Selection on phenotypic plasticity favors thermal canalization

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Abstract

Climate change affects organisms worldwide with profound ecological and evolutionary consequences, often increasing population extinction risk. Climatic factors can increase the strength, variability or direction of natural selection on phenotypic traits, potentially driving adaptive evolution. Phenotypic plasticity in relation to temperature can allow organisms to maintain fitness in response to increasing temperatures, thereby "buying time" for subsequent genetic adaptation and promoting evolutionary rescue. Although many studies have shown that organisms respond plastically to increasing temperatures, it is unclear if such thermal plasticity is adaptive. Moreover, we know little about how natural and sexual selection operate on thermal reaction norms reflecting such plasticity. Here, we investigate how natural and sexual selection shape phenotypic plasticity in two congeneric and phenotypically similar sympatric insect species. We show that the thermal optima for longevity and mating success differ, suggesting temperature-dependent trade-offs between survival and reproduction. Males in these species have similar thermal reaction norm slopes but have diverged in baseline body temperature (intercepts), being higher for the more northern species. Natural selection favoured reduced thermal reaction norm slopes at high ambient temperatures, suggesting that the current level of thermal plasticity is maladaptive in the context of anthropogenic climate change and that selection now promotes thermal canalization and robustness. Our results show that ectothermic animals also at high latitudes can suffer from overheating and challenge the common view of phenotypic plasticity as being beneficial in harsh and novel environments.

Significance Statement

Organisms are increasingly challenged by increasing temperatures due to climate change. In insects, body temperatures are strongly affected by ambient temperatures, and insects are therefore expected to suffer increasingly from heat stress, potentially reducing survival and reproductive success leading to elevated extinction risks. We investigated how ambient temperature affected fitness in two insect species in the temperate zone. Male and female survivorship benefitted more from low temperatures than did reproductive success, which increased with higher temperatures, revealing a thermal conflict between fitness components. Male body temperature plasticity reduced survival, and natural and sexual selection operated on such thermal plasticity. Our results reveal the negative consequences of thermal plasticity and show that these insects have limited ability to buffer heat stress.

Introduction

Ongoing climate change due to anthropogenic activities affects organisms across the globe, often causing local extinctions(1–3) and changing selection pressures on phenotypic traits(4, 5). Ectothermic animals such as reptiles, amphibians and arthropods are especially sensitive to climate change, as their body temperatures are strongly influenced by ambient temperatures, with strong effects on life-history traits such as growth, survival and reproduction(6). Sensitivity to ambient temperatures is thought to be especially high in low-altitude tropical ectotherms living in shaded habitats such as forests, as these organisms are adapted to rather stable and narrow thermal zones(7–9). When critical thermal limits are exceeded in such organisms(10), extinction risk is thought to increase dramatically(2, 11). Recent studies have shown that upper critical thermal limits are strongly conserved phylogenetically(12) but also that ectothermic animals at high latitudes can experience overheating(10).

Increasing local, regional and global temperatures could therefore elevate population extinction risk, but an alternative outcome is that natural or sexual selection(5, 13) instead promote thermal adaptation(6). This could increase the ability to withstand high temperatures, resulting in evolutionary rescue(14). Evolutionary rescue through genetic adaptation would take time, due to a delay between the onset of selection and genetic evolutionary responses. However, phenotypic plasticity (environment-dependent expression of alternative phenotypes by the same genotype) and thermal plasticity, in particular, could “buy time” for organisms before establishment of evolutionary adaptation by genetic means(15–18). Recent research on endothermic organisms like birds have revealed between-generation plastic changes in life-history traits in response to increasing temperatures, through earlier breeding phenologies(19, 20). However, the adaptive significance of such thermal plasticity is largely unknown and in some cases plasticity might be non-adaptive or even maladaptive(21–24). A recent review on

96 the limited evidence for selection on thermal plasticity found only eight examples of selection
97 studies on thermal plasticity, from four species of plants and two species of birds (in the latter
98 case no direct measurements of temperature were used but indirect proxies such as year or
99 climate index)(25). Notably, recent meta-analyses have not documented a single selection study
100 on thermal plasticity in insects, the most species rich animal class and a group to be strongly
101 affected by increasing temperatures(20, 25). A recent experimental evolution study in a
102 laboratory setting suggested that sexual selection could facilitate evolutionary rescue in
103 response to increasing temperatures(26). However, there is not yet any field study to our
104 knowledge, on how natural and sexual selection operate on thermal plasticity and how such
105 selection might promote thermal adaptation.

106

107 Here, we quantified fitness effects of temperature and natural and sexual selection on thermal
108 plasticity in natural populations of two sexually dimorphic insect species: the banded
109 demoiselle (*Calopteryx splendens*) and the beautiful demoiselle (*C. virgo*). These two closely
110 related species belong to the odonate superfamily Calopterygoidea, which has a tropical origin
111 in southeast Asia(27, 28). Wing pigmentation in these and other damselflies and dragonflies
112 have previously been shown to be important in intra- and intersexual selection as well as in
113 speciation and species recognition and does also play a role in thermal adaptation(13, 27, 29).
114 The tropical origin of the genus *Calopteryx* would presumably make these species well adapted
115 to high temperatures and sensitive to low temperatures. Yet, these two species are the only
116 members of this clade that have successfully expanded into northern Europe and Scandinavia,
117 where this study took place. Results in this study are also of general for those who wish to
118 understand sex differences in the fitness effects of ambient temperatures and how sexual
119 selection on males might increase thermal adaptation and contribute to evolutionary rescue (14,
120 30–32).

