

LETTER

Temperature shapes the costs, benefits and geographic diversification of sexual coloration in a dragonfly

Michael P. Moore,^{1*} Cassandra Lis,² Iulian Gherghel¹ and Ryan A. Martin¹

¹Department of Biology Case Western Reserve University Cleveland, OH 44106, USA ²Hathaway Brown School Shaker Heights, OH 44120, USA

*Correspondence:
E-mail: mpm116@case.edu

Abstract

The environment shapes the evolution of secondary sexual traits by determining how their costs and benefits vary across the landscape. Given the thermal properties of dark coloration generally, temperature should crucially influence the costs, benefits and geographic diversification of many secondary sexual colour patterns. We tested this hypothesis using sexually selected wing coloration in a dragonfly. We find that greater wing coloration heats males – the magnitude of which improves flight performance under cool conditions but dramatically reduces it under warm conditions. In a colder region of the species' range, behavioural observations of a wild population show that these thermal effects translate into greater territorial acquisition on thermally variable days. Finally, geo-referenced photographs taken by citizen scientists reveal that this sexually selected wing coloration is dramatically reduced in the hottest portions of the species' range. Collectively, our results underscore temperature's capacity to promote and constrain the evolution of sexual coloration.

Keywords

Citizen science, flight performance, sexual selection, territorial success, thermoregulation, wing pigmentation.

Ecology Letters (2018)

INTRODUCTION

Many animals produce elaborate, sex-specific traits to attract mates and intimidate rivals (Darwin 1871). While more exaggerated characters typically improve mate acquisition (Andersson 1994), reproductive interactions occur against a complex backdrop of environmental factors that can modify the relative costs and benefits of these traits (Cornwallis & Uller 2010; Maan & Seehausen 2011; Miller & Svensson 2014). Some environments magnify the reproductive benefits of elaboration, as when only the loudest vocalisations can be transmitted through the noisiest habitats and detected by females (Paticelli & Blickley 2006; Halfwerk *et al.* 2011). Other environments increase the costs of elaboration, for example when more intense sexual signalling increases detection by parasites and predators (Endler 1980; Zuk *et al.* 2006). In the many cases where the prevailing conditions of the habitat govern the magnitude of the costs and benefits of a secondary sexual trait, environmental variation across space and time will promote the phenotype's diversification (Andersson 1994; Wiens 2001). However, despite the wide array of environmental factors that could plausibly regulate the fitness effects of these traits (Cornwallis & Uller 2010), our understanding of the environmental causes of their diversification remains largely limited to variation in the signalling environment and/or species' enemies (Maan & Seehausen 2011; Miller & Svensson 2014).

For the sexually selected colour patterns displayed by many animals, temperature could be one widely overlooked driver of their costs, benefits and ultimately diversification. As darker colours absorb more emitted light and lead to greater heating for a given level of solar radiation (Watt 1968; Clusella Trullas *et al.* 2007; Stuart-Fox *et al.* 2017), coloration for signalling rivals and potential mates could also raise body temperatures above ambient environmental temperatures.

Such effects of sexual coloration may confer pronounced reproductive benefits at low environmental temperatures by raising an individual's body temperature closer to its optimum for fighting rivals and courting potential mates (e.g. Huey & Kingsolver 1989; Punzalan *et al.* 2008). In contrast, at high environmental temperatures, thermal effects of sexual coloration could increase an individual's body temperature beyond the optimum for mate acquisition and/or for other vital functions like feeding or predator avoidance (West & Packer 2002). Given the considerable differences in environmental temperature between many populations and between many closely related species (Sunday *et al.* 2012, 2014; Kingsolver *et al.* 2013), such effects could underlie geographic patterns of sexually selected coloration in a diverse suite of animals. Furthermore, these potential thermal effects may have outsized evolutionary consequences as global temperatures continue to rise (IPCC 2014). Yet, while temperature is often linked to the evolution of colour patterns that are produced by both sexes (Watt 1968; Kingsolver 1995; Clusella Trullas *et al.* 2007; Stuart-Fox *et al.* 2017), its role in the geographic diversification of sexually selected coloration has received far less attention (West & Packer 2002; Punzalan *et al.* 2008; Svensson & Waller 2013).

In this study, we explored temperature's potential to influence the costs, benefits and geographic diversification of sexual coloration in a North American dragonfly (*Pachydiplax longipennis* Burmeister). Males of this medium-sized species produce dark, condition-dependent coloration on their wings (Moore & Martin 2018), which intimidates rivals and improves territorial success (Moore & Martin 2016). As territorial success determines a substantial amount of mating success in this (Sherman 1983) and similar species (reviewed in Suhonen *et al.* 2008), such advantages strongly increase male fitness. Males exhibit continuous variation in wing coloration

within populations across the northern and eastern portions of the species' range (e.g. Moore & Martin 2016). However, males possess drastically reduced coloration in western regions (e.g. Paulson 2009). This geographic variation makes *P. longipennis* well suited for testing temperature's ability to modify the costs and benefits of sexually selected coloration. Previous work in odonates also suggests that the evolution of sexually selected wing coloration can be sensitive to thermal conditions (Outomuro & Ocharan 2011; Svensson & Waller 2013), making them good candidates for such tests generally. If temperature indeed has the capacity to determine the relative costs and benefits of wing coloration in *P. longipennis*, and ultimately shapes its diversification across the species' range, then at least four predictions should be supported. First, greater wing coloration should increase male body temperatures. Second, any colour-induced heating should meaningfully affect male flight performance. Third, in a cold portion of the species' range, the territorial advantages of greater wing coloration should be largest on the overall coolest days and/or on the most thermally variable days (because of frequent drops below requisite temperatures for territorial acquisition and defence). Fourth, male wing coloration should be substantially reduced in warm regions.

METHODS

Wing coloration in Pachydiplax longipennis

Males can display dark brown coloration across their entire wing surface, with particularly dense regions of pigmentation between the nodus and pterostigma, as well as at the base of the wing. Females sometimes, though not always, produce a small amount of basal wing pigmentation. Unless otherwise noted, we report wing coloration as the per cent of the total wing area with pigmentation, as this metric is a known target of intrasexual selection (Moore & Martin 2016). The Supporting Information Methods and Results provides detailed methods for measuring this trait from digitised photographs in ImageJ (Rasband 2012). While distal wing coloration is much more variable than basal wing coloration (coefficients of variation: distal = 0.324, basal = 0.191), they are fairly strongly correlated ($r = 0.668$, $P < 0.001$), suggesting they are unlikely to have completely independent evolutionary consequences. The total proportion of wing coloration is not correlated with total wing area (Moore & Martin 2016), total body mass ($r = -0.092$, $P = 0.669$) or dorsal thorax darkness ($r = -0.269$, $P = 0.175$).

