

Sexual and natural selection on thermal plasticity and thermal canalization in insects at high latitudes

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Abstract

The evolution of phenotypic plasticity is of much principal interest, but there are few empirical studies in natural populations demonstrating how natural or sexual selection favour either increased plasticity or its reverse (increased canalization). Insects and other ectotherms are highly influenced by ambient temperatures and thermal plasticity might be beneficial to maintain optimal body temperatures and thereby maximize survival and reproduction. Here, we estimated the strength of natural and sexual selection on thermal plasticity (heating rate) and thermal canalization in two congeneric and sympatric species of damselflies (genus *Calopteryx*). Thermal plasticity is an important trait insects since they must largely rely on sunlight and ambient temperature in order to reach and maintain optimal body temperatures for survival and reproduction, particularly after nights or cold periods. In natural populations of these damselflies, the temperature fitness optimum for survival and mating rate is between 23 °C and 26 °C. We hypothesized that selection on thermal plasticity would increase outside this temperature range. Consistent with this prediction, we found that thermal plasticity was selectively beneficial when temperature conditions fell outside this range. Our results further suggest that when ambient temperatures are close to optimal body temperatures, sexual selection favours thermal canalization and disfavors maladaptive thermal plasticity since individuals might already be at optimal body temperatures most of the time. These results also have important implications for the evolution of thermal traits in relation to climatic factors, as ectotherms in more stable environments, such as the tropics, might experience weak selection on

thermal plasticity, which might affect their ability to respond evolutionarily or adjust plastically to rapidly changing thermal environments.

Introduction

Evolutionary biologists are increasingly directing their focus towards the evolution of phenotypic plasticity in temporally and spatially heterogeneous environments, as exemplified by both empirical studies in natural populations (Charmantier et al. 2008; Vedder et al. 2013) and theoretical models of the evolution of reaction norm slopes and intercepts (Lande 2009; Chevin et al. 2010; Chevin and Lande 2011). Research on the thermal plasticity is not only of interest for increasing our basic understanding of how traits evolve and why, but are also of practical concern in conservation biology, in particular the question if, when and how plasticity can lead to evolutionary rescue of populations (Chevin et al. 2013; Ashander et al. 2016). One form of phenotypic plasticity that is currently subject to much interest is thermal plasticity, or how organisms respond plastically in relation to ambient temperatures (Angilletta 2009). Understanding how genetic adaptation in thermal adaptations evolve is crucial for our understanding of how organisms will respond to increasing global or regional temperatures (Hoffmann et al. 2003; Deutsch et al. 2008; Kellermann et al. 2009; Sinervo et al. 2010; Wiens 2016).

Insects and other small ectotherms are traditionally considered to be thermal conformers because they allow their body temperatures to closely match ambient temperatures (Sanborn 2004; Angilletta 2009). In ectotherms, important physiological processes are at the mercy of ambient temperature. From an enzymatic point of view, being an obligate thermal conformer might not always be beneficial (Huey and Berrigan 2001; Blouin-Demers et al. 2005; Martin et al. 2008). Enzymes are less efficient at any non-optimal temperatures (Martin et al. 2008), and even though ectotherms will tend to match ambient temperatures without immediate mortality, we might still expect better thermoregulatory ability to be selected for in certain contexts, especially in extreme thermal environments (Huey et al. 2009). While ectotherms have less control over their internal body temperatures than endotherms, they typically still have some ability to thermoregulate to some degree via behavioural and/or physiological adjustments (Huey et al. 2003). Variation in thermoregulatory ability typically differs between species as well as between individuals within single populations (Hoffmann et al. 2011; Somero 2010; Kearney et al. 2009; Buckley et al. 2015). Therefore, the dichotomy of being a true thermoregulator versus a thermal conformer is as false one, and even groups considered to be thermal conformers there are fitness

incentives to regulate their internal body temperatures by behavioral means (Huey and Slatkin 1976, Martin et al. 2008).

Ongoing and anticipated future climate change will most likely increase seasonal variation in temperature in many areas, making the ability to thermoregulate increasingly important for ectotherms (Deutsch et al. 2008). However, some temperate ectotherms appear to have maximum thermal tolerances that are 10–20 °C higher than required to withstand the average summer air temperatures where they currently live, which might reflect their evolutionary origin in tropical environments (Sanborn 2004; Sunday et al. 2011). There are thus many behavioral, morphological, and physiological adaptations that might allow ectotherms to survive unfavourable thermal environments outside their current thermal range (May 1979; Heinrich 2013), and in some more extreme cases the body temperatures of some insects have been recorded more than 35°C above ambient temperature (Sanborn 2004).

A large source of heat in flying insects comes from the activity of their flight muscles, and flying insects can increase heat generation through increased muscle activity (May 1979). Basking or changing body orientation to the sun is also another common thermoregulatory behavioral strategy that minimizes the exposed surface area and heat uptake (Porter 1982; Heinrich 2013). Body color such as melanism, provides yet another means by which the amount of radiation that gets turned into heat energy can be increased (True 2003; Trullas 2007; Svensson and Waller 2013). Finally, simply evolving a larger body size will increase the surface-to-volume ratio and thermal inertia (Kingsolver and Huey 2008; Angiletta 2009).

Thermal plasticity is a measure of an individual's ability to change its body temperature in relation to ambient temperature (Trotta et al. 2006; Waller et al. 2017). Thermal plasticity is a specific form of a reaction norm in relation to a specific environmental variable (temperature)(Angiletta 2009). Such reaction norms can be quantified in terms of both their slopes (plasticity) and their intercept (Angiletta 2009). Mechanistically, thermal plasticity can be quantified by estimating the slope parameter of how body temperature changes in relation to ambient temperature (Angiletta 2009). This measure of thermal plasticity captures any physiological differences between individuals, but also might capture any behavioral adjustments such as habitat selection, orientation, and muscle activity.

