

# Intrasexual selection favours an immune-correlated colour ornament in a dragonfly

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## Abstract

Sexual signalling is predicted to shape the evolution of sex-specific ornamentation, and establishing the costs and benefits of ornamentation and the information that ornamentation provides to receivers is necessary to evaluating this adaptive function. Here, we assessed the adaptive function of a common colour ornament in insects, melanin wing ornamentation, using the dragonfly *Pachydiplax longipennis*. We hypothesized that greater ornamentation would improve territory-holding success by decreasing aggression that males receive from territorial rivals, but that more ornamented males may have shorter lifespans. Using mark–recapture field observations, we found that more ornamented males had greater territory-holding success and that viability selection did not act on wing melanization. We then compared the aggression of territorial rivals to decoy males before and after experimentally augmenting wing melanization, finding that males significantly reduced aggression following the manipulation. We next hypothesized that wing melanization would signal fighting ability to territorial rivals by reflecting condition via investment in the costly melanin synthesis pathway. We observed a positive relationship between ornamentation and the likelihood of winning territorial disputes, suggesting that wing melanization provides information about fighting ability to rivals. We also found a positive relationship between melanin-based immune defence and ornamentation, supporting a link between the signal and condition. We conclude that wing melanization is a condition-related signal of fighting ability and suggest that this may be a common mechanism promoting the evolution of melanin ornamentation.

## Introduction

Sex-specific ornamentation is predicted to affect reproductive success through sexual signalling (Darwin, 1871; Andersson, 1994; Maynard Smith & Harper, 2003). Specifically, sexual signals in animals functionally shape mating success by providing information about the individual to rivals or potential mates, thereby directly moderating the outcomes of behavioural interactions over territories or copulation opportunities (Maynard Smith & Harper, 2003; Searcy & Nowicki, 2005; Lailvaux & Irschick, 2006). For example, sex-specific ornamentation may influence

competition over territories by signalling the bearer's fighting ability to rivals and facilitating the resolution of disputes quickly and without injury (Maynard Smith & Harper, 2003; Arnott & Elwood, 2009). As sexual selection should therefore strongly favour individuals with large, conspicuous ornaments, the adaptive value and evolutionary maintenance of signal function crucially depends on its ability to convey reliable information (Maynard Smith & Harper, 2003). Understanding the adaptive function of sex-specific ornaments thus necessitates directly evaluating the relationship between ornamentation and components of reproductive success, as well as characterizing the information being signalled to rivals and potential mates. However, investigations of the adaptive function of sex-specific ornamentation rarely integrate both approaches (Lailvaux & Irschick, 2006).

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Melanin is a phylogenetically widespread pigment underlying sex-specific ornaments in many animals, yet its role in sexual signalling has been relatively controversial (Stoehr, 2006; Roulin, 2016). While any positive covariance between ornamentation and aspects of condition may promote sexual signalling functions, insects use the melanin synthesis enzymatic pathway not only for colouration but also for directly mounting immune responses and repairing wounds (Schmid-Hempel, 2005; Siva-Jothy *et al.*, 2005), providing a strong proximate link by which ornamentation may intrinsically reflect aspects of condition (Hill, 2011; Roulin, 2016). For instance, allocation trade-offs of melanin precursors (e.g. tyrosine) may promote signal reliability such that only males in the best condition can invest precursors in both immunocompetence and ornamentation (i.e. Y-shaped acquisition–allocation model; Stoehr, 2006; Hill, 2011). Additionally, the high energetic costs of melanin synthesis may maintain signal reliability (e.g. Moret & Schmid-Hempel, 2000; Fedorka *et al.*, 2004; Schwarzenbach & Ward, 2006), whereby only males with the greatest energetic reserves can activate and maintain high overall levels of melanin synthesis (González-Santoyo & Córdoba-Aguilar, 2012). As a consequence of this proximate link, melanin ornamentation may often reliably signal information about an individual's condition, directly moderating the outcomes of interactions with rivals or potential mates, and causally shaping reproductive outcomes (Hooper *et al.*, 1999; Siva-Jothy, 2000; Wittkopp & Beldade, 2009). However, the relatively few tests of this sexually selected function of melanin colouration in insects have been equivocal (Lawniczak *et al.*, 2007; Punzalan *et al.*, 2008b; Izzo & Tibbetts, 2012), and its explicit function in intrasexual selection has received little attention in particular. Given this, and the growing recognition of similar pleiotropic effects in invertebrates and vertebrates (Ducrest *et al.*, 2008; Roulin, 2016), studies that directly estimate phenotypic selection on, and the information content of, melanin ornaments remain critical to understanding the adaptive function of this widespread pigment.