Prediction 1: Wing coloration increases male body temperatures

We first compared thoracic heating between males with naturally high and low levels of wing coloration. Over the course of 5 days between 10:00 and 11:30 EDT, we captured 22 mature males from a population in north-eastern Ohio, USA (Hunting Valley, OH, USA). To best approximate the large differences in wing coloration observed across the species' range, we compared males with as divergent extents of coloration as possible (mean % coloration \pm SD: high = $57.9 \pm 7.6\%$, $n = 11$; low = $25.5 \pm 7.9\%$, $n = 11$). We placed captured males in plastic

bags on ice to ease handling and brought them into a field room at the Squire Valleevue Farm (Hunting Valley, OH, USA). Once a male had cooled enough to safely handle without injuring it, we tied a loop of nylon line around its legs and placed it back in its bag on ice until its heating trial began. After each male's trial, we removed one of its middle legs to prevent recapturing and released it.

We also conducted an experimental manipulation of wing coloration and compared male heating. Over 2 days, we captured 20 males with low coloration. We then visually paired each male with another captured on the same day that was a similar size and had similar natural extent of wing coloration. On one male within the pair, we experimentally augmented its extent of coloration to that of a high-coloration male by colouring its wings with a brown felt tip marker (c. 60%, Crayola® *Cuppa' Cappuccino*). On the other male within each pair, we coloured in the same wing area with a colourless blending marker (Copic® 0-S), which has the chemical properties of marker ink but lacks dye. This controlled for any effect of the marker on the wing. Males were otherwise captured and handled as described above. Males in each treatment did not differ in mass ($F_{1,19} = 0.46$, $P = 0.507$) or basal wing coloration ($F_{1,19} = 0.18$, $P = 0.677$).

For each heating trial, we carefully removed the male from its bag, touching only the nylon line, and tethered it to a metal eyelet in a white Styrofoam box (LxWxD: 17 cm \times 17 cm \times 20 cm), which had the top and one side removed. Using a 60-W lamp positioned 20 cm above the eyelet (Punzalan *et al.* 2008; Hegna *et al.* 2013), we heated each male and recorded the surface temperature of the lateral side of its thorax every 30 seconds for five minutes with a thermal imaging camera (FLIR® C2). Trials were always performed between 12:00 and 15:00 EDT, when room temperatures vary little (mean \pm sd: 23.10 ± 0.40 °C). While surface temperature recordings in odonates follow internal temperatures taken with thermal probes very precisely ($R^2=0.988$, Samejima & Tsubaki 2010), they somewhat exaggerate the magnitude of warming (slope of internal temperature regressed on surface temperature = 0.836, Samejima & Tsubaki 2010). Surface temperature differences therefore may overstate the true magnitude, but not the presence or direction, of internal temperature differences.

We then assessed how natural and experimental differences in wing coloration affected males' maximum temperatures and heating rates. We first fit an asymptotic nonlinear model to each male's heating curve, including each temperature recording as the response ('nlme', Pinheiro *et al.* 2015, Table S1). Using the parameter estimates from each male's fitted heating curve, we then compared the asymptotes (maximum temperature) and rate constants (heating rate) between the groups using likelihood ratio tests of models with and without each term. We fit models using 'metafor' (Viechtbauer 2010) to directly incorporate the standard error of the parameter estimates into the analyses. We included date as random effect in these models to account for any non-independence among trials performed on the same day (e.g. acclimation to ambient temperature). We also included pair as a random effect in the analyses of the experimental males to explicitly test for differences between each manipulated male and its control counterpart. Because comparisons between males with naturally high

and low levels of coloration are inherently correlative, we included mass as a covariate in this analysis. Conversely, we did not include mass in analyses of experimental males because statistical comparisons are between individuals that were already paired by size. Significant differences between groups in the asymptote or rate constant indicate differences in maximum temperatures or heating rate, respectively. Initial temperature was not strongly associated with asymptotic temperatures in either comparison (both $t < 2.1$, $P > 0.058$).

Prediction 2: Thermal consequences of wing coloration translate into performance variation

We next used a modification of the protocol described by Samejima & Tsubaki (2010) to measure how temperature influenced lifting force, a known performance target of intra-sexual selection in dragonflies (Marden & Cobb 2004). Over the course of 10 days, we captured 70 males and acclimated them to one of seven temperatures (25, 29, 33, 37, 41, 45 and 49 °C, $n = 10$ males each) for 90 minutes in lighted incubators (DigiTherm® DT2-MP-47L). To each male, we then attached a nylon string with evenly spaced bundles of small sequins (*c.* 0.030 g per bundle). We perched the male on a plastic cylinder that was positioned vertically, induced it to take off and recorded how many bundles it lifted. Because males must take off from perches frequently over short time spans to engage territorial intruders, we used the average mass lifted across three trials as a proxy of a male's potential flight performance at a temperature at an ecologically relevant task (see also Marden 1995). All males acclimated to 49 °C surpassed their critical thermal maxima before performance trials, and we scored their performance as 0 (Kingsolver *et al.* 2013). Results are similar if these males are excluded (*c.f.* Tables S2, S3). We fit an exponentially modified Gaussian model to the relationship between acclimation temperature and flight performance ('nlme'; Table S2 compares potential thermal performance curves). From this thermal performance curve, we estimated the maximum average performance across all temperatures (P_{MAX}), the temperature of P_{MAX} (thermal optimum) and the range of temperatures at which males perform at least 80% P_{MAX} (performance breadth, *sensu* Huey & Kingsolver 1989). To explore how a plausible magnitude of colour-induced heating may influence performance, we quantified how a 1–2 °C increase (see Results) alters performance relative to P_{MAX} at: (1) 25 °C; (2) the thermal optimum; and (3) the upper and lower bounds of the performance breadth. While nonlinear models of flight performance that directly included body size would not converge, several supplemental analyses demonstrated that any effects of body size did not confound our results (see Supporting Information Methods and Results).

Prediction 3: Wing coloration provides its greatest territorial advantages on the coldest and/or most thermally variable days

To assess whether temperature modifies the relationship between wing coloration and territorial success, we re-analysed a data set described in Moore & Martin (2016). During the 2015 reproductive season, we captured, marked, phenotyped (Supporting Information Methods and Results) and

observed the daily territorial interactions of mature males at a pond in north-eastern Ohio (perimeter = 140.2 m, area = 0.248 ha). Prior to release, we marked each male's abdomen with a unique combination of acrylic paint colours. Then, every sunny day throughout the species' flight period that year ($n = 18$ days), one observer (MPM) slowly circled the pond during peak activity hours (10:00–16:30 EDT) and recorded each sighted male's location, territorial behaviour and the time of the observation.

