

# Ultraviolet wing signal affects territorial contest outcome in a sexually dimorphic damselfly



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Ultraviolet (UV) reflectance and UV vision are both common among animals and are known to function in mate choice and male–male competition among numerous vertebrates. In comparison, examples of functional UV reflectance among invertebrates are scarce. In a territorial damselfly (*Megaloprepus caerulatus*), data from natural territorial contests indicated that males assessed the male wing band of rivals. We investigated the functions of (1) UV reflectance of the male-specific white wing band in territorial contests by staging contests between size- and age-matched, control and UV-reduced males, and (2) UV reflectance of the female-specific wing tip in sex recognition by presenting control and UV-reduced females to territorial males. Results showed that males whose UV reflectance of the white wing bands was reduced were more likely to lose contests. This effect dissipated late in the reproductive season, when breeding sites typically decrease in value. UV reflectance of the female wing tips did not affect male sex recognition, nor did it affect the detectability of a female at a male territory. Our study provides the first direct evidence from invertebrates, and one of the few among all animals, that UV reflectance of wing ornamentation affects the outcome of male contests in the field.

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Coloration of organisms was recognized by early naturalists as a key target of natural and sexual selection (Bates, 1862; Darwin, 1859). Today, we have a much better understanding of the diverse roles that human-visible colour traits play in animal communication systems, such as the cryptic coloration of peppered moths (Cook, 2003) to avoid detection, the warning colour of poisonous frogs to facilitate predator learning (Maan & Cummings, 2012), the nuptial colours of fish to attract mates (Kodric-Brown, 1998) and coloration as signals of individual, sex or species identity (Coultridge & Alexander, 2002; Sætre & Slagsvold, 1992; Tibbetts, 2002). However, unlike many animals, humans do not see ultraviolet (UV) light (wavelengths between 200 and 400 nm; Bennett, Cuthill, & Norris, 1994). Although UV reflectance was described in insects more than four decades ago (Silberglied, 1979; Silberglied & Taylor, 1973), only recently has it been demonstrated that UV reflection (Eaton & Lanyon, 2003) and sensitivity to UV (Briscoe & Chittka, 2001; Douglas & Jeffery, 2014; Jacobs, 1992; Marshall & Oberwinkler, 1999) are much more widespread in animals than previously thought.

A growing body of work has documented diverse functions of UV reflectance in different signalling systems. Birds and bees are known to use the UV reflectance of fruits and flowers as a guide in foraging (Chittka, Shmida, Troje, & Menzel, 1994; Jones & Buchmann, 1974; Siitari, Honkavaara, & Viitala, 1999; Wheelwright & Janson, 1985). UV reflectance can also be used as a private communication channel by prey. For example, male northern swordtails (*Xiphophorus*) use conspicuous UV reflectance to attract females, while remaining cryptic to their major predator, the Mexican tetra, *Astyanax mexicanus*, which cannot detect UV (Cummings, Rosenthal, & Ryan, 2003). On the other hand, predators, such as the web-building spider *Argiope argentata*, are known to use their UV-reflective dorsal surface to attract flower-seeking pollinator prey (Craig & Ebert, 1994). Finally, UV reflectance is an important sexual signal, used in sex recognition (Guillermo-Ferreira, Therezio, Gehlen, Bispo, & Marletta, 2014; Lim, Land, & Li, 2007; Ries, Spaethe, Sztatecsny, Strondl, & Hödl, 2008) and female mate choice among both invertebrates (Detto & Backwell, 2009; Kemp, 2008; Knüttel & Fiedler, 2001; Lim, Li, & Li, 2008; Robertson & Monteiro, 2005; Silberglied & Taylor, 1978) and vertebrates (Bajer, Molnar, Torok, & Herczeg, 2010; Bennett, Cuthill, Partridge, & Lunau, 1997; Hunt, Cuthill, Bennett, & Griffiths, 1999; Johnsen, Andersson, Ornborg, & Lifjeld, 1998; Liu, Siefferman, & Hill, 2007; Robertson & Monteiro, 2005; White, Partridge, &

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Church, 2003; but see Kurvers, Delhey, Roberts, & Peters, 2010) among a broad range of taxa.

Less clear is whether UV reflectance affects male–male competition (Stapley & Whiting, 2006). Several examples from vertebrates indicate that UV reflectance influences the escalation and duration of male–male competition in the damselfish *Pomacentrus amboinensis* (Siebeck, 2004), threespine stickleback, *Gasterosteus aculeatus* (Rick & Bakker, 2008), Augrabies flat lizard, *Platysaurus broadleyi* (Stapley & Whiting, 2006; Whiting et al., 2006), blue tit, *Cyanistes caeruleus* (Remy, Gregoire, Perret, & Doutrelant, 2010), and eastern bluebird, *Sialia sialis* (Siefferman & Hill, 2005). Yet only a handful of studies have shown that UV reflectance affects the outcome of male–male competition in vertebrates, including the European green lizard *Lacerta viridis* (Bajer, Molnar, Torok, & Herczeg, 2011) and blue tit (Vedder, Schut, Magrath, & Komdeur, 2010), and few studies have been conducted in field populations.