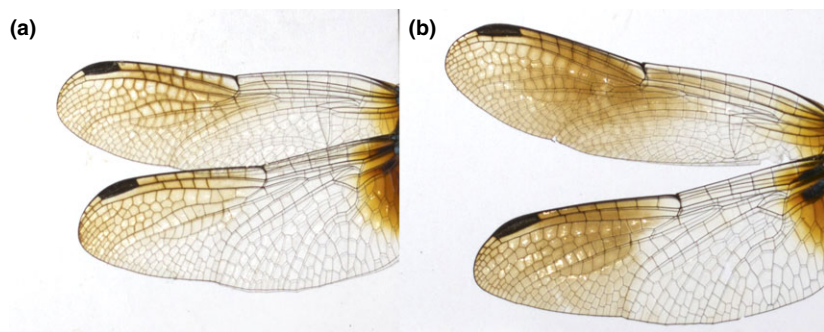
Using the sexually dimorphic dragonfly, *Pachydiplax longipennis* (Burmeister), we tested the adaptive

function of a common melanin ornament in arthropods, wing melanization (Fig. 1). Similar to damselflies that display red wing ornaments (e.g. *Hetaerina americana*, Grether, 1996b), we hypothesized that wing melanization improves territory-holding success, a vital component of mating success in odonates (reviewed in Koenig, 2008; Suhonen *et al.*, 2008), by decreasing energetically costly and potentially injurious aggressive interactions with rivals. We also predicted that males with greater wing melanization would be more susceptible to predators and have higher mortality (e.g. Grether, 1997; Kuchta & Svensson, 2014) and therefore have shorter reproductive lifespans. We further hypothesized that wing melanization signals fighting ability to rivals by proximately reflecting energetic reserves via the shared melanin synthesis pathway. We thus predicted that ornamentation would be positively associated with the likelihood of winning territorial contests and with melanin-based immune defence.

## Materials and methods

### Study system

*Pachydiplax longipennis* (Burmeister) is a medium-sized dragonfly distributed broadly across North America. Females are brown with longitudinal yellow stripes, whereas males have blue abdomens and, in the eastern extent of their range where our study was conducted, express melanin colouration on the distal portion of their wings (Fig. 1; Paulson, 2012). Detailed descriptions of the mating system can be found elsewhere (Johnson, 1962; Robey, 1975; Fried & May, 1983; Sherman, 1983). Briefly, on sunny days between mid-June and early August, males arrive at the pond between 0800 and 0900 EST and defend territories on emergent vegetation around the perimeter of the pond until approximately 1530 EST. Males spend time in their territories perching on emergent vegetation and patrolling. Males frequently encroach upon rivals' territories, engaging in aggressive disputes where the winner retains the territory and the loser either searches for a new territory or leaves the pond entirely. Once males have established at a pond for breeding, they rarely disperse (McCauley,



**Fig. 1** Wings of two mature males with relatively low (a) and high (b) wing melanization. Note that the pigmentation at the base of the wing is less variable among males and is also expressed in females. Pigmentation in the distal portion of the wing is sexually dimorphic and highly variable among males.

2010). Females arrive at the pond throughout the day, mate with a male, and then oviposit in his territory while he hovers above her (Sherman, 1983). There are seldom more than one or two females on a pond at a given time, and copulations are very short, rarely lasting more than 2 min (Sherman, 1983; Paulson, 2012).

### Study site, population monitoring and phenotypic measurements

We conducted this study at a small (perimeter = 140.2 m) research pond at Case Western Reserve University's Squire Valleevue Farm (Hunting Valley, OH, USA). Males defend territories along two regions of emergent vegetation on opposite sides of the pond (primarily *Typha* sp.; 13.8 and 35.7 m, respectively). We captured males and uniquely marked their abdomens with four dots of acrylic paint (randomly chosen from among five different colours; Anderson *et al.*, 2011). We kept males on ice in plastic bags before processing to slow their movement and facilitate safe handling (McCauley, 2010). To assess variation in wing melanization, body size and wing size (two traits associated with odonate reproductive success; Koenig, 2008), we took digital photographs (Canon G15; Canon, USA, Inc., Lake Success, NY, USA) of each male's wings and body. We standardized the lighting conditions by taking pictures of males against a standard white background (DGK Color Tools®; Fig. 1) in a dark box that excluded ambient light. We attempted to include only males with little wing wear and fully developed abdomen colouration to minimize potential variation due to age differences (Grether, 1996a; Contreras-Garduño *et al.*, 2008; McCauley, 2010). In two cases, we recaptured and reprocessed males that had not developed their full abdomen colouration. Following processing, we released males from a common location approximately 10 m from the pond. While several ponds were within the average dispersal distance of breeding males (430 m, McCauley, 2010), no marked males were ever observed at any of these other ponds.

