Natural and sexual selection on phenotypic plasticity favour 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 also 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 by phenotypic plasticity to increasing temperatures, it is unclear if such plasticity is adaptive and how natural and sexual selection operate on such thermal reaction norms. 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 male mating success differ between these species, with temperature-dependent trade-offs between natural and sexual selection. These species have similar thermal reaction norm slopes but have diverged in baseline body temperature (intercepts), being higher for the more northern species. Natural and sexual selection therefore favour reduced plasticity, which is currently maladaptive at high ambient temperatures. 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.

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 particularly thermal plasticity, 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 is non-adaptive or even maladaptive[21,22]. A recent

review on the limited literature on evidence for selection on thermal plasticity found only eight examples of selection studies on thermal plasticity, from four species of plants and two species of birds (in the latter case no direct measurements of temperature were used but indirect proxies such as year or climate index)[23]. Notably, recent meta-analyses have not documented a single selection study on thermal plasticity in insects, the most species rich animal class and one of the groups expected to suffer the most due to increasing temperatures[20,23]. A recent experimental evolution study in a laboratory setting suggested that sexual selection could facilitate evolutionary rescue in response to increasing temperatures[24]. However, there is not yet any study in any natural population, to our knowledge, of how sexual selection operates on thermal plasticity and how sexual and natural selection might promote thermal adaptation in nature.

Here, we quantified natural and sexual selection on thermal plasticity in natural populations of two sexually dimorphic insects: the banded demoiselle (*Calopteryx splendens*) and the beautiful demoiselle (*C. virgo*)(Fig. 1). These two closely related species belong to the odonate superfamily Calopterygoidea, which has a tropical origin in southeast Asia[25,26]. Their tropical origin would presumably make them well adapted to high temperatures and sensitive to low temperatures. Yet, these two species are the only members of this clade that have successfully expanded into northern Europe and Scandinavia, where this study took place.

Results

Males in these temperature-sensitive insects are strongly affected by ambient temperatures in both mesocosm settings (Fig. 1A-B) and under field conditions (Fig. 1C- F), in terms of both survivorship and mating success, although the effects differed between species and fitness

98 components (Table S1). The temperature effects on survivorship were qualitatively similar
99 between the mesocosm settings (Fig. 1A-B) and the field (Fig. 1C-D), which suggest that
100 confounding factors that could covary with temperature (e. g. avian predators[27]) are unlikely
101 to explain these results, as predators were experimentally excluded from the mesocosms.

102

103 The more melanised species (*C. virgo*), which has entirely dark wings, experienced a steeper
104 decline in longevity with increasing ambient temperature (Fig. 1A, C) compared to the less
105 melanised *C. splendens,* which has only 50 % of its wings covered with melanin (Fig. 1B, D;
106 Table S1). Interestingly, *C. virgo* males (Fig. 1E), and to a lesser extent *C. splendens* males
107 (Fig. 1F), had higher probability of mating at higher temperatures (Table S1). Thus, whereas
108 survivorship was higher at low temperatures, mating success instead increased with higher
109 ambient temperatures, consistent with a thermally mediated trade-off between natural and
110 sexual selection.

111

112 To investigate how natural and sexual selection operate on thermal plasticity[6] we used
113 thermal imaging, a novel non-invasive tool to quantify body surface temperatures in insects and
114 other animals[25]. Thermal imaging estimates surface temperatures of animals, and for small
115 organisms like insects these surface temperatures are highly correlated with internal body
116 temperatures[25] (Fig. S1). We estimated variation between individual males of both species
117 in heating rates of their thorax (containing the wing muscles, which are essential for flight)
118 following a cold challenge (Fig. 2A). This experimental setup simulates the effects of a cold
119 night, after which the males are gaining heat in the early morning hours before they start
120 searching for females and food under natural conditions in the field. We estimated thermal
121 plasticity as the slope of the thermal reaction norms (°C/second) and intercept (baseline

122 temperature in °C) at the start of the experiment, controlling for the ambient temperature.
123 Although, these two aspects of reaction norms (slope and intercepts) are the two classical
124 parameters in theoretical models for the evolution of plasticity and canalization[6,15,16],
125 selection on such parameters have seldom been quantified[23].

