RESEARCH ARTICLE

Negative body size-dependent resource allocation underlies conspicuous sexual ornaments in a territorial damselfly

Mingzi Xu[1,2] | Ola M. Fincke[1]

[1]Ecology and Evolutionary Biology Program, Department of Biology, University of Oklahoma, Norman, Oklahoma, USA

[2]Department of Ecology, Evolution and Behavior, University of Minnesota, St Paul, Minnesota, USA

Correspondence
Mingzi Xu, Department of Ecology, Evolution and Behavior, University of Minnesota, St Paul, MN, USA.
Email: xu000574@umn.edu

Abstract

Sexual ornaments, signalling individual quality to choosy females or rival males, often show steeper body size scaling compared with non-sexually selected traits. Theory posits such steeper body size scaling is the result of differential resource allocation, reflecting trade-offs between different components of fitness. Yet, the process of resource allocation towards body size-dependent sexual ornaments has been rarely understood empirically. Using the Neotropical territorial damselfly *Megaloprepus caerulatus*, whose males and females carry wax-based, sex-specific white wing bands and white wing tips respectively, we investigated nutrition sensitivity and body size scaling of both traits by manipulating larval food availability and directly quantified both the fat allocated to wing ornaments and the fat reserve from which allocations are made. Both colour traits exhibited sensitivity to food availability during larval development and steeper body size scaling compared with control traits. Although the absolute amount of fat invested in developing the colour ornaments increased with body size, the proportion of total fat allocated to the ornaments decreased with body size, making exaggerated ornaments less affordable for smaller individuals. Our data demonstrate that measuring the proportion of resource pool from which an individual's ornaments are derived (i.e. its affordability) is essential for understanding the maintenance of honesty of sexual signals.

KEYWORDS
allometry, damselfly, differential cost, handicap, honest signal, insect, structural colour, territorial contest, trade-off

1 | INTRODUCTION

Sexual ornaments can reveal information to conspecifics about how good a male is as a partner, competitor or parent (Andersson, 1994). Such sexual signals often correlate with body size, with larger males carrying larger and more conspicuous ornaments (Kodric-Brown et al., 2006). Static allometric scaling (*sensu lato*) of sexual ornaments with body size is widespread and has been documented across a broad range of taxa (reviewed by Bonduriansky, 2007; Kodric-Brown et al., 2006). As body size is also commonly associated with viability and outcome of male-male competition (Benson & Basolo, 2006; Kingsolver & Huey, 2008; Mitchem et al., 2019; Sokolovska et al.,

2000), body size-dependent sexual ornaments are often used in assessment of potential mates or rivals in mate choice and male-male competition.

Body size dependence, however, is not unique to sexual ornaments because nearly all structural traits scale with body size. Theory posits that a signalling trait under sexual selection should exhibit steeper isometric, or sometimes, allometric scaling with body size than morphological traits that are not under sexual selection (Bonduriansky & Rowe, 2005). This is because when a sexual ornament is under directional sexual selection, the fitness return of the signal is expected to increase with resource allocation to it, which in turn, favours individuals that allocate as much of their resource

pool as possible towards the sexual ornament until it is countered by natural selection (Bondurianksy & Day, 2003; Rowe & Houle, 1996). In comparison, a non-signalling trait would have lower fitness return from increased allocation, hence, is expected to receive a base-line level of resource allocation and exhibit a base-line degree of body size scaling (Bonduriansky, 2007). In addition, ornaments with a steeper scaling relationship with body size often show a higher level of phenotypic variance (Cotton et al., 2004), making such ornaments more effective in accentuating variation in the underlying signalling content (Emlen & Nijhout, 2000).

Resource allocation plays a central role in the expression of sexual ornaments, especially when resources required for developing the ornaments are limiting. Yet, most of our understanding on resource allocation of sexual ornaments comes from indirect empirical data on phenotypic trade-offs between sexual ornaments and other traits (e.g. Baeta et al., 2008; Contreras-Garduño et al., 2008; Simmons & Emlen, 2006), likely because the specific resource needed for expressing sexual ornaments is not conducive to direct quantification. Whereas phenotypic data on trade-offs can reveal a competing relationship between the expression of sexual ornaments and other traits, such evidence only offers indirect insights into the mechanistic causation between resource allocation and expression of sexual ornaments. In addition, a comprehensive understanding of resource allocation requires knowledge of both the amount of resource allocated to the trait of interest and the total available resource pool. The latter is important because it provides a context within which affordability of a specific amount of investment can be interpreted. Using a simple hypothetical example, assume it costs one thousand dollars to add a bedroom to a house. Although the absolute marginal cost of expanding the house is the same across homeowners with different incomes, the affordability of adding a bedroom varies with income: A thousand dollars is more affordable for someone with an income of one million dollars than for someone with an income of five thousand dollars. By analogy, understanding the affordability of a signal enhancement requires knowing the resource pool, which is often elusive or difficult to quantify.

One outstanding question about resource allocation of sexual ornaments concerns allocation to the ornaments in relation to the resource pool (i.e. 'affordability' of the ornament). Although it is generally agreed that larger males are expected to allocate more towards expressing sexual ornaments, the resource pool from which the allocation is made is also expected to increase with body size. Thus, whether relative resource allocation (i.e. the proportion of total resource allocated to sexual ornaments) changes with body size remains an open question. If allocation to sexual ornaments and total resource increase with body size at the same rate, then the proportion should be invariable with body size (Figure 1). Alternatively, if allocation to sexual ornaments increases at a faster rate than the resource pool, we expect the proportion of resource directed towards sexual ornaments to increase with body size. Conversely, if allocation to sexual ornaments increases at a slower rate than the resource pool, we expect the proportion of resource directed to sexual ornaments to decrease with body size. These scenarios have
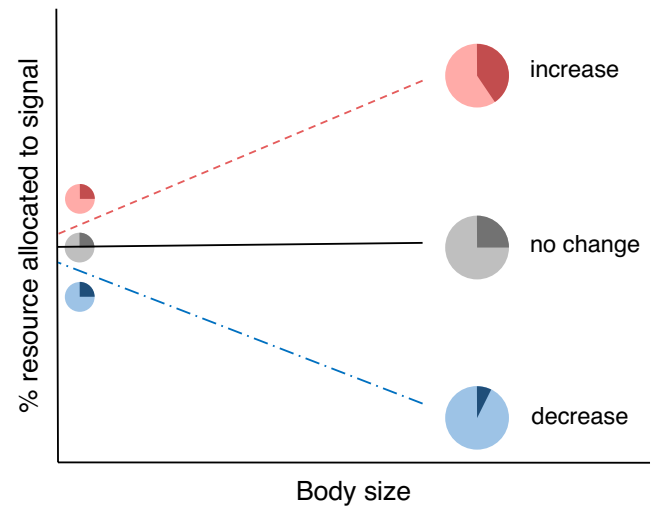

FIGURE 1   Diagram of three hypothetical examples where proportion of resource allocated to sexual ornaments increases, remains the same or decreases with body size. The light-coloured circles indicate resource pools and the dark-coloured pie sections represent proportions of resource allocated to sexual ornaments

contrasting implications for the fitness payoff as well as the maintenance of honesty of sexual ornaments. For example, increasing relative allocation should reflect an increasing fitness return and decreasing affordability of the signal as body size increases. Yet, empirical data on relative allocation remain scant (Kotiaho, 2001).

