) Colour, fat, and social status in male damselflies, *Calopteryx maculata*. *Animal Behaviour*, **60**, 851–855.
- Fox, D.L. (1976) *Animal Biochromes and Structural Colours*. University of California Press, Berkeley.
- Gilbert, L.E., Forrest, H.S., Schultz, T.D. & Harvey, D.J. (1988) Correlations of ultrastructure suggest how genes control development of wing scales of *Heliconius* butterflies. *Journal of Research on Lepidoptera*, **26**, 141–160.
- Gorb, D.J., Kesel, A. & Berger, J. (2000) Microsculpture of the wing surface in Odonata: evidence for cuticular wax covering. *Arthropod Structure and Development*, **29**, 129–135.
- Grant, R.H., Heisler, G.M., Gao, W. & Jenks, M. (2003) Ultraviolet leaf reflectance of common urban trees and the prediction of reflectance from leaf surface characteristics. *Agricultural and Forest Meteorology*, **120**, 127–139.
- Grether, G.F. (1996) Intrasexual competition alone favors a sexually dimorphic ornament in the rubyspot damselfly *Hetaerina americana*. *Evolution*, **50**, 1949–1957.
- Grether, G.F., Kolloru, G.R. & Nerisissian, K. (2004) Individual colour patches as multicomponent signals. *Biological Review*, **79**, 583–610.
- Hilton, D.F.J. (1986) A survey of some Odonata for ultraviolet patterns. *Odonatologica*, **15**, 335–345.
- Hogue, C.L. (1993) *Latin American Insects and Entomology*. University of California Press, Berkeley.
- Kemp, D.J. (2002) Shedding new light on nature's brightest signals. *Trends in Ecology and Evolution*, **17**, 298–300.
- Kemp, D.J. (2006) Heightened phenotypic variation and age-based fading of ultraviolet butterfly wing coloration. *Evolutionary Ecology Research*, **8**, 515–527.
- Kemp, D.J. (2007a) Female butterflies prefer males bearing bright iridescent ornamentation. *Proceedings of the Royal Society B, Biological sciences*, **274**, 1043–1047.
- Kemp, D.J. (2007b) Female mating biases for bright ultraviolet iridescence in the butterfly *Eurema hecabe* (Pieridae). *Behavioral Ecology*, **19**, 1–8.
- Kemp, D.J. & Macedonia, J.M. (2006) Structural ultraviolet ornamentation in the butterfly *Hypolimnas bolina* L. (Nymphalidae): visual, morphological and ecological properties. *Australian Journal of Zoology*, **54**, 235–244.
- Lavoie-Dornik, J., Pilon, J.-G., Gogala, M. & Li, M.A. (1988) Étude électrophysiologique de la croissance de l'oeil composé de *Enallagma cyathigerum* (Charpentier) et *E. clausum* Morse (Zygoptera: Coenagrionidae). *Odonatologica*, **17**, 337–355.
- Lencioni, F.A.A. (2005) *Damselflies of Brazil: An Illustrated Identification Guide 1- Non-Coenagrionidae Families*. All Print Editora, São Paulo.
- Loyau, A., Gomez, D., Moureau, B., Thery, M., Hart, N.S., Saint Jalme, M., Bennett, A.T.D. & Sorci, G. (2007) Iridescent structural based coloration of eyespots correlates with mating success in the peacock. *Behavioral Ecology*, **18**, 1123–1131.
- Marshall, N.J., Jennings, K., McFarland, W.N., Loew, E.R. & Losey, G.S. (2003) Visual biology of Hawaiian coral reef fishes. III. Environmental light and an integrated approach to the ecology of reef fish vision. *Copeia*, **2003**, 467–480.
- Meyers, J.J., Irschick, D.J., Vanhooydonck, B. & Herrel, A. (2006) Divergent roles for multiple sexual signals in a polygynous lizard. *Functional Ecology*, **20**, 709–716.
- Morehouse, N.I., Vukusic, P. & Rutowski, R. (2007) Pterin pigment granules are responsible for both broadband light scattering and wavelength selective absorption in the wing scales of pierid butterflies. *Proceedings of the Royal Society B, Biological Sciences*, **274**, 359–366.
- Neville, A.C. (1975) *Biology of the Arthropod Cuticle*. Springer-Verlag, New York.