Among invertebrates, and insects especially, we know surprisingly little about the role of UV in male–male competition (Detto & Backwell, 2009; Lim & Li, 2006; but see Kemp & Macedonia, 2006; Rutowski, 1992), with no direct evidence that UV reflectance affects contest outcome. Therefore, our knowledge is taxonomically biased towards vertebrates (Detto & Backwell, 2009). Even among invertebrates, studies have mainly focused on butterflies and jumping spiders. Consequently, the question of whether UV reflectance functions in agonistic interactions among invertebrates remains largely unanswered.

The broad-winged Neotropical damselfly *Megaloprepus caerulatus* exhibits sexually dimorphic wing coloration and flight kinematics that are well adapted for sexual signalling (Hilfert-Rüppell & Rüppell, 2013; Schultz & Fincke, 2009). In Panama, both males and females possess iridescent blue bands on the wings, whereas males have a sex-specific white band proximal to the blue band. In contrast, females have white tips distal to the blue band. The female white tips signal sexual identity (Schultz & Fincke, 2009), yet it was unclear which colour component has the signalling function. The white bands and tips are structural colours that reflect strongly in the UV (Schultz & Fincke, 2009). Like most insects, odonates can detect UV (Huang, Chiou, Marshall, & Reinhard, 2014; Schultz, Anderson, & Symes, 2008; Yang & Osorio, 1991).

Male *M. caerulatus* defend forest light gaps that contain large, water-filled tree holes where females come to mate and lay eggs (Fincke, 1984, 1992a). Females do not exert direct mate choice of male phenotype, but by mating only at territories, they indirectly select larger males; body size predicts fight outcome (Fincke, 1992b). The proportion of the wing surface covered by the white band is positively correlated with male body size (Schultz & Fincke, 2009), suggesting a potential signalling role.

In the present study, we first determined whether rivals assess the white wing band and the type of assessment they use in natural contests (i.e. self-assessment, opponent-assessment, mutual assessment; Arnott & Elwood, 2009; Elwood & Arnott, 2012). In terms of receiver psychology, we hypothesized that assessment is mutual and that longer contests are more escalated. Here, we define “escalation” as contests that involve more behavioural elements and more energetically expensive behaviours (Enquist, Leimar, Ljungberg, Mallner, & Segerdahl, 1990). We subsequently investigated the potential functions of the UV reflectance of male white wing bands and of female white wing tips in two field experiments. Specifically, we hypothesized that the UV reflectance of male white wing band signals male fighting ability during the territorial contests. In addition, we hypothesized that the UV reflectance of female white wing tips is an indispensable component of the sexual identity signal used in sex recognition by territorial males. Furthermore, because UV reflectance is conspicuous against

UV-absorptive vegetation background (Schultz & Fincke, 2009), we hypothesized that the UV reflectance of female wing tips may also enhance the detectability of a female to a territorial male.

## METHODS

### Study Site

The study was conducted in the lowland moist forest on Barro Colorado Island (hereafter BCI), Panama (9.15°N, 79.85°W), where the dry season begins in December and continues until late April (Windsor, 1990). The 9-month reproductive season of *M. caerulatus* begins in the wet season after tree holes fill with water, and continues through mid dry season. Most holes dry up by the end of March (Fincke, 1992b).

### Rival Assessment during Natural Contests

We quantified male agonistic behaviours and the durations of 50 natural contests from unique dyads of unmanipulated males between July 1996 and February 1997, July 1997 and February 1998, and December 2010 and February 2011. We marked each male with a unique number on the left hindwing with a permanent black Sharpie marker (Sanford Corp., Newell Rubbermaid Office Products, Oak Brook, IL, U.S.A.) and measured left forewing length as a proxy for body size (Fincke & Hadrys, 2001), width of the white band (details in Schultz & Fincke, 2009) and territorial status (i.e. resident or intruder). Male behaviours during the contests were noted as ‘chase’, ‘contact’, ‘face-off’ and ‘spiral flight’. During a ‘face-off’, each male beats its wings synchronously while directly facing the opponent. In an upward ‘spiral’, two males circle upward while facing each other. Face-off and spiral flight are energetically more expensive than forward flight (i.e. chase) and contact, and spiral flight is the most energetically expensive (Dudley, 2000; Rüppell & Fincke, 1989). We assigned each behaviour an escalation score between 1 (low) and 4 (high). We used the criteria that energetically more expensive behaviours are more escalated, and among low-cost behaviours, contact behaviour is more escalated than noncontact behaviour (i.e. chase). This resulted in the following escalation scores: 1 = chases; 2 = contacts; 3 = face-offs; 4 = spiral flight. Total contest duration was the sum of all interaction durations (not including perching time) that occurred before the loser left the forest light gap and did not return for at least 30 min. All contests occurred in the absence of females. Male morphological characteristics were measured only from marked males, whereas contest duration and behavioural components of contests were quantified from both marked and unmarked males.