The rate of evolution in response to selection on thermal plasticity will depend the additive genetic variances and covariances underlying variation of this trait and the strength of selection on these traits (Lynch and Walsh 1998). Some previous studies have demonstrated that there is indeed genetic variation in the slope of thermal reaction norms (Gutting et al. 2007; van Asch et al. 2007; Winterhalter and Mousseau 2007), whereas others have failed to find any such genetic variation (Waller et al. 2017). Under some environmental conditions, we might expect the evolution of thermal canalization towards an optimal body temperature that is associated with high fitness (Liefting et al. 2009). Conversely, classic theory for

the evolution of phenotypic plasticity suggests that plasticity should be high in variable environments, so that individuals could express their optimum phenotype even if environmental conditions change radically (Lande 2014; Chevin and Hoffmann 2017). How these classic theories for the evolution of phenotypic plasticity in general apply to the more specific case of thermal plasticity is yet largely unknown, as there are few empirical studies in natural populations in this area.

Here, we studied how natural and sexual selection operate on thermal reaction norms in two congeneric European species of damselflies (the banded demoiselle *Calopteryx splendens* (Harris 1780) and the beautiful demoiselle *Calopteryx virgo* Linnaeus 1758). The genus *Calopteryx* did not evolve in Europe, but has a Asiotropical origin, from which they expanded in to temperate regions at high latitudes (Misof et al. 2000; Svensson and Waller 2013). These two species of *Calopteryx* damselflies invaded southern Sweden approximately 12,000 years ago after the last glacial period (Misof et al. 2000; Clark et al. 2009). Their tropical origin and relatively recent establishment in the temperate areas at high latitudes make these damselflies interesting in terms of thermal adaptation and thermal plasticity, as they might potentially not yet be fully adapted to the temperature regimes in the north.

In *Calopteryx*, males are highly territorial (Marden and Waage et al. 1990; Plaistow et al. 1996; Córdoba-Aguilar 2002), and they rely on basking behaviour to reach optimal body temperatures after cool nights (Marden et al. 1996). These damselflies usually spend most of their active period on perches and take flights when feeding or defending mating territories, so maintaining optimal body temperatures is a challenge for them. *Calopteryx* males have large wing patches composed of melanin, and these dark wing patches might also help males to achieve higher body temperatures (Svensson and Waller 2013). Because mating success in these damselflies is largely dependent on a male's ability to hold a territory, males that are able to maintain optimal body temperatures over a wide range of ambient environmental temperatures would be expected to be more successful in obtaining matings.

Here, we define thermal plasticity as the slope of the thermal reaction norm for individuals that warm up after a cold period, such as after a night. If an individual has a high slope (i.e. a high heating rate), it means they are more affected by ambient temperature, but they might also more quickly reach their optimal body temperature after thermally stressful period. Therefore, a high heating rate might be either a fitness advantage or a fitness disadvantage to a male damselfly, depending on environmental conditions. For instance, if the thermal environment is below the thermal optimum of a male damselfly, it might be advantageous to quickly heat up. Conversely, if the ambient temperature is above the thermal optimum, it might be advantageous to heat up more slowly or not at all. In other words, under some conditions there could be selection for thermal canalization

that buffers the organism against the stressful thermal environment (cf. Huey et al. 2003). Finally, if ambient temperatures are on average close or near to the thermal fitness optimum, selection on the thermal reaction norms would be expected to become relaxed or at least weakened, which could potentially result in the accumulation of high phenotypic and genetic variation thermal reaction norms in such environments, such as in relatively stable tropical thermal environments. On the other hand, thermal plasticity could also be expected to increase in variable temperate environments for adaptive reasons, if such plasticity is more beneficial at higher compared to lower latitudes (Mitchell et al. 2011; Lancaster et al. 2015). The latitudinal hypothesis predicts increasing thermal plasticity as one moves from the equator to the poles as seasonality increases. This hypothesis would be supported by observations of either reduced plasticity in organisms in extreme environments or increased plasticity in less extreme environments. For a trait that is initially canalized, a major change in the environment can select for increased plasticity (Gavrilets and Scheiner 1993; Lande 2009; Lancaster et al. 2015).

Here, we show that sexual selection on thermal plasticity overall favors canalization and shallower thermal reaction norm slopes, suggesting that even at these high latitudes, insects can suffer from overheating, at least when temperatures are high. However, the strength of sexual selection for thermal canalization also depends on ambient temperatures and interacts with morphological variation, albeit in different ways in these two phenotypically very similar species.

Methods

Field site and general field work procedures

Our field studies were performed along the river Klingavälsån in the province of Skåne in southern Sweden at Sövdemölla Gård (Latitude: 55.601492, Longitude: 13.657340). These field studies were conducted each summer (June and July) during four seasons (2013 - 2016). In each year, we collected morphological data, fitness data (survival and mating success), and thermal performance measures on two sympatric damselfly species (*Calopteryx splendens* and *Calopteryx virgo*). Field work took place between 10.00 and 15.00 when activity levels were the highest. Damselflies at our field site have very low activity levels when temperature are below 15° C and during rainy and windy days (i.e. they do not fly or mate under these such conditions). Therefore, we only captured, processed, and re-sighted individuals on the relatively warm and calm days when they were

active, whereas on cold and rainy days, such data could not be collected, as animals were not active and could not be found.

Morphological measurements

Males and females of both *C. splendens* and *C. virgo* were captured with handnets and processed in the field. Individual damselflies were measured by placing their wings flat against two layers of transparency paper. Digital scans were taken with a known scale and the body and wings traits were measured in ImageJ (Schneider et al. 2012; Waller and Svensson 2016; 2017). We measured the following morphological traits: 1) forewing length and width; 2) total body length; 3) abdomen length; 4) thorax length; 5) fore- and hindwing melanin patch length (for *C. splendens* males only); 6) fore- and hindwing melanin patch width (for *C. splendens* males only); 7) mass (to the nearest ± 0.002 g) and 8) melanin wing patch darkness based on the mean intensity of a grayscale image of the patch (larger values meaning darker wing patches).

Thermal imaging

A series of thermal images (NEC Avio Infrared Technologies H2640) were taken of each field-caught individual (Fig. 1). Individuals were first allowed to cool for 3 minutes in a cooler box at approximately 5°C before being placed back into ambient temperature, each within an individual cup, under a thermal imaging camera. Thermal images were taken every 10 seconds for 3 minutes (i.e. up to 18 frames per individual, except for those that flew away within 3 minutes). This experiment aimed to simulate how these ectothermic insects heat up with increasing ambient temperature, e.g. after a cold night in the morning hours.