126

127 We found that the slopes of the thermal reaction norms between these two closely related
128 species were similar and almost identical (Fig. S2). Thus, thermal plasticity is conserved
129 between these two phylogenetically closely related congeneric species (Fig. 2B; Table S2). In
130 contrast, the reaction norm intercept was significantly higher in *C. virgo* than in *C. splendens*
131 (Fig. 2C; Table S2). Thus, the more melanised species *C. virgo* is more cold-tolerant and better
132 able to keep a high body temperature at low ambient temperatures (Fig. 2C).

133

134 Next, we estimated natural and sexual selection on thermal reaction norm slopes and intercepts
135 in males of both species using field data on male longevity and mating success. We found
136 evidence for significant natural and sexual selection on thermal reaction norm slopes and
137 intercepts in both species (Fig. 3). In *C. splendens,* natural and sexual selection on reaction
138 norm slopes and intercepts had opposite signs consistent with a trade-off, with longevity
139 decreasing with increasing slopes and intercepts, whereas mating success increased (Fig. 3A).
140 In contrast, in *C. virgo* this selective trade-off was less pronounced (Fig. 3B, Table S3).

141

142 Both natural and sexual selection on thermal reaction norm slopes and intercepts were strongly
143 temperature-dependent in both species (Fig. 4A-F; Table S4). Specifically, the interaction terms
144 between ambient temperature and both slope and intercepts were all significant and negative,
145 meaning that lower slopes (lower thermal plasticity) and lower intercept (lower baseline body

temperature) were beneficial at high temperatures (Table S4), whereas higher slopes and higher intercepts were beneficial at low or intermediate temperatures (Figs. 4A-D). 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 S4). Notably, in *C. virgo* we found evidence for sexual selection on the intercept, as mating success was maximized at intermediate temperatures and for intermediate levels of the intercept (Fig. 4E).

Discussion

The novel results presented here is one of few examples where natural and sexual selection on phenotypic plasticity have been quantified in natural populations. Decreasing survival with increasing temperature in the more melanised species (*C. virgo*) fits with previous phylogenetic comparative work and field studies in this insect group[13,25,27,28]. The new results presented here suggest negative thermal fitness consequences of melanisation as an ecological cost of sexual selection, as wing pigmentation in these and other damselflies and dragonflies have previously been shown to be important in intra- and intersexual selection as well as in speciation and species recognition [13,25].

We found that in the strongly melanised species *C. virgo*, the thermal optimum for mating success was higher than that for survivorship (cf. Fig. 4D vs. 4E; see also Fig. 1A, C and E). At high ambient temperatures, natural and sexual selection on slopes and intercepts become largely concordant, whereas at lower ambient temperatures, the natural and sexual selection optima became increasingly divergent. Concordance between natural and sexual selection in thermally

169 harsh environments can have some important implications for evolutionary rescue by natural
170 and sexual selection, respectively [24,29,30].

171

172 These results are consistent with previous research showing that the more melanised species,
173 *C. virgo,* has higher cold tolerance and a more northerly geographic range limit in Scandinavia
174 than *C. splendens*[25,31]. The results presented here suggest that this cold tolerance might have
175 come at the cost of being less tolerant to high temperatures (Figs. 1A, C). Therefore, thermally
176 mediated trade-offs between natural and sexual selection therefore seem to differ between these
177 otherwise phenotypically similar species, and these trade-offs might be partially mediated by
178 interspecific differences in the wing melanisation[25,31].

179

180 Our results challenge a common view that phenotypic plasticity is adaptive, enabling organisms
181 to survive in novel and harsh environments (Fig. 4A-D, Table S4). Thermal plasticity would
182 become increasingly maladaptive with increasing ambient temperatures increase during the
183 summer months, which raises some concern about the future persistence of these thermally
184 sensitive species in the face of ongoing climate change. We find evidence that selection
185 favoured reduced thermal plasticity, presumably to counteract the increase in body
186 temperatures with increasing ambient temperatures ((Fig. 4A-D, Table S4). The reason for this
187 selection is presumably the risk of overheating, which is known to have many negative fitness
188 consequences in insects and other organisms, both endo- and ectotherms[6,7]. We underscore
189 that male fertility might be even more sensitive to high temperatures [32] than are mating
190 success and survival. Our results might therefore be conservative regarding the total fitness
191 costs of overheating. These data therefore strongly suggest that selection favours thermal
192 canalization and environmental robustness against high temperatures, and that current level of

thermal plasticity is maladaptive, consistent with other recent studies which have found evidence for non-adaptive or even maladaptive plasticity[21,22].