Here, we present evidence for negative body size-dependent resource allocation underlying the development of positive body size-dependent wax-based sexual ornaments in the neotropical giant damselfly *Megaloprepus caerulatus*, a rare system where both resource allocation to sexual ornaments and the resource pool from which the allocation is made can be directly measured. In *M. caerulatus*, both males and females have iridescent blue wing bands (Figure 2). This structural colour trait provides a visual signal that flashes with each wing stroke. Whereas the blue band showed negative allometry, the flashing feature of this signal likely serves both sexes in attracting the attention of conspecifics (Schultz & Fincke, 2009). In addition to the blue wing band, females have white tips, a cue of their sexual identity, whereas males have relatively clear wing tips (Schultz & Fincke, 2009). Additionally, males have a sex-specific white wing band proximal to the blue band (Figure 2a). The male white wing band and female white wing tip are structural colours made of nano-sized wax crystals secreted by epidermal cells and transferred to the wing surface by a canal system (Gorb et al., 2000; Schultz & Fincke, 2009). These wax filaments can cause Mie scattering, giving the appearance of the white colour (Schultz & Fincke, 2009). Fatty acids stored in fat reserves serve as a precursor for wax synthesis in insects (Arrese & Soulages, 2010). Therefore, fat reserve stored in the body represents the resource pool from which wax used to produce the male white wing bands and female white wing tips is allocated. Interestingly, newly emerged immature males have female-like white wing tips, which significantly dim within 36 h
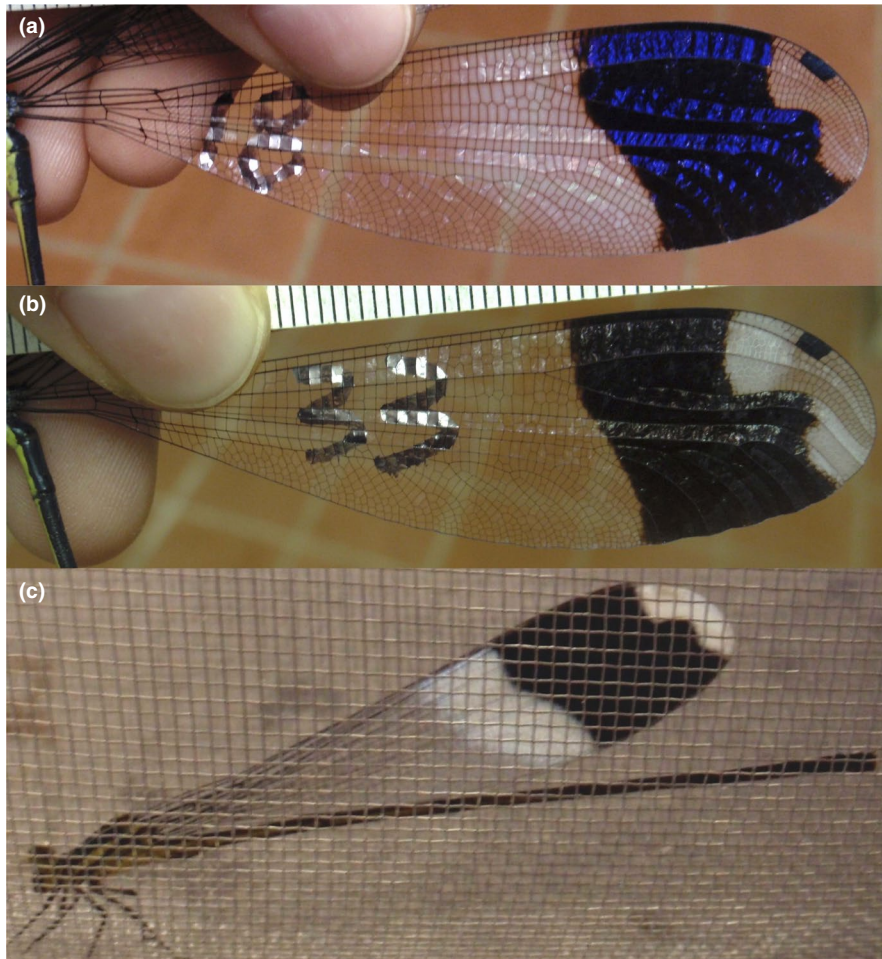
FIGURE 2 Wing morphology of (a) mature male, (b) mature female and (c) newly emerged male showing the irredescent blue wing band, the white wing band proximal to the blue wing band, common to mature and immature males, and the white wing tip similar to that of mature females. Note that the white wing tips in (c) disappear shortly after emergence, becoming almost transparent as in (a)

(herein), rendering most of the male wing tips almost transparent in sexually mature adult males (Fincke et al., 2018 and herein).

In *M. caerulatus*, males defend water-filled tree holes in forest light gaps where females come to mate and lay eggs (Fincke, 1984, 1992a). The male white wing band functions as a signal of fighting ability in territorial contests (Xu & Fincke, 2015). The size of the male white wing band increases with body size, which in turn, correlates with territory ownership and mating success (Fincke, 1992a). Information from both size and colouration of the white wing band is used by males to settle territorial contests (Fincke, 1992a; Xu & Fincke, 2015). Controlling for body size, males with larger white wing bands are more likely to win territorial contests and contest duration decreases with size difference in the wing bands between two contestants (Xu & Fincke, 2015). In addition, males with more UV reflectance from the white wing band are more likely to win contests. In comparison, female white wing tips signal sexual identity to territorial males (Schultz & Fincke, 2009). Males react sexually to individuals with white wing tips and experimentally blackening a female's white wing tips leads to aggressive reaction from territorial males (Schultz & Fincke, 2009).

Water-filled tree holes are a detritus-based food web where nutrients come from organic matter that falls into or is deposited in the tree holes. In such an ecosystem, nutrients are usually extremely limited (Kitching, 2000; Yanoviak, 2001). Male body size, which is under strong sexual selection, is positively correlated with the volume of the natal tree hole (Fincke, 1992a), which in turn, positively correlates with nutrient availability (Paradise, 2004) and the time available for larvae to develop before tree holes dry up seasonally (Fincke, 1992a; Yanoviak, 1999). Development of a larva to adulthood requires a water-filled tree hole of at least 0.1 L in volume (Fincke, 1992a) and tree species with sufficiently large tree holes are relatively rare (Fincke, 2006). Therefore, food availability from this detritus food web is a major limiting resource during larval development.

In this paper, we first investigate the relationship among adult body size, ornament size and larval food availability in a rearing experiment where we manipulated food availability of full-sib larvae, simulating differences in tree hole volume that correlate with the amount of total nutrient available in nature. We also compare body size scaling between male white wing band area and two control traits, blue wing band area and squared length of the left middle leg femur, neither of which are expected to be under strong selection for size. For the wing tips in females and males, we used femur as a control trait. We then examine whether the male white wing band advertises any signalling content independent of body size. We hypothesized that the white wing band may reflect flight muscle mass and/or fat reserves, both of which play a role in performance during aerial territorial contests (Contreras-Garduño et al., 2008; Marden &

Waage, 1990). Lastly, we quantified both the wax deposited on the wings that forms the white wing band and tip and the fat reserve inside the body. Using these data, we test three alternative predictions: that the proportion of fat allocated to the two wax-based, sex-specific ornaments (1) does not change, (2) increase or (3) decrease with body size.

2 | MATERIALS AND METHODS

2.1 | Egg collection and food manipulation

We mated eight field-caught female *M. caerulatus* with unique males at natural territories in the lowland moist forest on Barro Colorado Island, Panama (9.15°N, 79.85°W) during the wet season between 24 September and 17 November 2010. After a mating was completed, the female was caught and placed in a plastic jar with wet filter paper as oviposition substrate to lay eggs (Fincke & Hadrys, 2001). Its lengthy and unique copulation behaviour indicates *M. caerulatus* uses repeated bouts of sperm removal and replacement to achieve last male sperm precedence; we assume that eggs laid immediately after a mating are full siblings (Fincke, 1984, 2011; Fincke & Hadrys, 2001).