We quantified all traits from digitized photographs in ImageJ (Rasband, 2012). Body size was calculated as the distance (mm) from the front of the head to the tips of the cerci. Wing area was scored as the total area (mm<sup>2</sup>) of all four wings. Wing area and body size are highly correlated ( $r = 0.834$ ,  $P < 0.001$ ), and thus, we calculated relative wing size by taking the standardized residuals from the linear regression of body size on total wing area. To quantify the extent of wing melanization, we identified the highest mean grey value (0–255, 0 = most opaque; 255 = most transparent) in ImageJ of the pigmented portion of each wing (i.e. least darkly pigmented), converted the photograph to binary black and white with this value as the threshold for black and calculated the size (mm<sup>2</sup>) of the digitized black area. Wing melanization was then estimated as

the proportion of the total wing area that was pigmented. To ensure the consistency of measurements, we calculated repeatability by randomly resampling a subset of 16 individuals and blindly rescored each trait. The repeatability was high for all traits (all  $R > 0.99$ , all  $F_{15,16} > 259.2$ , all  $P < 0.001$ ; Lessells & Boag, 1987). We evaluated the phenotypic variation in the population by considering how wing melanization varied with body size and relative wing size using Pearson's product-moment correlation coefficients, with individuals included as the unit of replication.

### *Do territory-holding success and longevity vary with wing melanization?*

We examined the fitness costs and benefits of our focal traits using behavioural observations of marked individuals. One observer (MPM) continuously circled the pond during the peak activity hours (0900–1530 EST) on all sunny days between 23 June and 24 July ( $n = 18$ ), recording each marked male's location, territorial behaviour and the time. On most days, approximately 50% of the territorial males at the pond were marked. Territorial males exhibit characteristic, unambiguous behaviours, such as perching in an obelisk position or chasing other males (see Johnson, 1962; Robey, 1975). We quantified a male's within-day territorial tenure as the amount of time (minutes) that it was territorial during that day. Males that were sighted only once were assigned a territorial tenure of zero minutes. We could not directly evaluate mate choice as we observed only four marked males copulating with females. However, as females choose among territorial males, a male's territorial tenure is strongly correlated with mating success (Sherman, 1983).

We assessed how daily territorial tenure varied with wing melanization, body size and relative wing size using a generalized linear mixed-effects model with a negative binomial error distribution to account for overdispersion. We did not test interactive effects among traits because models including these terms did not converge, and preliminary visual assessment of the interactions suggested that there were no strong effects between any combinations of the traits. To account for multiple territorial tenures of the same male among days, and the nonindependence among males within a given day, we included random intercepts for individual identity and observation date, respectively. As territorial tenures of zero minutes were potentially misidentified males, we excluded these observations from analyses. However, our results are qualitatively robust to their inclusion. Using individuals' daily territorial tenures, as opposed to total time spent defending a territory over the flight season, reduces bias resulting from variation in longevity (Hamon, 2005).

We evaluated how wing melanization, body size and relative wing size varied with minimum-estimated longevity using a generalized linear model with a

quasipoisson error distribution to account for overdispersion. Each male's minimum-estimated longevity (hereafter: 'longevity') was calculated as the number of days between marking and the last day it was observed. As we conducted field observations regularly through the end of the flight period, this sample of days reflects a realistic period over which viability selection may act through differences in longevity. This metric is commonly used in odonate studies (e.g. Grether, 1996a; Córdoba-Aguilar, 2002) and is relatively robust to low resighting probabilities (Waller & Svensson, 2016). We only included males marked prior to 5th July to control for differences in the available number of days between marking date and the end of the flight season. We only considered males that were observed at least twice after marking to ensure that we exclusively included resident males.

To estimate the strengths of intrasexual and viability selection on our focal traits, we used standard regression techniques to calculate selection gradients (Lande & Arnold, 1983), dividing each individual's fitness component (territorial tenure or longevity) by the population mean (i.e. relative fitness) and converting phenotypic values to mean of 0 and unit variance. To compare overall fitness variation acting through intrasexual selection vs. viability selection, we also calculated the opportunities for sexual and viability selection by dividing the variance in each fitness component by its squared mean (Arnold & Wade, 1984).

#### *Do males with greater wing melanization receive less aggression from territorial rivals?*

To assess whether wing melanization has direct effects on the aggression received from rival males, we presented decoys (previously frozen males) to territorial males, experimentally augmented the ornamentation of the decoys, presented them to new males and compared the aggression received before and after the experimental manipulation (see also Anderson & Grether, 2010; Guillermo-Ferreira *et al.*, 2015). We tethered decoys to a 2-m aluminium pole using clear nylon line (diameter = 0.18 mm), and presented them to territorial males. Typical of natural encounters between rivals (Suhonen *et al.*, 2008; McCauley, 2010), males engaged the decoys by aggressively making direct, physical contact multiple times (mean number of strikes  $\pm$  SD =  $4.4 \pm 5.1$ ) over short durations (mean number of seconds  $\pm$  SD =  $6.2 \pm 4.6$ ). For each presentation, we evaluated the following: whether the territorial male engaged the decoy ('engagement',  $y/n$ ); the time between the territorial male engaging the decoy and returning to a perch in its territory ('engagement duration', seconds); how many times it struck the decoy ('strikes',  $n$ ); and the number of strikes per second ('aggression rate', strikes per second). After presenting the decoy to several different territorial males (median: 4, range: 2–5), we augmented the