### Design of UV Manipulation Experiments

UV manipulation experiments were conducted over two dry seasons (13 December 2008–27 February 2009 and 25 December 2010–24 February 2011) at one natural tree hole and five artificial tree holes (i.e. plastic basins tied to the trunk of fallen trees in forest light gaps). Throughout the experiment, water volume in all experimental holes was kept at 4 litres. All individuals used in the experiments were collected in the field, and marked and measured as above. Age category of an individual (i.e. young, medium or old) was scored by the wear on the wings.

For tests of both hypotheses on UV function, we compared a UV-reduced treatment with a control treatment. Individuals in the UV-reduced treatment were gently painted with a thin layer of Neutrogena ultra sheer dry-touch sunscreen (SPF45, Neutrogena, Los Angeles, CA, U.S.A.) on the white bands or tips. We had two types of controls: natural controls were individuals whose white bands or

tips were intact, and sham controls were individuals that were painted with a thin layer of UV-reflective zinc white gouache paint (Winsor & Newton, Piscataway, NJ, U.S.A.) on the white wing bands or tips. To assess the effectiveness of the sunscreen treatment and how closely the zinc paint matched the natural wing colour, we measured the relative reflectance of three individuals of each sex before and after the sunscreen treatment and the zinc paint treatment with an OceanOptics SD 2000 spectrometer and a PX-2 xenon light source (OceanOptic, Dunedin, FL, U.S.A.). We interpolated the spectrum and calculated brightness in the UV and human-visible range for each individual in Avicol v.6 (Gomez, 2006). As expected, the zinc paint did not significantly change reflectance in either the UV range (natural control:  $7.84 \pm 0.64\%$ ; zinc-painted:  $8.07 \pm 0.56\%$ ; paired  $t$  test:  $t_5 = 0.63$ ,  $P = 0.56$ ; Fig. 1b) or the human-visible range (natural control:  $6.12 \pm 0.64\%$ ; zinc-painted:  $6.69 \pm 0.56\%$ ;  $t_5 = 1.50$ ,  $P = 0.19$ ). The sunscreen treatment significantly decreased UV reflectance of the white bands and tips compared to those of the natural controls (natural control:  $7.58 \pm 1.19\%$ ; UV-reduced:  $2.99 \pm 0.68\%$ ; paired  $t$  test:  $t_5 = 16.65$ ,  $P < 0.001$ ; Fig. 1a), but it also significantly increased brightness in the human-visible range (wavelength 400–700 nm, natural control:  $5.74 \pm 0.94\%$ , UV-reduced:  $7.13 \pm 1.36\%$ ;  $t_5 = 2.67$ ,  $P = 0.04$ ). To specifically compare the brightness in the human-visible region of natural, sunscreen- and zinc-painted colour patches, we measured relative reflectance of an additional 10 females. For each female, the natural reflectance of the left hindwing tips was measured. We then treated the left and right hindwing tips with zinc paint and sunscreen, respectively, and

measured the reflectance of both treatments. The brightness in the human-visible region of natural, sham control and UV-reduced treatments did not differ significantly (natural:  $9.82 \pm 1.08\%$ ; sunscreen:  $10.73 \pm 1.47\%$ ; zinc paint:  $9.97 \pm 1.29\%$ ; repeated measures ANOVA:  $F_{2,8} = 0.78$ ,  $P = 0.49$ ). Post hoc multiple comparisons revealed that the visible brightness did not differ between natural and UV-reduced treatments (paired  $t$  test with Bonferroni correction:  $\alpha' = 0.017$ ,  $t_9 = 1.00$ ,  $P = 0.35$ ), nor did it differ between sham control and UV-reduced treatment ( $\alpha' = 0.017$ ,  $t_9 = 1.32$ ,  $P = 0.22$ ).