121

122 ## Results

123 Both males and females in these temperature-sensitive insects are strongly affected by ambient
124 temperatures under field conditions, in terms of both survivorship and mating success (Fig. 1A-
125 H; Tables S1-S2). Longevity showed a curvilinear relationship with temperature, and typically
126 peaked between 15 and 20 °C for males and females in both *C. virgo* and *C. splendens* (Figs.
127 1A, B, E, F,Table S1-S2). Mating success also showed significant curvilinear relationship with
128 temperature for all phenotypes, except *C. virgo* females (Fig. 1C, D, G, H; Tables S1-S2). In
129 contrast to longevity where the thermal optima were found between 15 and 20 °C, the fitness
130 peaks for mating success were located between 20 and 25 °C for *C. virgo* males (Fig. 1C) and
131 between 25 and 30 °C for the other three phenotypes (Fig. 1D,G,H). Thus, the thermal optima
132 differed for longevity and mating success, with mating success benefitting from higher
133 temperatures whereas survivorship being negatively affected (Fig. 1A-H).

134

135 To investigate how natural and sexual selection operate on thermal plasticity and thermal
136 reaction (6) in males, we used thermal imaging, a novel non-invasive tool to quantify body
137 surface temperatures in insects and other animals(27). Thermal imaging estimates surface
138 temperatures of animals(33). For small organisms like insects, with their low thermal inertia,
139 surface temperatures are highly correlated with internal body temperatures(27) (Fig. S1). We
140 estimated variation between individual males of both species in heating rates of their thorax
141 (containing the wing muscles, which are essential for flight) following a cold challenge (Fig.
142 2A). This experimental setup simulates the effects of a cold night, after which the males are
143 gaining heat in the early morning hours before they start searching for females and food under
144 natural conditions in the field. We estimated thermal plasticity as the slope of the thermal

145 reaction norms (°C/second) and intercept (baseline temperature in °C) at the start of the
146 experiment, controlling for the ambient temperature. Although, these two aspects of reaction
147 norms (slope and intercepts) are the two classical parameters in theoretical models for the
148 evolution of plasticity and canalization(6, 15, 16), selection on such parameters have seldom
149 been quantified(25).

150

151 We found that the slopes of the thermal reaction norms between these two closely related
152 species were similar and almost identical (Fig. S2). Thus, thermal plasticity (slopes) are
153 conserved between these two phylogenetically closely related congeneric species (Fig. 2B;
154 Table S3). In contrast, the reaction norm intercept (baseline temperature) was significantly
155 higher in *C. virgo* than in *C. splendens* (Fig. 2C; Table S3). Thus, the more melanised species
156 *C. virgo* seemed to be more cold-tolerant and better able to keep a high body temperature at
157 low ambient temperatures (Fig. 2C).

158

159 Next, we estimated natural and sexual selection on thermal reaction norm slopes and intercepts
160 in males of these two species by combining data on individual thermal reaction norms with
161 field data on male longevity and mating success. We found evidence for significant natural and
162 sexual selection on thermal reaction norm slopes and intercepts in both species (Fig. 3; Table
163 S4). In *C. splendens,* natural and sexual selection on reaction norm slopes and intercepts had
164 opposite signs consistent with a trade-off, with longevity decreasing with increasing slopes and
165 intercepts, whereas mating success increased (Fig. 3A). In *C. virgo*, this selective trade-off was
166 less evident and natural and sexual selection were more concordant (Fig. 3B, Table S4). Natural
167 selection operated on the thermal slopes and intercepts in both these species, but in different
168 ways, revealing species-specific selection on thermal reaction norms (Fig. 3A-B; Table S4). In

contrast, sexual selection favored higher reaction norm intercepts in both species but did not operate on reaction norm slopes (Fig. 3; Table S4).

Natural selection on thermal reaction norm slopes and intercepts were also strongly temperature-dependent in both species (Fig. 4A-F; Table S5). Specifically, all four interaction terms between ambient temperature and the thermal slopes and thermal intercepts were significant and negative in both species (Fig. 4A-D; Table S5). Thus, lower slopes (lower thermal plasticity) and lower intercept (lower baseline body temperature) were beneficial to survival at high ambient temperatures (Fig. 4A-D; Table S5). In contrast, at low and intermediate temperatures, higher slopes and higher intercepts were associated with high survival (Figs. 4A-D; Table S5). Hence, thermal plasticity was maladaptive when ambient temperature was high, and selection then instead favoured thermal canalization and lower body temperatures, presumably to avoid overheating (Figs. 4A-D; Table S5). Finally, mating success increased with higher thermal intercept in both species (Fig. 3; Table S4), and in *C. virgo* it increased with both temperature and intercept (Fig. 4E).

Discussion

The novel results presented here is one of few empirical examples where the fitness effects of ambient temperature on both males and females and natural and sexual selection on phenotypic plasticity have been quantified in natural populations. We found that the thermal optima for survival and mating success differed, with survivorship generally being highest at lower ambient temperatures, and with mating success increasing with ambient temperature, suggestive of a trade-off between survival and reproduction (Fig. 1; Tables S1-S2). Moreover, the different thermal optima between survival and reproduction were largely concordant

193 between the sexes and across the species. This suggests that this thermally mediated trade-off
194 between survival and reproduction is general, shared between the sexes and accordingly we
195 found no evidence of sexual antagonism (Fig. 1; Tables S1-S2). These results are broadly in
196 agreement with previous studies on other insects, including both other species of damselflies
197 and butterflies, which generally have found that longevity is highest at lower temperature
198 whereas reproductive output increases with higher temperatures (34–39). For ectothermic
199 animals in general, population growth rate seem to peak between 25 and 30 °C (7), which
200 largely coincides with the temperature peak in mating activity for *Calopteryx* (Fig. 1).