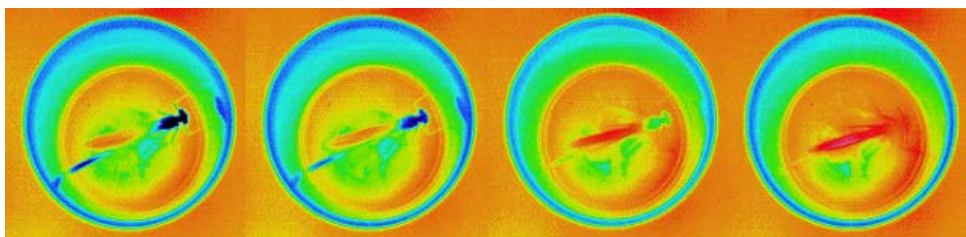


Figure 1. A series of thermal images.

Thermal images of a damselfly heating up over 3 minutes. Warm colors (red, orange) represent higher temperatures and cooler colors (blue, green) represent cooler colors.

Batches of 6 individuals were cooled and imaged simultaneously. After the thermal images were taken, individuals were released. Because individuals were exposed to ambient temperature and sunlight, variability in these two parameters were controlled for before analysis of heating rates (thermal plasticity) of each individual. Images were then analysed to retrieve the body temperature of each individual at each time point.

Estimating slopes for thermal reaction norms

All statistical analyses were done in R unless otherwise stated. We defined thermal plasticity as the slope of the heating rate of individuals after a cooling stress event. This slope was calculated by fitting a linear model of body temperature (obtained from thermal images) against ambient temperature. After this, we fit an additional linear model accounting for daily variation in solar radiation and temperature, and the residuals from this model were used in any downstream analysis of thermal performance. Solar radiation data was taken from the STRÅNG model of solar radiation from the Swedish Meteorological and Hydrological Institute (SMHI) (<http://strang.smhi.se/>). Ambient temperature was extracted from the iButton temperature loggers at various damselfly neighborhoods at our field site (described below).

Measuring thermal microenvironmental variation

We placed approximately 30 iButton thermologgers (1-Wire; DS1921G-F5) at different locations within our field site to measure thermal microenvironmental variation. Such temperature loggers are able to record temperature at set intervals for several months. We recorded ambient temperature every hour for the duration of our field study each year. The field site was divided into 10 neighborhoods (sections; three iButtons per neighborhood) along our study site. Neighborhoods were marked with colored tape, so that when individuals were recaptured, they could always be linked to a particular neighborhood. When ambient temperature is used in our statistical models, we are using temperature data from these temperature loggers.

Longevity and mating success

In conjunction with our recordings of morphological and thermal plasticity data, all individuals were marked with a unique code with three colors on the last three segments of the abdomen. These unique markings were used to identify

individuals and record their subsequent survival (longevity) in the field. Apart from catching and re-capturing single individuals, we also caught as many copulating pairs in the field as possible so that we could obtain estimates of sexual selection through male mating success. We tried to capture as many mating pairs as possible during days with high mating activity, however, we could of course not capture all observed pairs. Nevertheless, we recorded all observed copulations in the field (both those that were captured and those that were not) to quantify mating activity during all days.

Selection on thermal plasticity in relation to ambient temperatures

To examine the factors that influence thermal plasticity (heating rate) in these damselflies we fit a series of mixed-effect models using the R package nlme. We estimated the following fixed effects: ambient temperature, solar radiation, body mass, body size, and wing patch density (darkness). All these models contained year as a random factor.

We estimated linear selection gradients of thermal plasticity (slopes of the thermal reaction norms) for both sexual selection (male mating success) and survival selection and related this to natural variation in temperature in the environment. We divided individuals into groups based month and year. We did this in order to capture as much natural variation in temperature as possible while still having reasonable statistical power to detect selection. Abundance data at this field site reveals that there are two different cohorts each summer, due to so-called cohort splitting (Norling 1984), which is common in many species of damselflies. These two seasonal cohorts are roughly divided between June and July. We analyzed the data using generalized linear mixed effect models. We calculated linear selection differentials and quadratic selection gradients (stabilizing and disruptive selection) on slopes of thermal reaction norms and morphological traits following the general statistical methodology of Lande and Arnold (1983). The quadratic regression coefficients were multiplied by two to obtain correct estimates of the strength of stabilizing and disruptive selection (Stinchcombe et al. 2008). All of our phenotypic traits were standardized to mean zero and unit variance and our two fitness components (minimum lifespan or male mating success) were divided by the population average (mean fitness) before being used as the response variables in these regression analyses of selection (Lande and Arnold 1983).

In the sexual selection analyses, we used a binary response variable as our measure of male mating success (males that obtained at least one mating = 1; non-mated males = 0). From our daily field counts and captures at our study site, we found 311 males mating, out of a total of 2945 observed ($311/2945 = 0.105$). Based on these numbers, we therefore conservatively assumed that only 10% of

males in this population successfully mated at least once, which we thus used as our measure of average male mating fitness (i.e. 0.10) in our regression analyses of selection (cf. Lande and Arnold 1983). This quite low mating success estimate of 10% males obtaining at least one copulation at this site is consistent with similar estimates from our previous independent selection studies at two other sites (“Klingavälsåns Naturreservat” and “Höje Å, Värpinge”), where we collected similar data from 2001-2003 (Svensson et al. 2004; 2006). This low mating success reflects the reproductive biology of *Calopteryx* damselflies, which are characterized by high mating skew and strong sexual selection. We continued to monitor the population until all of our marked individuals had died. The average lifespan of an adult *C. splendens* males in the field is around 3-4 days, although some males can live for more than two weeks (Svensson et al. 2006; Waller and Svensson 2016).