#### Does UV Reflectance Predict the Outcomes of Male Territorial Contests?

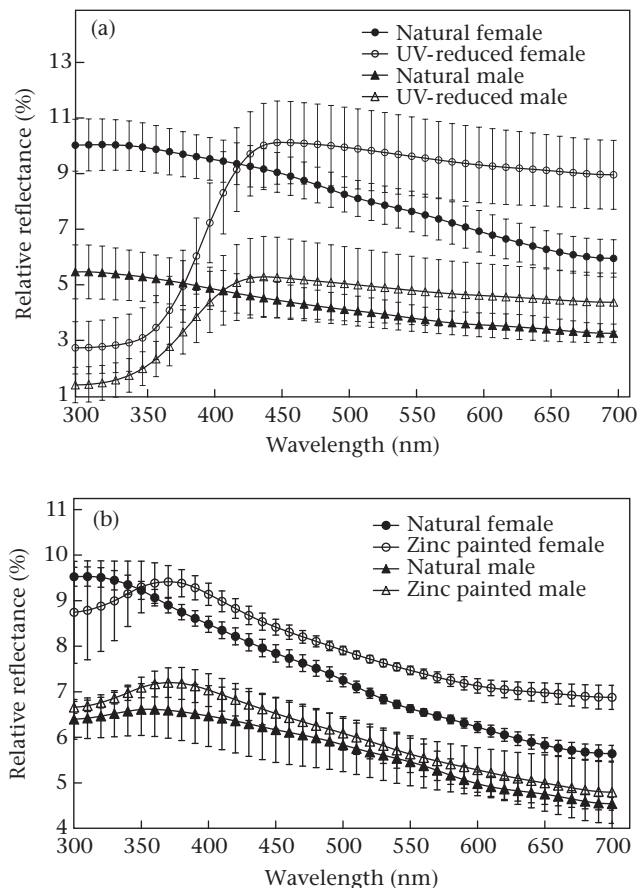
We paired males of similar size and within the same age category into 26 dyads. In each dyad, one male was randomly assigned to the UV-reduced treatment and the other male was either left intact as a natural control ( $N = 18$ ) or sham-painted with zinc paint ( $N = 8$ ). One male in a dyad was the original resident male at the tree hole and the other was a territorial resident collected from another defended site. To arrange a contest, we released the nonresident male at the experimental hole either while the original resident was out of the area foraging, or after it was gently chased away from the forest light gap. When the introduced male failed to perch near the tree hole, we allowed a tethered female to fly near the hole until she attracted the male's attention, but before any interaction occurred. A dyad was used in the analysis only if the introduced male displayed territorial behaviour (e.g. fluttered at the water surface and perched directly above the hole) before a fight occurred such that both males had a chance to establish residency. A territorial contest began when the original resident returned to the forest light gap and engaged the new male. Contest duration and outcome were determined as above. All dyads used a unique combination of males. Five males were used in two different dyads, but they were assigned to different treatments and different territorial status (i.e. the original or the new resident).

#### Does UV Reflectance of Females Affect Male Sex Recognition?

We presented 17 territorial males with a UV-reduced female and a control female ( $N = 12$  natural controls,  $N = 5$  sham controls) from the same age category. The females were presented sequentially with the sequence randomly assigned. The females were tethered with  $8 \times (0.076 \text{ mm diameter})$  fly-fishing line (Black Knight Industries, Inc., Oil City, PA, U.S.A.) around the groove between the head and the thorax, anterior to the mesostigmal plates such that it did not interfere with tandem formation. The other end of the line was tied to a twig held by the experimenter. A presentation began by fluttering a female 1 m directly in front of a perched male. Male responses were noted as hover, grab, tandem attempt or tandem. Hover was regarded as a nonsexual reaction, whereas the rest were regarded as sexual reactions. The presentation ended if the male reacted sexually; otherwise, the presentation was repeated three times and the most extreme reaction was used for analysis. In addition, for a subset of 11 focal males, we recorded reaction time towards females. Male reaction time, a proxy for the detectability of females, was defined as the time elapsed from the beginning of the presentation to when a focal male took off from the perch to fly towards the female. Afterwards, females were released from the tethers with no fishing line attached, and all flew away. No female was injured from tethering.

#### Statistical Analyses

Durations of natural contests were log transformed for all analyses. We examined the correlation between contest duration and



**Figure 1.** Relative reflectance of *Megaloprepus caerulatus* (a) natural and sunscreen-treated male wing bands ( $N = 3$ ) and female wing tips ( $N = 3$ ) and (b) natural and zinc-painted male wing bands ( $N = 3$ ) and female wing tips ( $N = 3$ ). Error bars represent standard errors.

the difference between rivals in body size and signal size (i.e. absolute value of each difference). We also investigated the correlation between contest duration and number of behavioural elements and the escalation level. Because body size decreases across the reproductive season (Fincke & Hadrys, 2001), we controlled for day of season (first day set as 1 May) using part (semipartial) correlations.

We examined whether males from the control or the UV-reduced treatment were more likely to win the contests using a binomial test. We also tested whether any possible confounding factors could have affected the contest outcomes. We used a binomial test to investigate whether males of different territorial status (i.e. original or new resident) differed in the probability of winning. Furthermore, we calculated the difference in the body sizes of contestants as the forewing length of the winner minus the forewing length of the loser, and tested whether this value was significantly different from zero with a one-sample *t* test. We examined the effect of year (2008–2009 or 2010–2011 study period), control type (natural or sham) and number of days since the approximate beginning of the dry season (1 December) on which treatment was more likely to win with generalized linear models (GLMs). We used the treatment of the winner (i.e. control or UV-reduced) as a binary dependent variable for the GLMs.