Results in this study call for greater appreciation for how selection can favour thermal canalization and question the efficiency of thermal plasticity as an efficient evolutionary rescue mechanism. The adaptive significance of phenotypic plasticity should therefore not be taken for granted and plastic traits should ideally be subject to as rigorous selection analyses as to those which have been carried out for non-plastic (fixed) phenotypic traits[4,5]. Finally, our results should lead to some concern, as the prospects of evolutionary rescue through selection on phenotypic plasticity[15,16,18,20] followed by genetic assimilation[15] seems limited for these temperature-sensitive insects. Selection in favour of thermal canalization, rather than plasticity, could potentially counteract or delay extinction risk, although this will also depend on the rate of environmental change and the amount of genetic variation in plasticity, among other factors[16,19,20].

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[27,28,33–38]. The field studies in the present study were carried out during the summers (June and July) of four years (2013 - 2016).

217 In each year, we collected individual fitness data (survival and mating success) and thermal
218 performance measures of two sympatric damselfly species (*Calopteryx splendens* and *C. virgo*).
219 Fieldwork took place between 10.00 and 15.00 when activity levels were the highest.
220 Damselflies are ectothermic animals which have very low activity levels when temperatures
221 are below 15° C and during rainy and windy days (i.e. they do not fly or mate under such
222 conditions)[39]. Therefore, we only captured, processed, and re-sighted individuals on
223 relatively warm and calm days when they were active.

224

225 **Recording longevity and mating success in the field**

226 In conjunction with our recordings of phenotypic and thermal plasticity data, all individuals
227 were marked with a unique code with three colors on the last three segments of the abdomen.
228 These unique markings were used to identify individuals and record their subsequent survival
229 (longevity) in the field. Apart from catching and re-capturing single individuals, we also caught
230 as many copulating pairs in the field as possible so that we could obtain estimates of sexual
231 selection through male mating success. We tried to capture as many mating pairs as possible
232 during days with high mating activity, although we could of course not capture all observed
233 pairs. Nevertheless, we recorded all observed copulations in the field (both those that were
234 captured and those that were not) to quantify mating activity (% male mating) during all field
235 days.

236

237 **Measuring thermal microenvironmental variation**

238 We placed 30 iButton thermologgers (1-Wire; DS1921G-F5) at different locations within our
239 field site to measure thermal microenvironmental variation. Such temperature loggers are able
240 to record temperature at set intervals for several months. We recorded ambient temperature
241 every hour for the duration of our field study each year. The field site was divided into 10

242 neighborhoods (sections; three iButtons per neighborhood) along our study site. Neighborhoods
243 were marked with coloured tape, so that when individuals were recaptured, they could always
244 be linked to a particular neighborhood. When ambient temperature is used in our statistical
245 models, we are using temperature data from these temperature loggers, averaged over days or
246 the life-time of individuals.
247
248 **Quantifying effects of ambient temperatures on longevity and mating rates**
249 To quantify the general effect of temperature on average lifespan of both species, we estimated
250 the relationship between individual longevity variation and the average daytime temperature
251 experienced by each male. The lifespan temperature of each male was calculated by taking the
252 average temperature experienced by each individual in its given microenvironment. We
253 performed this analysis for males only, as the recapture rates for females were very low. We
254 also used the average daily temperature and the number of mating pairs counted each day in the
255 field to quantify the population-level effect of temperature on mating activity. Linear models
256 with quadratic terms were then fit to the separate datasets of *C. splendens* and *C. virgo*.
257
258 **Mesocosm studies on temperature-dependent longevity and mating success**
259 We also carried out mesocosm experiments, where we measured longevity of individual *C.*
260 *splendens* and *C. virgo* males in large outdoor cages (3 x 3 x 3 m^3) at Stensoffa Ecological Field
261 Station, about 20 km east of Lund, Sweden. This work was carried out in parallel with our
262 previous experimental studies of how intra- and interspecific interactions influenced male
263 mating harassment of females and the role of negative frequency-dependence (rare-species
264 advantage), and the general results have been presented and discussed elsewhere [40,41]. These
265 mesocosm studies were performed in the summers of 2014 and 2015, and we also recorded
266 ambient temperatures in the cages using iButtons (see section above) in conjunction with daily

visits to record male survival rates and individual longevities[41]. Here, we present longevity data in relation to ambient temperature, using individual male *C. splendens* and *C. virgo* data from only the allopatric experimental treatments[41], where survivorship cannot (per definition) be influenced by the confounding effects of male-male interference competition and interspecific interactions. These mesocosm longevity data complemented our field estimates of survivorship recorded on free-living individuals at Sövdemölla (see above).