ornamentation by homogeneously colouring the wings distally from the nodus (Fig. S2) with a felt-tip marker chosen to approximate the natural colour (Crayola® *Cuppa' Cappuccino*; *sensu* Anderson & Grether, 2010; Guillermo-Ferreira *et al.*, 2015). This degree of ornamentation is within the natural phenotypic range. We then presented the decoys to a different set of territorial males and again evaluated the interactions. While the same territorial males were often presented multiple decoys, many of these males were presented post-manipulation decoys before premanipulation decoys, and thus, any observed effects of the manipulation on territorial male aggression are not confounded with territorial males becoming acclimated to the decoys.

We compared interactions before and after the manipulation using mixed-effects models with decoy identity as a random effect to account for multiple presentations of the same decoy to the different males. For error distributions, we specified the binomial for engagement, negative binomial for number of strikes and gaussian for engagement duration and aggression rate. Engagement duration was natural log-transformed to improve normality of the residuals. Significance was assessed with likelihood ratio tests of models with and without the fixed effect for generalized linear mixed-effects models and *F*-tests with the Kenward–Roger degrees of freedom approximation for linear mixed-effects models (Kenward & Roger, 1997).

#### *Is wing melanization associated with fighting ability?*

To assess the potential for wing melanization to signal fighting ability to territorial rivals, we observed naturally occurring, aggressive interactions (e.g. chasing, striking) between males during the field observations described above. We defined the winner of a territorial contest as the male that succeeded in forcing the other male out of the territory. Many contests included marked males, and we attempted to capture all unmarked males from contests. However, as contest losers frequently fly to another location on the pond or off the pond altogether, we rarely knew the phenotypic values of both males in a contest.

We analysed the likelihood of winning a territorial contest using a generalized linear mixed-effects model with a binomial error distribution and contest outcome (win = 1, lose = 0) fitted as the response variable. We included wing melanization, body size and relative wing size as fixed effects. To account for repeated measures of some individuals across disputes, and the nonindependence of two individuals in the same contest when both were known, we included random intercepts for both individual and contest identity, respectively.

#### *Does wing melanization correlate with melanin-based immune responses?*

To evaluate whether wing melanization proximately signals an individual's condition via investment in the



shared melanin synthesis pathway (*sensu* Siva-Jothy, 2000), we considered the relationship between a male's wing melanization and its ability to mount a melanin-based immune response. We captured and assayed the melanin-based immune responses of 33 territorial males over 2 days (28 July and 3 August). To assay immunocompetence, we inserted a piece of sterilized nylon monofilament (mean length  $\pm$  SD =  $2.70 \pm 0.38$  mm, diameter = 0.18 mm) into the body cavity dorsally between the fifth and sixth abdominal segments and allowed the males' immune systems to react to it for 24 h. A pilot study indicated that 24 h provided the strongest and most variable immune responses in this species (M. P. Moore, unpublished). During this period, males were kept in plastic bags in a dark climate chamber set to 8.2 °C. While this treatment may have slowed the rate of melanin immune responses, it was necessary to prevent adults from injuring themselves during or after the surgical implantation, and we do not expect that any temperature effect will have differentially affected males with different phenotypes. We then dissected out the implants and stored them in a freezer set to -22.8 °C. In four cases, the implant fell out or was accidentally inserted into the midgut, and these males were not considered further.

We quantified the melanin-based immune responses from digitized photographs of the implants. Using a dissecting microscope with a bright field background, we took one photograph of the implant, rotated the implant 90° and then took another photograph. In every photograph, we also had a nylon monofilament that was not inserted into any males as a negative control. We used ImageJ to assess the mean grey values (0 = opaque, 255 = transparent) of each implant. To calculate an immune response score for each male, we subtracted the mean grey values of the implant from the control nylon for each picture and averaged the two pictures. Higher scores indicate a darker implant and therefore a stronger melanin-based immune response, and previous work indicates this standard technique reflects resistance to naturally occurring pathogens (Rantala & Roff, 2007). Using the procedure described above (see also Lessells & Boag, 1987), the repeatability of this metric was high ( $R = 0.995$ ,  $F_{15,16} = 404.00$ ,  $P < 0.001$ ).

We used a linear model to consider how immune response scores varied with wing melanization, body size and their interaction. To account for potential variation between the two dates when males were captured, we also initially included date and its interactions in the model. As these interactions were not significant (all  $F < 1.75$ ,  $P > 0.201$ ), we removed them from the model and retested effects. The immune score from one very large male was an outlier and was not included in the analysis to improve the normality of model residuals. However, our results are qualitatively robust to its inclusion.