We used a generalized linear mixed model (GLMM) with binomial error distribution to investigate whether UV reflectance of the wing tips signalled a female's sexual identity to males. In this model, focal male reaction (i.e. sexual versus nonsexual) was used as a binary dependent variable; focal male was treated as a random effect and female treatment (control or UV-reduced) was nested within focal male. In addition, we investigated whether control and UV-reduced females differed in their detectability to territorial males by comparing male reaction times towards the two female treatments. Because male reaction time was not normally distributed even after a log transformation (Shapiro–Wilk test: control:  $W = 0.51$ ,  $P < 0.001$ , UV-reduced:  $W = 0.76$ ,  $P = 0.002$ ), we used the Wilcoxon signed-ranks test for the comparison.

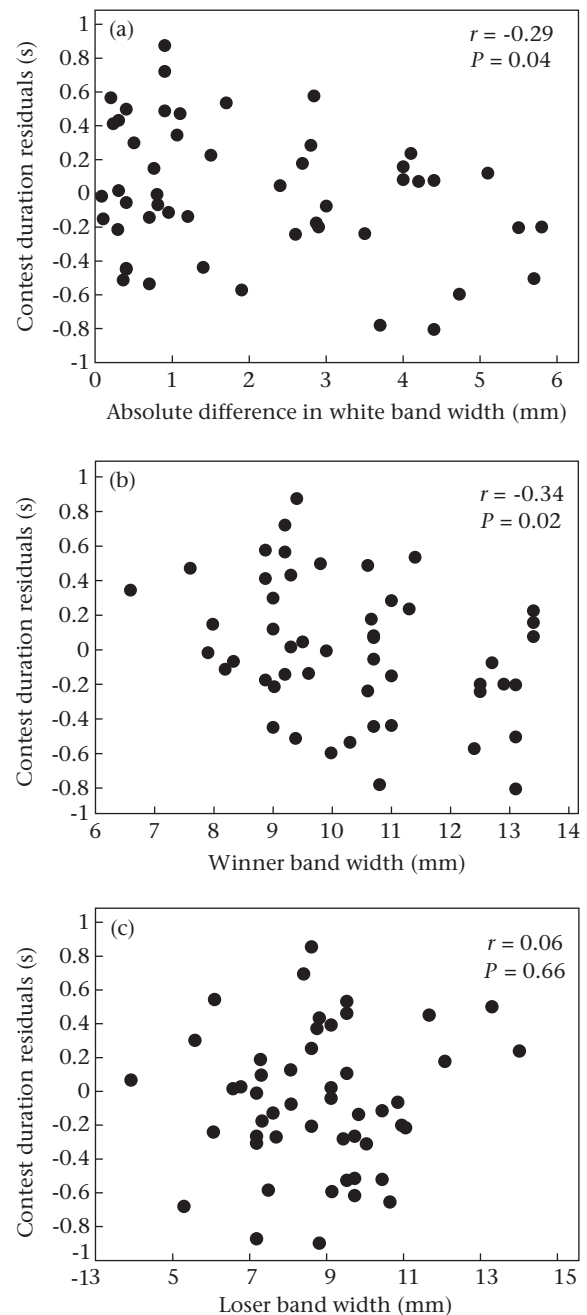
Statistical tests were performed in R (version 2.15.1, R Core Team, 2012) and SAS (version 9.1, SAS Institute Inc., Cary, NC, U.S.A.). Throughout, means are shown with standard errors.

## RESULTS

### Male Assessment of Rivals in Natural Contests

Contest duration decreased as the reproductive season progressed (Pearson correlation:  $r_{48} = -0.44$ ,  $P = 0.001$ ). Controlling for day of the reproductive season, contest duration decreased with the difference in white band size between the two males (part correlation:  $r_{48} = -0.29$ ,  $P = 0.04$ ; Fig. 2a), but not with the difference in body size ( $r_{48} = -0.20$ ,  $P = 0.17$ ). Contest duration was negatively correlated with winner band size (part correlation:  $r_{48} = -0.34$ ,  $P = 0.02$ ; Fig. 2b) but not with loser band size ( $r_{48} = 0.06$ ,  $P = 0.66$ ; Fig. 2c).