Effect of ambient temperature on longevity and mating rate

To quantify the general effect of temperature on average lifespan of both species, we estimated the relationship between individual longevity variation and the average daytime temperature experienced by each male individual. The lifespan temperature of each individual male was calculated by taking the average temperature of experienced by each individual in its given microenvironment. We performed this analysis for males only, as the recapture rates for females were very low. We also used the average temperature and the number of mating pairs each day in the field to quantify the population-level effect of temperature on mating activity. Linear models with quadratic terms were then fit to the datasets of both *C. splendens* and *C. virgo*.

Results

Effect of temperature on lifespan and mating rate

To assess the general sensitivity of *C. splendens* and *C. virgo* individuals to temperature, we modeled survival and mating rate as a function of temperature (Fig. 2; Table 1). In both *C. splendens* and *C. virgo* mating activity peaked somewhere between 20 – 25°C, whereas higher or lower temperatures reduced the population level mating activity (Table 1; Fig. 2; A,B). Although plots of mating rate of the two species appear to be different (Fig. 2 A,B), there is no statistical difference between the peak mating rates of the two species (Table 1:

Temperature² x Species (ref. *C. virgo*), $P = 0.897$). Survivorship in *C. splendens* males peaked at temperatures between 20 – 25°C, whereas higher or lower temperatures reduced male lifespan in the field (Fig. 1; C). Similarly, *C. virgo* males had reduced lifespan at high and low temperatures, with a tendency for a somewhat increased mortality with increasing temperatures, compared to *C. splendens* which seemed less heat sensitive in terms of survival (Table 1: Temperature² x Species; $P = 0.087$).

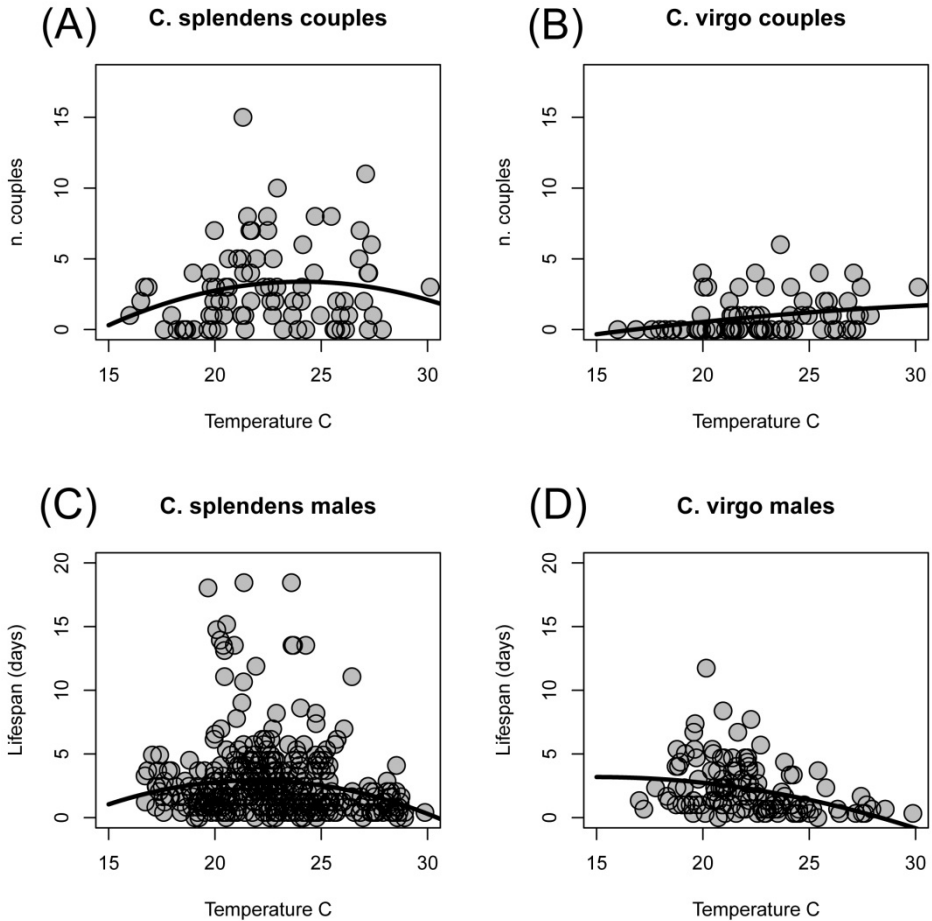


Figure 2. Effect of ambient temperature on survival and mating rate.

The effect of ambient temperature on mating activity and lifespan in *C. splendens* and *C. virgo* at the sympatric site at Klingavälsån near Sövdemölla. In *C. splendens*, mating activity peaks between 20 – 25°C, but declines at higher or lower temperatures. Mating rate for *C. virgo* individuals, may increase at higher temperatures (B). Survival in *C. splendens* males peaked at temperatures between 20 – 25°C, whereas higher or lower temperatures reduces lifespan in the field (C). Similarly, *C. virgo* males had low lifespan at higher or lower temperatures, with the peak at lower temperature than *C. splendens* (D). See Table 1 for statistics.

Table 1. Modeling mating rate and survival with temperature.

Modeling mating rate and survival in our population of *C. virgo* and *C. splendens*. Both daily mating rate and survival were modeled as with a negative binomial generalized linear model assuming an over-dispersed count outcome. The intercept term was estimated, but is omitted from the table for brevity. Mating was taken as the total number of matings each day and Temperature in this model was mean ambient temperature of that day between 10:00-15:00 hours. Species is a two level factor of both species. Survival is the number of days observed in the field of males in the population. temperature in this model is the mean temperature over the lifespan of each individual male. Significant effects are shown in **bold**.