Thermal imaging and quantifying individual variation in thermal reaction norms

A series of thermal images using a thermal image camera (NEC Avio Infrared Technologies H2640)**[25]** were taken of each field-caught individual male. The accuracy of temperature measurements from this thermal image camera is 0.1 °C. From such thermal images, one can estimate body surface temperatures, which is usually highly correlated with internal body temperatures, especially in small ectothermic animals like insects[42,43](Fig. S1). We quantified individual body temperatures using our previously developed procedures which are described in detail elsewhere[25]. The thermal image camera was regularly calibrated during the use in the field against a black body (black plastic lens cover), with emissivity set to 1.0. To quantify thermal reaction norms (heating rates), individual males were first allowed to cool for three minutes in a cooler box at 5° C and thermal equilibrium. They were then placed back into ambient temperature, each within an individual cup, under a thermal imaging camera. Thermal images were then recorded every ten seconds for three 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 spell or after a night with low temperatures in the early morning hours. Batches of six individuals were cooled and images were taken simultaneously as they warmed up. After the thermal images were taken, individuals were allowed to fly off and their subsequent survivorship was recorded and later used in the selection analyses (see further below). Images

were analysed to estimate the body temperature of each individual at each time point, controlling for ambient temperature. We defined thermal plasticity as the slope of the heating rate (° C/second) of individual males after being cooled down in the fridge box. The intercepts (slope elevation) of these reaction norms is the baseline temperature they had immediately after being released from the cool box, before starting to heat up.

Selection on thermal plasticity in relation to ambient temperatures

We estimated linear selection gradients from models incorporating the two thermal traits (slopes and intercepts of the thermal reaction norms described above). We carried out these analyses for both sexual selection (male mating success) and survival selection (longevity) and subsequently related this to natural variation in ambient temperature. We analyzed the data using general and generalized linear mixed effect models. Univariate linear selection differentials were estimated separately for the slopes and intercepts of thermal reaction norms following the general statistical methodology of Lande and Arnold[44], as these two variables are highly correlated and are therefore difficult to estimate simultaneously in a multiple regression framework. The two thermal traits (slopes and intercepts of individual thermal reaction norms) 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[44]. Since significance levels of regressions with Poisson or binomially distributed data can be unreliable, we also carried out a second generalized mixed effect model using non-relativized fitness components. We used binomial distribution for mating success and Poisson distribution for longevity. We then present the significance levels from these regressions. Apart from estimating selection gradients on thermal slopes and thermal intercepts, we also visualized

317 the fitness surfaces of how these two thermal traits and ambient temperature influenced survival
318 and mating success, using standard procedures[45].
319
320 In the sexual selection analyses, we used a binary response variable as our measure of male
321 mating success (males that obtained at least one mating = 1; non-mated males = 0). From daily
322 field counts and captures at our study site, we found 311 males mating, out of a total of 2945
323 observed (311/2945 = 0.105). Based on these numbers, we conservatively assume that 10% of
324 males in this population successfully mated at least once. This estimate is likely to be somewhat
325 conservative, as we certainly were not able to record all matings in the field, e. g. those that
326 took place when we did not scan the stream. We used our conservative measure of average male
327 mating fitness (i.e. 0.10) in our regression analyses of selection. This low mating success
328 estimate of 10% males obtaining at least one copulation at this site is consistent with similar
329 low estimates from our previous independent selection studies at two other sites
330 ("Klingavälsåns Naturreservat" and "Höje Å, Värpinge"), where we collected similar data from
331 2001-2003[28,34]. Low mean mating success reflects the reproductive biology of *Calopteryx*
332 damselflies, which are characterized by high mating skew and strong sexual selection. We
333 continued to monitor the population until all of our marked individuals had died. The average
334 lifespan of an adult *C. splendens* males in the field is around 3-4 days, although some males
335 can live for more than two weeks [28,33].
336
337 **Statistical procedures**
338 All statistical analyses were carried out using statistical packages in R[46], unless otherwise
339 stated. We carried out general and generalized linear models with different error structures,
340 which are specified under each analysis. We visualized the fitness surfaces (Fig. 4) using splines
341 obtained from Generalized Additive Models (GAM:s). In the mesocosm experiments, we

342 incorporated cage number as a random factor to account for statistical non-independence due
343 to several estimates from the same cage.