Larger males and males with larger white bands were more likely to win contests (binomial test: body size:  $P < 0.001$ ; band size:  $P < 0.001$ ). The resident male in a dyad was more likely to win the contest (binomial test:  $P < 0.001$ ), but was on average  $3.2 \pm 1.08$  mm larger than the intruder rival (one-sample *t* test:  $t_{49} = 3.17$ ,  $P = 0.003$ ). Of the 40 residents winning contests, seven were smaller or the same size as their rival, whereas of the 10 intruders that won contests, three were smaller than their resident rival. Hence, a resident had no inherent advantage over larger rivals (Fisher's exact test:  $P = 0.40$ ). After controlling for day of reproductive season and white band size difference, the duration of



**Figure 2.** After controlling for day of reproductive season, relationship between contest duration of 50 natural fights between unmanipulated *Megaloprepus caerulatus* males and (a) absolute difference in white band size between contestants, (b) winner white band size and (c) loser white band size. Standard residuals from the regression between contest duration and day of reproductive season were used as the dependent variable.

contests won by residents did not differ from those won by intruders (ANCOVA:  $F_{1,45} = 1.08$ ,  $P = 0.31$ ).

Territorial contests were stereotypic and acrobatic. Chases were the most common, occurring in 90% of the fights, followed by contacts (55%), spiral flights (31%) and face-offs (27%). The number of behavioural elements increased with contest duration ( $r_{71} = 0.32$ ,  $P = 0.006$ ). The level of escalation attained in a contest also increased with contest duration ( $r_{71} = 0.28$ ,  $P = 0.017$ ). Controlling for the difference in size of white bands, contests with one or more face-offs tended to be shorter than those without face-offs (ANCOVA:  $F_{2,43} = 3.24$ ,  $P = 0.08$ ).



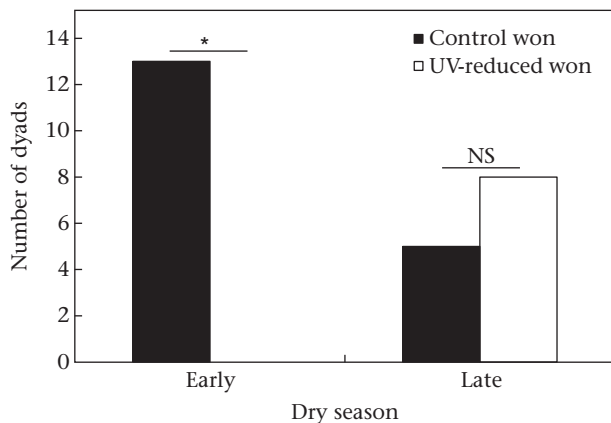
### The Function of UV Reflectance of Male White Bands

Year and control type had no effect on which treatment won the contest (GLM: year:  $\chi^2_1 = 0.005$ ,  $P = 0.95$ ; control type:  $\chi^2_1 = 0.24$ ,  $P = 0.62$ ). Therefore, we pooled results from the 2 years and the two control types. Thirteen out of 26 contests were won by the smaller males in the dyads. The body size difference between the winner and the loser ( $0.26 \pm 0.78$  mm) was not significantly different from zero (one-sample  $t$  test:  $t_{25} = 0.33$ ,  $P = 0.74$ ), and the larger male in a dyad did not enjoy a higher probability of winning than the smaller male (binomial test:  $P = 1.00$ ). In addition, original residents and new residents were equally likely to win contests (binomial test:  $P = 0.33$ ).

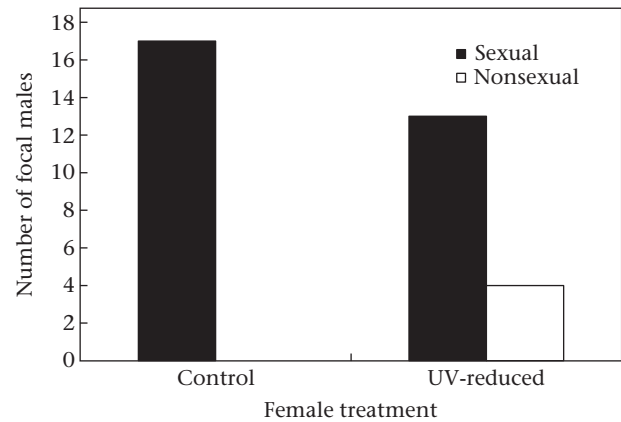
Among the 26 dyads, control males won in 18 dyads. Overall, control males and UV-reduced males did not differ in their probability of winning the contests (binomial test:  $P = 0.08$ ). However, the number of days since 1 December had a significant effect on which treatment won the contest (GLM:  $\chi^2_1 = 6.29$ ,  $P = 0.01$ ). Thus, we divided each study period into roughly two halves in number of days: early dry season (13 December–24 January, 13 dyads) and late dry season (25 January–27 February, 13 dyads). All eight cases where the UV-reduced males won the contests occurred during the late season, whereas during early season, control males won all contests; this seasonal effect was repeatable for two field seasons. In early dry season, control males were more likely to win the contests (binomial test:  $P < 0.001$ ; Fig. 3). In contrast, in late season, control males and UV-reduced males did not differ in their likelihood of winning the contests (binomial test:  $P = 0.58$ ; Fig. 3). Moving the dividing date  $\pm 10$  days did not change the above conclusions. After size matching, winners and losers did not differ significantly in body size in either the early season (absolute size difference:  $0.20 \pm 1.30$  mm; one-sample  $t$  test:  $t_{12} = 0.16$ ,  $P = 0.88$ ) or the late season (absolute size difference:  $0.32 \pm 0.94$  mm;  $t_{12} = 0.34$ ,  $P = 0.74$ ).