## Statistical analyses

All statistical analyses were conducted using R v. 3.1.2 (R Development Core Team, 2014). Mixed-effects models were fit using the 'lme4' package (Bates *et al.*, 2014). To account for large scaling differences among three focal phenotypes, wing melanization, body size and relative wing size were z-transformed for all analyses (Schielzeth, 2010). All model parameter estimates and selection gradients are reported as estimate  $\pm$  SE.

## Results

### Phenotypic variation

We first assessed the phenotypic variation of males in our population. The mean body size  $\pm$  SD of males was  $38.06 \pm 2.18$  mm, and the mean wing melanization  $\pm$  SD was  $0.41 \pm 0.09$  (proportion of melanized win area;  $n = 115$ ). There was no relationship between body size and wing melanization ( $r = -0.090$ ,  $t_{114} = -0.96$ ,  $P = 0.339$ ; Fig. S1a), or between relative wing size and wing melanization ( $r = -0.143$ ,  $t_{114} = -1.54$ ,  $P = 0.126$ ; Fig. S1b).

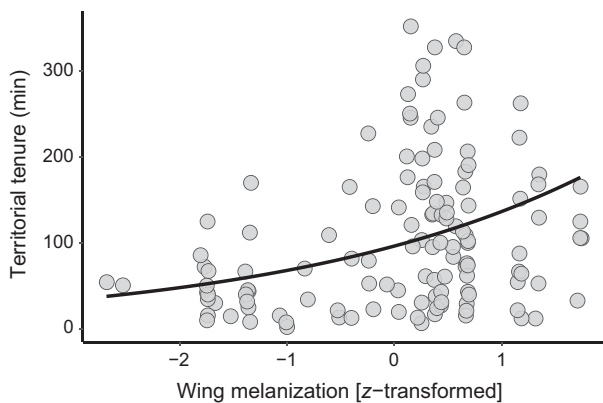
### Do territory-holding success and longevity vary with wing melanization?

In total, we observed 126 territorial tenures across 45 males (mean  $\pm$  SD per individual:  $2.8 \pm 2.2$  tenures), with a mean tenure  $\pm$  SD of  $104.9 \pm 84.2$  min. Males with greater wing melanization had longer territorial tenures ( $0.348 \pm 0.090$ ;  $\chi^2 = 13.99$ , d.f. = 1,  $P = 0.002$ ; Fig. 2). In contrast, territorial tenure did not vary with body size ( $-0.023 \pm 0.077$ ;  $\chi^2 = 0.09$ , d.f. = 1,  $P = 0.767$ ) or relative wing size ( $-0.057 \pm 0.088$ ;  $\chi^2 = 0.43$ , d.f. = 1,  $P = 0.515$ ). Overall, the opportunity for intrasexual selection was 0.707, and the estimated strength of intrasexual selection on wing melanization was relatively strong ( $\beta = 0.326 \pm 0.104$ ; c.f. Kingsolver *et al.*, 2012). We report nonsignificant intrasexual selection gradients in Table S1.

Of the 24 males included in the longevity analyses, the mean longevity  $\pm$  SD was  $17.4 \pm 5.4$  days. Longevity was not associated with wing melanization ( $0.078 \pm 0.066$ ,  $\chi^2 = 1.43$ , d.f. = 1,  $P = 0.232$ ), body size ( $-0.096 \pm 0.079$ ,  $\chi^2 = 1.49$ , d.f. = 1,  $P = 0.222$ ) or relative wing size ( $-0.022 \pm 0.093$ ,  $\chi^2 = 0.05$ , d.f. = 1,  $P = 0.817$ ). Overall, the opportunity for viability selection was 0.097, and we report the nonsignificant viability selection gradients in Table S1.

### Do males with greater wing melanization receive less aggression from territorial rivals?

Regardless of experimental treatment, decoys were equally likely to be engaged by territorial males (before



**Fig. 2** Territorial tenure increased with wing melanization. Each point represents the duration of time within a day that an individual held a territory, and points are jittered horizontally by 0.05 to improve visual clarity. The regression line is fitted from the mixed-effects model reported in the Results, and wing melanization was z-transformed to improve scaling among explanatory variables.

vs. after:  $0.719 \pm 0.479$ ,  $\chi^2 = 2.31$ , d.f. = 1,  $P = 0.129$ ). However, engagement durations were shorter following the manipulation ( $-0.429 \pm 0.201$ ,  $F_{1,46.9} = 4.46$ ,  $P = 0.040$ ). Similarly, decoys received fewer strikes ( $-0.951 \pm 0.367$ ,  $\chi^2 = 6.12$ , d.f. = 1,  $P = 0.0134$ ; Fig. 3a) and lower aggression rates ( $-0.441 \pm 0.121$ ,  $F_{1,43.7} = 12.98$ ,  $P < 0.001$ ; Fig. 3b) after augmenting the decoy's ornamentation.