201

202 Whereas the relationship between mating success and fitness is straightforward in males, the
203 situation in females could be more complex if there are also fitness costs of mating and
204 preceding male mating harassment (40). However, in *Calopteryx* damselflies, female mating
205 success is likely to be strongly correlated with female fecundity and total reproductive output,
206 at least in the temperate zone where oviposition is often constrained by the number of warm
207 and sunny days (37, 38) and short windows of reproductive opportunities in between many
208 rainy and cold days. In the damselfly *Coenagrion puella*, for instance, life-time female egg
209 production increases with 12 eggs for each 1 °C in ambient temperature (38). For these reasons,
210 male and female reproductive fitness increase in a similar fashion with temperature (Fig. 1C-
211 D, G-H), and temperature therefore seems to have concordant effects on the fitness of both
212 sexes. This implies that thermally mediated selection on one sex (e. g. males) could positively
213 influence fitness also for the other sex (e. g. females), consistent with recent research on desert-
214 dwelling *Drosophila* (30). Hence, sexually concordant selection could facilitate climatic
215 adaptation in both males and females. Concordance between male and female fitness in
216 thermally harsh environments can have some important implications for evolutionary rescue by
217 natural and sexual selection, respectively (26, 41, 42). There is increasing interest to link

research fields such as thermal adaptation, sex differences, sexual selection and sexual antagonism to achieve a bitter understanding of how these factors interact over various spatial and biogeographic scales and shape climatic adaptation of organisms (30, 43–47).

Our results challenge a common view that phenotypic plasticity is often adaptive, enabling organisms to survive in novel and harsh environments. We found that natural selection on thermal slopes and intercepts became stronger at higher ambient temperatures, when thermal canalization and robustness was instead favored (Fig. 4A-D, Table S5). This suggests that thermal plasticity would become increasingly maladaptive as ambient temperatures increase during the summer months. This should cause some concern about the future persistence of these thermally sensitive species in the face of ongoing climate change. The mechanisms behind such selection against thermal slopes and intercepts could be the risks of overheating, which can lead to several negative physiological consequences and fitness costs, caused by elevated metabolic rates (48) or increased water loss (4, 12, 49).

We underscore that male fertility might be even more sensitive to high temperatures (50) than male mating success and survival, and the female fitness consequences of high temperatures are likely to be similar to the effects on males (Fig. 1). Potentially, natural selection on males could therefore also have a positive effect also on female fitness and contribute to evolutionary rescue, given the lack of any evidence for sexual antagonism in this study (Fig. 1). Therefore, our results might be conservative regarding the total fitness costs of overheating, considering the role of other male fitness component as well as female fitness.. Our data therefore strongly suggest that natural selection favours thermal canalization and increased environmental robustness at high temperatures, and that current level of thermal plasticity is maladaptive. Our

242 conclusions are broadly in agreement with some other recent studies which have found evidence
243 for non-adaptive or even maladaptive plasticity(21–24). The population consequences of
244 natural and sexual selection on males deserves future consideration in studies of evolutionary
245 rescue (14, 42), especially as selection on males has been considered to be of minor importance
246 in the evolution local adaptations, given female demographic dominance (31) and the common
247 view that it is only females who contribute to population growth.

248

249 Results in this study call for greater appreciation for how natural and sexual selection can favour
250 thermal canalization and question the efficiency of thermal plasticity as an evolutionary rescue
251 mechanism. The adaptive significance of phenotypic plasticity should therefore not be taken
252 for granted and plastic traits should ideally be subject to as rigorous selection analyses as to
253 those which have been carried out for non-plastic (fixed) phenotypic traits(4, 5). Finally, our
254 results should lead to some concern, as the prospects of evolutionary rescue through selection
255 on phenotypic plasticity(15, 16, 18, 20) followed by genetic assimilation(15) seems limited for
256 these temperature-sensitive insects. Selection in favour of thermal canalization, rather than
257 plasticity, could potentially counteract or delay extinction risk, although this will also depend
258 on the rate of environmental change and the amount of genetic variation in plasticity, among
259 other factors(16, 19, 20). On a more optimistic note, the positive effect of increasing
260 temperatures on reproductive success in these insects (Figs. 1C-D, G-H) might partly
261 compensate for the negative temperature effects on longevity (Figs. 1A-B, E-F), leading
262 towards a thermally-mediated life-history shift with shorter life spans but with elevated
263 reproduction (51).

264

265

266

Methods

Field site and general field work procedures

Our field studies were performed at Sövdemölla Gård along the river Klingavälsån in the province of Skåne in southern Sweden (Latitude: 55.601492 N, Longitude: 13.657340 E). We have carried out field studies at this site and along this river since the year 2000. General field work procedures and basic natural history of the study organisms and ecology of this and other study populations are described in detail elsewhere(52–59). The field studies in the present study were carried out during the summers (June and July) of four years (2013 - 2016). In each year, we recorded individual fitness data of males and females (survival and mating success) and obtained thermal performance measures (estimated individual reaction norms) in males of two sympatric damselfly species (*Calopteryx splendens* and *C. virgo*). Fieldwork took place between 10.00 and 15.00 when activity levels were the highest. Damselflies are ectothermic animals which have low activity levels when temperatures are below 15° C and during rainy and windy days (i.e. they do not fly or mate under such conditions)(60). Therefore, we mainly captured, processed, and re-captured individuals on relatively warm and calm days when they were active and visible, and when it was possible to carry out field work.