We assessed two components of territorial success from these field observations: territorial acquisition and the duration of territorial defence. For every day between a male's capture and its last-known day alive (its last resighting), we assessed whether or not it acquired a breeding territory ($n = 45$ males, 309 potential male-by-day combinations). A male was designated as having acquired a territory for a day if we observed it perching or patrolling within one of two regions of emergent vegetation (13.8 and 35.7 m, respectively) where males always set up territories. As we captured and marked exclusively territorial males, each male's first recorded territorial acquisition was always the day on which it was captured. Male *P. longipennis* do not come to the pond except for reproduction (Fried & May 1983), and 'sneaker' or 'satellite' tactics were never observed. For males that were subsequently resighted in an acquired territory, we also scored the duration (mins) over which they were able to defend the territory for that day ($n = 126$ territorial tenures). This duration was estimated as the time between the first and last observation of the male in the territory. Then, for the activity period of each day that males were observed defending territories, we obtained the means and SDs of temperature (°C) and solar radiation ($W \cdot m^{-2}$) from a weather station (HOBO® U30 Station) that was located *c.* 1.5 km away in a similarly open habitat. Mature males rarely migrate and defend territories at new ponds within a single reproductive season (McCauley 2010), making territoriality elsewhere after the last resighting unlikely.

We used mixed-effects models ('lme4', Bates *et al.* 2015) to test whether the daily means and SDs of temperature and/or solar radiation altered the relationships between wing coloration and our measures of territorial success. In each model, we included individual as a random effect to account for the repeated measures of individuals across days. As fixed effects in both models, we included a male's total wing coloration and body length (each scaled to mean of 0 and SD of 1; Lande & Arnold 1983), mean temperature of the day, SD of temperature of the day, mean solar radiation of the day, SD of solar radiation of the day and all trait by environment two-way interactions. We modelled the probability of acquiring a territory for a given day as a binary response (1 = did acquire territory, 0 = did not acquire territory) and tested the significance of terms with likelihood ratio tests of models with versus without the effect. We \log_e -transformed the duration of territorial defence and tested the significance of terms using *F*-tests with the Kenward–Rogers degrees of freedom approximation (Kenward & Roger 1997). In these analyses, a significant trait by environment interaction indicates that the environmental factor is modifying the relationship between wing coloration and the aspect of territorial success (MacColl 2011). Mean daily temperature was the only environmental

variable to show a temporal trend across the season (Table S4), and previous analysis of this population showed no relationship between longevity and either male trait (Moore & Martin 2016).

Prediction 4: Reduced wing coloration in hottest parts of range

We used photographs taken by citizen scientists to characterise environmental correlates of the reported variation in male wing coloration. We examined the first 480 geo-referenced photographs of male *P. longipennis* that had been uploaded to iNaturalist.org and scored if: (1) the male was mature (indicated by a waxy blue prunescence over its abdomen) and (2) it had produced distal wing coloration (1 = yes, 0 = no). While both distal and basal wing coloration can vary, we used this qualitative measure of wing colour variation because it can be reliably assessed from the unstandardised photographs of wild males. Importantly, because the regions of pigmentation are correlated, and distal coloration is much more variable, this metric should enable us to generally assess if wing coloration is indeed reduced in warm regions. As males can also produce wing coloration in this region of the wing prior to maturation (Moore & Martin 2018; Moore *et al.* 2018), we positively scored males that were not mature but exhibited such coloration (*c.* 2% of all observations). Immature males without any wing coloration were excluded. We also haphazardly chose and scored 55 additional photographs in regions that were underrepresented in our initial sample. Similar procedures have been validated for examining clines in other colour polymorphisms (Leighton *et al.* 2016). No duplicated photographs were observed. We also extracted the annual mean temperature and precipitation of the driest quarter (aridity) for the location of each observation from WorldClim 1.4 (Hijmans *et al.* 2005). We computed annual mean solar radiation with ArcGIS using standard techniques (Böhner & Antonić 2009). Results are similar for environmental data from WorldClim 2 (Fick & Hijmans 2017; Tables S5, S6). We used annual means of temperature and solar radiation because differences in the duration of the flight season across the species' range may expose adults of some populations and not others to subsets of year (e.g. hottest or coldest quarters). As *P. longipennis* requires at least some permanent standing water to complete its life cycle, we included precipitation of the driest quarter as our measure of aridity because it should best reflect the input into ponds necessary for maintaining water levels during larval development than annual means.

We used a mixed-effects logistic regression ('lme4', Bates *et al.* 2015) to explore how the production of wing coloration varies with annual mean air temperature, aridity, annual mean solar radiation and their interactions. We z-transformed each variable prior to analyses to make parameter estimates more comparable among these environmental factors (Schielzeth 2010). Correlations among environmental variables were modest (all $r < 0.242$), indicating that multicollinearity should not be a large concern (Graham 2003). Models including the interaction between solar radiation and aridity did not converge and were not tested. We modelled each geo-referenced observation as a response. Because population boundaries are not discernable from these geo-referenced observations, we

included a random term for municipality (county or its equivalent) nested within state (USA, Mexico) or province (Canada). This nesting structure accounts for non-independence of observations of males from within the same populations and also for observations within a single state being more correlated to each other than observations from different states (Outomuro & Ocharan 2011). We tested the significance of fixed effects by comparing models with and without the effect using likelihood ratio tests. We also conducted a spatially explicit logistic regression using the 'gwr' function from the 'spgwr' package in R (Bivand & Yu 2017). As test statistics have not yet been implemented for this package, we compared the parameter estimates of this model to those generated by the nested mixed-effects model. In all cases, the parameter estimates between the two approaches were similar (Tables S5, S6). We therefore report on only the nested mixed-effects model in the main text.

RESULTS

Prediction 1: Wing coloration increases male body temperatures

Males with naturally greater wing coloration reached higher asymptotic temperatures than males with less wing coloration, but heating rates did not differ between them (Fig. 1a, Table 1). Heavier males also reached marginally higher asymptotic temperatures ($\beta = 9.646 \pm 5.147$, $\chi_1^2 = 3.7$, $P = 0.054$) and heated marginally slower ($\beta = -5.876 \pm 3.325$, $\chi_1^2 = 3.1$, $P = 0.077$). Males with experimentally augmented coloration both reached higher asymptotic temperatures and heated faster than their size-matched, control counterparts (Fig. 1b, Table 1).

Prediction 2: Thermal consequences of wing coloration translate into performance variation

Lifting force increased with body temperature, reaching 0.252 g (P_{MAX}) at 39.4 °C, before precipitously declining (Fig. 2). Flight performance was 80% of P_{MAX} between 33.4 and 42.5 °C (performance breadth, P_{BR}). At 25 and 33.4 °C, colour-induced heating of 1–2 °C (Table 1) improves relative flight performance by 2.6–5.4% and 3.9–7.9%, respectively. In contrast, at 39.4 and 42.5 °C, that same range of heating reduces relative flight performance by 1.9–8.1% and 15.3–32.3%, respectively.

Prediction 3: Wing coloration provides its greatest territorial advantages on the coldest and/or most thermally variable days

The probability of a male acquiring a territory on a day increased strongly with wing coloration on the most thermally variable days and slightly decreased on the least variable days ($\chi_1^2 = 7.6$, $P = 0.006$; Fig. 3). This relationship was unaffected by body size, daily mean temperature or solar radiation (all $\chi_1^2 < 2.6$, $P > 0.108$, Table S7). Of the males that acquired territories on a given day, those with more wing coloration were able to defend them for longer ($F_{1,26.4} = 10.4$, $P = 0.003$, see also Moore & Martin 2016), but this relationship was not modified by body size, temperature or solar radiation (all $F < 2.6$, $P > 0.107$, Table S7).