### Is wing melanization associated with fighting ability?

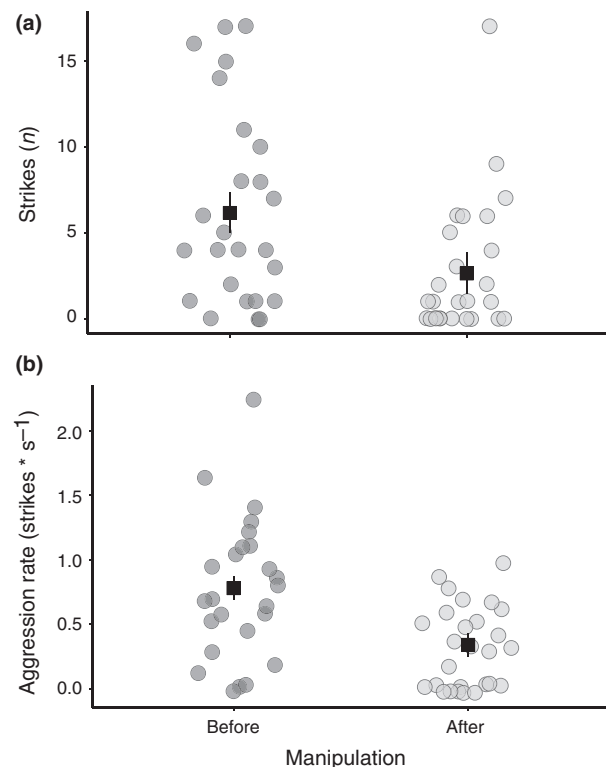
We observed 155 territorial contests (94 winners, 73 losers; mean contests  $\pm$  SD per individual:  $2.8 \pm 3.1$ ). Males with greater wing melanization ( $0.484 \pm 0.181$ ;  $\chi^2 = 6.50$ , d.f. = 1,  $P = 0.011$ ) and larger body sizes ( $0.433 \pm 0.176$ ;  $\chi^2 = 5.39$ , d.f. = 1,  $P = 0.020$ ) were more likely to win contests (Fig. 4). Relative wing size was not associated with the probability of winning contests ( $0.213 \pm 0.166$ ;  $\chi^2 = 1.72$ , d.f. = 1,  $P = 0.189$ ).

### Does wing melanization correlate with melanin-based immune responses?

Immune response scores increased with wing melanization and marginally decreased with body size (Table 1). Immune response scores were also marginally different between the two capture dates (Table 1).

## Discussion

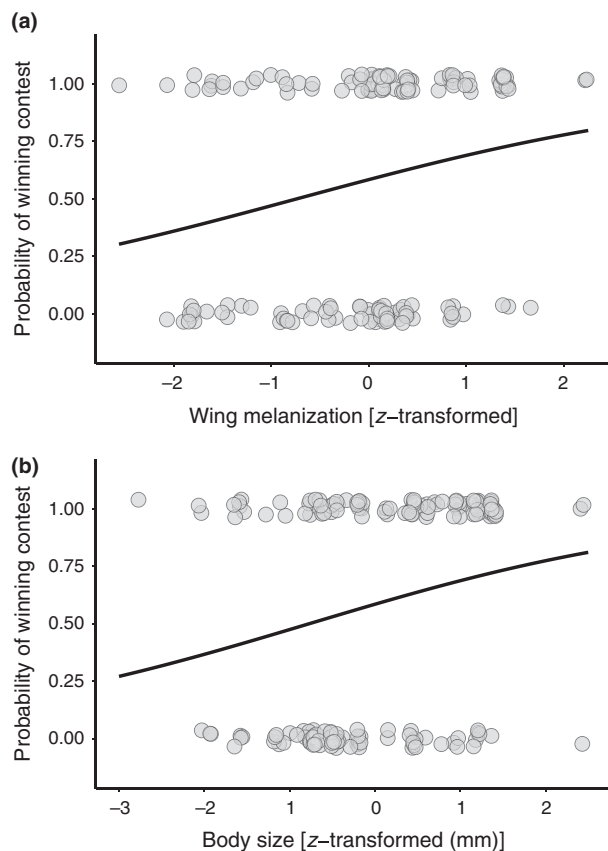
We investigated the adaptive function of melanin ornamentation in the sexually dimorphic dragonfly, *P. longipennis*. Following patterns in damselflies with



**Fig. 3** Territorial males exhibited reduced aggression (a: total number of strikes; b: number of strikes per second) to decoy males following experimental increase of wing melanization. Each circle represents a presentation of a decoy to a territorial male, and points in panel (a) are jittered vertically by 0.1 to improve visual clarity. Squares represent the model-estimated means  $\pm$  SE.

red wing ornaments (Grether, 1996b), we hypothesized that greater wing melanization would improve territory-holding success by decreasing aggression that males receive from territorial rivals, but that benefit may come at the cost of a shorter reproductive lifespan (Grether, 1997). We also hypothesized that wing melanization would signal fighting ability to territorial rivals by proximately reflecting condition via investment in the costly melanin synthesis pathway (Hooper *et al.*, 1999; Rantala *et al.*, 2000; Siva-Jothy, 2000). Overall, our results provide broad support for the hypothesized adaptive function of male wing melanization as a sexual signal of fighting ability to territorial rivals.