Recording longevity and mating success in the field

In conjunction with our recordings of phenotypic and thermal plasticity data, all captured 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 to 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, although we could of course not capture all observed

293 pairs. Nevertheless, we recorded all observed copulations in the field (both those that were
294 captured and those that were not) to quantify mating activity (% individuals found mating out
295 of all individuals) during all field days.
296
297 **Measuring thermal microenvironmental variation**
298 We placed 30 iButton thermologgers (1-Wire; DS1921G-F5) at different locations within our
299 field site to measure thermal microenvironmental variation. Such temperature loggers are able
300 to record temperature at set intervals for several months. We recorded ambient temperature
301 every hour for the duration of our field study each year. The field site was divided into 10
302 neighborhoods (sections; three iButtons per neighborhood) along our study site. Neighborhoods
303 were marked with coloured tape, so that when individuals were recaptured, they could always
304 be linked to a particular neighborhood. When ambient temperature is incorporated in our
305 statistical models on male and female longevities, we used temperature data from these
306 temperature loggers, averaged between 10.00 and 15.00 (the main activity period of these
307 damselflies) of all days these individuals were alive. The time period chosen (10.00-15.00)
308 reflects the warmest period during the day, when either too hot or too cold temperatures are
309 influencing the reproductive activities of these damselflies and therefore also when short-term
310 thermal plasticity is therefore presumably of critical importance.
311
312 **Quantifying effects of ambient temperatures on longevity and mating success**
313 To quantify the general effect of temperature on male and female longevity in both species, we
314 estimated the relationship between individual longevity (minimum life-span; last day of capture
315 minus first day of capture(52) and the average daytime temperatures experienced by each
316 individual male or female during their lifespans. We also used average daytime temperatures
317 from the day of capture and in models where we estimated how the probability of being found
318 in copula (our measure of mating success: 0 = not copulating; 1 = copulating) was affected by

319 average daytime temperature on the day of capture. We used general linear mixed models
320 (negative binomial for longevity and binomial for mating success) with year as random factor
321 and the main effect of temperature and squared temperatures were incuded as fixed predictor
322 variables, using the four different datasets of the two species (*C. virgo* males and females and
323 *C. splendens* males and females).

324

325 **Thermal imaging and quantifying individual variation in thermal reaction norms**

326 A series of thermal images using a thermal image camera (NEC Avio Infrared Technologies
327 H2640)**(27)** were taken of each field-caught individual male. The accuracy of temperature
328 measurements from this thermal image camera is 0.1 °C. From such thermal images, one can
329 estimate body surface temperatures, which is usually highly correlated with internal body
330 temperatures, especially in small ectothermic animals like insects**(33, 61)**(Fig. S1). We
331 quantified individual body temperatures using our previously developed procedures which are
332 described in detail elsewhere**(27)**. The thermal image camera was regularly calibrated during
333 the use in the field against a black body (black plastic lens cover), with emissivity set to 1.0. To
334 quantify thermal reaction norms (heating rates), individual males were first cooled for three
335 minutes in a cooler box at 5° C and thermal equilibrium. They were then placed back into
336 ambient temperature, each within an individual cup, under a thermal imaging camera. Thermal
337 images were then recorded every ten seconds for three minutes (i.e. up to 18 frames per
338 individual, except for those that flew away within 3 minutes). This experiment aimed to
339 simulate how these ectothermic insects heat up with increasing ambient temperature, e.g. after
340 a cold spell or after a night with low temperatures in the early morning hours. Batches of six
341 individuals were cooled and images were taken simultaneously as they warmed up. After the
342 thermal images were taken, individual males were allowed to fly off to the field site and their
343 subsequent survivorship was recorded through recaptures and later used in the selection

344 analyses (see further below). Images were analysed to estimate the body temperature of each
345 individual at each time point, controlling for ambient temperature. We defined thermal
346 plasticity as the slope of the heating rate (° C/second) of individual males after being cooled
347 down in the fridge box. The intercepts (slope elevation) of these reaction norms is the baseline
348 temperature these males had immediately after being released from the cool box, before starting
349 to heat up (Table S3).
350
351 **Selection on thermal plasticity in relation to ambient temperatures**
352 We estimated linear selection gradients from models incorporating the two thermal traits
353 (slopes and intercepts of the thermal reaction norms described above). We carried out these
354 analyses for both sexual selection (male mating success) and survival selection (longevity) and
355 subsequently related this to natural variation in ambient temperature. We analyzed the data
356 using general and generalized linear mixed effect models with year as random factor. Univariate
357 linear selection differentials were estimated separately for the slopes and intercepts of thermal
358 reaction norms following the general statistical methodology of Lande and Arnold(62). The
359 two thermal traits (slopes and intercepts of individual thermal reaction norms) were
360 standardized to mean zero and unit variance and our two fitness components (minimum lifespan
361 or male mating success) were divided by the population average (mean fitness) before being
362 used as the response variables in these regression analyses of selection(62). Since significance
363 levels of regressions with Poisson or binomially distributed data can be unreliable, we also
364 carried out complementary generalized mixed effect models using non-relativized fitness
365 components. We used binomial distributions for mating success and Poisson distribution for
366 longevity. We then present the significance levels from these regressions. Apart from
367 estimating selection gradients on thermal slopes and thermal intercepts, we also visualized the

