

1 **Temperature drives pre-reproductive selection and shapes**
2 **the biogeography of a female polymorphism**

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14 **AUTHORSHIP STATEMENT**

15 E.I.S. conceived the idea of this study, planned the mesocosm experiments and maintained the
16 long-term population study. E.I.S., BW and MCD collected field data and carried out the
17 mesocosm experiments. E.I.S., BW and LL performed the statistical analyses. E.I. wrote the
18 first draft of this paper. All the other authors read and contributed to the writing of the final
19 manuscript.
20

21 **DATA ACCESSIBILITY STATEMENT**

22 Original data behind all the analyses in this paper and associated R-code will be uploaded on
23 Dryad (<https://datadryad.org/>). Correspondence and requests for additional materials should
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25

26 **Abstract:**

27 Conflicts of interests between males and females over reproduction is a universal feature of
28 sexually reproducing organisms and has driven the evolution of intersexual mimicry, mating
29 behaviors and reproductive polymorphisms. Here we show how temperature drives pre-
30 reproductive selection in a female colour polymorphic insect that is subject to strong sexual
31 conflict. This species has three female colour morphs, one of which is a male mimic. This
32 polymorphism is maintained by frequency-dependent sexual conflict caused by male mating
33 harassment. The frequency of female morphs varies geographically, with higher frequency of
34 the male mimic at higher latitudes. We show that differential temperature sensitivity of the
35 female morphs and faster sexual maturation of the male mimic increases the frequency of this
36 morph in the north. These results suggest that sexual conflict during the adult stage is shaped
37 by abiotic factors and frequency-independent pre-reproductive selection that operate earlier
38 during ontogeny of these female morphs.

39

40 **Keywords:** biogeography, climate, colour polymorphism, frequency-dependent selection,
41 ontogeny, pre-reproductive selection, sexual conflict, temperature

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49 INTRODUCTION

50 Sexual conflict is a major force that shapes male and female sexual interactions and in many
51 species females incur fitness costs of mating or mating harassment (Rice 1996; Arnqvist &
52 Rowe 2005). Experimental studies and comparative analyses (Arnqvist & Rowe 2002a, b)
53 suggest that sexual conflict could promote the rapid evolution of female and corresponding
54 male traits, contributing to speciation (Arnqvist *et al.* 2000; Gavrilets 2000), but sexual conflict
55 could also elevate extinction risk (Rankin *et al.* 2011), since female fitness is closely linked to
56 population performance and stability (Le Galliard *et al.* 2005; Harts *et al.* 2014; Takahashi *et*
57 *al.* 2014). However, evidence for a strong link between sexual conflict and speciation remains
58 equivocal (Ritchie 2007). Theory also shows that an alternative outcome of sexual conflict is
59 the evolution of distinct female (and sometimes also male) genetic clusters (or morphs)
60 (Gavrilets & Waxman 2002; Svensson *et al.* 2005; Le Rouzic *et al.* 2015; Iversen Lars
61 Lønsmann *et al.* 2019). An increasing number of examples of such polymorphisms that have
62 evolved as a response to sexual conflict have now been demonstrated in several insect taxa
63 (Svensson *et al.* 2005, 2009; Reinhardt *et al.* 2007; Karlsson *et al.* 2013).

64

65 One form of such mating polymorphisms is intersexual mimicry, in which some male or female
66 individuals express a phenotype resembling the opposite sex (Gosden & Svensson 2009; Neff
67 & Svensson 2013). Intersexual mimicry provides a fitness advantage either for males to obtain
68 ‘sneaky’ matings through deception by looking like females (Neff & Svensson 2013), or, in the
69 case of females, to avoid excessive male mating harassment by looking like males, especially
70 when male densities are high and mating harassment of females is intense (Gosden & Svensson
71 2009; Svensson *et al.* 2009). Such intraspecific and intersexual mimicry are expected to show
72 negative frequency-dependency, similar to more well-known interspecific Batesian mimicry

73 systems, where benefits of mimicry are expected to break down if mimics becomes very
74 common (Finkbeiner *et al.* 2018).

75

76 Many genera of dragonflies and damselflies are characterized by strong sexual conflict,
77 intersexual mimicry and several genetic female colour morphs that coexist locally within
78 populations (Corbet 1999; Svensson *et al.* 2009). In many species of pond damselflies in the
79 globally distributed genus *Ischnura*, for example, one male-like female morph (androchrome
80 females, hereafter called “male mimic”) almost always occurs at lower frequency than the
81 alternative female morph (gynochrome females)(Sanchez-Guillen *et al.* 2011). Thus, most
82 species have two female colour morphs: a minority male mimic (androchrome) and a majority
83 female-like (gynochrome) morph (Sanchez-Guillen *et al.* 2011). Some species like the widely
84 distributed common bluetail damselfly (*Ischnura elegans*) even has three such female colour
85 morphs, of which only one is a male mimic (Fig. 1)(Svensson *et al.* 2005; Le Rouzic *et al.*
86 2015). In *I. elegans*, and in several other species of *Ischnura*, colour morph development is
87 governed by a single autosomal locus with three alleles in a dominance hierarchy and with sex-
88 limited expression to females (Fig. 1)(Sanchez-Guillen *et al.* 2005; Svensson *et al.* 2009).
89 However, such female morphs differ also in several other traits, apart from colour, including
90 parasite loads, female fecundity, cold acclimation ability, mating behaviours and resistance and
91 tolerance towards male mating harassment (Gosden & Svensson 2009; Lancaster *et al.* 2017;
92 Willink & Svensson 2017).