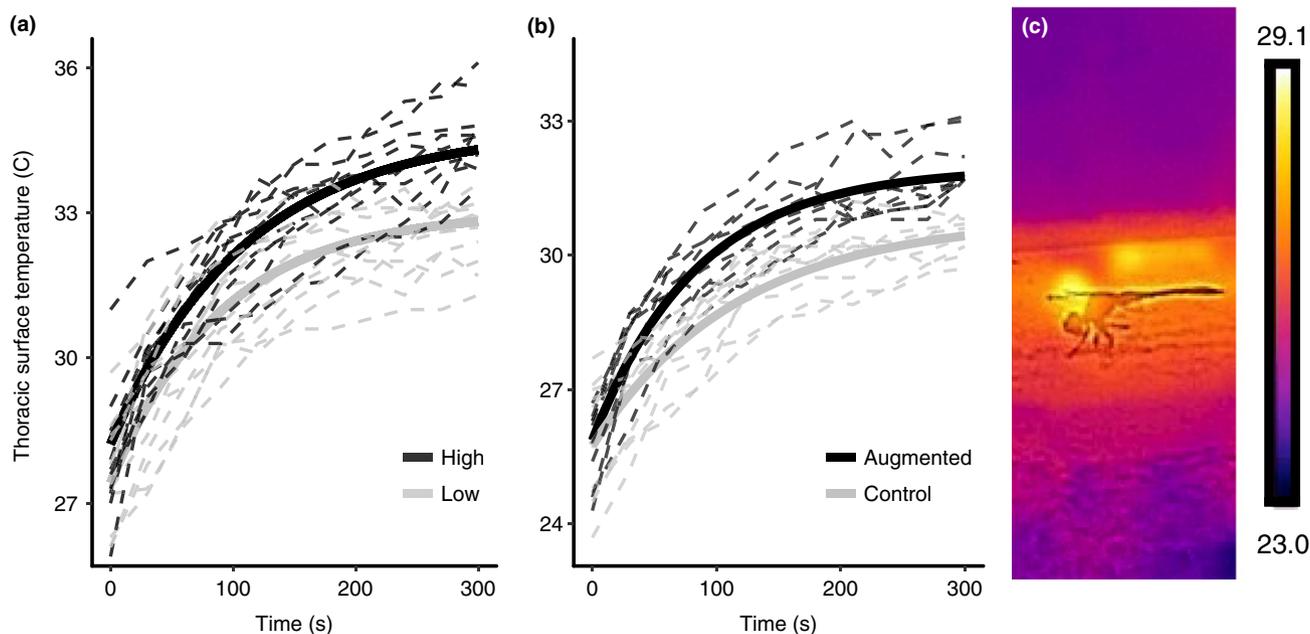


Figure 1 Wing coloration increases male body temperatures. (a) Males with naturally greater coloration reached higher thoracic surface temperatures ($n = 11$) than those with less coloration ($n = 11$) when warmed under a lamp (Table 1). (b) Males with experimentally augmented coloration ($n = 10$) also reached higher thoracic surface temperatures ($n = 10$) than control males when warmed (Table 1). In both panels (a) and (b), dotted lines represent individual males, and solid lines illustrate heating curves with the mean asymptotes and heating constants that were estimated from mixed-effects models. (c) Thermal image of male *P. longipennis* with naturally high coloration being warmed under a lamp. Temperature gradient is in °C.

Table 1 Least-squares mean asymptotes and rate constants (\pm SE) from asymptotic models fit to each male's heating response. Comparisons between high vs low natural coloration (a) and experimentally augmented and control coloration (b) were made using the R package 'metafor'. A higher asymptote indicates greater maximum thoracic temperature. A larger rate constant indicates faster heating. We also included each male's body mass as a covariate in analyses of natural variation, and we report these effects in the main text.

Comparison	Parameter	High/Augmented	Low/Control	χ^2	P
a) Natural Variation	Asymptote (°C)	33.16 ± 0.87	31.38 ± 0.87	51.6	< 0.001
	Log_e - Rate Constant (°C * s ⁻¹)	-3.74 ± 0.55	-3.50 ± 0.56	2.1	0.143
b) Experimental Manipulation	Asymptote (°C)	31.93 ± 0.20	30.83 ± 0.23	18.8	< 0.001
	Log_e - Rate Constant (°C * s ⁻¹)	-4.40 ± 0.12	-4.78 ± 0.14	4.2	0.040

Prediction 4: Reduced wing coloration in hottest parts of range

Consistent with reports in field guides (e.g. Paulson 2009), males typically produce at least some coloration across their wing area in northern and eastern portions of the species' range, but coloration is usually reduced in western regions (Fig. 4a). These observations also revealed that mature males frequently exhibit reduced coloration in southern regions. After controlling for the spatial non-independence of observations, males typically had reduced wing coloration in regions with the greatest annual mean air temperatures ($\beta = -0.502 \pm 0.103$, $\chi_1^2 = 21.4$, $P < 0.001$, Fig. 4c). Reduced wing coloration was also common in the driest areas ($\beta = 0.273 \pm 0.101$, $\chi_1^2 = 6.5$, $P = 0.011$). There were no effects of annual mean solar radiation ($\beta = -0.089 \pm 0.069$, $\chi_1^2 = 1.8$, $P = 0.184$) or any interaction (all $\chi_1^2 = 1.0$, $P > 0.328$).

DISCUSSION

Given its role in the evolution of coloration shared by both sexes (Watt 1968; Clusella Trullas *et al.* 2007; Stuart-Fox *et al.* 2017), temperature should also have important effects on the costs, benefits and ultimately diversification of coloration produced by one sex to attract mates and intimidate rivals (West & Packer 2002; Ellers & Boggs 2003; Svensson & Waller 2013). Here, we found that: (1) the sexually selected wing coloration of *P. longipennis* increases male body temperatures; (2) heating, like that associated with greater wing coloration, modestly enhances flight performance under cool conditions and dramatically reduces it under warm conditions; (3) wing coloration most greatly improves territory acquisition on thermally variable days in a cold region of the species' range; and (4) male wing coloration is greatly reduced in the hottest parts of the species' range. These results collectively

support temperature's capacity to modify the strength and direction of selection on wing coloration and, therefore, to shape this trait's evolution across the landscape.