368 fitness surfaces of how these two thermal traits and ambient temperature influenced survival
369 and mating success, using standard procedures(63).
370
371 In the sexual selection analyses, we used a binary response variable as our measure of male
372 mating success (males that obtained at least one mating = 1; non-mated males = 0). From daily
373 field counts and captures at our study site, we found 902 males mating, out of a total of 5902
374 observed (902/5902 = 0.152). Based on these numbers, we conservatively assume that 15% of
375 males in this population successfully mated at least once. This estimate is likely to be somewhat
376 conservative, as we certainly were not able to record all matings in the field, e. g. those that
377 took place when we did not scan the stream. We used our conservative measure of average male
378 mating fitness (i.e. 0.15) in our regression analyses of selection. This low mating success
379 estimate of 15% males obtaining at least one copulation at this site is consistent with similar
380 low estimates from our previous independent selection studies at two other sites
381 ("Klingavälsåns Naturreservat" and "Höje Å, Värpinge"), where we collected similar data from
382 2001-2003(53, 54). Low mean mating success reflects the reproductive biology of *Calopteryx*
383 damselflies, which are characterized by high mating skew and strong sexual selection. We
384 continued to monitor the population until all of our marked individuals had died. The average
385 lifespan of an adult *C. splendens* males in the field is around 3-4 days, although some males
386 can live for more than two weeks (52, 54).
387
388 **Statistical procedures**
389 All statistical analyses were carried out using standard functions in the statistical packages lme4
390 (64), MASS (65) and mcgv (66) in the R statistical environment (67). Code and datasets will
391 be made available after publication at Dryad Digital Repository **(https://datadryad.org/).** We
392 carried out general and generalized linear mixed models with different error structures, which

are specified under each analysis. We include thermal plasticity traits (slopes and intercepts), ambient temperature and its squared component as fixed predictor variables (to account for curvilinear relationships; see Fig. 1) and year as random effect. We visualized the fitness surfaces (Fig. 4) using splines obtained from Generalized Additive Models (GAM:s)(63).

Supporting Information

Table S1. Effects of ambient temperatures on fitness components in male and female *Calopteryx* damselflies.

Table S2. Comparisons (within sexes and between species) of the effects of ambient temperatures on fitness components in male and female *Calopteryx* damselflies.

Table S3. Species differences in thermal slopes and intercepts of male *Calopteryx.*

Table S4. Natural and sexual selection on thermal reaction norm slopes and intercepts in *Calopteryx* males across all seasons and temperatures.

Table S5. Natural and sexual selection on thermal reaction norm slopes and intercepts in *Calopteryx* males in relation to ambient temperature.

Fig. S1. Fig. S1. External and internal body temperature recordings of individual *Calopteryx* males.

Fig. S2. Individual and species variation in thermal reaction norms of male *Calopteryx* damselflies.

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Author Contributions

E.I.S. conceived the idea of this study, obtained funding and planned the field experiments in collaboration with J.W. and M.G.L. J.W., M.G.L. and E.I.S. carried out the field studies and experiments under the guidance from E.I.S. J.W. and M.G.L. performed the statistical analyses with input from E.I.S. E.I.S. wrote the paper. All authors contributed to the finalization of the manuscript.

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609 ## Legends to figures

610

611 **Fig. 1. Effects of ambient temperatures on fitness components in male and female**
612 ***Calopteryx* damselflies.** We quantified how survivorship (longevity) and mating success was
613 affected by ambient temperature in a natural field population (Klingavälsån at Sövdemölla in
614 Southern Sweden) along a slowly running stream. Inserted are pictures of the four phenotypes:
615 males and females of *Calopteryx virgo* (left column) and males and females of *C. splendens*
616 (right column). **A:** Longevity in *C. virgo* males. **B:** Longevity in *C. splendens* males. **C:** Mating
617 success in *C. virgo* males. **D:** Mating success in *C. splendens* males. **E:** Longevity in *C. virgo*
618 females. **F:** Longevity in *C. splendens* females. **G:** Mating success in *C. virgo* females. **H:**
619 Mating success in *C. splendens* females. See Tables S1 and S2 for statistical tests.

620

621 **Fig. 2. Using thermal imaging (infrared camera) to quantify species-specific thermal**
622 **reaction norm slopes and intercept in male *Calopteryx* damselflies. A:** We recorded
623 individual variation in heating rates using thermal image camera of male *Calopteryx*
624 damselflies in the field, to quantify variation in thermal reaction norms (slopes and intercepts).
625 Shown is the same individual male that is heating up under ambient temperature, after being
626 cooled down in a fridge box (c. a. 5 °C; Upper left shows first picture). Warmer colours (red,
627 orange) show higher surface body temperature. We recorded a thermal image every 10th second
628 for three minutes until he flew off, which usually happened before the three minutes (180
629 seconds) were over. **B-C:** individual and species variation in thermal reaction norms of *C.*
630 *splendens* (left; n = 778) and *C. virgo* (n = 179; see also Fig. S2). These two species do not
631 differ in thermal reaction norm slopes (**B**), but they differ significantly in thermal reaction norm
632 intercepts (**C**). Statistical tests refer to t-tests, using each individual-level reaction norm slopes

633 and intercepts as individual data points (see Fig. S2 for range of variation). The higher intercept
634 of *C. virgo* means that its baseline body temperature is higher at low ambient temperatures.
635 This might explain its more northernly distributional range as it could become active at lower
636 temperatures. See Table S3 for statistical tests.