93

94 The maintenance of these female colour polymorphisms is believed to be due to balancing
95 selection (Svensson 2017), with empirical evidence for negative frequency-dependent selection
96 in several species (Svensson *et al.* 2005; Takahashi *et al.* 2010; Le Rouzic *et al.* 2015) and with

97 some additional and complementary role for density-dependent selection (Gosden & Svensson
98 2009; Galicia-Mendoza *et al.* 2017). However, all of the proposed mechanisms for how
99 negative frequency-dependent selection arises in these polymorphic systems rely on arguments
100 for evolutionary change arising from selection driven by male mating harassment on females
101 during the adult (reproductive) stage (Robertson 1985; Van Gossum *et al.* 2001; Fincke 2004;
102 Takahashi & Watanabe 2009; Takahashi *et al.* 2010, 2014; Iserbyt *et al.* 2011; Gering 2017).
103 However, we know little about how selection might operate on earlier life-stages, such as during
104 the pre-reproductive development period of females, in spite of the fact that we know that
105 opposing selection pressures during ontogeny is common in populations of many other
106 organisms (Schluter *et al.* 1991; Barrett *et al.* 2008; Sinervo & McAdam 2008).

107

108 Recently, researchers interested in various forms of sexual conflict have turned their attention
109 to the role of temperature in modulating such conflict in some laboratory insect systems (Berger
110 *et al.* 2014; García-Roa *et al.* 2019). The aim of this study was to investigate how temperature
111 might influence and explain geographic variation in female colour morph frequencies in the
112 polymorphic species *I. elegans*. Specifically, we combined field studies of natural populations
113 with mesocosm studies, to investigate how frequency-independent pre-reproductive selection
114 operating early in female colour development could influence adult morph frequencies. Such
115 frequency-independent pre-reproductive selection could potentially oppose the well-known and
116 previously demonstrated frequency-dependent selection, which is driven by sexual conflict and
117 which is operating on the adult female morphs later in ontogeny (Svensson *et al.* 2005;
118 Takahashi *et al.* 2014; Le Rouzic *et al.* 2015).

119

120

121 **METHODS**

122 **Study organism**

123 The common bluetail damselfly (*Ischnura elegans*) is a common insect in Europe with a broad
124 geographic distribution, ranging from the UK in the west to Japan and several other countries
125 in Asia (Indonesia, Malaysia, India and Pakistan) in the east
126 (<http://www.iucnredlist.org/details/165479/0>). In Europe, *I. elegans* occurs from Cyprus,
127 Greece, Italy and Spain in the south to Scandinavia in the north (Askew 1988; Gosden *et al.*
128 2011). Southern populations of *I. elegans* are multivoltine with several generations per year,
129 whereas populations in northern Europe are generally univoltine, with only one generation per
130 year (Parr 1970; Shama *et al.* 2011; Fitt *et al.* 2019). *I. elegans* is characterized by three female-
131 limited colour morphs, one of which is typically a male mimic (Fig. 1). The genetic basis of
132 female colour polymorphism in *I. elegans* and its close relatives (*I. graellsii* and *I. genei*) has
133 been revealed in controlled breeding experiments over several generations and arises from
134 genetic variation at a single autosomal locus with three alleles in a dominance hierarchy and
135 with sex-limited expression to females (Cordero 1990; Sanchez-Guillen *et al.* 2005; Sanmartín-
136 Villar & Cordero-Rivera 2016). In addition to fixed genetic colour differences among adult
137 female colour morphs (Fig. 1), there are also pronounced ontogenetic colour changes within the
138 three female morphs during their sexual maturation period (Supporting Information, Fig. S1).

139

140 The three female morphs can be distinguished during their immature stage based on the melanin
141 patterning on their blue abdomen patches (Supporting Information, Fig. S1). Specifically, the
142 two female gynochrome (female-like) morphs (I- and O-females) cover their blue abdominal
143 patch with pigment as they become sexually mature, whereas the androchrome female morph
144 (A-females, male mimic) retains this blue abdomen patch (Supporting Information, Fig. S1).

145 This blue abdomen patch is also present on males throughout their entire lives (Fig. 1).
146 Retaining of this male colour signal in androchrome females is an important part of male
147 mimicry as it causes female resemblance to males and experimental phenotypic manipulations
148 have revealed that the presence of this blue patch reduces male mating harassment (Willink *et*
149 *al.* 2019a). Thorax colour also changes in these morphs during the course of their ontogeny
150 (Cordero *et al.* 1998). Thorax colour changes from violet to blue in A-females, from violet to
151 green in I-females and from pink to brown in O-females (Fig. 1)(Cordero *et al.* 1998; Svensson
152 *et al.* 2009). Female colour maturation is tightly correlated with female reproductive maturity,
153 as immature-coloured females of all morphs have high levels of reproductive failure and lay
154 few eggs, if any at all, if given the opportunity (Willink *et al.* 2019a).

155

156

157 **General field work procedures**

158 We performed field work around Lund (southern Sweden) in a series of natural populations of
159 *I. elegans* that are part of a long-term population study that was initiated in the year 2000 by
160 E.I. Svensson (Svensson & Abbott 2005; Svensson *et al.* 2005; Abbott *et al.* 2008; Gosden &
161 Svensson 2008, 2009; Le Rouzic *et al.* 2015; Willink & Svensson 2017). Field work for this
162 study was carried out during three reproductive seasons of *I. elegans* in the summers (June and
163 July) of 2015-2017. This field observational study was complemented with a mesocosm study
164 at Stensoffa Ecological Field Station (see further below). Field data on morph frequency
165 variation and changes in morph frequencies during generations come from a total of 16
166 populations that are part of our core long-term population study (Le Rouzic *et al.* 2015). General
167 field work routines are described in detail elsewhere (Svensson & Abbott 2005; Svensson *et al.*
168 2005; Le Rouzic *et al.* 2015; Willink & Svensson 2017). Briefly, we visited and re-visited each

169 of our populations at weekly intervals to catch and record densities, female colour morph
170 frequencies, sex ratios and age class frequencies (teneral, immatures and adults) and used
171 standardized (time-recorded) sampling sessions to take into account total catching effort
172 (Willink & Svensson 2017). By using data from these standardized field-sampling routines, we
173 quantified morph frequency variation between populations and recorded morph frequency
174 changes during the course of ontogeny, from the sexually immature stage to the adult
175 reproductive stage (Supporting Information, Fig. S1). All populations were visited multiple
176 times across all field seasons in an effort to minimize any potential biases due to possible
177 morph-differences in catchability or detectability linked to specific weather conditions on
178 specific days. Our previous work has revealed that population densities estimated using this
179 approach are not biased by daily temperature fluctuations (Fitt & Lancaster 2017).