Eggs from different families were placed in separate plastic containers with 1 L aerated water to develop. Hatchlings from a family that hatched on the same day were placed in plastic containers in groups of 5 with 250 ml aerated water and *ad lib.* brine shrimp eggs, which hatch readily in freshwater. Once a larva reached 4 mm in body length, it was randomly assigned to one of three food treatments. Larvae from the high, medium and low food treatments were fed every one, two, and four days respectively. Live one-day-old *Physalaemus pustulosus* tadpoles and mosquito larvae roughly 5 mm in length were used as food sources, both natural prey of *M. caerulatus* (Fincke, 1992b). The mean dry weight of the tadpoles and the mosquito larvae used were 0.75 ± 0.02 mg ($n = 29$) and 0.47 ± 0.03 mg ($n = 18$) respectively. The amount of food per feeding was adjusted for larval size across all three treatments: 4–7 mm larvae received two tadpoles or 6 mosquito larvae, 7–10 mm larvae received four tadpoles or 8 mosquito larvae, larvae between 10 and 15 mm received six tadpoles or 10 mosquito larvae, and larvae > 15 mm received 8 tadpoles. Larvae < 15 mm in body length were kept individually in small plastic containers with 200 ml water, whereas those >15 mm were kept individually in large plastic containers with 1 L water. All containers had dead leaves as perching sites. We changed water and cleaned the containers after each larval moult. All larvae were reared in an outdoor screened shed with natural light cycle, humidity and temperature.

2.2 | Adult trait measurements

Once a final instar larva with swollen wing pads stopped feeding, signalling imminent emergence, we moved its container into a cylindrical emergence cage made of window screen. A newly emerged adult was kept in its cage for 36 h until its wings dried, after which it was frozen. Loss of white colouration on the male wing tips occurred within 36 h. We photographed the left hindwing of male and female offspring with a ruler in view and measured the area of the hindwing, the male white wing band, the male blue wing band, and both male and female wing tips (i.e. the area between the outer edge of the blue band and the edge of the wing) in ImageJ (National Institutes of Health, Bethesda, USA). Hindwing area was used as a proxy for body size (Fincke & Hadrys, 2001). The femur length of the left middle leg was measured with digital callipers. In addition, we obtained relative reflectance spectra of the male white wing band and the female white wing tip from left hindwing using an OceanOptics SD 2000 spectrometer with a PX-2 xenon light source (OceanOptic, Dunedin, USA). In the relative spectrum, 0 was calibrated by taking a measurement in a dark room and 100 was calibrated using a WS-1-SL diffuse reflectance standard (OceanOptic, Dunedin, USA). We measured brightness (i.e. total relative reflectance) of the white wing traits from the reflectance spectra interpolated between 300 and 700 nm.

We measured the amount of thoracic muscle in male offspring by dissolving the muscle tissue in 0.35 mol/L NaOH solution according to a method described by Marden (1987). In addition, we measured the amount of wax on the wings that made up the white bands and tips and fat reserve inside the body using a Soxhlet extraction procedure (Marden, 1989). Briefly, we soaked samples in chloroform under room temperature for 8 h and then extracted fat in a Soxhlet apparatus with boiling chloroform for 8 h. After the extraction, the male white wing bands and the female white wing tips completely disappeared, confirming that our fat extraction protocol was effective in extracting wax from the wings. Because 36 h after emergence when we sacrificed adults, the males' white tips had almost completely disappeared (see Figure 2a,c), and the wax extracted from male wings was primarily from the male white wing bands.

2.3 | Statistical analyses

In all statistical analyses below, we used linear mixed models (LMMs) with family as a random effect variable. Individual ID was used as an additional random effect variable nested within family when multiple measures from one individual were used in a model. We examined the relationship between larval food availability and body size as well as wing traits using LMMs with body size (i.e, hindwing area) or area of the white wing band, the blue wing band and female wing tips as the dependent variable. Because larval developmental time decreased with more food fed (see Supplementary Material), we used total dry weight fed, instead of treatment, as a continuous fixed effect variable in the above LMMs.

To examine body size scaling of the wing traits, we used LMMs with wing ornament area as the dependent variable and body size as a fixed effect variable. To account for the large difference in the scale of the area of different wing traits, we standardized trait area

by taking the difference between trait area measurement and the mean trait area and then dividing it by the mean trait area. To investigate whether the male white wing band and female white wing tip showed different degrees of body size scaling from control traits, we tested for a significant trait by body size interaction. In those models, we used the squared femur length to make it comparable with area measures of wing colour traits. Relationships between the brightness of the white wing band and larval food availability as well as body size were also examined using similar LMMs with brightness as the dependent variable and body size as a fixed effect variable.

To test whether the male white wing band signalled quality independent of body size, we first calculated the standardized residuals from regressing white wing band area on hindwing wing area and then performed LMMs with the residual as the dependent variable and fat reserve or thoracic muscle mass as the independent variable. A significant relationship would signify that fat reserve or thoracic muscle mass can explain additional variance in white band area that body size fails to explain and hence would indicate that the white band signals either fat reserve or thoracic muscle mass independent of body size. In the model for fat reserve, we excluded one individual representing an outlier (see grey arrow in Figure S1a). Including this individual did not change any conclusion.

To investigate resource allocation towards the male white wing band and the female white wing tip, we first confirmed that more total dry weight fed resulted in more total fat in adults. We then tested whether fat reserve inside the body and wax deposit on the wings increased with body size using LMMs with fat reserve or wing wax as the dependent variable and wing area as a fix effect variable. We also examined whether wax density on the white wing band varied with body size to understand the marginal production cost of this signal. Wax density was calculated as the amount of wax on the wing divided by the area of the white band. To examine whether individuals of different body size allocated fat differently, we used LMM to test the effect of wing area on arcsine transformed fraction of total fat deposited on the wings.

Linear mixed models were run using package lme4 in R (version 3.6.3, R Core Team, 2020). Throughout, means are shown with standard errors.

3 | RESULTS

3.1 | Nutrition sensitivity, body size scaling and information content of the male white wing band

Among 42 F_1 males, hindwing area increased with total dry weight fed ($\chi^2_1 = 11.29, p = 0.001$, Figure 3a). Similarly, the area of the white band, blue band and squared middle femur length also increased with total dry weight fed (white band: $\chi^2_1 = 7.95, p = 0.005$, blue band: $\chi^2_1 = 6.54, p = 0.01$, middle femur: $\chi^2_1 = 22.19, p < 0.001$, Figure 3b-d). Standardized area of white band, blue band and squared middle femur length increased with wing area (white band: $\chi^2_1 = 19.25, p < 0.001$, blue band: $\chi^2_1 = 49.23, p < 0.001$,

middle femur: $\chi^2_1 = 35.46, p < 0.001$, Figure 4a). The three male traits differed significantly in their isometric scaling with body size ($\chi^2_1 = 34.2, p < 0.001$, Figure 4a). Specifically, the white band area increased with body size at a steeper slope (b = 0.002) than both the blue band (b = 0.001, $\chi^2_1 = 4.49, p = 0.03$) and femur (b = 0.001, $\chi^2_1 = 26.84, p < 0.001$). In addition, the brightness of the white band increased with wing area ($\chi^2_1 = 5.25, p = 0.02$, Figure 5a).