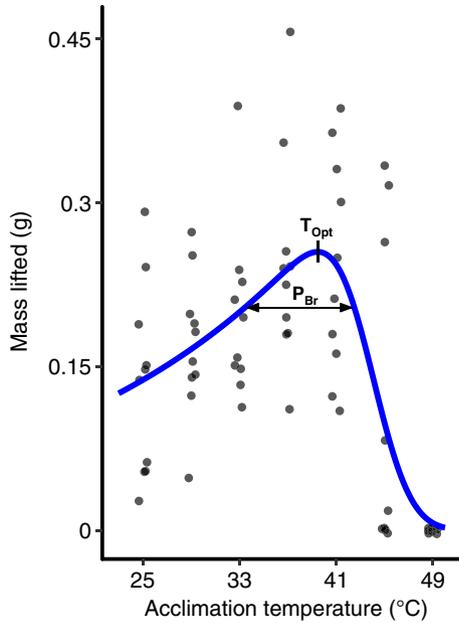


Figure 2 Thermal flight performance of male *P. longipennis*. Circles are the mean mass lifted by individual males that were acclimated to one of seven temperatures ($n = 10$ males per temperature). The blue line is the best-fitted nonlinear relationship. T_{OPT} is the temperature at which performance is maximised, and P_{BR} is the range of temperatures at which performance is at least 80% of the maximum.

When sexual coloration raises male body temperatures, any fitness effects will depend on environmental temperatures, especially in ectotherms (Clusella Trullas *et al.* 2007; Stuart-Fox *et al.* 2017). Both our correlative and manipulative experiments indicate that the presence of dark coloration across the entire wing surface can elevate male temperatures, consistent with direct and indirect evidence from other Holarctic odonates (e.g. Outomuro & Ocharan 2011; Svensson & Waller 2013) and some lepidopterans (e.g. Hanley *et al.* 2013; Hegna *et al.* 2013; Brashears *et al.* 2016). Beyond governing the physiological consequences of their thermal effects, environmental temperature may also affect how secondary sexual colour patterns function during behavioural interactions. For example, in the many other territorial animals that display coloration to signal fighting ability during male–male competition (Cuthill *et al.* 2017), body temperature could be one component of the information that is signalled to rivals. If this is the case, the way that males respond to a rival's coloration in cool regions, where colour-induced heating improves male fighting ability, should differ from warm regions, where such heating diminishes it. Consequently, sexual coloration may signal strength and deter territorial intruders in one part of the range, but signal weakness and invite them in others. While the possible signalling of male body temperatures remains to be explored, our results nevertheless underscore the potential thermal consequences of sexually selected coloration (see also West & Packer 2002; Punzalan *et al.* 2008).

For temperature to determine the reproductive benefits and performance costs of sexually selected traits, their thermal effects must translate into variation in performance. We found that heating, like that induced by sexually selected wing

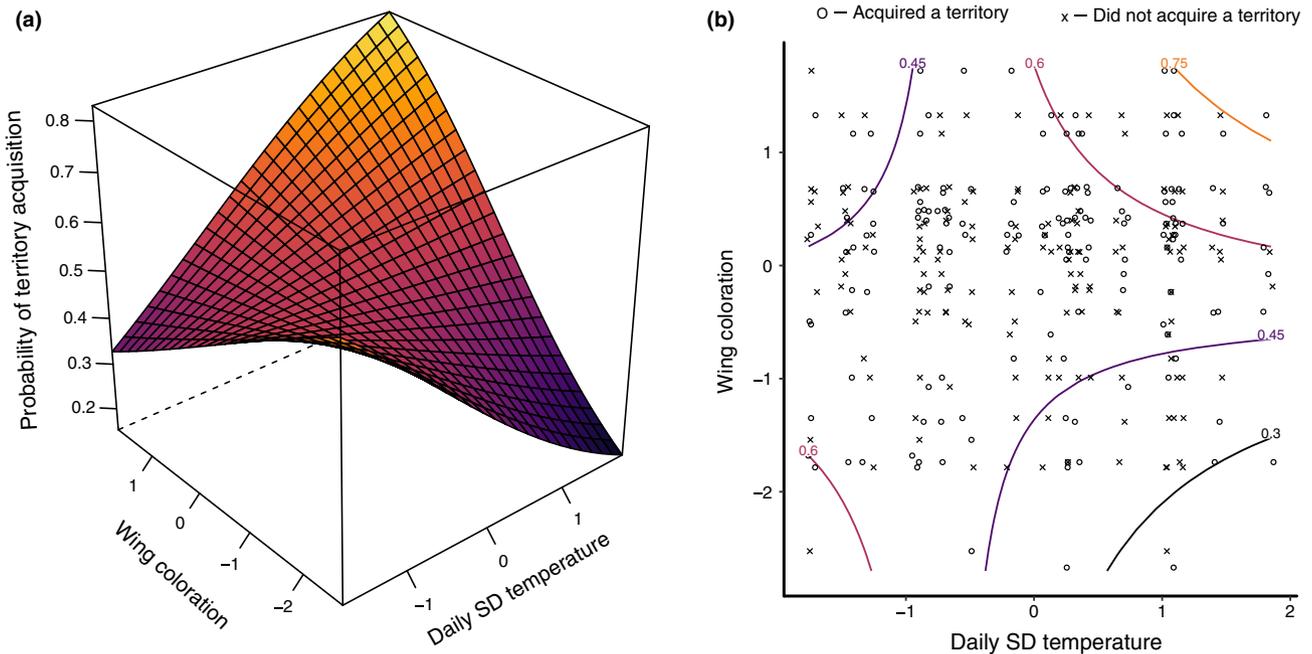


Figure 3 Wing coloration conferred the greatest improvements to territorial acquisition on the most thermally variable days. Probabilities in both (a) surface and (b) contour plots are those estimated from the generalised linear mixed-effects model. Points (b) represent whether a male of known wing coloration did (circles) or did not (x's) acquire a territory on a given day ($n = 309$ male-by-day combinations).