180

181 In addition to field work in southern Sweden, we also compiled a large biogeographic dataset
182 on female colour morph frequency variation across a number of populations along a latitudinal
183 South-North gradient in Europe. We updated a previous biogeographical survey which
184 contained data on adult female morph frequencies across all of Europe (Gosden *et al.* 2011) by
185 adding eleven more populations (three from France, five from Cyprus) to this dataset. This large
186 biogeographic dataset contained information from 129 populations across 11 countries in
187 Europe with a total of 15 714 morphotyped adult females of *I. elegans* (Supporting Material).
188 All these populations were georeferenced and contained longitudinal and latitudinal locality
189 information. We used this spatial information to extract temperature information from
190 bioclimatic data (Hijmans *et al.* 2005) for each locality and relate such local temperature
191 information to local morph frequencies (Fig. 2). We used standard temperature measures from
192 the Bioclim database (Hijmans *et al.* 2005) and related these to geographic variation in morph
193 frequencies. Of these temperature variables, Bio1 (“Annual mean temperature”) has previously

194 been shown by us to be the predictor with the highest contribution in Maxent species
195 distribution modelling of the geographic range of *I. elegans* (62.1 % contribution to the model)
196 (Lancaster *et al.* 2015), and both Bio1 and to an even higher degree Bio5 (“Maximum
197 temperature during the warmest month”) are both associated with significant molecular genetic
198 differentiation and local adaptation along a latitudinal cline from southern Sweden, up to its
199 northern range limit (Dudaniec *et al.* 2018). However, we were also interested in the effects of
200 more temporally fine-grained temperature measures that might be relevant to the sexual
201 maturation period during summer and the timing of colour development of the female colour
202 morphs. We therefore investigated how all the eleven temperature variables in Bioclim (Bio1-
203 B11) performed in explaining geographic variation in morph frequencies of *I. elegans*. As these
204 different temperature measures are typically highly correlated with each other (see Supporting
205 Material), we only present the relationship with the variable that performed best in explaining
206 geographic variation in morph frequencies, as judged by the lowest value of the Akaike
207 Information Criterion (AIC).

208

209 **Mesocosm study**

210 Beginning in the summer of 2015 and continuing in 2016 and 2017, we performed mesocosm
211 studies, in which we marked individuals with unique numbers on their wings, recaptured them
212 daily and measured morph-specific female colour maturation rates and survivorship from the
213 immature stage to the final colour stage that is reached at sexual maturity (Fig. 1; Supporting
214 Information, Fig. S1) (Cordero *et al.* 1998; Svensson *et al.* 2009). These mesocosm studies
215 were performed in large outdoor insectaries ($3 \times 3 \times 3 = 27 \text{ m}^3$) at Stensoffa Ecological Field
216 Station, outside Lund. These large outdoor insectaries mimicked natural conditions. Cages were
217 covered with nets with a mesh size that enabled small insects (primarily moths and flies) to
218 enter, which provided natural food in the form of live prey for the damselflies (Takahashi *et al.*

219 2014; Svensson *et al.* 2018). Each cage also contained 2-3 small and shallow plastic water tanks
220 with resting substrate in the form of floating vegetation, where the damselflies could perch and
221 obtain water.

222

223 We individually marked and released immature female *I. elegans* of these three female colour
224 morphs. All survivors were recaptured every day until they either died or reached the adult
225 colour-maturation stage. We recorded time (in days) until the different individuals of the three
226 different morphs reached the final colour-maturation stage or were no longer found alive. In
227 total, we marked and released 450 immature individual *I. elegans* females from five populations
228 in these cages, during 2015-2017. Of these marked individuals, 144 (32 %) belonged to the A-
229 morph, 215 (48 %) belonged to the I-morph and 91 (20 %) belonged to the O-morph, and a total
230 of 162 (36 %) survived until sexual maturity. Males were not present in these female-only
231 mesocosms as we were interested in intrinsic female morph differences in pre-reproductive
232 females and the presence of males might confound our findings. This is justified as males do
233 typically avoid mating with such immature females, when given a choice in experimental
234 settings (Willink *et al.* 2019a). To ensure that males did not affect our results, our mesocosm
235 cages were not in the vicinity of any natural pond, meaning that females did not encounter any
236 males, neither physically nor visually during their colour maturation process.

237

238 We compared the three female morphs with respect to development time until maturity and
239 survival until maturity (binomial variable: 1=survived, 0=did not survive) using previously
240 published information about the colour developmental stages (Cordero *et al.* 1998; Svensson *et*
241 *al.* 2009; Willink *et al.* 2019a). We incorporated information about the daily mean temperatures
242 during the entire colour development period of individual females during the summers 2015-

243 2017, which we obtained from the closest weather station in Malmö (southern Sweden:
244 <http://opendata-download-metobs.smhi.se/explore/>). In addition to the daily mean
245 temperatures that females experienced during their colour development period, we also
246 incorporated information about the daily mean temperatures they experienced the three days
247 preceding their captures in the field, following our previous study in which we found that this
248 time period influenced individual acclimation (Lancaster *et al.* 2017).