Fitness	Term	Estimate	SE	P-value	N
Mating	Species (ref. <i>C. virgo</i>)	-5.945	11.777	0.613	163
	Temperature	0.981	0.470	0.037*	
	Temperature ²	-0.020	0.010	0.049*	
	Temperature x Species (ref. <i>C.virgo</i>)	0.268	1.013	0.791	
	Temperature ² x Species (ref. <i>C.virgo</i>)	-0.002	0.021	0.897	
Survival	Species (ref. <i>C. virgo</i>)	9.987	4.333	0.021*	957
	Temperature	0.555	0.128	<0.001***	
	Temperature ²	-0.013	0.002	<0.001***	
	Temperature x Species (ref. <i>C.virgo</i>)	-0.768	0.379	0.043*	
	Temperature ² x Species (ref. <i>C.virgo</i>)	0.014	0.008	0.087	

Factors influencing thermal plasticity

To examine what morphological and environmental factors influence intercepts and slopes of the thermal reaction norms (thermal plasticity = slopes of thermal reaction norms) in *C. splendens* males, we constructed a series of linear mixed-effect models using all of the data from 2013 to 2016, while controlling for the random effect of year (Table S1). These models incorporated two environmental factors (ambient temperature and solar radiation) and two morphological traits (body mass and wing color patch darkness). The best performing model was a model that included that included ambient temperature, solar radiation, and body mass (Table S1; Model 4). A model that also included wing patch darkness was the second best performing model in terms of AIC (Table 1; Model 6), with a small but positive effect of wing patch darkness on thermal reaction norm slope (Table 1).

Sexual selection on the slopes of thermal reaction norms

We modelled the effect of the morphological traits and thermal variables (slope) on sexual selection in *C. splendens* and *C. virgo* males (Tables 2-3). Data from 2013 – 2016 was used, while we controlled for the random effect of year in a mixed effect model. For *C. splendens* males, a model that included a linear effect of wing length and wing patch darkness was the best performing model in terms of AIC (Table 2; Model 5). Model 6, was the second best performing model and included a non-significant overall negative effect of the slope of the thermal reaction norm (thermal plasticity) on male mating success. Thermal plasticity had an overall negative effect on mating success even after including body mass and forewing length in the analysis (Table 2; Model 7). In contrast, in *C. virgo*, we found evidence for sexual selection against thermal plasticity across all years, (Table 3).

Table 2. Thermal plasticity and morphology on mating success in *C. splendens*.

Models of how variation in morphology and thermal physiology influence mating success in *C. splendens* males. All models use data from all years (2013-2016; N = 2133), while controlling for year as a random factor. Across years, thermal plasticity was found to have an overall non-significant negative effect on mating success, even after controlling for the effect of body size (mass and wing length) (model 6). The best performing model (model 5), included only wing length and wing patch darkness, indicating that black color might primarily function as a sexual signal rather than in primarily serving in thermoregulation. Model effects that are significant or nearly significant ($P < 0.10$) models are indicated in **bold**.

Model	Term	Estimate	SE	P-value	AIC	Rank
1	thermal plasticity	-0.100	0.0685	0.1429	10974.46	8
2	wing length	0.136	0.0342	< 0.0001	10962.27	5
3	patch darkness.	0.321	0.0682	< 0.0001	10954.44	4
4	mass	0.2242	0.0683	0.0011	10965.88	6
5	wing length	0.1327	0.0341	0.0001	10946.24	1
5	patch darkness	0.3163	0.0680	< 0.0001		
6	wing length	0.1306	0.0341	0.0001	10949.82	2
6	patch darkness	0.3182	0.0679	< 0.0001		
6	thermal plasticity	-0.0952	0.0680	0.1615		
7	wing length	0.1166	0.0348	0.0008	10951.49	3
7	patch darkness	0.3042	0.0683	< 0.0001		
7	thermal plasticity	-0.0856	0.0681	0.2092		
7	mass	0.1366	0.0700	0.0511		
8	wing length	-0.1337	0.8265	0.8715	10969.85	7
8	patch darkness	0.3254	0.0900	0.0003		
8	thermal plasticity	-0.1455	0.0869	0.0943		
8	mass	0.1982	0.0763	0.0095		
8	wing length ²	0.0082	0.0281	0.7697		
8	patch darkness ²	0.0473	0.0701	0.4997		
8	thermal plasticity ²	0.0455	0.0435	0.2952		
8	mass ²	-0.1150	0.0495	0.0202		

Table 3. Thermal plasticity and morphology influence on mating success in *C. virgo*.

Modelling morphology and physiology influencing mating success in *C. virgo* males. All models use data from all years (2013-2016; N = 427), while controlling for the random effect of year. Thermal plasticity was found to have an overall negative effect on mating success, even after controlling for the effect of body size (mass and wing length)(model 5). The best performing model (model 1), included only thermal plasticity, which might indicate that heating rate is a composite trait that reflects variation in several underlying traits such as body size, mass, or physiology. Significant effects are indicated in **bold**.

Model	Term	Estimate	SE	P-value	AIC	Rank
1	thermal plasticity	-0.4811	0.1789	0.0074	2333.606	1
2	wing length	0.1261	0.0844	0.1360	2340.065	5
3	mass	-0.2824	0.1799	0.1173	2338.320	4
4	wing length	0.1222	0.0838	0.1456	2336.603	3
4	thermal plasticity	-0.4766	0.1787	0.0080		
5	wing length	0.1764	0.0874	0.0442	2335.774	2
5	thermal plasticity	-0.4708	0.1780	0.0085		
5	mass	-0.3894	0.1864	0.0373		
6	wing length	-0.3938	0.8293	0.6351	2348.984	6
6	thermal plasticity	-0.5183	0.2144	0.0160		
6	mass	-0.4209	0.1897	0.0271		
6	wing length ²	0.0187	0.0271	0.4901		
6	thermal plasticity ²	0.0579	0.1541	0.7074		
6	mass ²	-0.1721	0.2233	0.4412		

Survival selection on thermal performance

We also modelled the effect of various morphological traits and thermal performance on survival in *C. splendens* and *C. virgo* males (Table 3.; Table S3). Data from 2013 – 2016 was used, while incorporating year as a random factor in a mixed-model framework. For *C. splendens* males, the model that included a linear effect of patch density (darkness) was the best performing model in terms of AIC (Table 3 ; Model 3). Model 1, with only a linear effect thermal performance also performed well in terms of AIC (ranked 2nd). Thermal performance in this model had a non-significant negative effect on survival in *C. splendens* males. Thermal performance also continued to have an overall negative effect on survival even after controlling for mass and wing length (Table 3; Model 7). *C. virgo* was also modeled in a similar way and is presented (Table S2).