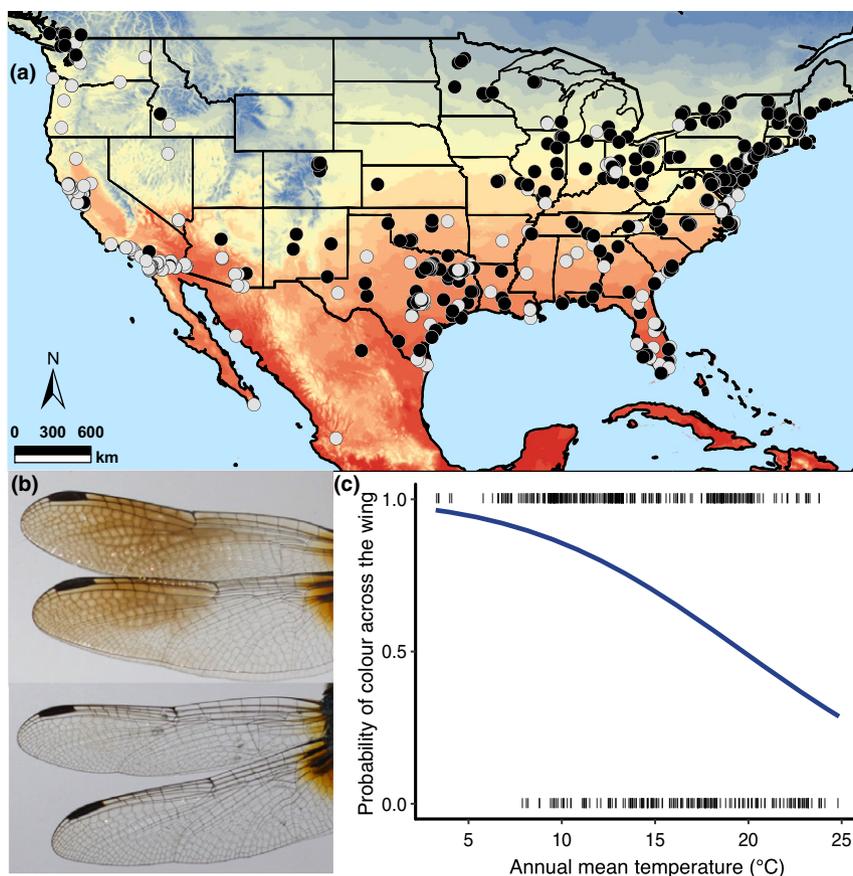


Figure 4 Geographic variation of wing coloration. (a) Males typically produce at least some coloration across their entire wing area (black circles) in the northern and eastern portion of the species range, but this coloration is often greatly reduced (grey circles) in the southern and western portions of the range ($n = 535$ observations). Annual mean temperature is shown as a colour gradient with the coldest regions being shown in blue and the hottest being shown in red. (b) Mature males with high wing coloration (top) and reduced coloration (bottom) captured in north-east Ohio. (c) After accounting for spatial non-independence, the probability of a mature male producing at least some distal wing coloration declines as annual mean temperature increases. Each tick mark corresponds to a geo-referenced photograph of a male from the citizen science website iNaturalist.org. The blue line shows the fitted line from the mixed-effects logistic regression.

coloration, modestly improves male flight performance under cool conditions and dramatically reduces it under hot conditions. As locomotor performance boosts mating success in dragonflies (Marden & Cobb 2004) and many other animals (Lailvaux & Irschick 2006), even small thermal effects on territorial defence or mate attraction should have substantial fitness consequences (Punzalan *et al.* 2008). Indeed, in one of the coolest portions of the species' range, we found that wing coloration most improved territorial acquisition on the most thermally variable days. Thus, at least one reproductive benefit of wing coloration depends on the thermal conditions, perhaps because colour-induced heating buffers against fluctuations below lower thermal thresholds for territorial activities. However, because males also behaviourally thermoregulate via adjustments of position and posture (May 1976), exploring how behaviour and coloration act together to affect male body temperatures in the field remains necessary to elucidating the precise mechanism underlying this pattern. Intriguingly, once a male successfully acquired a territory for a day, the benefits of wing coloration to the duration of territorial defence did not depend on thermal conditions, indicating that not all of this trait's territorial benefits are temperature-dependent.

Nonetheless, these observations collectively reveal that the thermal effects of wing coloration can translate into variation in intrasexually selected performance in the wild.

In contrast to the territorial benefits conferred under the coolest conditions, wing coloration likely imposes intense thermal costs to both reproduction and survival in the warmest regions. For example, in parts of the American Southwest where wing coloration is reduced (e.g. Sonoran Desert, USA, 32.8°N, 112.8°W), one to two months every year have maximum daily temperatures that average over 41.5 °C (Supporting Information Methods and Results). Thus, on most days for long stretches of the summer, colour-induced heating of 1–2 °C would cause males to reach or exceed the upper limit of their performance breadth. This in turn would force males to abandon territories and seek shade during the peak activity hours (i.e. reproductive cost; Huey *et al.* 2009; Sunday *et al.* 2014) and/or induce unsustainably high metabolic rates (i.e. survival cost; Fried & May 1983; Gillooly *et al.* 2001; Dillon *et al.* 2010). As animal performance commonly plummets at high temperatures (Huey & Kingsolver 1989), similar thermal costs may often cause both sexual selection and viability selection to disfavour secondary sexual coloration in warm regions

(see also West & Packer 2002; Svensson & Waller 2013). These thermal costs should also intensify with the rising global temperatures, potentially making them an important force on the future evolutionary trajectories of sexual coloration in many animals as well.

Environmental variation in the costs and benefits of a sexually selected trait will promote its diversification across the species' range (Wiens 2001; Maan & Seehausen 2011). Consistent with the thermal modifications to the relative costs and benefits of wing coloration that we observed, we also found substantial reductions in this sexually selected trait in the hottest portions of the species' North American range. While the relative importance of evolved, constitutive differences versus temperature-induced plasticity to this geographic pattern remains unknown (Watt 1969), any such plasticity would still likely be a signature of temperature-mediated differences in selection (Kingsolver 1995). Additionally, recent work in butterflies shows that genetic variation and plasticity combine to facilitate adaptation to temperature (Kingsolver & Buckley 2017), demonstrating that their evolutionary effects are not mutually exclusive. Our analysis also cannot exclude other factors that are themselves correlated with temperature, but several alternatives seem unlikely. For instance, defending against infection inhibits wing colour production in odonates (Siva-Jothy 2000; Moore *et al.* 2018). The observed geographic pattern could then plausibly reflect variation in parasite load. However, as odonates' primary parasites have additional aquatic hosts and/or free-swimming life stages (Corbet 1999; Forbes & Robb 2008), this explanation seems at odds with the pronounced wing colour reduction occurring in the most arid regions. Interspecific character displacement of wing coloration could also potentially explain these patterns (Tynkynen *et al.* 2004; Anderson & Grether 2010; Hassall 2014), yet no similarly colourful dragonfly inhabits only the regions where *P. longipennis* typically lack distal wing coloration (Paulson 2009). Thus, although other explanations cannot yet be definitively rejected, the observed geographic variation is broadly consistent with temperature-mediated variation in selection on wing coloration translating into diversification across the landscape.

A complex interaction of environmental factors ultimately controls the magnitude of the reproductive benefits conferred by secondary sexual characters, as well as the intensity of any performance costs that they inflict (Miller & Svensson 2014). The optimal extent of sexually selected traits is thus expected to track spatial or temporal variation in any of these abiotic or biotic features (Maan & Seehausen 2011). Our results broadly support temperature's capacity to shape the performance costs, reproductive benefits and geographic diversification of sexually selected colour patterns. Given the pervasiveness of sexual coloration (Cuthill *et al.* 2017) and the well-known thermal effects of dark coloration (Clusella Trullas *et al.* 2007), similar effects could readily drive geographic patterns of sexual selection and diversification across the animal kingdom. Additionally, in the light of the central role that sexually selected colour patterns play in assortative mating and other mechanisms of pre-zygotic reproductive isolation (Servedio & Boughman 2017), variation in temperature-mediated selection could have underappreciated effects on the promotion and/or maintenance of current reproductive

boundaries between species. Moreover, if rising global temperatures further modify the costs and benefits of sexually selected traits, subsequent evolutionary changes in these traits may also promote novel pre-zygotic barriers between populations that currently can interbreed (Boughman 2001) or weaken barriers between those that presently cannot (Seehausen *et al.* 1997).