249

250 **Quantifying pre-reproductive survival and estimating selection coefficients of female** 251 **colour morphs in field populations**

252 We estimated relative fitness on a per-morph basis using a cross-sectional selection analysis
253 approach (Lande & Arnold 1983), where we compared the morph frequencies among immature
254 females (prior to sexual maturity) and sexually mature females (judged by their colour
255 maturation stage) (Cordero *et al.* 1998; Svensson *et al.* 2009; Willink *et al.* 2019a) (Fig. 4A,B).
256 An increase in the relative frequency of one morph from the immature to the mature stage within
257 a local population would be consistent with higher pre-reproductive survival, compared to
258 morphs that decrease in frequency (within-generation selection). Based on the within-
259 generation frequency changes for these three morphs, we calculated two closely related
260 parameters: relative morph fitness and pre-reproductive selection coefficient on each morph,
261 using classical approaches in ecological genetics (Linnen & Hoekstra 2009). We estimated
262 these two parameters using data from 2015 to 2017 in 16 intensively monitored field
263 populations, that are part of a long-term population study of *I. elegans* initiated in the year
264 2000 (Le Rouzic *et al.* 2015). In total, we sampled and identified 4625 females to colour-
265 development category and morph. Of the total, 3078 were A-females (67 %), 1310 were I-
266 females (28 %) and 237 were O-females (5 %).

267

268 **Statistical analyses**

269 All statistical analyses in this paper were performed using various packages (nlme, lme4,
270 MCMCglmm) in the “R” statistical environment (R Development Core Team 2014). We used
271 generalized linear mixed models when analyzing geographic variation in morph frequencies
272 (Fig. 2C), differences in development time between the morphs (Fig. 3A) and morph-specific
273 survivorship to maturity in relation to temperature in mesocosm cages (Fig. 3B; Supporting
274 Information, Tables 1-2). In the mesocosm studies (Fig. 3; Tables 1-2), we included the year,
275 population of origin and experimental cage as random factors, and female colour morph and
276 temperature variables were fixed effects. When we analyzed geographic variation in
277 androchrome frequencies (Figs. 2A,B) and how local microclimatic variation (temperature)
278 affected androchrome frequencies (Fig. 2C), we also used a mixed model with population
279 (Supporting Information, Table S1) and population and latitude block (Supporting Information,
280 Table S2) to control for spatial autocorrelation and statistical non-independence of closely
281 located populations within the same latitudinal band. Europe was divided in to seven latitudinal
282 blocks of equal length (each block encompassing 5°), ranging from Cyprus in the south to
283 Sweden in the north (Supporting Information, Table S2).

284

285 Female morph frequencies at the pre-reproductive and reproductive developmental stages were
286 estimated using a categorical (i.e. multinomial) mixed-effect model fitted by MCMC. We used
287 a flat Kronecker prior for the two-way marginal probabilities (i.e. A- vs I-females, I- vs O-
288 females and A- vs O females) in each developmental stage (Hadfield 2015). In this model, we
289 included a random interaction between the latent morph-frequency variable and the sampling
290 population, thereby allowing natural populations to differ in their morph frequencies. Relative

291 fitness was calculated as the frequency ratio between the reproductive and immature stages,
292 across the entire posterior distribution of morph frequency estimates. Cross sectional selection
293 coefficients for each morph were also calculated across posterior estimates following standard
294 ecological genetics literature (Linnen & Hoekstra 2009) as:

$$295 \quad \frac{\Delta p}{(p' * (1 - p) - p * q * r)}$$

296
297 where Δp is the change in frequency of the focal morph between the immature and mature
298 stages, p' and p are the focal morph frequencies at the reproductive and immature stages
299 respectively, and q and r are the frequencies of the two other morphs during the immature
300 developmental stage. We considered the relative fitnesses and selection coefficients of two
301 female morphs as significantly different if the values for one morph were higher than for the
302 other in more than 95 % of the posterior distribution of model estimates. All data and computer
303 code behind the statistical analyses in this paper will be made openly available and uploaded
304 on the Dryad data repository.

305 **Results**

306 **Geographic variation in colour morph frequencies**

307 Analysis of geographic variation in the local population frequencies of the male mimic in *I.*
308 *elegans* across Europe reveals that the male mimics reach their highest frequencies near the
309 species' northern range limit in Scandinavia, where they are often the majority morph with 60
310 – 80 % of all females (Fig. 2 A,B; Supporting Information, Table S1). Although there is also
311 considerable regional variation between populations within latitudinal bands (Lancaster *et al.*
312 2017), this overall large-scale geographic pattern is robust to such local deviations (Fig. 2, A,B;
313 Supporting Information, Table S1). We further found a strong and highly significant positive

314 relationship between local temperatures and the frequency of the male mimic across the
315 biogeographic range in Europe (Fig. 2C; Supporting Information, Table S2). Of the different
316 temperature variables, the best predictor of the local frequency of the male mimic was
317 maximum temperature during the warmest month (Bio5: Supporting Information, Table S2),
318 which probably best captures the temperatures that females experience in the summers during
319 colour maturation (Fig. 3A-B). Notably, temperature during the warmest month showed a better
320 statistical fit to the data (as judged by lower AIC-values) than the annual mean temperature
321 (Bio1), which also showed a highly significant relationship with the local frequency of the male
322 mimic (Supporting Information; Table S2, Fig S2). Interestingly, mean temperature of the
323 coldest month (Bio6) - which reflects temperature during winter when these damselflies are in
324 their aquatic larval stage - was not significantly related to the local frequency of the male mimic
325 (Supporting Information, Table S2). This might suggest that thermal conditions during the adult
326 stage are more important than thermal conditions earlier in ontogeny in shaping biogeographic
327 variation in morph frequencies.

328

329 **Survivorship to maturity in mesocosms**

330 We performed mesocosm studies in the northern part of the geographic range of *I. elegans*, and
331 complemented these studies by tracing within-generation morph frequency dynamics across a
332 series of populations that have been subject to a long-term study, starting in the year 2000
333 (Svensson *et al.* 2005; Le Rouzic *et al.* 2015). We found that the male mimic had significantly
334 shorter development time to sexual maturity than the two gynochrome morphs, which did not
335 differ significantly from each other (Fig. 3A, Table 1). We also found that the rate and
336 probability of reaching sexual maturity was dependent on temperature, morph and the
337 interaction between morph and temperature (Fig. 3B, Table 2). Whereas the male mimic
338 showed evidence of being relatively canalized and less sensitive to thermal conditions in terms

339 of reaching sexual maturity, the two gynochrome female morphs showed higher thermal
340 sensitivity with increasing survival at high temperatures (Fig. 3B, Table 2).