Selection on thermal performance compared to monthly temperature variation

We plotted the relationship between linear selection on survival and monthly mean temperature at our field site (Fig. 3). In *C. splendens* and *C. virgo* males, we see that there is increasing sexual selection on thermal plasticity at extreme temperatures (at around 22°C and 28°C) (Fig. 3A, C), and no to negative selection on thermal plasticity at medium monthly mean temperatures (23°C – 26°C). We also show survival selection on thermal plasticity in *C. virgo* and *C. splendens* males (Fig. 3 B, D). Modelling the sexual selection data using mixed effect models (controlling for the random effect of year) rather than splitting the data into months, revealed two nearly significant positive interaction terms between thermal plasticity and ambient temperature (Table 4; term: thermal plasticity x temp.²) in both *C. splendens* and *C. virgo* males. This significant interaction indicates that there is a underlying positive curvature to the fitness surface that is temperature dependent. There was no such significant effects of thermal plasticity in terms of survival selection (Table 4).

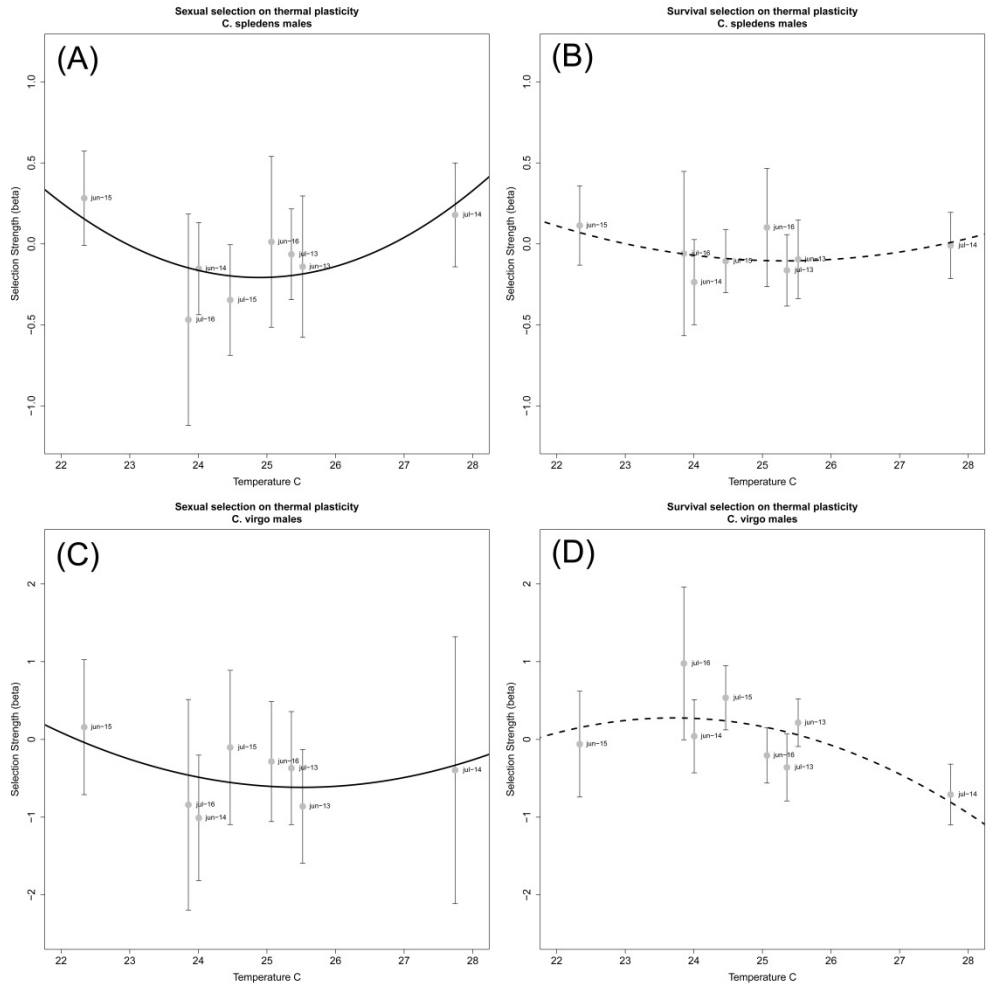


Figure3. Effect of ambient temperature on natural and sexual selection.

The relationship between linear selection on male mating success and male longevity against with monthly mean temperatures at our field site. In *C. splendens* and *C. virgo* males, the strength of sexual selection on thermal plasticity was higher at extreme temperatures (at around 22°C and 28°C) (A and C), and weak negative selection on thermal plasticity at intermediate temperatures (23°C – 26°C). The pattern for survival selection in both *C. virgo* and *C. splendens* males was less clear and not significant (B and D). See Table 4 for statistics.

Table 4. Effect of ambient temperature on natural and sexual selection.

Modeling the interactive effect between ambient temperature and sexual and natural selection on thermal plasticity in *C. splendens* and *C. virgo* males (CSM and CVM, respectively). We modeled the effect of ambient temperature, thermal plasticity and curvature of the plasticity fitness surface in relation to ambient temperature. All terms with $P < 0.1$ are shown in bold. The relationship between temperature and survival selection in *C. splendens* and *C. virgo* males is less clear, compared to the effect on sexual selection. Significant or near-significant ($P < 0.10$) terms are shown in bold.