On average, fat reserve and flight muscle made up $8.5 \pm 0.3\%$ and $25.8 \pm 0.2\%$ of total dry weight of male offspring respectively. The residual white band size explained neither fat reserve ($\chi^2_1 = 2.34, p = 0.13$, Figure S1a) nor flight muscle mass ($\chi^2_1 = 0.97, p = 0.32$, Figure S1b).

3.2 | Nutrition sensitivity and body size scaling of the wing tip

Among 63 F_1 females, body size and white tip size increased with total dry weight fed (body size: $\chi^2_1 = 31.20, n = 63, p < 0.001$, wing tip: $\chi^2_1 = 8.92, n = 63, p = 0.002$, Figure S2). The area of both the female white wing tip and male's nearly transparent wing tip increased with body size (female: $\chi^2_1 = 65.23, p < 0.001$, male: $\chi^2_1 = 70.06, p < 0.001$, Figure 4b), and the slopes did not differ between the sexes (female: b = 0.0017, male: b = 0.0019, $\chi^2_1 = 1.45, p = 0.23$, Figure 4b). Unlike the male white band, the brightness of the female white wing tip did not change with body size ($\chi^2_1 = 0.19, p = 0.66$, Figure S3). Using standardized trait value, the area of female and male wing tip increased with body size at steeper slopes than the female squared middle femur length (b = 0.0008) and male squared middle femur length (b = 0.001) respectively (female: $\chi^2_1 = 10.75, p = 0.001$, male: $\chi^2_1 = 8.56, p = 0.003$, Figure 4b).

3.3 | Resource allocation to the male white wing band and female white wing tip

We extracted fat from a total of 42 and 52 male and female offspring respectively. Total dry fat mass increased with total dry weight fed in both sexes (male: $\chi^2_1 = 11.66, p = 0.001$, female: $\chi^2_1 = 39.04, p < 0.001$). On average, males and females allocated $23.1 \pm 0.7\%$ and $23.8\% \pm 0.8\%$ of total fat towards white wing bands and white wing tips respectively. The absolute amount of fat reserve and wax on the wings both increased with body size for both males and females (males: fat reserve: $\chi^2_1 = 23.60, p < 0.001$, wing wax: $\chi^2_1 = 27.03, p < 0.001$, Figure 6a, females: fat reserve: $\chi^2_1 = 34.40, p < 0.001$, wing wax: $\chi^2_1 = 32.09, p < 0.001$, Figure 6b). Wax density of the male white wing band did not change with body size ($\chi^2_1 = 0.32, p = 0.57$), whereas wax density of the female white wing tip increased with body size ($\chi^2_1 = 33.61, p < 0.001$). The proportion of fat allocated to the wings decreased with body size for both males ($\chi^2_1 = 4.11, p = 0.04$, Figure 7a) and females ($\chi^2_1 = 5.75, p = 0.02$, Figure 7b). In male offspring, the highest proportion of fat allocated to the wing was 31.8% in males, made by the smallest male (wing area of
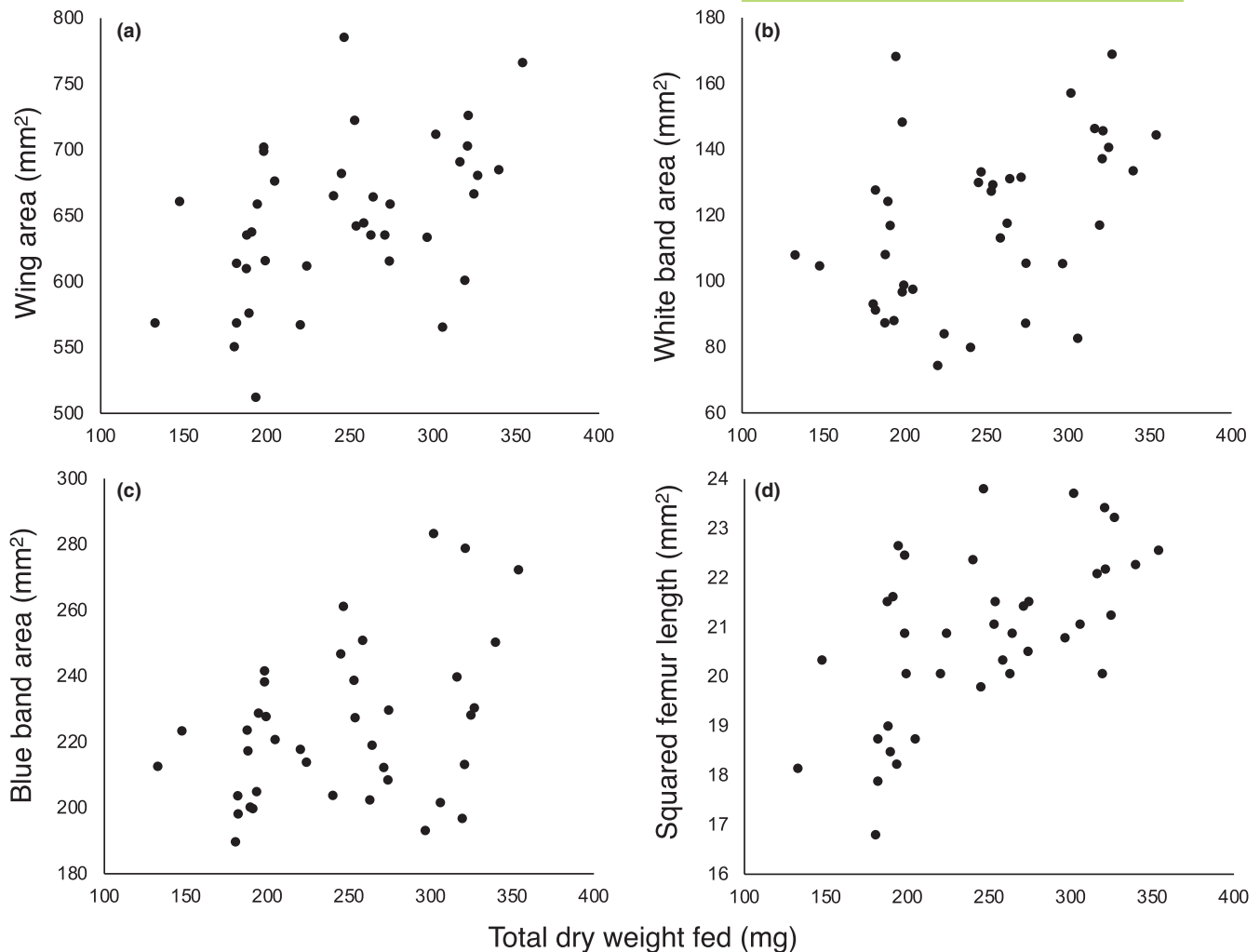
FIGURE 3 Positive relationships between total dry weight fed and area of (a) hind wing, (b) the white wing band, (c) the blue wing band and (d) squared femur length in male offspring

512.3 mm^2), compared with the lowest proportion of 14.9% made by the fifth largest male (wing area of 712.0 mm^2, Figure 6a). The allocation by the smallest male resulted in a white band of 88.05 mm^2 with a relative brightness of 4.31%, compared with a band of 157.2 mm^2 with a relative brightness of 7.01% of the male allocating only 14.9% of his fat.

4 | DISCUSSION

Conspicuous sexual ornaments are common in nature yet systems that offer a direct view of the physiological mechanisms underlying the production and maintenance of such signals are rare (Warren et al., 2013). Here, by taking advantage of the damselfly *M. caerulatus*, where both investment in the sexual ornaments and the size of the resource pool from which investment is allocated can be directly assessed, we found negative body size-dependent relative resource allocation underlies heightened body size scaling of wax-based sexual ornaments.