341

342 **Pre-reproductive selection in the field**

343 We complemented our mesocosm experiments with a cross-sectional selection study in our
344 field populations, in which we estimated the relative fitnesses of the three female morphs and
345 associated selection coefficients, based on morph frequency changes during the ontogeny. This
346 cross-sectional selection analysis across three seasons and 16 populations revealed that the male
347 mimic increased in frequency from the sexually immature to the sexually mature adult stage,
348 and we thus found evidence for positive pre-reproductive selection in favour of the A-females
349 (Fig. 4A,B).

350

351 **Discussion**

352 Our analyses of the large-scale geographic variation in female morph frequencies of *I. elegans*
353 across its range in Western Europe (Fig. 2; Supporting Information Table S1-S2) strongly
354 suggested that the male mimicking female morph is better adapted to cooler microclimates than
355 the two other female morphs. Consistent with this interpretation, male mimics have a higher
356 acclimation ability in response to recent cold spells than the two other female morphs (Lancaster
357 *et al.* 2017). A similar latitudinal geographic pattern with a higher frequency of the male mimic
358 at higher latitudes has also been documented in the closely related subtropical species *I.*
359 *senegalensis* in Japan (Takahashi *et al.* 2011). These observational data in combination with
360 our mesocosm studies (Fig. 3) suggest that the colour morph locus might interact with other
361 loci influencing thermal adaptation in this ectothermic insect species. Our recent experimental
362 gene expression studies have revealed that genes governing the production of heat shock

363 proteins (*Hsp*) are differentially upregulated between morphs in relation to heat- and cold
364 shocks, and in complex and different ways depending on geographic range position (Lancaster
365 *et al.* 2016). Further, our recent gene expression studies performed during ontogenetic colour
366 maturation of these female colour morphs (Fig. 1, Fig. S1) have revealed that the morph locus
367 shows extensive pleiotropy and interacts with many other genes behind vital physiological and
368 reproductive functions during development (Willink *et al.* 2019b). This previous work of ours
369 in combination with the novel results in this study suggests that the morph locus might also be
370 involved in epistatic relationships with loci for thermal adaptation, something that we plan to
371 investigate in the future. We also note that the best predictor of geographic variation in local
372 morph frequencies was the maximum temperature during the warmest month (Bio5; Fig. 2C),
373 rather than annual mean temperature (Bio1; Fig. S2) or mean temperature during the coldest
374 month (Bio6), which was not significant (Table S2). This suggests that it is temperature
375 conditions during adult stage, rather than during the much longer larval stage, that are most
376 important in affecting morph frequencies, consistent with the results from our mesocosm and
377 field studies (Figs. 3-4).

378

379 The biogeographic patterns we present here and the link to local temperature suggest that the
380 male mimic has increased in frequency at higher latitudes due to higher physiological and
381 reproductive performance in colder climates, whereas it is kept at low frequency at lower
382 latitudes where temperatures are higher (Fig. 2). The morph differences in the rate of sexual
383 development and colour maturation (Fig. 3A) might partly reflect the costs of pigment
384 production such as pterins and maybe also melanin (Roff & Fairbairn 2013; Henze *et al.* 2019),
385 since the male mimicking females do not deposit as much pigment on their abdomen tips as the
386 gynochrome morphs (Fig. S1). Thus, the male mimicking females may become sexually mature
387 more quickly and reach their final reproductive colour stage faster, compared to the

388 gynomorphs (Table 1). We note, however, that there are also many other physiological,
389 developmental and reproductive differences between the male mimicking females and the
390 gynomorphs, making it difficult to exactly pinpoint the mechanism behind the faster
391 development of the A-females (Gosden & Svensson 2009; Willink & Svensson 2017; Willink
392 *et al.* 2019b).

393

394 Consistent with the large scale biogeographic link between the frequency of the male mimic
395 and mean temperature of the warmest month (Fig. 2C), both the rate and probability of reaching
396 maturity depended on temperature, morph and their interaction in our mesocosm studies (Fig.
397 3B; Table 2). Specifically, the probability of reaching maturity for the male mimic was
398 independent across a broad temperature range, whereas the two gynochrome female morphs
399 showed higher thermal sensitivity and increasing survivorship with higher temperature (Fig.
400 3B; Table 2). Taken together, these data suggest that low temperatures at high latitudes have
401 favoured the male mimic and its frequency has increased in the north, due to its shorter
402 development time, lower developmental sensitivity to temperature, and greater acclimation
403 ability in response to cold-weather (Lancaster *et al.* 2017).

404

405 If low temperatures at high latitudes in combination with shorter development time of the male
406 mimic has increased its frequency (Figs. 2-3), we predicted that there should be a within-
407 generation selective increase in frequency of the male mimic in these cool northern field
408 populations, due to the higher probability of A-females to reach the adult reproductive stage.
409 Our cross-sectional selection analysis strongly supported this prediction, as the male mimic
410 increased in frequency from the sexually immature to the mature adult stages and hence
411 experienced significant and positive pre-reproductive survival selection (Fig. 4A,B). Such pre-

412 reproductive selection and conflicting selection pressures between different ontogenetic stages
413 of the life-history has been documented in other organisms (Schluter *et al.* 1991; Barrett *et al.*
414 2008; Sinervo & McAdam 2008) and might be a general feature of many natural populations,
415 including *I. elegans*.