344

345 Supporting Information

346

347 **Table S1. Effects of ambient temperatures on fitness components in male *Calopteryx***
348 **damselflies.**

349

350 **Table S2. Species differences in thermal slopes and intercepts.**

351

352 **Table S3. Natural and sexual selection on thermal reaction norm slopes and intercepts**
353 **across all seasons and temperatures**.

354

355 **Table S4. Natural and sexual selection on thermal reaction norm slopes and intercepts in**
356 **relation to ambient temperature.**

357

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

359

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

361

362

363

Acknowledgements

We are grateful to the many field assistants, PhD-students and postdocs who have participated in the field work and thereby directly and indirectly contributed to this study over many years since it started in 2013. Funding for this study have been provided by research grants from The Swedish Research Council (VR: grant no. 2016-03356), Carl Tryggers Foundation (CTS), Gyllenstiernska Krapperupstiftelsen (grant no. KR2018-0038), Lunds Djurskyddsfond, The Royal Physiographic Society in Lund, Stiftelsen Olle Engqvist Byggmästare and Stina Werners Foundation. We are grateful to the late Jonathan "Jackie" Brown, Adam Hasik, Andreas Nord and Adam Siepielski for critical feedback and comments on the first draft of this manuscript.

Author Contributions

E.I.S. conceived the idea of this study, obtained funding and planned the field experiments in collaboration with J.W. and the mesocosm experiments in collaboration with M.G.L. J.W. and M.G.L. carried out the field studies and mesocosm experiments under the guidance from E.I.S. and 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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The authors declare no competing financial interests. Correspondence and requests for materials should be directed to E.I.S. (*erik.svensson@biol.lu.se*). Original data behind all the analyses in this paper and associated R-code will be uploaded on Dryad (**https://datadryad.org**/).

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531 ## Legends to figures

532

533 **Fig. 1. Effects of ambient temperatures on fitness components in male *Calopteryx***
534 **damselflies.** We quantified how male survivorship (longevity) and mating success was affected
535 by ambient temperature in both mesocosm cages and in a natural field population (Klingavälsån
536 at Sövdemölla in Southern Sweden) along a stream. **A-B:** In the mesocosms, longevity of both
537 the beautiful demoiselle (*C. virgo*; left column) and the banded demoiselle (*C. splendens*, right
538 column) are low at high temperatures, although in the latter species it also decreases at very
539 low temperatures, peaking at intermediate temperatures. **C-D:** A qualitatively similar pattern
540 was found for longevity in the field. **E-F:** In contrast, mating success in both species increases
541 with ambient temperature in the field, suggestive of a temperature-dependent trade-off between
542 natural and sexual selection. See Table S1 for statistical tests.

543

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

555 tests refer to t-tests, using each individual-level reaction norm slopes and intercepts as
556 individual data points (see Fig. S2 for range of variation). The higher intercept of *C. virgo*
557 means that its baseline body temperature is higher at low ambient temperatures. This might
558 explain its more northernly distributional range as it could become active at lower temperatures,
559 but it might also lead to higher sensitive to overheating than *C. splendens* (cf. fig. 1). See Table
560 S2 for statistical tests.

561

562 **Fig. 3. Natural and sexual selection on reaction norm slopes and intercepts across all**
563 **seasons and ambient temperatures.** Field estimates (standardized selection gradients) of
564 natural selection (longevity) and sexual selection (mating success) on reaction norm slopes and
565 intercepts across all years and temperature ranges for *C. splendens* males (longevity, n = 778;
566 mating success, n = 2160) (**A**) and *C. virgo* males (longevity, n = 179; mating success, n = 413)
567 (**B**). Shown is the standard error around the mean. See Table S3 for statistics.