We found that both body size and the size of the male white wing band are sensitive to food availability during larval development. Since adult body size does not change after emergence in this species, this common relationship with larval nutrition renders the male white wing band an appropriate trait to signal body size. Indeed, the male white band is positively correlated with body size but not fat reserve or thoracic muscle mass controlling for body size, indicating this ornament is primarily a signal of body size but not fat or muscle content. The relationship between the male white wing band size, larval nutrition and body size, which is a primary determinant of male territorial ownership (Fincke, 1992a), reflects a causal link between larval development and reproductive success. In addition, the lack of a relationship between the white band and fat reserves of immature males, controlling for body size, contrasts with findings from *Calopteryx* damselflies where fat reserves of mature males play a large role in determining contest outcome (Marden & Rollins, 1994; Marden & Waage, 1990). Whereas those contests are frequent, highly escalated, and prolonged, sometimes lasting for hours, contests in *Megaloprepus* are less frequent, typically much shorter,
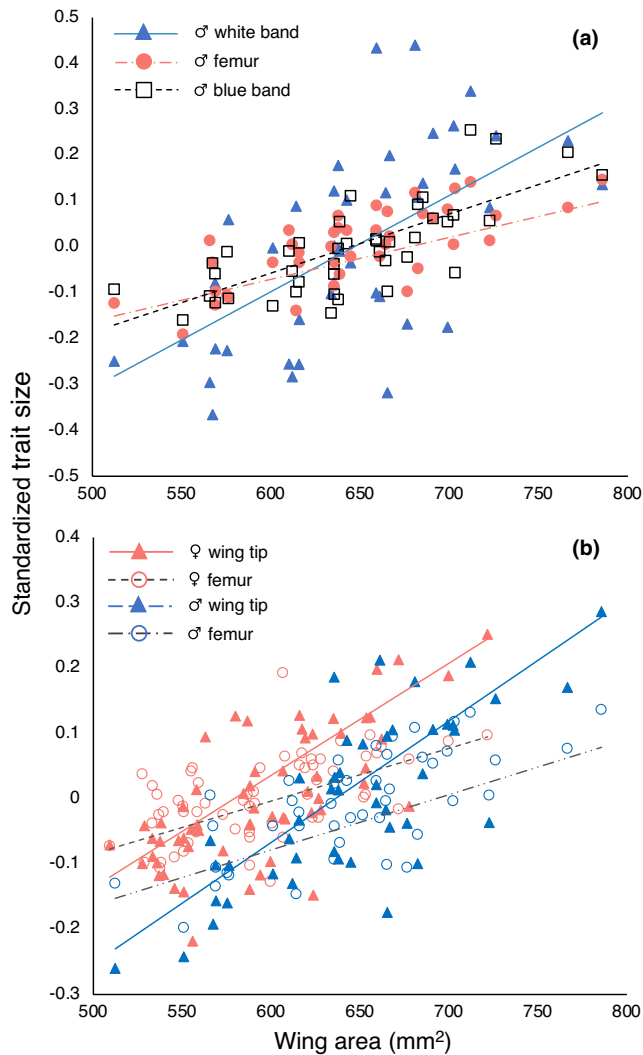
FIGURE 4 Relationship between body size, measured as hindwing area, and standardized trait size for the (a) white wing band, the blue wing band and squared femur length in male offspring and (b) wing tip and squared femur length in male and female offspring from eight families. Trait size was standardized by taking the difference between a trait size measure and the mean trait size, divided by the mean trait size. Here, intercepts estimated from all families pooled and slope coefficients estimated from the linear mixed models are shown in the figure. Coefficients from the linear mixed models allowing family specific intercepts are reported in Results

FIGURE 5 Positive relationship between the brightness of the white wing band and body size, measured as hindwing area in male offspring

band is conspicuous against the dark, UV absorbing forest background (Endler, 1993, also see Rüppell & Fincke, 1989, video online at https://doi.org/10.3203/IWF/E-2976 eng#t=0,00:36), offering an easily perceivable advertisement of body size. Furthermore, when initiating a territorial contest, males typically face off with each other, during which the wings of the contestants are displayed while hovering, a behaviour that should facilitate perception and comparison of the wing bands. The steeper slope of the white wing band effectively augments the difference in body size compared with control traits, rendering the white wing band an amplifier (Emlen & Nijhout, 2000), facilitating the comparison of body size during aerial contests.

Like the male white wing band, the sexually dimorphic white wing tip of females also showed sensitivity to food availability during larval development and steeper body size scaling in adults. This trait signals sexual identity to territorial males, who use the information to decide whether to fight or mate with the individual coming to the territory (Schultz & Fincke, 2009; Xu & Fincke, 2015). Although it is unclear whether and how the size of the female trait affects its function, the steeper body size scaling of the female white wing tip suggests that this trait may be also under selection for size, perhaps because larger size improves the detectability of a potential mate for territorial males. On the contrary, the white wing tip in newly emerged males, which disappears within 36 h, is not expected to be under selection for its size, but exhibited similar steeper body size scaling, likely due to genetic and/or developmental correlation with the female wing tip. The fact that males of *M. brevistylus*, the most ancestral species of the genus *Megaloprepus* (Feindt, 2019), along with the two other congeners, have more female-like white wing tips (Fincke et al., 2018) and that the white wing tips of *M. caerulatus* males disappears shortly after emergence is consistent with a developmental constraint.

lasting on the scale of seconds, and are often resolved without escalation (Fincke et al., 2018; Xu & Fincke, 2015). The frequency and nature of contests by mature *M. caerulatus* suggest that selection on fat reserve and flight muscle mass in the context of territorial contests may have been relaxed compared with species with escalated and prolonged contests.

The finding that the male white wing band showed steeper body size scaling than control traits has implications for the efficacy of this sexual ornament. Without coloured wing ornaments, the otherwise completely transparent wings of *M. caerulatus* would be difficult to see and compare by conspecifics. The white, UV reflective wing
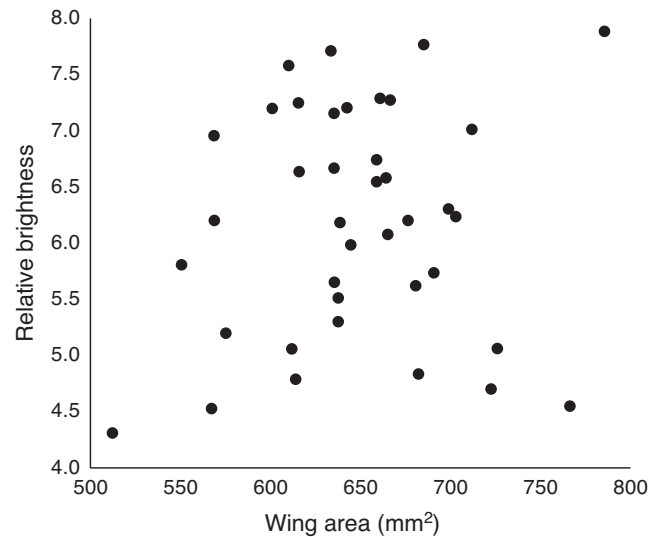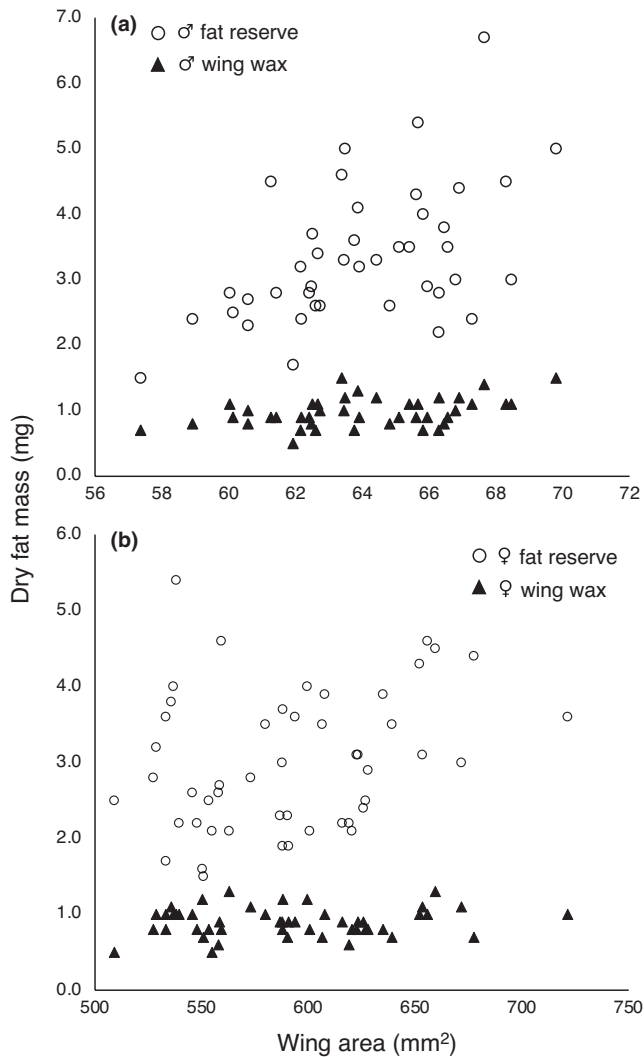
FIGURE 6 Positive relationship between the dry fat reserve mass and dry mass of wax on the wing and body size in (a) male offspring and (b) female offspring