416

417 Colour morphs in *I. elegans* and other taxa often differ in multiple traits apart from colour,
418 including thermal physiology, fecundity, behavior and parasite loads (Gosden & Svensson
419 2009; Lancaster *et al.* 2017; Willink & Svensson 2017). For instance, the male mimic has lower
420 fecundity than the two gynochrome female morphs (Willink *et al.* 2019a), which could reflect
421 costs of mimicry due to the more male like shape of these male mimics (Gosden & Svensson
422 2009). The lower fecundity of the male mimic could also be a cost of their earlier sexual
423 maturity and faster colour development that we have demonstrated in this study (Fig. 3A).
424 These and other phenotypic morph differences might have partly evolved as a result of
425 correlational selection for different adaptive trait combinations in the different morphs (Sinervo
426 & Svensson 2002). When the benefits of different trait combinations in the different morphs
427 differ across geographic space, frequency-independent directional selection in favor of one trait
428 (such as thermal physiology) can interfere with or can even partly counteract frequency-
429 dependent selection on the female polymorphism at the adult stage, especially when colour is
430 genetically or phenotypically correlated with other traits (Lancaster *et al.* 2017). When such
431 episodes of frequency-dependent selection that operate early in the ontogeny either opposes or
432 is aligned with the direction of the later frequency-dependent selection during reproduction, it
433 can have large consequences for population mean fitness, maladaptation and the prospects of
434 evolutionary rescue (Svensson & Connallon 2018). In the case of *I. elegans* in northern Europe,
435 the male mimic has apparently increased to higher frequencies than in any other *Ischnura*
436 species or population that has been studied to date (Sanchez-Guillen *et al.* 2011). This increase

437 of male mimics is most likely the result of its higher cold weather-responsive acclimation
438 capacity and shorter maturation time, although this thermal benefit and shorter development
439 advantage might be partly counteracted by their lower fecundity (Willink *et al.* 2019a).

440

441 The high frequency of the male mimic in the north might also decrease the efficacy of male
442 mimicry, as expected from theory and from research on interspecific mimicry systems
443 (Finkbeiner *et al.* 2018). As a consequence, we expect higher male mating harassment on male
444 mimics and potentially also higher population extinctions rates in the north. Our previous
445 manipulations of morph frequencies in mesocosm experiments did indeed show evidence for
446 reduced population fitness when female morph frequencies become very biased (Takahashi *et*
447 *al.* 2014) and population morph frequencies are also increasingly biased at the range limits of
448 *I. elegans* (Gosden *et al.* 2011), consistent with such a scenario. Our data suggest that these
449 high-latitude populations are not at their evolutionary equilibrium but are torn between the
450 opposing forces of directional and frequency-independent selection for thermal adaptation that
451 favors the male mimic (Fig. 4A,B). This pre-reproductive selection is in a tension against
452 negative frequency-dependent selection at the adult stage that maintains the polymorphism and
453 which prevents the male mimic from going to fixation (Svensson *et al.* 2005; Le Rouzic *et al.*
454 2015). As a result of these opposing selection pressures, our populations might experience a
455 fitness load due to a conflict between pre-reproductive frequency-independent selection that
456 increases the frequency of the male mimic with the progress of ontogeny, but where male
457 mating harassment among adults might ultimately prevent the fixation of this morph. These
458 results suggest that abiotic factors experienced during development might influence the
459 dynamics of sexual conflict among adults and illustrates that adopting a biogeographic
460 perspective can be fruitful to understand such evolutionary dynamics. The study of sexual
461 conflict has been heavily dominated by laboratory studies of model organisms in controlled and

462 stable laboratory environments (Rice 1996; Arnqvist & Rowe 2005) but recently the role of
463 temperature, climate and other ecological factors in modulating various forms of sexual conflict
464 has gained increased attention (Berger *et al.* 2014; Perry *et al.* 2017; De Lisle *et al.* 2018;
465 García-Roa *et al.* 2019). The novel results in this study suggests that temperature can also
466 influence the morph frequency composition among adults through frequency-independent pre-
467 reproductive selection that operates earlier in ontogeny.

468

469 **Conclusions**

470 Our integrative study links climatic factors and biogeographic patterns to this female colour
471 polymorphism with strong implications for the dynamics of sexual conflict and frequency-
472 dependent selection on the adult morphs. Our results underscore the need to incorporate abiotic
473 factors and pre-reproductive selection that operates earlier in development and the possibility
474 of conflicting selection pressures during the course of ontogeny, which can have profound
475 consequences for the evolutionary dynamics of sexual conflict among adults.

476

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484

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490

491 **COMPETING INTERESTS**

492 The authors declare no competing interests.

493

494 **ETHICAL STATEMENT**

495 All the experiments carried out in this study are in accordance with Swedish legislation, and
496 research on insects do not require any ethical permits in Sweden.

497

498 **SUPPORTING INFORMATION**

499 **Fig. S1-S2**

500 **Tables S1-S2.**

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843 **Table 1.** Development time from the immature stage to final colour maturation stage of the
 844 three female colour morphs in mesocosm experiments.

845	Fixed effects	D.f.	SS	F	χ^2	<i>P</i>
846	Female colour morph	2	25.417	12.709	29.462	< 0.001 ***
847	Developmental temperature	1	8.454	8.454	8.454	0.004 **
848						
849	Random effects	Levels		Variance		Standard Deviation
850						
851	Cage	7		0.02641		0.16251
852	Population	5		0.00000		0.00000
853	Year	3		0.00665		0.08155
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855

856 Generalized linear mixed models were used to examine how the duration and success of development to colour
 857 maturity (sexual maturity) depends on female colour morph and temperature in our mesocosm experiments with
 858 females of *I. elegans* (Fig. 3A,B). Experimental cage number, population origin and year were included as random
 859 factors in these models (Tables 1-2). In the analysis of development time, the interaction between female colour
 860 morph and developmental temperature was not significant, and a model included this interaction had a higher AIC
 861 than the present model, hence it is not shown here. We also constructed a model incorporating past temperature
 862 temperature prior to capture as we did in the next model (Table 2), but this variable was not significant on its own
 863 or through any of the interactions with female colour morph and developmental temperature, and hence this
 864 variable was not included in the final model shown above. The male mimic (A-females) had significantly shorter
 865 development time than the two gynochrome female morphs (Fig. 3A; Tukey posthoc tests: A vs. I: $P < 0.001$; A
 866 vs. O: $P = 0.003$), whereas these two gynochrome female morphs did not differ significantly from each other
 867 (Tukey posthoc test: I vs. O: $P = 0.23$).