ACKNOWLEDGEMENTS

Logistical assistance, research accommodations and weather station data were provided by A. Locci, J. Koonce and the Squire Valleevue Farm staff, as well as M. Willis and P. Kalyanasundaram. We also appreciate coordination assistance from C. Miller and the Hathaway Brown School Science Research & Engineering Program. Critical feedback from L. Chick, S. Diamond, M. Benard, P. Lorch, H. Rollins, A. Perez, the CWRU Ecology & Evolution reading group, J. Chase, G. Grether and three anonymous referees greatly improved the design and presentation of the study. This project was funded by a Theodore Roosevelt Award from the American Museum of Natural History to MPM, as well as two Oglebay Grants from the Department of Biology at CWRU to MPM. MPM was also funded in part by a GAANN fellowship during the completion of this project.

AUTHOR CONTRIBUTIONS

MPM conceived and designed study; MPM and CL collected the data; MPM and IG analysed the data and prepared the figures; and MPM and RAM wrote and revised the manuscript.

DATA ACCESSIBILITY STATEMENT

Data available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.g9v97vq>.

REFERENCES

- Anderson, C.N. & Grether, G.F. (2010). Character displacement in the fighting colors of *Hetaerina* damselflies. *Proc. R. Soc. B*, 277, 3669–3675.
- Andersson, M. (1994). *Sexual Selection*. Princeton University Press, Princeton, NJ.
- Bates, D., Machler, M., Bolker, B. & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *J. Stat. Softw.*, 61, 1–48.
- Bivand, R. & Yu, D. (2017). 'spgwr'. Geographically weighted regression. R package version 0.6-32.
- Böhner, J. & Antonić, O. (2009). Land-surface parameters specific to topo-climatology. In: *Developments in Soil Science* (eds Hengle, T. & Reuter, H.I.). Elsevier, Amsterdam, pp. 195–226.
- Boughman, J.W. (2001). Divergent sexual selection enhances reproductive isolation in sticklebacks. *Nature*, 411, 944–947.
- Brashears, J., Aiello, A. & Seymoure, B.M. (2016). Cool bands: wing bands decrease rate of heating, but not equilibrium temperature in *Anartia fatima*. *J. Therm. Biol.*, 56, 100–108.
- Clusella Trullas, S., Van Whyk, J.H. & Spotila, J.R. (2007). Thermal melanism in ectotherms. *J. Therm. Biol.*, 32, 235–245.
- Corbet, P.S. (1999). *Dragonflies: Behavior and Ecology of Odonata*. Cornell University Press, Ithaca, NY.
- Cornwallis, C.K. & Uller, T. (2010). Towards an evolutionary ecology of sexual traits. *Trends Ecol. Evol.*, 25, 145–152.