FIGURE 7 Negative relationship between the proportion of total fat that was deposited on the wings as wax and body size in (a) male offspring and (b) female offspring

Although sexual ornaments are widespread, the physiological mechanism underlying their development remains unclear in most cases. Perhaps, the only other direct empirical data to date come from carotenoid-based colour ornaments where the precursor for the pigments cannot be synthesized de novo and come exclusively from an individual's diet (Blount & McGraw, 2008; Pérez-Rodríguez, 2009; Peters, 2007; Svensson & Wong, 2011). Our results offer another rare and direct view into resource allocation for the development of a sexual ornament. Data showed that smaller males, not larger males, invested disproportionally more resource towards the white wing band; a similar pattern is also seen for the female white wing tip. In fact, compared with the male that spent the lowest proportion of the total fat on white wing band, the smallest male allocated more than twice his proportion of total fat to the white wing band (Figure 7a), but still his white wing band was roughly only half the size and 40% less bright (Figure 5) than the male investing the lowest proportion of fat to his ornament. These results highlight that
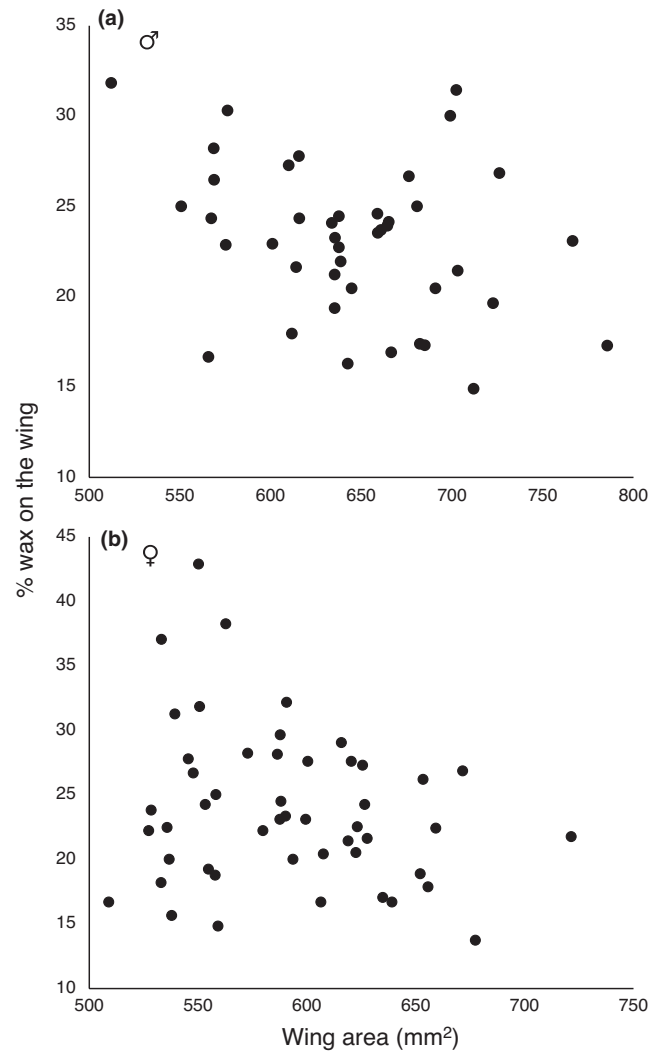
knowing the affordability of a sexual signal is key to understanding the mechanism underlying its honesty.

Importantly, this finding demonstrated that 'affordability' of exaggeration should not be interpreted outside the context of the resource pool. We found that wax density did not change with male body size, indicating that the absolute amount of wax needed to exaggerate one unit area of wing band (i.e. marginal production cost of cheating) was the same for small and large males. Yet because smaller males begin with small fat reserves and spend a disproportionally larger proportion of the fat reserve to develop the white wing bands, the extra fat required for exaggeration, although the same absolute amount as for larger males, is less affordable and may involve higher survival risk. Condition-dependent cost of exaggeration is, in fact, the crux of the condition-dependent handicap hypothesis for the maintenance of signal honesty (Grafen, 1990; Iwasa & Pomiankowski, 1994, 1999; Iwasa et al., 1991; Zahavi, 1977). This hypothesis proposes that the expression of the sexual signal changes

according to condition and that differential cost associated with exaggerating the signal for individuals in low and high conditions offers a mechanism to maintain signal honesty. Although the male white wing band is not condition-dependent, our findings of both positive body size scaling of and negative body size-dependent resource allocation to the wing ornaments are analogous to the mechanism for the maintenance of signal honesty in condition-dependent handicap hypothesis. Moreover, our data highlight an important but implicit nuance for empiricists: an appropriate interpretation of the cost (or conversely, affordability) of exaggeration requires knowledge about the size of the resource pool and lack of this knowledge can result in misleading conclusions.

Differential fat allocation in males also offers insights into fitness payoff and trade-offs of this sexual ornament. Previous data showed that when the difference in the white band size between rivals is large, territorial contests are resolved without escalating to energetically expensive aerial fights (Xu & Fincke, 2015), which dramatically decrease fat reserves in odonates (Marden, 1989). Therefore, a one-time investment in this signal of fighting ability at emergence has the potential to repeatedly generate payoffs during adulthood. On the contrary, fat is a primary fuel for flight in odonates (Marden, 1989). Like many odonates in seasonal habitats, *M. caerulatus* are not active during extreme dry season or extensive rains in wet season (Fincke, 1992b). Hence, initial fat reserves can be crucial for survival for adults emerging during such inclement weather (Arrese & Soulages, 2010; Marden, 1989). The differential fat allocation strategy by males of different sizes thus reflects, to some extent, a trade-off between investment into reproductive success (white wing band) and investment into survival (fat reserve). In contrast to sexual ornaments that continue to develop in adults (e.g. Grether, 1996; Guillermo-Ferreira et al., 2014; Moore, 2021), the size of the white wing band and wing tip cannot be augmented in adults, nor can individuals make them brighter because wax cannot be added to the wing after emergence (Gorb et al., 2009). Therefore, the only time an individual can invest in this signal is before emergence. In comparison, fat reserves vary among adults, depending on their foraging success (Anholt et al., 1991; Fitzstephens & Getty, 2000; Marden, 1989). This may explain, at least partially, why smaller individuals prioritize ornament over fat reserve at the time of emergence. This also raises interesting questions such as whether the resource allocation pattern we observed is common to ornaments that stop developing at the time of emergence or maturity, and whether animals whose sexual ornaments continue to grow follow a different allocation pattern.