### The Function of UV Reflectance of Female Wing Tips

Focal male reactions towards sham control females did not differ from reactions towards natural control females (GLM:  $\chi^2_1 = 0$ ,  $P = 1.00$ ). Therefore, we pooled results from the two types of controls in the following analyses. Among 17 focal males tested, 13 males reacted sexually towards both control and UV-reduced females; four males reacted sexually towards control females and nonsexually towards UV-reduced females (Fig. 4). There was no



**Figure 3.** Numbers of size- and age-matched dyads of *Megaloprepus caerulatus* that were won by control or UV-reduced males in the early dry season (December 13–January 20) and the late dry season (January 21–February 27) over two field seasons. \* $P < 0.001$ .



**Figure 4.** Number of sexual and nonsexual responses by focal male *Megaloprepus caerulatus* in paired presentations of control and UV-reduced females. Responses in the control and the UV-reduced treatments are from the same focal males.

difference in focal male reactions towards the control and UV-reduced females (GLMM:  $\chi^2_1 = 0$ ,  $P = 0.997$ ). Male reaction time towards control females (mean = 1.5 s, median = 1.0 s, interquartile range = 0.0 s) did not differ from that towards UV-reduced females (mean = 2.5 s, median = 1.0 s, interquartile range = 4.0 s; Wilcoxon signed-ranks test:  $W_{10} = 4.5$ ,  $P = 0.25$ ).

## DISCUSSION

Our results indicated that UV reflectance of a male's wing colour ornament can affect the outcome of male territorial contests in a damselfly. This is, to our knowledge, the first evidence from an invertebrate that UV reflectance functions in resolving male–male competition. At least two previous studies have investigated the function of UV reflectance per se in the display component of male agonistic interactions among invertebrates. In a fiddler crab (*Uca mjoebergi*), results showed that the UV reflectance of the male major claw was used only by females in mate choice, not by male competitors to choose with whom to fight (Detto & Backwell, 2009). In contrast, in a salticid spider (*Cosmophasis umbratica*), males showed more agonistic elements in their display of body coloration under a UV light source than when ambient UV light was absent (Lim & Li, 2006). Although these studies offered pioneering insights into the function of UV in the dynamics of male contests, they could not tell us whether UV reflectance affects contest outcomes, because males in these studies were either tethered or kept in separate compartments in the arena and thus could not actually fight. In another study on the effect of UV-green iridescence on *C. umbratica* male contests, the band separation of UV and green coloration (i.e. the difference in wavelengths between the UV and the green reflectance peaks), instead of the hue (i.e. the wavelength at the reflectance peak), correlated with which male initiated the fight and how long a male persisted, but neither band separation nor the hue of UV affected contest outcome (Lim & Li, 2013). By isolating the UV reflectance from other wavelengths and by staging male contests between size- and age-matched males in nature, our results provide the first direct evidence from an invertebrate that males use information from UV reflectance in settling their territorial contests. This result is consistent with findings from vertebrates that stronger UV reflectance gives males an advantage in winning male–male agonistic interactions (e.g. Bajer et al., 2011; Siefferman & Hill, 2005; Vedder et al., 2010; but see Stapley & Whiting, 2006).

Natural contest duration was correlated with the difference in male white band size, but surprisingly, not with the difference in

male body size measured by wing length, although larger males, independent of territorial status, were more likely to win contests (see also Fincke, 1992b). Thus, our results strongly suggest that males use information from the white wing band of rivals in settling their territorial conflicts. Because the area of the wing covered by the white band increases disproportionately with body size (Schultz & Fincke, 2009), the conspicuous white band may serve as a 'biological billboard' (sensu "amplifier", "exaggerator": Hasson, 1991; Maynard Smith & Harper, 2003) to facilitate the assessment of body size that could otherwise be difficult to perceive in the scramble of the fight. In contrast with predictions of self-assessment or mutual assessment, contest duration did not increase with the size of the white band of losers. Rather, our results (Fig. 2) were most consistent with opponent-only assessment (Arnott & Elwood, 2009; Elwood & Arnott, 2012). As expected, the longer a contest lasted, the more escalated it became as the number of behaviours and more costly behaviours increased. The most energetically expensive behaviours (face-offs and spirals) were relatively rare. Such contests are akin to those of cichlid fish, which assess rivals sequentially (Enquist et al., 1990). Face-offs, the most obvious wing displays, seemed to decrease contest duration (a marginally significant result after controlling for differences in white band size between rivals).