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871 **Table 2.** Survivorship to maturity in mesocosm experiments in relation to female colour morph
 872 and temperature during colour development.

873	Fixed effects	D.f.	SS	F	χ^2	<i>P</i>
874	Female colour morph (FM)	2	2.548	1.274	2.806	0.25
875	Developmental temperature (DT)	1	3.961	3.961	4.153	0.041*
876	Past temperature (PT)	1	0.273	0.273	0.441	0.51
877	FM x DT	2	5.260	2.630	10.744	0.005**
878	FM x PT	2	11.005	5.503	13.518	0.001**
879	DT x PT	1	12.391	12.391	12.391	0.0004***
880						
881	Random effects	Levels		Variance		Standard Deviation
882						
883	Cage	7		0.04226		0.2056
884	Population	5		0.06402		0.2530
885	Year	3		0.16944		0.4116
886						
887						

888 We investigated how the probability of reaching maturity (binomial variable: 0=did not reach maturity; 1=did
 889 reach maturity) depends on female colour morph (FM), temperature during colour development (DT), past
 890 temperature experienced prior to capture (“acclimation”; PT) and all the three two-way interactions between these
 891 three variables. The three-way interaction (FM x DT x PT) was not significant and such a complete model had
 892 significantly higher AIC than the model presented above. The significant interaction between female colour morph
 893 and temperature during colour development in the mesocosm experiments is illustrated in Fig. 3B, and shows that
 894 the survivorship of I-females increased significantly with temperature, in contrast to the two other female morphs.
 895 Posthoc tests (*P*-value adjustment method: holm) of morph differences in the slopes of how probability of reaching
 896 maturity was affected by developmental temperature showed that A-females differed significantly from I-females,
 897 which showed a positive relationship with temperature (Fig. 3B; *P* = 0.003), whereas A- and O-females did not
 898 differ significantly from each other (*P* = 0.37). There was a non-significant tendency for I-females to have a steeper
 899 slope than O-females (*P* = 0.08).

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902 **LEGENDS TO FIGURES**

903

904 **Figure 1.** Sexual conflict and female colour polymorphism in *Ischnura* damselflies. Upper
905 picture: the common bluetail damselfly (*I. elegans*) is characterized by strong sexual conflict
906 over mating between males and females. During mating, the male (upper) and the female
907 (lower; a male mimic, or “A-female”, see below) can remain attached to each other for several
908 hours. Lower picture: as a result of sexual conflict over mating, many species of *Ischnura*
909 damselflies exhibit female colour polymorphisms, with two or (in the case of *I. elegans*) three
910 heritable female colour morphs. Such colour polymorphisms are maintained by frequency-
911 dependent sexual conflict among adult females, in which common females suffer from
912 excessive male mating harassment, resulting in lower female fitness. Females show age-related
913 colour changes during the course of their ontogeny (Supporting Information, Fig. S1), but the
914 three adult female morphs at maturity is the result of a single autosomal locus with three alleles
915 in a dominance hierarchy, with expression limited to females (males are monomorphic and do
916 not express this polymorphism). Androchrome females (A-females) are male mimics and carry
917 at least one *p*-allele, which is dominant over the *q* and *r*-alleles. The three female morphs are
918 denoted A, I and O throughout this paper.

919

920 **Figure 2.** Biogeographic variation in female colour morph frequencies of *I. elegans* and local
921 microclimatic factors. **A.** Across Europe, the frequency of the male mimic (A-females)
922 increases at higher latitudes and near the northern range limit (Supporting Information, Table
923 S1) **B.** Morph frequency variation in Europe divided in to three latitudinal bands. Each corner
924 in the ternary plot represents a hypothetical population fixed for one morph (100 %), but no
925 such populations have been found, and the majority of populations are trimorphic, with a few
926 dimorphic populations. **C.** The local population frequency of the male mimic (A-females) is
927 negatively related to maximum temperature during the warmest month (Bio5; Table S2),

928 suggesting that these females are more cold-tolerant (or less heat-tolerant) than the two other
929 female colour morphs.

930

931 **Figure 3.** Mesocosm studies revealing differences between female colour morphs in
932 development time and differential temperature sensitivity. The three female colour morphs
933 were kept in large outdoor cages from their immature stage and the colour development time to
934 reach sexual maturity was recorded, as well as temperature and the probability of reaching
935 maturity. **A.** The male mimics (A-females) had significantly shorter colour development time
936 and reached sexual maturity faster than the two other female colour morphs, which did not
937 differ from each other (Supporting Information, Table S2). **B.** The male mimic is less sensitive
938 to temperature in terms of its probability of reaching sexual maturity (left panels), compared to
939 the two other female morphs (middle and right panels) whose survivorship increases with
940 higher temperatures (Table 1). Data comes from 450 individually marked females that were
941 followed throughout their entire lives in these mesocosm cages. Temperatures ranged between
942 7.5 °C and 32.1 °C in these cages, and the average daily mean temperature experienced by the
943 individual females varied between 14.1 °C and 21.2 °C.

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946 **Figure 4.** Pre-reproductive survivorship and selection prior to sexual maturity in natural
947 populations of *I. elegans* in northern Europe, near the species range limit. **A.** Probability density
948 functions of relative fitnesses of the three female morphs, based on a cross-sectional selection
949 analysis where we compared the frequency changes between the immature age class and the
950 sexually mature females. Shown are the posterior densities of the estimates based on a
951 MCMCglmm analysis. **B.** The same dataset as in A, but expressed as selection coefficients. The
952 relative fitnesses and selection coefficients differ significantly between the morphs, with the

953 male mimics experiencing positive survival selection prior to sexual maturity, resulting in a
954 higher frequency among the adult females than the two other morphs (see Methods and
955 Results).