Male fitness is determined by the combined effects of intrasexual, intersexual and viability selection (Arnold & Wade, 1984; Hamon, 2005). Intrasexual selection via territorial occupancy is crucial to male reproductive success in territorial odonates (Moore, 1990; Grether, 1996b) and especially in *P. longipennis* (Sherman, 1983). Among the traits considered, only wing melanization improved territory-holding success



**Fig. 4** The likelihood of winning a territorial contest increased with wing melanization (a) and body size (b). Each point represents the outcome of a contest for an individual (0 = contest lost, 1 = contest won), and points were jittered vertically by 0.1 to improve visual clarity. Regression lines are fitted from the mixed-effects model reported in the Results, and both explanatory variables were z-transformed.

**Table 1** Variation in immune response scores of 28 territorial males as a function of wing melanization, body size and date (multiple  $R^2 = 0.306$ ,  $F_{3,24} = 3.53$ ,  $P = 0.030$ ). Model estimates ( $\pm$  SE) were obtained after removing the nonsignificant wing melanization  $\times$  size interaction. Wing melanization and body size were z-transformed prior to analysis, and date represents the difference between the 2 days over which males were captured. All partial  $F$ -tests were on 1 and 24 degrees of freedom.

Effect	Estimate	$F$	$P$
Wing melanization	$7.381 \pm 3.110$	5.69	0.026
Body size	$-5.418 \pm 2.808$	3.76	0.065
Date	$9.466 \pm 5.414$	2.94	0.100
Wing melanization $\times$ body size		1.26	0.274

(Fig. 2), and our selection analysis indicated strong intrasexual selection on this ornament (c.f. Kingsolver *et al.*, 2012). Although we are unable to assess the strength of intersexual selection in this study, other

selection analyses in odonates have found positive (e.g. Córdoba-Aguilar, 2002) or no (e.g. Grether, 1996b) intersexual selection on wing ornamentation. Contrary to many studies of other odonates (reviewed in Koenig, 2008), and despite a positive relationship between body size and the likelihood of winning territorial disputes (Fig. 4b), we did not observe selection on body size. As small males are likely to lose fights and never acquire a territory, it is possible that, by including primarily males that had already acquired territories, our estimates of selection on body size (and all other traits) may be conservative. While we also predicted that increased melanization may come at the cost of reproductive lifespan (e.g. Grether, 1997; Kuchta & Svensson, 2014), we found no evidence for viability selection against wing melanization. However, the opportunity for viability selection (0.097) was considerably lower than the opportunity for intrasexual selection (0.707), and therefore, intrasexual selection may be relatively more important for generating variance in male fitness in this population, further suggesting strong overall fitness benefits of ornamentation.

Our results indicate that the functional benefit of wing melanization in *P. longipennis* is through decreased aggression received from rival males. Territorial disputes in odonates are extremely energetically costly, and consume most of an individual's daily energy budget (Fried & May, 1983; Koskimäki *et al.*, 2004). As males do not feed while they are defending territories (Fried & May, 1983), any decrease in the energy expended on battling intruders may increase the time over which a male holds its territory, and therefore also increase the likelihood of mating (Suhonen *et al.*, 2008). Moreover, in contrast with many other odonates, *P. longipennis* males make physical contact during territorial disputes, greatly increasing the chances of severe injury or death (Sherman, 1983; McCauley, 2010; Paulson, 2012). Our results suggest that rivals challenge males with greater ornamentation less frequently and with lower intensity (Fig. 3), potentially improving a male's ability to remain in a territory and/or avoid injury. Similar to damselflies expressing red wing ornaments (Grether, 1996b; Guillermo-Ferreira *et al.*, 2015), it seems likely that a primary functional advantage of greater melanin ornamentation is through decreased aggression from territorial rivals.