Interestingly, *Megaloprepus caerulatus* belongs to a genus of four closely related species (Feindt et al., 2014, Feindt et al.2019) where the other three lack the male white wing band (Fincke et al., 2018). Compared with *M. caerulatus*, congeneric species in Mexico, the Pacific side of Costa Rica, and Nicaragua exhibited lower population density, more undefended suitable territories and lower male-male interaction rates, all of which made male territorial contests relatively rare, suggesting the male white wing band is a response to sexual selection imposed by greater frequency of male territorial contests (Fincke et al., 2018). We suggest that the evolution of this wing signal may have altered contest dynamics in *M. caerulatus* by discouraging prolonged escalation. Although lacking the white wing band, males of congeneric *Megaloprepus* species have wax-based white wing tips, which are not under apparent selection for size or brightness. It thus would be particularly interesting to compare fat allocation strategy among species lacking the white bands as doing so may provide clues to whether the negative body size-dependent allocation strategy found in *M. caerulatus* is adaptive. Wax-based sexually dimorphic signals that occur in the males of other territorial odonates, such as those in the families Polythoridae (Sánchez-Herrera et al., 2010) and Libellluidae (Paulson, 2009), offer further tests for a resource allocation mechanism underlying development of conspicuous sexual ornaments.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

AUTHOR CONTRIBUTIONS

MX designed and conducted research. MX analysed data and wrote the manuscript. OMF provided feedback in experimental design and manuscript writing.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/jeb.13963.

DATA AVAILABILITY STATEMENT

The Supplementary Material, data and R codes used to run all statistical analyses are available for downloading at https://datadryad.org/stash/dataset/doi:10.5061/dryad.ncjsxkswf.

ORCID

Mingzi Xu https://orcid.org/0000-0001-7947-9138
Ola M. Fincke https://orcid.org/0000-0002-4456-0136

REFERENCES

Andersson, M. (1994). *Sexual selection*. Princeton Univ. Press.
Anholt, B. R., Marden, J. H., & Jenkins, D. M. (1991). Patterns of mass gain and sexual dimorphosm in adult dragonflies (Insecta: Odonata). *Canadian Journal of Zoology*, 69, 1156–1163.
Arrese, E. L., & Soulages, J. L. (2010). Insect fat body: energy, metabolism, and regulation. *Annual Review of Entomology*, 55, 207.
Baeta, R., Faivre, B., Motreuil, S., Gaillard, M., & Moreau, J. (2008). Carotenoid trade-off between parasitic resistance and sexual display:

an experimental study in the blackbird (*Turdus merula*). *Proceedings of the Royal Society B: Biological Sciences*, 275, 427–434.

Benson, K. E., & Basolo, A. L. (2006). Male–male competition and the sword in male swordtails, Xiphophorus helleri. *Animal Behavior*, 71, 129–134.

Blount, J. D., & McGraw, K. J. (2008). Signal functions of carotenoid colouration. In G. Britton, S. Liaaen-Jensen, & H. Pfander (Eds.), *Carotenoids, Vol. 4: Natural functions* (pp. 213–236). Birkhäuser.

Bondurianky, R. (2007). Sexual selection and allometry: a critical reappraisal of the evidence and ideas. *Evolution*, 61, 838–849. https://doi.org/10.1111/j.1558-5646.2007.00081.x

Bondurianky, R., & Day, T. (2003). The evolution of static allometry in sexually selected traits. *Evolution*, 57, 2450–2458. https://doi.org/10.1111/j.0014-3820.2003.tb01490.x

Bondurianky, R., & Rowe, L. (2005). Sexual selection, genetic architecture, and the condition dependence of body shape in the sexually dimorphic fly *Prochyliza xanthostoma* (Piophilidae). *Evolution*, 59, 138–151. https://doi.org/10.1111/j.0014-3820.2005.tb00901.x

Contreras-Garduño, J., Buzatto, B. A., Serrano-Meneses, M. A., Nájera-Cordero, K., & Córdoba-Aguilar, A. (2008). The size of the red wing spot of the American rubyspot as a heightened condition-dependent ornament. *Behavioral Ecology*, 19, 724–732. https://doi.org/10.1093/beheco/arn026

Cotton, S., Fowler, K., & Pomiankowski, A. (2004). Condition dependence of sexual ornament size and variation in the stalk-eyed fly *Cyrtodiopsis dalmanni* (Diptera: Diopsidae). *Evolution*, 58, 1038–1046. https://doi.org/10.1111/j.0014-3820.2004.tb00437.x

Emlen, D. J., & Nijhout, H. F. (2000). The development and evolution of exaggerated morphologies in insects. *Annual Review of Entomology*, 45, 661–708. https://doi.org/10.1146/annurev.ento.45.1.661

Endler, J. (1993). The color of light in forests and its implications. *Ecological Monographs*, 63(1), 1–27. https://doi.org/10.2307/2937121

Feindt, W. (2019). *Conservation genomics: Speciation of the neotropical damselfly species* Megaloprepus caerulatus–*as a model for insect speciation in tropical rainforests*. (Doctoral dissertation, Hannover: Institutionelles Repositorium der Leibniz Universität Hannover).

Feindt, W., Fincke, O., & Hadrys, H. (2014). Still a one species genus? Strong genetic diversification in the world's largest living odonate, the Neotropical damselfly *Megaloprepus caerulatus*. *Conservation Genetics*, 15, 469–481. https://doi.org/10.1007/s10592-013-0554-z

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). Consequences of larval ecology for territoriality and reproductive success of a Neotropical damselfly. *Ecology*, 73, 449–462. https://doi.org/10.2307/1940752

Fincke, O. M. (1992b). Behavioural ecology of the giant damselflies of Barro Colorado Island, Panama (Odonata: Zygoptera: Pseudostigmatidae). In D. Quintero, & A. Aiello (Eds.), *Insects of panama and mesoamerica* (pp. 102–113). Oxford Univ. Press.

Fincke, O. M. (2006). Forest and tree species use and dispersal by giant damselflies (Pseudostigmatidae): their prospects in fragmented forests. In A. Cordero Rivera (Ed.), *Forests and dragonflies* (pp. 103–125). Sofia.

Fincke, O. M. (2011). Excess offspring as a maternal strategy: constraints in the shared nursery of a giant damselfly. *Behavioral Ecology*, 22, 543–551. https://doi.org/10.1093/beheco/arr015

Fincke, O. M., & Hadrys, H. (2001). Unpredictable offspring survivorship in the damselfly, *Megaloprepus coerulatus*, shapes parental behavior, constrains sexual selection, and challenges traditional fitness estimates. *Evolution*, 55, 762–772.