Sp.Sex	Selection	Term	Estimate.	Std. Error.	P	Fig.
CSM	Sexual	thermal plasticity.	-0.2531	0.1032	0.0143	3A
		temperature	0.2714	0.0689	0.0001	
		thermal plasticity ²	0.0133	0.0225	0.5530	
		temperature ²	0.0461	0.0579	0.4264	
		thermal plasticity x temperature	-0.0664	0.0622	0.2854	
		thermal plasticity x temperature. ²	0.0941	0.0498	0.0589	
CSM	Survival	thermal plasticity	-0.0448	0.0711	0.5289	3B
		temperature	-0.1226	0.0475	0.0099	
		thermal plasticity ²	-0.0069	0.0155	0.6553	
		temperature ²	0.0177	0.0399	0.6570	
		thermal plasticity x temperature	-0.0238	0.0428	0.5786	
		thermal plasticity x temperature ²	0.0015	0.0343	0.9662	
CVM	Sexual	thermal plasticity	-0.9033	0.2955	0.0024	3C
		temperature	0.6804	0.1773	0.0001	
		thermal plasticity ²	0.0707	0.0803	0.3787	
		temperature ²	0.0219	0.1543	0.8873	
		thermal plasticity x temperature	-0.3934	0.1543	0.0367	
		thermal plasticity x temperature ²	0.2993	0.1543	0.0529	
CVM	Survival	thermal plasticity	0.1411	0.1404	0.3155	3D
		temperature	-0.0592	0.0843	0.4829	
		thermal plasticity ²	0.0400	0.0381	0.2954	
		temperature ²	-0.0443	0.0733	0.5460	
		thermal plasticity x temperature	0.0447	0.0892	0.6167	
		thermal plasticity x temperature ²	-0.1056	0.0892	0.1502	

Discussion

Ectotherms like damselflies and many other insects are highly dependent on ambient temperature to maintain beneficial internal body temperatures for flight and function (Angilletta 2009; Heinrich 2013). Here, we quantified natural and sexual selection on thermal plasticity in two sympatric and closely related species of damselflies.

We find that *C. virgo* and *C. splendens* individuals have an optimal mating rate between 20°C and 25°C (Table 1; Fig. 2; A,B). Although plots of mating rate of the two species appear to be different (Fig. 2; A,B), we found no statistically significant difference in how these two species responded to ambient temperature in terms of mating rate (Table 1: i.e. no significant Temperature² x Species-term). Survivorship in *C. splendens* males peaked at temperatures between 20 – 25°C, whereas higher or lower temperatures reduced male lifespan in the field (Fig. 1C). Similarly, *C. virgo* males had reduced lifespan at high and low temperatures, with a near-significant tendency for decreasing survival rate with increasing temperature (Table 1: Temperature² x Species: $P = 0.087$).

We quantified how selection operated on the thermal reaction norms in these two phenotypically similar species (Tables 2-3). We found that, across all the years of this study, there was some evidence of sexual selection against thermal plasticity in both *C. splendens* and *C. virgo* (Tables 2-3). Thus, males of both species which had shallower thermal reaction norms had the highest mating success, suggesting sexual selection for thermal canalization. In other words, sexual selection against thermal plasticity. In contrast, there was no evidence that natural selection through adult longevity favored either increased or decreased thermal plasticity (Tables S2-S3). These results might suggest that mating success, and potentially reproductive traits in general, are more sensitive to thermally stressful environments, which can lead to selection for thermal canalization. These results might also suggest that high thermal sensitivity and thermal plasticity could be a maladaptive form of plasticity that is currently opposed by sexual selection for thermal canalization.

Some evolutionary biologists have argued that phenotypic plasticity is mostly adaptive, and plasticity is often invoked as a creative factor in evolution that could enhance long-term adaptation, diversification and speciation (West-Eberhard 2003; Pigliucci et al. 2006; Pfennig et al. 2010; Moczek et al. 2011). These optimistic views about the adaptive nature of phenotypic plasticity do, however, largely rely on verbal arguments rather than on formal models. Rigorous theoretical and empirical studies demonstrating the adaptive nature of phenotypic plasticity in natural populations are far outnumbered by verbal arguments in many reviews claiming that plasticity is adaptive. More formal mathematical models of the evolution of phenotypic plasticity emphasize the need to directly quantify

selection on reaction norms themselves and relate such selection to patterns of environmental variation (Lande 2009; Chevin et al. 2010; Chevin and Lande 2011).

Selection on plastic traits, such as thermal reaction norms, can be formalized as selection on function-valued traits (Kingsolver et al. 2001; Stinchcombe et al. 2012). One could even argue that it is only when the slopes of a thermal reaction norms are subject to stabilizing selection around the population mean that the current level of plasticity should be viewed as adaptive. The fact that we did not find any evidence for stabilizing selection on reaction norm slope for either species in this study (Tables 2-3), suggests that the observed level of plasticity is not adaptive. Instead of stabilizing selection we found evidence for directional sexual selection *against* thermal plasticity and for environmental canalization (Tables 2-3). Our results should caution against assuming that just because traits exhibit phenotypic plasticity, that such plasticity must be necessarily adaptive. Instead, selection can operate to reduce plasticity and favor increased canalization. Other examples of maladaptive plasticity have recently been found or suggested for guppies (Ghalambor et al. 2015) and soapberry bugs (Cenzer 2017).

We hypothesized that selection on thermal plasticity would increase if individuals found themselves in environments which were on average higher or lower than their optimal range. In both *C. splendens* and *C. virgo* males, we found that, selection for increased thermal plasticity occurs when temperature conditions are less than around 23°C and greater than 26°C (Fig. 3A-C; Table 4). This implies that individuals that are better able to heat up in cold environments and cool down in warm environments will have higher mating success.

In environments that are already close to optimal body temperatures, there should be weak or negative selection on thermal plasticity, since individuals will already be at optimal body temperatures. If an individual is already at an optimal body temperature, any sudden change in ambient temperature should be resisted. However, if an individual is at a non-optimal temperature, that individual should quickly adapt to a sudden change in ambient temperature because that change will likely be in the direction towards an optimal temperature. We found less strong patterns when looking at survival selection on thermal plasticity, which implies that thermoregulatory ability might not be as important for survival (Fig 3; B, D and Table 4). From a life history perspective, thermal performance might be less important in survival selection. Those individuals at risk of dying from overheating, will likely seek out cooler micro-environments, and damselflies can typically survive cold nights of less than 10°C in Sweden.

Whether selection in extreme environments will lead to a change in mean thermal plasticity depends on the quantitative genetic architecture of thermal plasticity and correlated traits. Recent experimental work shows that there might be some limited amount of genetic variation in heating rate but not in thermal performance curves for mating rate in *Drosophila melanogaster* (Waller et al.