- Cuthill, I.C., Allen, W.L., Arbuckle, K., Caspers, B., Chaplin, G., Hauber, M.E. *et al.* (2017). The biology of color. *Science*, 357, eaan0221.
- Darwin, C. (1871). *The Descent of Man, and Selection in Relation to Sex*. John Murray, London UK.
- Dillon, M.E., Wang, G. & Huey, R.B. (2010). Global metabolic impacts of recent climate warming. *Nature*, 467, 704–706.
- Ellers, J. & Boggs, C.L. (2003). The evolution of wing color: male mate choice opposes adaptive wing color divergence in *Colias* butterflies. *Evolution*, 57, 1100–1106.
- Endler, J.A. (1980). Natural selection on color patterns in *Poecilia reticulata*. *Evolution*, 34, 76–91.
- Fick, S.E. & Hijmans, R.J. (2017). WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. *Int. J. Climat.*, 37, 4302–4315.
- Forbes, M.R. & Robb, T. (2008). Testing hypotheses about parasite-mediated selection using odonate hosts. In *Dragonflies & Damselflies: Model Organisms for Ecological and Evolutionary Research*. (ed Córdoba-Aguilar, A.). Oxford University Press, Oxford, UK, pp. 175–188.
- Fried, C.S. & May, M.L. (1983). Energy expenditure and food intake of territorial male *Pachydiplax longipennis* (Odonata: Libellulidae). *Ecol. Entomol.*, 8, 283–292.
- Gillooly, J.F., Brown, J.H., West, G.B., Savage, V.M. & Charnov, E.L. (2001). Effects of size and temperature on metabolic rate. *Science*, 293, 2249–2251.
- Graham, M.H. (2003). Confronting multicollinearity in ecological multiple regression. *Ecology*, 84, 2809–2815.
- Halfwerk, W., Bot, S., Bukx, J., van der Velde, M., Komdeur, J., ten Cate, C. *et al.* (2011). Low-frequency songs lose their potency in noisy urban conditions. *Proc. Natl Acad. Sci. USA*, 108, 14549–14554.
- Hanley, D., Miller, N.G., Flockhart, D.T.T. & Norris, D.R. (2013). Forewing pigmentation predicts migration distance in wild-caught migratory monarch butterflies. *Behav. Ecol.*, 24, 1108–1113.
- Hassall, C. (2014). Continental variation in wing pigmentation in *Calopteryx* damselflies is related to the presence of heterospecifics. *PeerJ*, 2, e438.
- Hegna, R.H., Nokelainen, O., Hegna, J.R. & Mappes, J. (2013). To quiver or to shiver: increased melanization benefits thermoregulation, but reduces warning signal efficacy in the wood tiger moth. *Proc. R. Soc. B*, 280, 20122812.
- Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G. & Jarvis, A. (2005). Very high resolution interpolated climate surfaces for global land areas. *Int. J. Climat.*, 25, 1965–1978.
- Huey, R.B. & Kingsolver, J.G. (1989). The evolution of thermal sensitivity of ectotherm performance. *Trends Ecol. Evol.*, 4, 131–135.
- Huey, R.B., Deutsch, C.A., Tewksbury, J.J., Vitt, L.J., Hertz, P.E., Pérez, H.J.Á. *et al.* (2009). Why tropical lizards are vulnerable to climate warming. *Proc. R. Soc. B*, 276, 1939–1948.
- IPCC (2014) Climate Change 2013: the physical science basis. Working Group I Contribution to the AR5. Cambridge University Press, Cambridge NY USA.
- Kenward, M.G. & Roger, J.H. (1997). Small sample inference for fixed effects from maximum likelihood. *Biometrics*, 53, 983–997.
- Kingsolver, J.G. (1995). Fitness consequences of seasonal polyphenism in western white butterflies. *Evolution*, 49, 942–954.
- Kingsolver, J.G. & Buckley, L.B. (2017). Evolution of plasticity and adaptive responses to climate change along climate gradients. *Proc. R. Soc. B*, 284, 20170386.
- Kingsolver, J.G., Diamond, S.E. & Buckley, L.B. (2013). Heat stress and the fitness consequences for terrestrial ectotherms. *Funct. Ecol.*, 27, 1415–1423.
- Lailvaux, S.P. & Irschick, D.J. (2006). A functional perspective on sexual selection: insights and future prospects. *Anim. Behav.*, 72, 263–273.
- Lande, R. & Arnold, S.J. (1983). The measurement of selection on correlated characters. *Evolution*, 37, 1210–1226.
- Leighton, G.R.M., Hugo, P.S., Roulin, A. & Amar, A. (2016). Just Google it: assessing the use of Google Images to describe geographical variation in visible traits of organisms. *Methods Ecol. Evol.*, 7, 1060–1070.
- Maan, M.E. & Seehausen, O. (2011). Ecology, sexual selection and speciation. *Ecol. Lett.*, 14, 591–602.
- MacColl, A.D.C. (2011). The ecological causes of evolution. *Trends Ecol. Evol.*, 26, 514–522.
- Marden, J.H. (1995). Large-scale changes in thermal sensitivity of flight performance during adult maturation in a dragonfly. *J. Exp. Biol.*, 198, 2095–2102.
- Marden, J.H. & Cobb, J.R. (2004). Territorial and mating success of dragonflies that vary in muscle power output and presence of gregarine gut parasites. *Anim. Behav.*, 68, 857–865.
- May, M.L. (1976). Thermoregulation and adaptation to temperature in dragonflies (Odonata: Anisoptera). *Ecol. Monogr.*, 46, 1–32.
- McCauley, S.J. (2010). Body size and social dominance influence breeding dispersal in male *Pachydiplax longipennis* (Odonata). *Ecol. Entomol.*, 35, 377–385.
- Miller, C.W. & Svensson, E.I. (2014). Sexual selection in complex environments. *Annu. Rev. Entomol.*, 59, 427–444.
- Moore, M.P. & Martin, R.A. (2016). Intrasexual selection favours an immune-correlated color ornament in a dragonfly. *J. Evol. Biol.*, 29, 2256–2265.
- Moore, M.P. & Martin, R.A. (2018). Trade-offs between larval survival and adult ornament development depend on predator regime in a territorial dragonfly. *Oecologia*, 188, 97–106.
- Moore, M.P., Lis, C. & Martin, R.A. (2018). Immune deployment increases larval vulnerability to predators and inhibits adult life-history traits in a dragonfly. *J. Evol. Biol.*, 31, 1365–1376.
- Outomuro, D. & Ocharan, F.J. (2011). Wing pigmentation in *Calopteryx* damselflies: a role in thermoregulation? *Biol. J. Linn. Soc.*, 103, 36–44.
- Patricelli, G.L. & Blickley, J.L. (2006). Avian communication in urban noise: causes and consequences of vocal adjustment. *Auk*, 123, 639–649.
- Paulson, D. (2009). *Dragonflies and Damselflies of the West*. Princeton University Press, Princeton, NJ.
- Pinheiro, J., Bates, D., DebRoy, S. & Sarkar, D., & R Core Team (2015) nlme: Linear and nonlinear mixed effects models. R package version 3.1-119
- Punzalan, D., Rodd, F.H. & Rowe, L. (2008). Sexual selection mediated by thermoregulatory effects of male colour pattern in the ambush bug *Phymata americana*. *Proc. R. Soc. B*, 275, 483–492.
- Rasband, W.S. (2012). ImageJ. Bethesda, MD: U.S. National Institutes of Health.
- Samejima, Y. & Tsubaki, Y. (2010). Body temperature and body size affect flight performance in a damselfly. *Behav. Ecol. Sociobiol.*, 64, 685–692.
- Schielzeth, H. (2010). Simple means to improve the interpretability of regression coefficients. *Methods Ecol. Evol.*, 1, 103–113.
- Seehausen, O., van Alphen, J.J.M. & Witte, F. (1997). Cichlid fish diversity threatened by eutrophication that curbs sexual selection. *Science*, 277, 1808–1811.
- Servedio, M.R. & Boughman, J.W. (2017). The role of sexual selection in local adaptation and speciation. *Annu. Rev. Ecol. Evol. Syst.*, 48, 85–109.
- Sherman, K.J. (1983). The adaptive significance of postcopulatory mate guarding in a dragonfly, *Pachydiplax longipennis*. *Anim. Behav.*, 31, 1107–1115.
- Siva-Jothy, M.T. (2000). A mechanistic link between parasite resistance and expression of a sexually selected trait in a damselfly. *Proc. R. Soc. B*, 267, 2523–2527.
- Stuart-Fox, D., Newton, E. & Clusella-Trullas, S. (2017). Thermal consequences of colour and near-infrared reflectance. *Phil. Trans. R. Soc. B*, 372, 20160345.
- Suhonen, J., Rantala, M.J. & Honkavaara, J. (2008). Territoriality in odonates. In *Dragonflies & Damselflies: Model organisms for ecological and evolutionary research* (ed Córdoba-Aguilar, A.). Oxford University Press, Oxford UK, pp. 203–217.
- Sunday, J.M., Bates, A.E. & Dulvy, N.K. (2012). Thermal tolerance and the global redistribution of animals. *Nat. Clim. Change*, 2, 686–690.

- Sunday, J.M., Bates, A.E., Kearney, M.R., Colwell, R.K., Dulvy, N.K., Longino, J.T. *et al.* (2014). Thermal-safety margins and the necessity of thermoregulatory behavior across latitude and elevation. *Proc. Natl Acad. Sci. USA*, 111, 5610–5615.
- Svensson, E.I. & Waller, J.T. (2013). Ecology and sexual selection: evolution of wing pigmentation in Calopterygid damselflies in relation to latitude, sexual dimorphism, and speciation. *Am. Nat.*, 182, E174–E195.
- Tynkkynen, K., Rantala, M.J. & Suhonen, J. (2004). Interspecific aggression and character displacement in the damselfly *Calopteryx splendens*. *J. Evol. Biol.*, 17, 759–767.
- Viechtbauer, W. (2010). Conducting meta-analyses in R with the metaphor package. *J. Stat. Softw.*, 36, 1–48.
- Watt, W.B. (1968). Adaptive significance of pigment polymorphisms in *Colias* butterflies. I. Variation in melanin pigment in relation to thermoregulation. *Evolution*, 22, 437–458.
- Watt, W.B. (1969). Adaptive significance of pigment polymorphisms in *Colias* butterflies. II. Thermoregulation and photoperiodically controlled melanin variation in *Colias eurytheme*. *Proc. Natl Acad. Sci. USA*, 63, 767–774.
- West, P.M. & Packer, C. (2002). Sexual selection, temperature and the lion's mane. *Science*, 297, 1339–1343.
- Wiens, J.J. (2001). Widespread loss of sexually selected traits: how the peacock lost its spots. *Trends Ecol. Evol.*, 16, 517–523.
- Zuk, M., Rotenberry, J.T. & Tinghitella, R.M. (2006). Silent night: adaptive disappearance of a sexual signal in a parasitized population of field crickets. *Biol. Lett.*, 2, 521–524.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Editor, Greg Grether

Manuscript received 3 July 2018

First decision made 26 July 2018

Manuscript accepted 10 November 2018