637

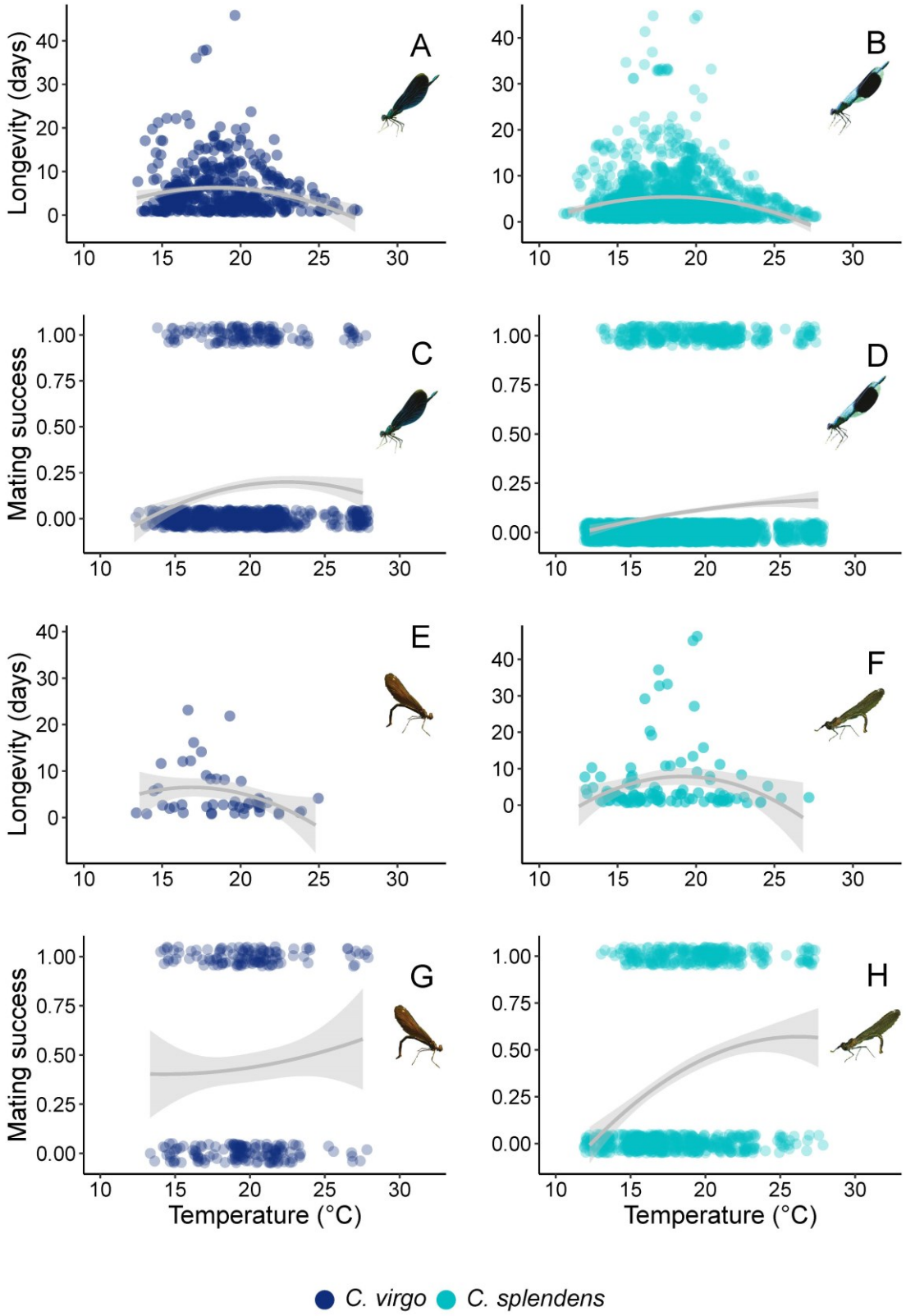
638 **Fig. 3. Natural and sexual selection on reaction norm slopes and intercepts in *Calopteryx***
639 **males across all seasons and ambient temperatures.** Field estimates (standardized selection
640 gradients) of natural selection (longevity) and sexual selection (mating success) on reaction
641 norm slopes and intercepts across all years and temperature ranges for *C. splendens* males
642 (longevity, n = 778; mating success, n = 2160) (**A**) and *C. virgo* males (longevity, n = 179;
643 mating success, n = 413) (**B**). Shown is the standard error around the mean. See Table S4 for
644 statistics.

645

646 **Fig. 4**. **Natural and sexual selection on thermal reaction norm slopes and intercepts in**
647 ***Calopteryx* males in relation to temperature.** Effects of thermal reaction norm parameters
648 (slopes and intercepts; see Fig. 2 and Fig S2) and ambient temperatures on male longevity (**A,C:**
649 *C. virgo,* n = 179; **B, D:** *C. splendens,* n = 778) and for male mating success (**E:** *C. virgo*, n =
650 413; **F:** *C. splendens*, n=2160). Fitness surfaces (splines) show the results from multivariate
651 generalized additive models (GAM's)(Table S5). In all four longevity subplots (**A-D**), the
652 interactions between ambient temperature and thermal reaction norm slope and intercept are
653 significant and negative (Table S5). This means that at higher temperatures, both lower
654 plasticity (slope) and lower intercepts are favored, suggesting that thermal plasticity leads to
655 overheating which reduces survival in these two ectothermic insects. In contrast, increasing
656 temperature had a positive effect on mating success in both species (Table S5; cf. Fig. 1C, D).