2017). Genetic covariance between thermal plasticity and other phenotypic traits might also affect expected evolutionary responses due to selection (Morrissey 2014). For example, it is known that body size in damselflies is under positive selection in the adult stage (Svensson and Waller 2017), and larger damselflies will also heat up slower (Table S1). The net effect of such selection for larger body size might restrict the evolution of thermal plasticity in damselflies because large body size will also increase thermal inertia (Table S1).

Conclusion

Thermal plasticity is an important trait in male damselflies for mating success in extreme temperature conditions. We found that, selection for increased thermal plasticity occurs when temperature conditions are on the extremes of this range (less than around 23°C and greater than 26°C). These results suggest that in environments that are already close to optimal body temperatures, there might even be selection against thermal plasticity, since individuals will already be at optimal body temperatures most of the time. Our results might also have some implications for the evolution of thermal traits in general and in less variable thermal environments, such as in the tropics. In thermally stable environments, selection might not remove shallow or steep thermal reaction norms, allowing the build-up of “cryptic” genetic variation that will only be exposed to selection following invasion in more thermally variable environments. Once organisms have invaded more variable environments, selection can then either favour reduced thermal plasticity and increased thermal inertia (as in this study), or increased thermal plasticity in other situations (see Lande 2009; 2014; Chevin and Lande 2010 for in-depth discussions).

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Supplementary Material

Table S1. Factors influencing heating rate in *C. splendens* males.

Modelling the factors affecting thermal plasticity (slope of thermal reaction norms) in *C. splendens* males. We used mixed models with data from all years (2013-2016; N = 2133), while controlling for the random effect of year. We tested models with two environmental factors (solar radiation and ambient temperature) and body mass and wing color patch darkness (patch density). All variables have been standardized to mean zero and unit variance. The best performing model (model 4) included a positive effect of solar radiation and ambient temperature and a negative effect of body mass. Wing patch darkness also had a positive effect on heating rate when included in the model (model 6).

Model	Term	Est.	SE	P-value	AIC	Rank
1	Temperature	0.0948	0.0064	< 0.0001	873.317	3
2	Solar radiation	0.0382	0.0067	< 0.0001	1049.35	5
3	Mass	-0.0094	0.0067	0.1616	1079.99	6
4	Temperature	0.0929	0.0064	< 0.0001	865.015	1
4	Solar radiation	0.0302	0.0064	< 0.0001		
4	Mass	-0.0205	0.0064	0.0014		
5	Temperature	0.0913	0.0064	< 0.0001	874.004	4
5	Solar radiation	0.0286	0.0064	< 0.0001		
5	Patch darkness	0.0070	0.0064	0.2725		
6	Temperature	0.0926	0.0064	< 0.0001	873.045	2
6	Solar radiation	0.0314	0.0064	< 0.0001		
6	Mass	-0.0217	0.0064	0.0008		
6	Patch darkness	0.0096	0.0064	0.1363		

Table S2. Thermal plasticity and survival in *C.splendens*.

Mixed models showing how morphology and thermal physiology influence survival in *C. splendens* males. All models use data from all years (2013-2016; N = 2133), while incorporating year as a random factor. Patch density had a large positive effect on survival in males (model 3), implying that it might be an honest indicator of condition. Thermal plasticity had an overall negative effect on survival (model 1), even after controlling for the effect of body size (wing length and mass) (models 6, 7, 8).

Model	Term	Est.	SE	P-value	AIC	Rank
1	thermal plasticity	-0.0634	0.0469	0.1769	9361.736	2
2	wing length	0.0232	0.0235	0.3243	9363.969	4
3	patch darkness	0.0832	0.0469	0.0762	9360.415	1
4	mass	-0.0148	0.0469	0.7527	9363.460	3
5	wing length	0.0223	0.0469	0.3421	9367.174	5
5	patch darkness	0.0823	0.0469	0.0796		
6	wing length	0.0210	0.0241	0.3728	9371.644	6
6	patch darkness	0.0835	0.0469	0.0751		
6	thermal plasticity	-0.0632	0.0469	0.1786		
7	wing length	0.0251	0.0241	0.2977	9377.193	7
7	patch darkness	0.0876	0.0472	0.0635		
7	thermal plasticity	-0.0660	0.0471	0.1613		
7	mass	-0.0396	0.0483	0.4124		
8	wing length	0.0912	0.5716	0.8732	9403.519	8
8	patch darkness	0.0827	0.0622	0.1839		
8	thermal plasticity	-0.0487	0.0601	0.4175		
8	mass	-0.0646	0.0528	0.2213		
8	wing length ²	-0.0021	0.0194	0.9122		
8	patch darkness ²	-0.0146	0.0485	0.7626		
8	therm. plasticity ²	-0.0127	0.0301	0.6738		
8	mass ²	0.0451	0.0342	0.1873		

Table S3. Mixed models of how morphology and thermal physiology influence survival (longevity) in *C. virgo* males.

Mixed models of how morphology and thermal physiology influence survival (longevity) in *C. virgo* males. All models use data from all years (2013-2016; N = 427), while incorporating year as a random factor. Body mass had a large positive effect on survival in males (model 3). Thermal plasticity had an overall positive effect on survival (model 1), even after controlling for the effect of body size (forewing length and body mass) (models 4, 5, 6).

Model	Term	Estimate	SE	P-value	AIC	Rank
1	thermal plasticity	0.0881	0.0832	0.2906	1683.223	2
2	wing length	0.0300	0.0391	0.4438	1685.268	3
3	mass	0.1307	0.0831	0.1165	1681.875	1
4	wing length	0.0307	0.0391	0.4328	1689.254	4
4	thermal plasticity	0.0892	0.0833	0.2847		
5	wing length	0.0138	0.0409	0.7357	1692.364	5
5	thermal plasticity	0.0874	0.0832	0.2941		
5	mass	0.1211	0.0871	0.1651		
6	wing length	-0.0533	0.3870	0.8904	1708.690	6
6	thermal plasticity	0.0029	0.1000	0.9768		
6	Mass	0.1120	0.0885	0.2067		
6	wing length ²	0.0022	0.0127	0.8614		
6	thermal plasticity ²	0.1067	0.0719	0.1387		
6	mass ²	-0.0661	0.1042	0.5263		