The information provided to rivals and potential mates is crucial to the evolution of sexual signals, and those involved in male–male competition are predicted to evolve to ensure that disputes are resolved as cheaply as possible for both parties (Maynard Smith & Harper, 2003; Searcy & Nowicki, 2005). In particular, territorial males should assess the fighting ability of their rival and avoid engaging them if they are unlikely to win the dispute or if the cost of winning the dispute is too high (Maynard Smith & Harper, 2003; Arnott & Elwood, 2009). As wing melanization is associated with a male's ability to win contests (Fig. 4), rivals would

benefit from avoiding costly and/or potentially injurious disputes with highly ornamented males (Junior & Peixoto, 2013; Guillermo-Ferreira *et al.*, 2015). Indeed, males with experimentally increased ornamentation received significantly lower aggression from rivals (Fig. 3). As the fitness benefits of displaying a large ornament are great, the signal must be reliable, on average, to be evolutionarily maintained, otherwise males would cease to respond (Maynard Smith & Harper, 2003). In many cases, melanin ornaments in arthropods may signal condition reliably because of the biochemical link between immune defence and colouration (Table 1). While a male's immunocompetence may not be specifically informative to rivals, the high costs of the melanin synthesis pathway (González-Santoyo & Córdoba-Aguilar, 2012) will ensure that only males in the best condition have the capacity to allocate sufficient resources (e.g. energy, precursors, enzymes) to support robust immune function and large wing ornaments (e.g. Hooper *et al.*, 1999; Rantala *et al.*, 2000; Siva-Jothy, 2000). The maintenance of melanin wing ornaments in insects also requires some degree of constant pigment deposition (Hooper *et al.*, 1999; True *et al.*, 1999), further enabling the ornament to reflect the individual's present physiological state. As there is a strong relationship between condition and fighting ability in insects (reviewed in Vieira & Peixoto, 2013), and investment in many other condition-related traits ends at metamorphosis or maturity (e.g. body size), the melanin synthesis pathway may be a common mechanism by which arthropods reliably signal proximate information about their condition and fighting ability to rivals.

While the shared biochemical pathway linking immune defence and colouration promotes signal reliability, intrasexual selection will also favour males that maximize signal efficiency (Badyaev, 2004; Stoehr, 2006), which will have consequences across the melanin synthesis pathway. Indeed, artificial selection experiments (Armitage & Siva-Jothy, 2005) and studies of natural populations experiencing divergent selection pressures (Fedorka *et al.*, 2013) have found that selection on melanin colouration often promotes the correlated evolution of melanin immune defence. As with most sexual signals (reviewed in Hill, 2011; Morehouse, 2014; but see Craig & Foote, 2001), the specific targets of selection for improving the efficiency of melanogenesis are unknown. However, two evolutionary outcomes for the melanin synthesis pathway seem most likely: (1) energetic resources may be more efficiently allocated to the production of melanin synthesis enzymes (e.g. phenoloxidase), and (2) amino acid precursors may be more readily available for conversion to melanin. It remains to be seen which of these two outcomes is more likely or whether either is general across species, as some studies of the melanin synthesis pathway indicate energetic limitations (e.g. Cotter *et al.*, 2010), while others report precursor limitations (e.g. Srygley

*et al.*, 2009). Importantly, as both immune defence and colouration depend on many of the same precursors and enzymes, selection for increased signalling efficiency may be unable to completely erode the association between colouration and condition, and the signal will remain reliable, on average. Nevertheless, if intrasexual selection favours greater efficiency of signal production, then populations exhibiting stronger intrasexual selection on wing melanization should express more efficient melanogenesis.

The evolution of sex-specific ornamentation depends in part on the functional mechanisms by which the ornament affects reproductive success (Andersson, 1994; Lailvaux & Irschick, 2006). We found strong support for a sexual signalling function shaping the evolution of sex-specific wing melanization in a territorial dragonfly. Melanin colouration exhibits a diverse suite of adaptive functions in arthropods (e.g. Punzalan *et al.*, 2008c; Fedorka *et al.*, 2013; Debecker *et al.*, 2015), but given its frequently observed condition dependence (e.g. Talloen *et al.*, 2004; Punzalan *et al.*, 2008a), the ability to act as a sexual signal of condition may commonly shape its evolution. Although any direct or indirect positive covariance between ornamentation and aspects of condition may ultimately facilitate condition-related sexual signalling of an ornament, linkages underlain by shared condition-dependent developmental pathways are predicted to readily evolve via sexual signalling functions (Hill, 2011; Roulin, 2016). As the melanin synthesis pathway links ornamentation and immunocompetence in arthropods, sexual signalling, like that observed here, may indeed prove to be a common adaptive function shaping the evolution of sex-specific melanin colouration.

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## Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article:

**Figure S1** There was no relationship between a male's wing melanization and its body size (a) or its relative wing size (b). Each point represents an individual.

**Figure S2** Rear wings of territorial *Pachydiplax longipennis* showing examples of weak natural melanization (top), experimentally manipulated melanization (middle) and strong natural melanization (bottom).

**Table S1** All variance-standardized selection gradients for intrasexual and viability selection (Lande & Arnold, 1983).

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