657

683 **Fig. 1**



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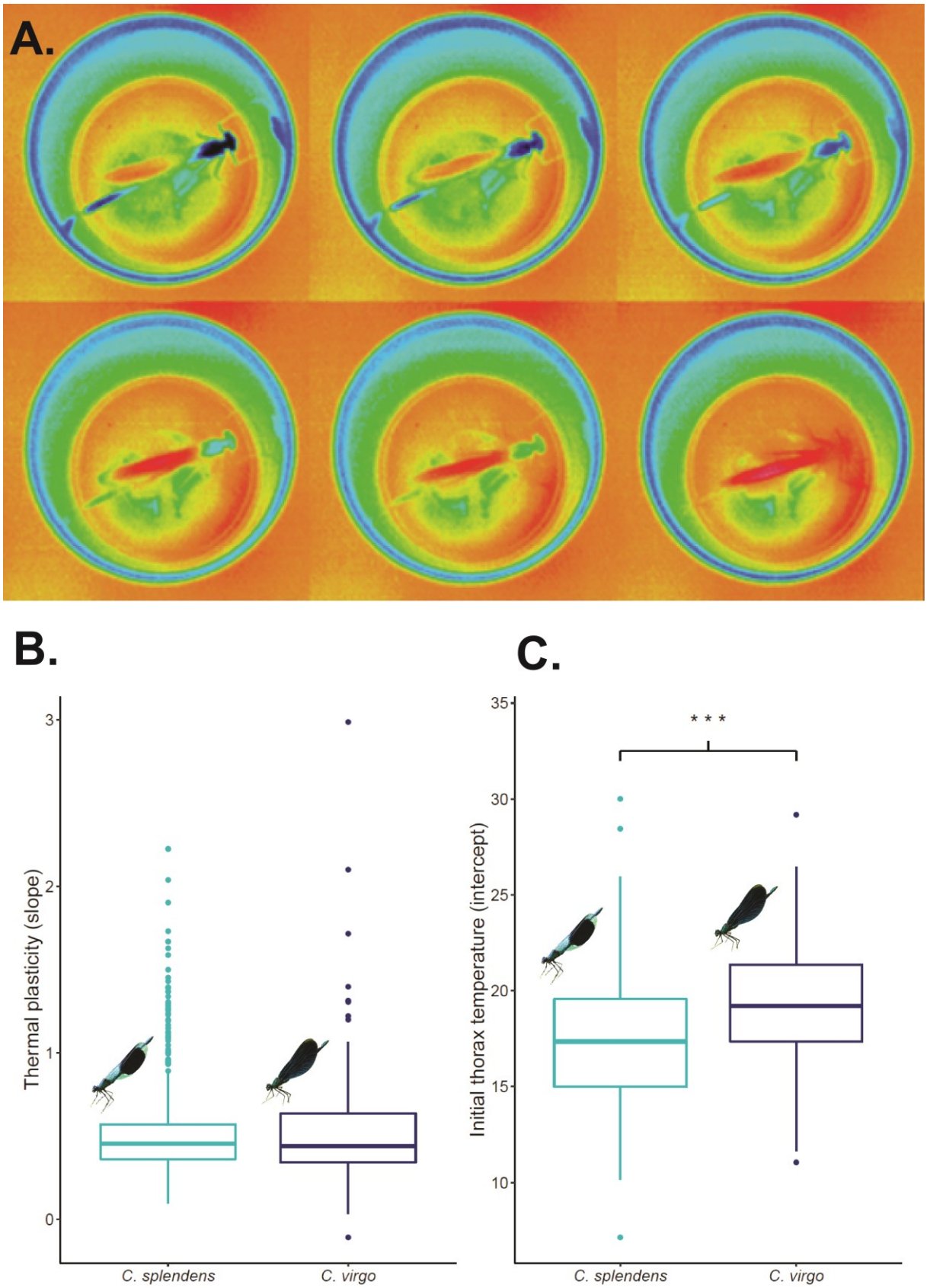
686 **Fig. 2**