- Parker, A.R. (1998) The diversity and implications of animal structural colours. *Journal of Experimental Biology*, **201**, 2343–2347.
- Robertson, K.A. & Monteiro, A. (2005) Female *Bicyclus anyana* butterflies choose males on the basis of their dorsal UV-reflective eyespot pupils. *Proceedings of the Royal Society B, Biological sciences*, **272**, 1541–1546.
- Rüppell, G. & Fincke, O.M. (1989) Film E 2976: *Megaloprepus coerulatus* (Pseudostigmatidae) Flug- und Fortpflanzungsverhalten. *Publikation zu Wissenschaftlichen Filmen (Biologie)*, **20**(10), 3–20.
- Rutowski, R.L. (1985) Evidence for mate choice in a sulphur butterfly (*Colias eurytheme*). *Zeitschrift für Tierpsychologie*, **70**, 103–114.
- Rutowski, R.L. (1997) Sexual dimorphism, mating systems and ecology in butterflies. *The Evolution of Mating Systems in Insects and Arachnids* (eds J.C. Choe & B.J. Crespi), pp. 257–272. Cambridge University Press, Cambridge.
- Rutowski, R.L., Macedonia, J.M., Kemp, D.J. & Taylor-Taft, L. (2007) Diversity in structural ultraviolet coloration among female sulphur butterflies (Coleadinae, Pieridae). *Arthropod Structure and Development*, **36**, 280–290.
- Schultz, T.D., Anderson, C.N. & Symes, L.B. (2008) The conspicuousness of colour cues in male pond damselflies depends on ambient light and visual system. *Animal Behaviour*, **76**, 1357–1364.
- Siefferman, L. & Hill, G.E. (2005) UV-blue structural coloration and competition for nestboxes in male eastern bluebirds. *Animal Behaviour*, **69**, 67–72.
- Silberglied, R.E. (1984) Visual communication and sexual selection among butterflies. *The Biology of Butterflies* (eds R.I. Vane-Wright & P.E. Ackery), pp. 207–223. Academic Press, London.
- Silberglied, R.E. & Taylor, O.R. (1978) Ultraviolet reflection and its behavioral role in the courtship of the sulphur butterflies *Colias eurytheme* and *C. philodice* (Lepidoptera, Pieridae). *Behavioral Ecology and Sociobiology*, **3**, 203–243.
- Siva-Jothy, M.T. (1999) Male wing pigmentation may affect reproductive success via female choice in a calopterygid damselfly (Zygoptera). *Behaviour*, **136**, 1365–1377.
- Siva-Jothy, M.T. (2000) A mechanistic link between parasite resistance and expression of a sexually selected trait in a damselfly. *Proceedings of the Royal Society B, Biological Sciences*, **267**, 2523–2527.
- Stavenga, D.G., Stowe, S., Siebke, K., Zeil, J. & Arikawa, K. (2004) Butterfly wing colours: scale beads make white pierid wings brighter. *Proceedings of the Royal Society B, Biological Sciences*, **271**, 1577–1584.
- Vukusic, P. & Stavenga, D. G. (2009) Physical methods for investigating structural colours in biological systems. *Journal of the Royal Society Interface*, **6**, S133–S148.
- Vukusic, P., Wootton, R.J. & Sambles, J.R. (2004) Remarkable iridescence in the hindwings of the damselfly *Neurobasis chinensis* (Linnaeus) (Zygoptera: Calopterygidae). *Proceedings of the Royal Society B, Biological sciences*, **271**, 595–601.



- Vukusic, P., Sambles, J.R., Lawrence, C.R. & Wootton, R.J. (1999) Quantified interference and diffraction in single *Morpho* butterfly scales. *Proceedings of the Royal Society B, Biological sciences*, **266**, 1403–1411.
- Waage, J.K. (1973) Reproductive behavior and its relation to territoriality in *Calopteryx maculata* (Beauvois)(Odonata: Calopterygidae). *Behaviour*, **47**, 240–256.
- Yang, E.C. & Osorio, D. (1991) Spectral sensitivities of photoreceptors and lamina monopolar cells in the dragonfly *Hemicordulia tau*. *Journal of Comparative Physiology A*, **169**, 663–669.

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