568

569 **Fig. 4**. **Natural and sexual selection on thermal reaction norm slopes and intercepts in**
570 **relation to temperature.** Effects of thermal reaction norms in relation to ambient temperature
571 for male longevity (*C. splendens,* n = 778*;* **A-B** and *C. virgo,* n = 179*;* **C-D**) and for male mating
572 success (**E:** *C. virgo* only, n = 413). Fitness surfaces (splines) show the results from multivariate
573 generalized additive models (GAM's)(Table S4). In all the five subplots (**A-E**), the interaction
574 between ambient temperature and thermal reaction norm slope or intercept are significant and
575 negative (Table S4), meaning that at higher temperatures, both lower plasticity (slope) and
576 intercepts are favoured, consistent with overheating in these two ectothermic insect species.

577

578

579

Fig. 1

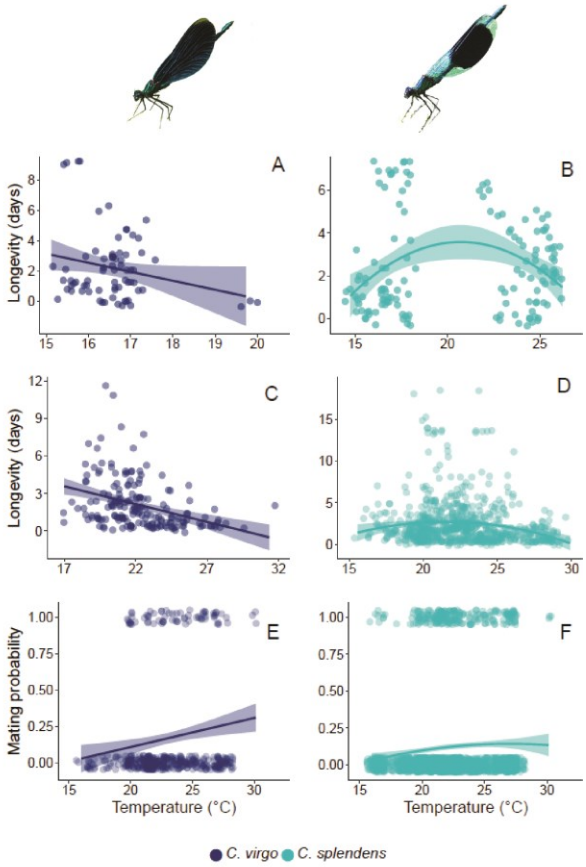

A
B
C
D
E
F
Longevity (days)
Mating probability
Temperature (°C)
C. virgo
C. splendens