687



688
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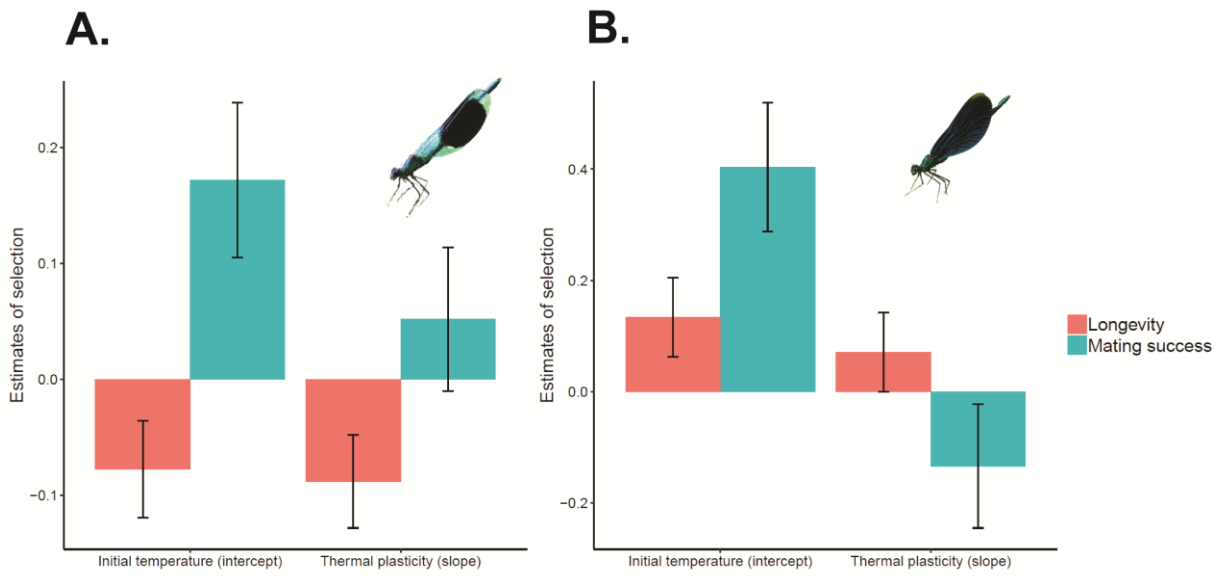
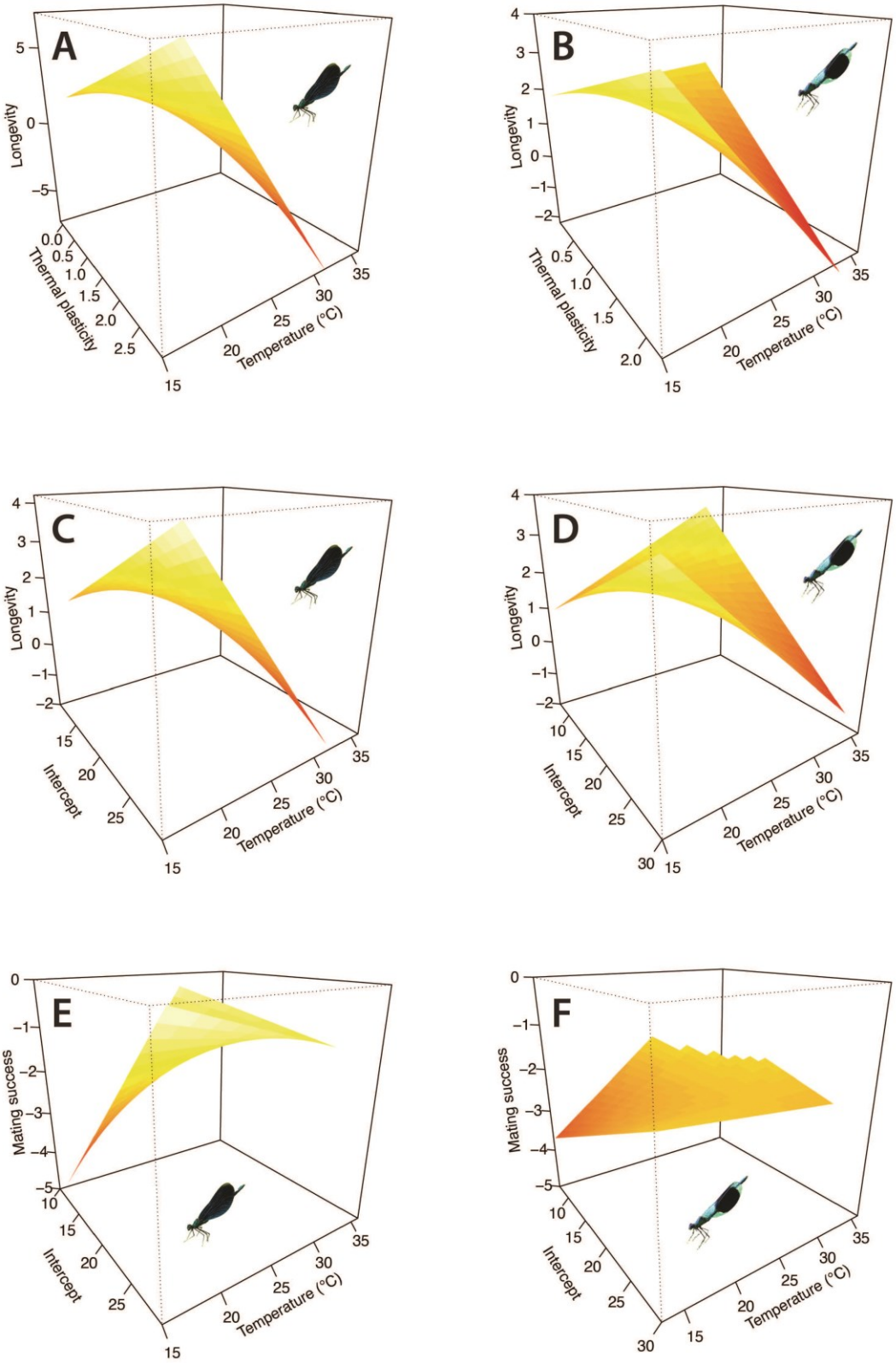
Fig. 3


A.
B.
Estimates of selection
0.2
0.1
0.0
−0.1
0.4
0.2
0.0
−0.2
Initial temperature (intercept)
Thermal plasticity (slope)
Longevity
Mating success

732
733
734 **Fig. 4**
735



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