Results from observations of natural contests indicated that it was the size, specifically, of the white wing band that males assessed during their territorial contests. The UV manipulation experiment further suggested that males specifically assessed the UV reflectance of the white band during territorial contests. As a side effect, our sunscreen treatment increased brightness in the human-visible region compared to the reflectance of natural male wing band, and this difference was significant for some individuals we tested, rendering brightness a potential confounding effect. However, evidence from another experiment showed that brighter wing bands are correlated with larger body size, more fat reserves and greater flight muscle mass, all making the male more likely to win a contest (Xu, 2014). Thus, increased brightness due to the sunscreen treatment would most likely increase the chance of a male winning the contest rather than decrease it; if this male still lost a contest, it suggests that the relative effect of reducing UV reflectance (i.e. sunscreen-painted males; Fig. 3) on fight outcomes was in fact underestimated. Therefore, it is unlikely that UV-reduced males were more likely to lose fights because of an increase in signal brightness in the human-visible region. In an experiment that varied food availability among full-sibling *M. caeruleatus* reared as larvae, UV reflectance of adult males' wings had higher signal variation than all other colour components (i.e. short wavelength: 400–500 nm; medium wavelength: 500–600 nm; long wavelength: 600–700 nm; Xu, 2014). In addition, UV showed the strongest response to food treatment and was the only colour component that was correlated with fat reserves, a body condition measure.

Interestingly, the effect of UV reflectance on contest outcome depended on the time during the field season: UV reflectance affected contest outcome early but not late in the dry season. One possible explanation for this effect is that late-season males do not pay as much attention to the signal as early dry season males do because late-season males are reluctant to fight as holes diminish in value late in the season (i.e. larvae that have yet to emerge will die when the tree holes dry up). Indeed, we found that the duration of natural contests decreased as the reproductive season progressed. Eventually, males abandon tree holes even when they are still full of water, because the high heat and low humidity of the late dry season become intolerable (Fincke, 1992b; Fincke & Hedström, 2008).

A contest can be settled by asymmetries in many factors, such as body size or mass, past experience, existence or size of weaponry,

physiological status, sensory cues (Arnott & Elwood, 2009), as well as territorial status (Jennions & Backwell, 1996; Kemp & Wiklund, 2001). Furthermore, the value of the resource is known to affect the cost, duration and outcome of contests (Enquist & Leimar, 1987). In this study, we explicitly controlled for the effect of body size, age and territorial status of the two males in a dyad; hence not surprisingly, none had a significant effect on contest outcome. Even in the late field season when UV reflectance did not affect contest outcome, the slight size difference between contestants after size matching did not affect contest outcome. Therefore, contests in late season may have been settled by other asymmetrical qualities or by a male's motivation to obtain a territory, if they were not settled completely randomly.

Although the female's white wing tips are signals of sexual identity to males (Schultz & Fincke, 2009), it is unclear which colour is the signalling component. Here, we demonstrated that reducing the UV reflectance of the wing tip signal had no effect on a male's sex recognition. Therefore, UV is likely not a functional component of the sex identity signal in this species. Our results differ from findings from another territorial damselfly, *Mnesarete pudica*, in which males were less likely to react towards mature, UV-reduced females relative to controls (Guillermo-Ferreira et al., 2014). In *M. pudica*, UV reflectance of wing coloration is likely a signal of sexual maturity because both juvenile males and females have significantly lower UV reflectance than sexually mature adults. Indeed, in *M. pudica*, male reactions towards UV-reduced females were identical to those towards juvenile females. In contrast, the UV reflectance of juveniles and sexually mature individuals in *M. caeruleatus* does not show such an 'all-or-none' qualitative difference (Xu, 2014), and so it was not surprising that we did not find a similar function of UV reflectance. In addition, although UV reflectance of the female's wing tips is conspicuous against the UV-absorptive forest background, males did not react faster to females with UV blocked wing tips, but our sample size was small ( $N = 11$ ).

Our study provides the first evidence from an invertebrate that UV reflectance of a colour trait is used by males to settle territorial contests, and it provides one of the few examples among animals that UV reflectance affects the outcome of contests in the field. Opponents likely use the size of a male's white band as an advertisement for overall male size. Application of sunscreen decreased UV reflectance without decreasing the size of the white colour signal, suggesting that males were responding to the decrease in the UV component. However, because sunscreen reduced roughly half of the UV reflectance at the wavelength of the UV receptor, it will be important to quantify natural variation in UV reflectance among males in the field and investigate whether difference in UV reflectance within the range of natural variation could also affect the outcome of contests. Finally, our experiment does not exclude the possibility that other wavelengths could play a role in sexual communication (Hunt, Cuthill, Bennett, Church, & Patridge, 2001). Hence, it would be interesting to manipulate other wavelengths and compare the relative effect of different colour components.

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