Fincke, O. M., Xu, M., Khazan, E., Wilson, M., & Ware, J. (2018). Tests of hypotheses for morphological and genetic divergence in *Megaloprepus* damselflies across Neotropical forests. *Biological Journal of the Linnean Society*, 125, 844–861. https://doi.org/10.1093/biolinnean/bly148

Fitzstephens, D. M., & Getty, T. (2000). Colour, fat and social status in male damselflies, *Calopteryx maculate*. *Animal Behavior*, 60, 851–855.

Gorb, S. N., Kesel, A., & Berger, J. (2000). Microsculpture of the wing surface in Odonata: evidence for cuticular wax covering. *Arthropod Structure & Development*, 29, 129–135. https://doi.org/10.1016/S1467-8039(00)00020-7

Gorb, S. N., Tynkkynen, K., & Kotiaho, J. S. (2009). Crystalline wax coverage of the imaginal cuticle in *Calopteryx splendens* (Odonata: Calopterygidae). *International Journal of Odonatology*, 12, 205–221.

Grafen, A. (1990). Biological signals as handicaps. *Journal of Theoretical Biology*, 144, 246–517. https://doi.org/10.1016/S0022-5193(05)80088-8

Grether, G. F. (1996). Sexual selection and survival selection on wing coloration and body size in the rubyspot damselfly *Hetaerina americana*. *Evolution*, 50, 1939–1948.

Guillermo-Ferreira, R., Therézio, E. M., Gehlen, M. H., Bispo, P. C., & Marletta, A. (2014). The role of wing pigmentation, UV and fluorescence as signals in a Neotropical damselfly. *Journal of Insect Behavior*, 27, 67–80. https://doi.org/10.1007/s10905-013-9406-4

Iwasa, Y., & Pomiankowski, A. (1994). The evolution of mate preferences for multiple sexual ornaments. *Evolution*, 48, 853–867.

Iwasa, Y., & Pomiankowski, A. (1999). Good parent and good genes models of handicap evolution. *Journal of Theoretical Biology*, 200, 97–109. https://doi.org/10.1006/jtbi.1999.0979

Iwasa, Y., Pomiankowski, A., & Nee, S. (1991). The evolution of costly mate preferences II. The "handicap" principle. *Evolution*, 45, 1431–1442.

Kingsolver, J. G., & Huey, R. B. (2008). Size, temperature, and fitness: three rules. *Evolutionary Ecology Research*, 10, 251–268.

Kitching, R. L. (2000). *Food webs and container habitats: The natural history and ecology of phytotelmta*. Cambridge Univ. Press.

Kodric-Brown, A., Sibly, R. M., & Brown, J. H. (2006). The allometry of ornaments and weapons. *Proceedings of the National Academy of Sciences of the United States of America*, 103, 8733–8738. https://doi.org/10.1073/pnas.0602994103

Kotiaho, J. S. (2001). Costs of sexual ornaments: a mismatch between theoretical considerations and the empirical evidence. *Biological Reviews of the Cambridge Philosophical Society*, 76, 365–376.

Marden, J. H. (1987). Maximum lift production during takeoff in flying animals. *Journal of Experimental Biology*, 130, 235–258. https://doi.org/10.1242/jeb.130.1.235

Marden, J. H. (1989). Bodybuilding dragonflies: costs and benefits of maximizing flight muscle. *Physiological Zoology*, 62, 505–521. https://doi.org/10.1086/physzool.62.2.30156182

Marden, J. H., & Rollins, R. A. (1994). Assessment of energy reserves by damselflies engaged in aerial contests for mating territories. *Animal Behavior*, 48, 1023–1030. https://doi.org/10.1006/anbe.1994.1335

Marden, J. H., & Waage, J. K. (1990). Escalated damselfly territorial contests are energetic wars of attrition. *Animal Behavior*, 39, 954–959. https://doi.org/10.1016/S0003-3472(05)80960-1

Mitchem, L. D., Debray, R., Formica, V. A., & Brodie, E. D. III (2019). Contest interactions and outcomes: relative body size and aggression independently predict contest status. *Animal Behavior*, 157, 43–49. https://doi.org/10.1016/j.anbehav.2019.06.031

Moore, M. P. (2021). Larval habitats impose trait-dependent limits on the direction and rate of adult evolution in dragonflies. *Biology Letters*, 17, 20210023. https://doi.org/10.1098/rsbl.2021.0023

Paradise, C. J. (2004). Relationship of water and leaf litter variability to insects inhabiting treeholes. *Journal of the North American Benthological Society*, 23, 793–805.

Paulson, D. (2009). *Dragonflies and damselflies of the west*. Princeton Univ. Press.

Pérez-Rodríguez, L. (2009). Carotenoids in evolutionary ecology: Reevaluating the antioxidant role. *BioEssays*, 31, 1116–1126. https://doi.org/10.1002/bies.200900070

Peters, A. (2007). Testosterone and carotenoids: An integrated view of trade-offs between immunity and sexual ornamenting. *BioEssays*, *29*, 427–430.

R Core Team. (2020). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Rowe, L., & Houle, D. (1996). The lek paradox and the capture of genetic variance by condition dependent traits. *Proceedings of the Royal Society B: Biological Sciences*, *263*, 1415–1421.

Rüppell, G., & Fincke, O. M. (1989). *Megaloprepus coerulatus (Pseudostigmatidae) Flug- und Fortpflanzungs verhalten (Flying and reproductive behaviour)*. Publikationen fur den Wissenschaftlichen Film, Gottingen, Ser. 20, Nr 10/E 2976.

Sánchez-Herrera, M., Realpe, E., & Salazar, C. (2010). A neotropical polymorphic damselfly shows poor congruence between genetic and traditional morphological characters in Odonata. *Molecular Phylogenetics and Evolution*, *57*, 912–917. https://doi.org/10.1016/j.ympev.2010.08.016

Schultz, T. D., & Fincke, O. M. (2009). Structural colours create a flashing cue for sexual recognition and male quality in a Neotropical giant damselfly. *Functional Ecology*, *23*, 724–732. https://doi.org/10.1111/j.1365-2435.2009.01584.x

Simmons, L. W., & Emlen, D. J. (2006). Evolutionary trade-off between weapons and testes. *Proceedings of the National Academy of Sciences of the United States of America*, *103*, 16346–16351. https://doi.org/10.1073/pnas.0603474103

Sokolovska, N., Rowe, L., & Johansson, F. (2000). Fitness and body size in mature odonates. *Ecological Entomology*, *25*, 239–248. https://doi.org/10.1046/j.1365-2311.2000.00251.x

Svensson, P. A., & Wong, B. B. M. (2011). Carotenoid-based signals in behavioural ecology: A review. *Behaviour*, *148*, 131–189. https://doi.org/10.1163/000579510X548673

Warren, I. A., Gotoh, H., Dworkin, I. M., Emlen, D. J., & Lavine, L. C. (2013). A general mechanism for conditional expression of exaggerated sexually-selected traits. *BioEssays*, *35*, 889–899. https://doi.org/10.1002/bies.201300031

Xu, M., & Fincke, O. M. (2015). Ultraviolet wing signal affects territorial contest outcome in a sexually dimorphic damselfly. *Animal Behavior*, *101*, 67–74. https://doi.org/10.1016/j.anbehav.2014.12.018

Yanoviak, S. P. (1999). Community structure in water-filled tree holes of Panama: Effects of hole height and size. *Selbyana*, *20*, 106–115.

Yanoviak, S. P. (2001). Predation, resource availability, and community structure in Neotropical water-filled tree holes. *Oecologia*, *126*, 125–133. https://doi.org/10.1007/s004420000493

Zahavi, A. (1977). The cost of honesty (further remarks on the handicap principle). *Journal of Theoretical Biology*, *67*, 603–605. https://doi.org/10.1016/0022-5193(77)90061-3

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.