Fig. 2

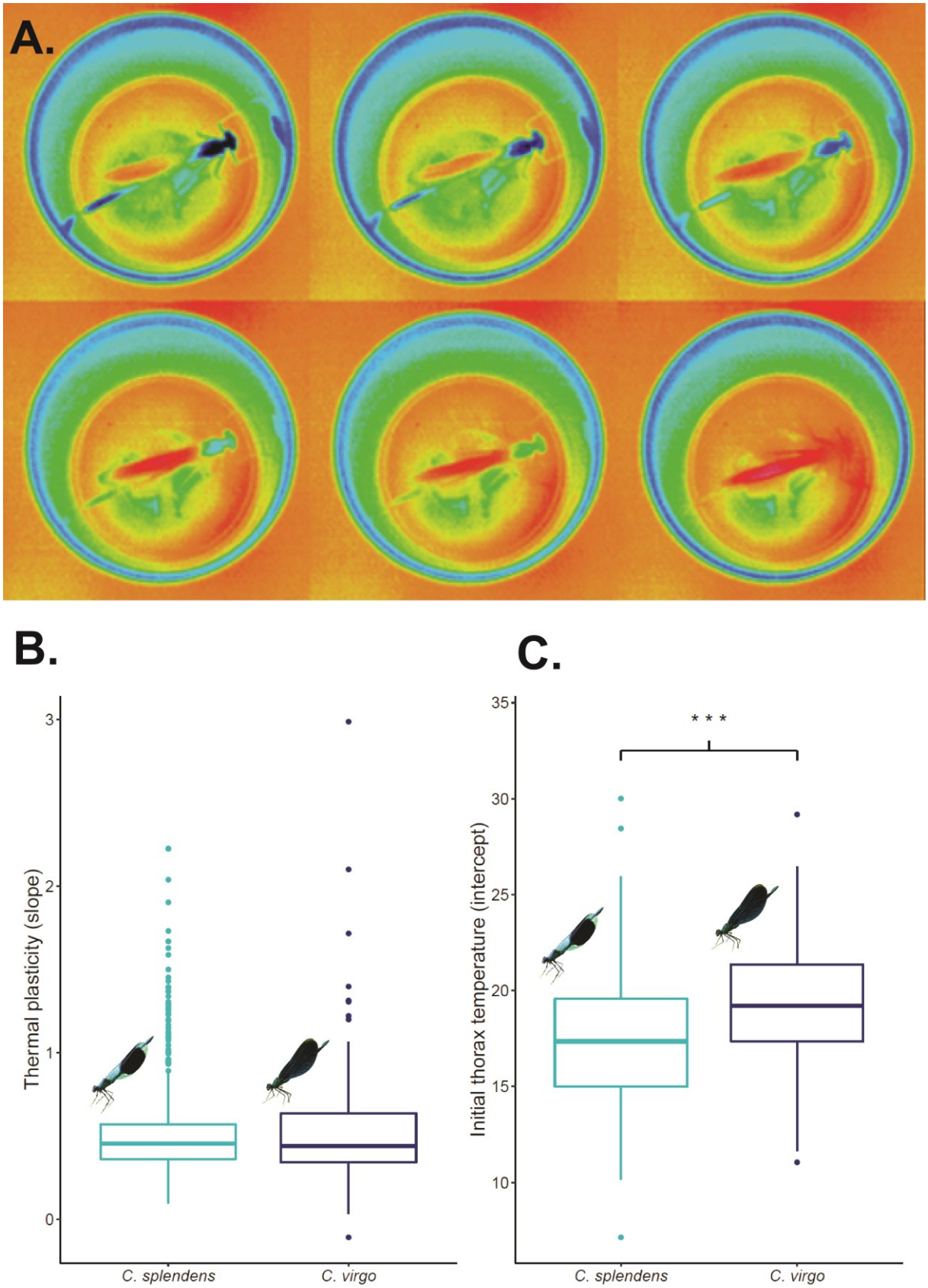
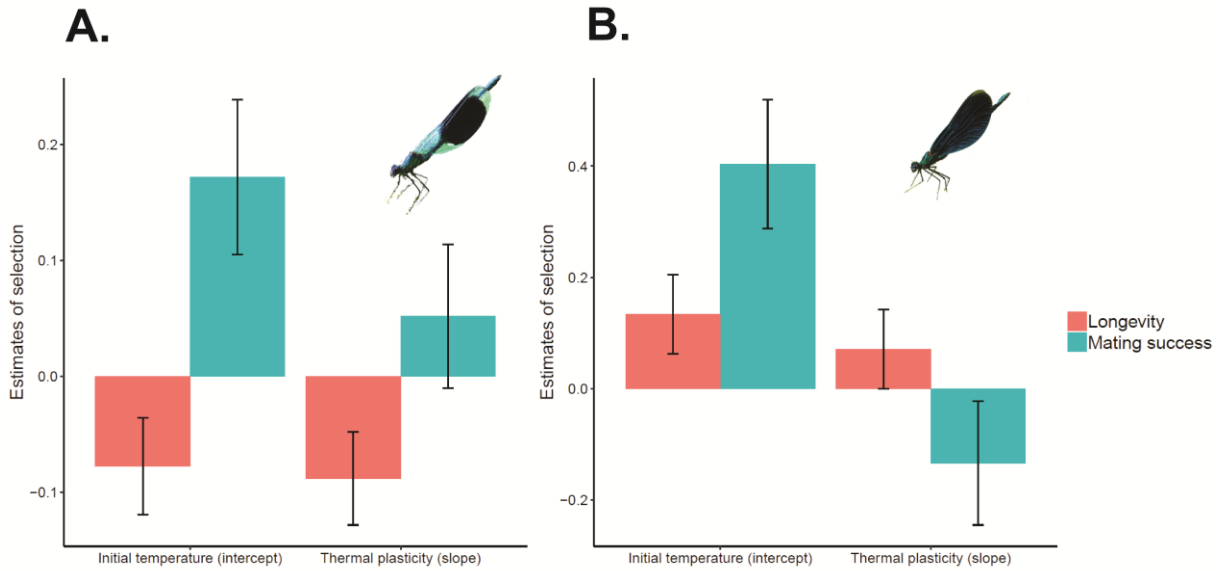
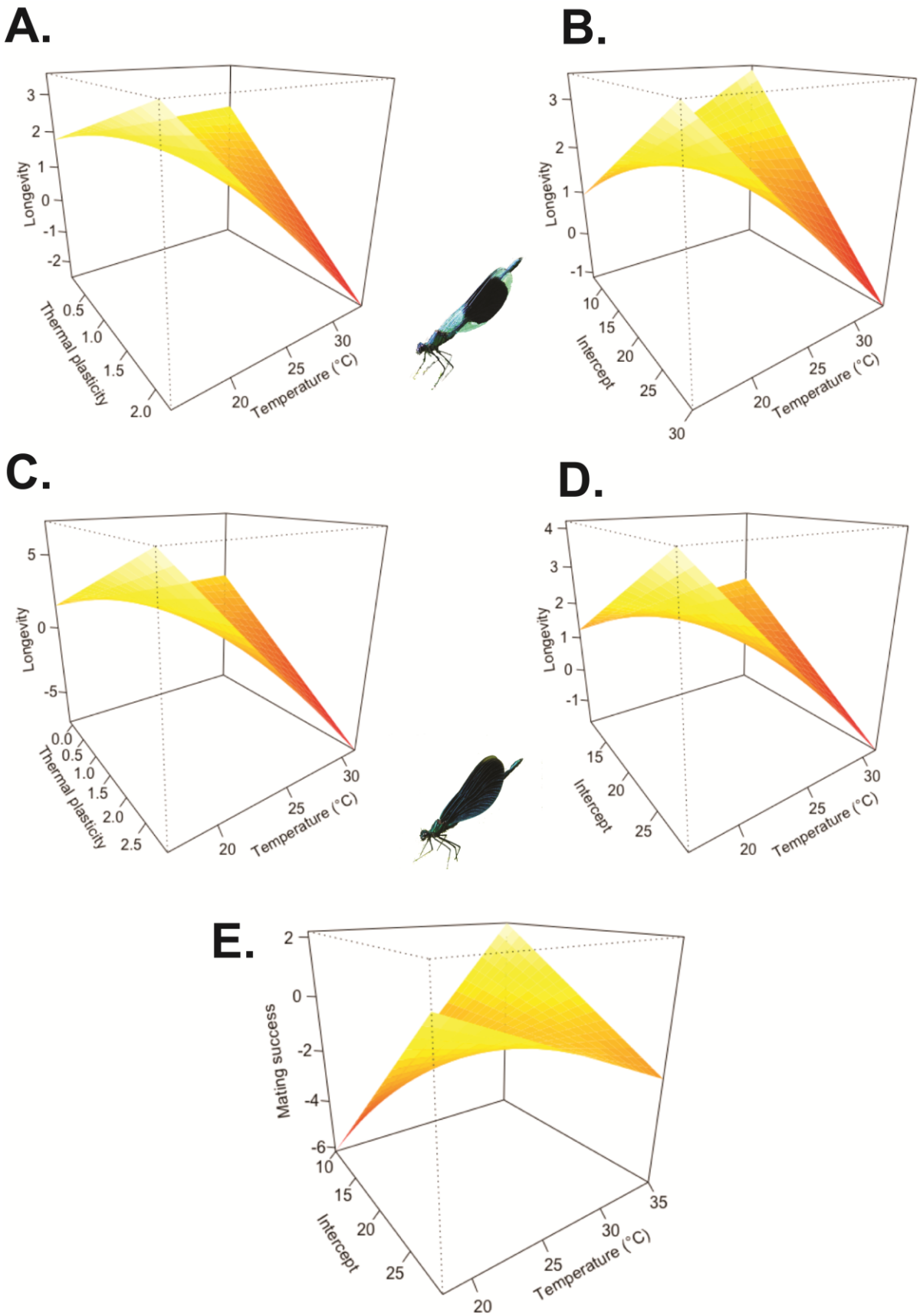

A.
B.
C.
Thermal plasticity (slope)
0
1
2
3
C. splendens
C. virgo
Initial thorax temperature (intercept)
10
15
20
25
30
35
* * *
C. splendens
C. virgo

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

656
657

Fig. 4



658