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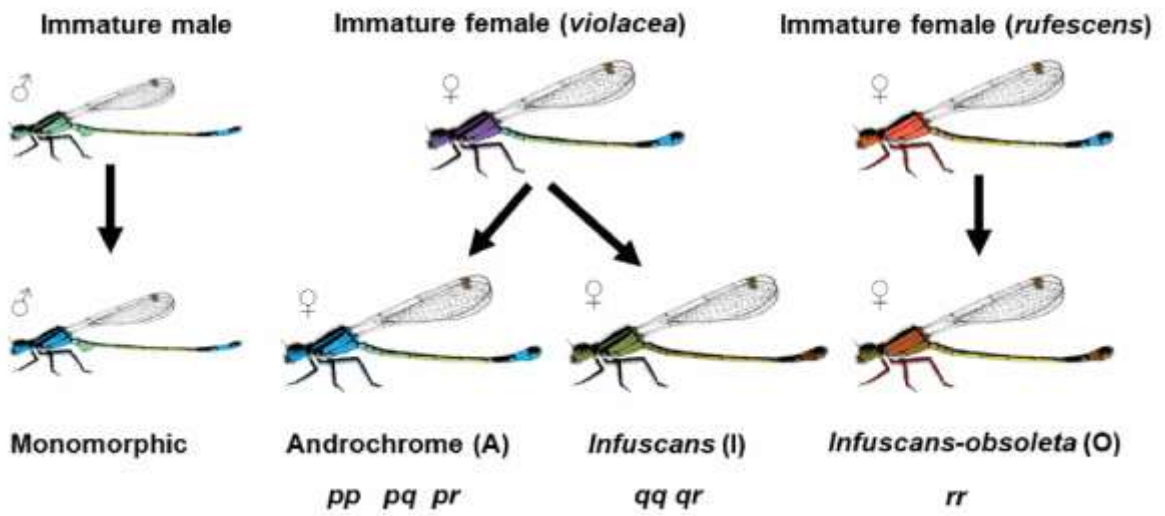
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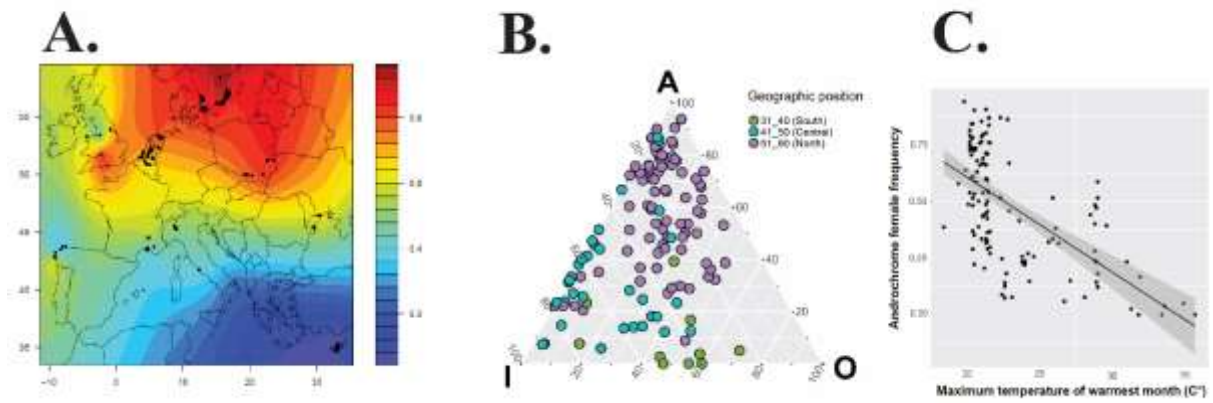
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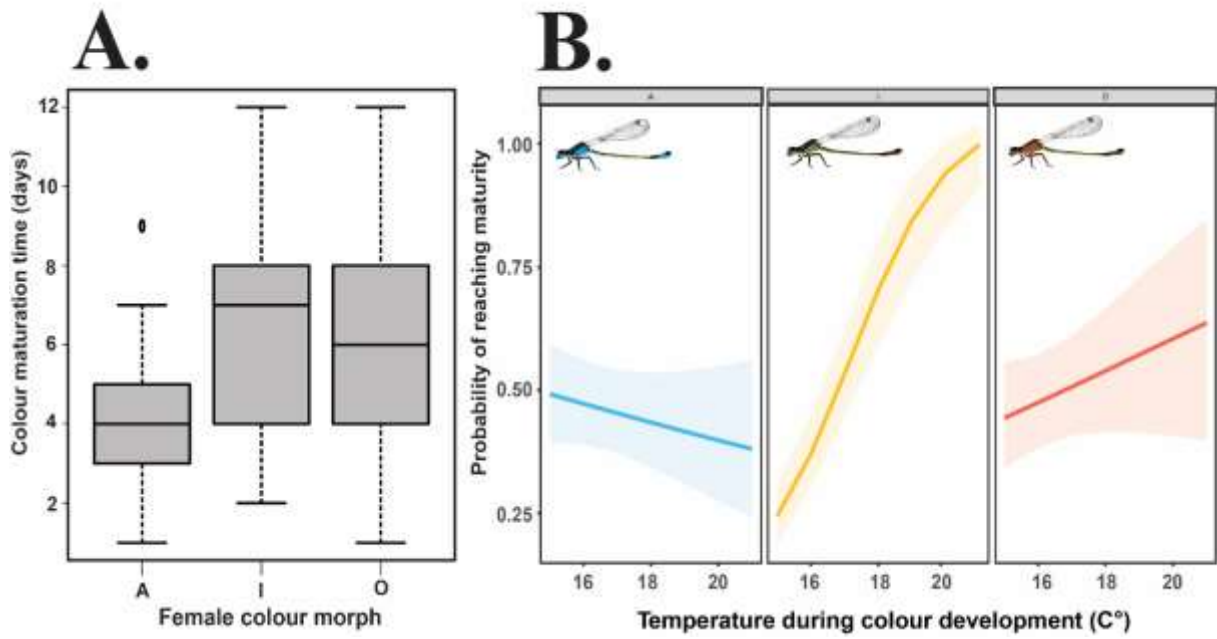
Fig. 2



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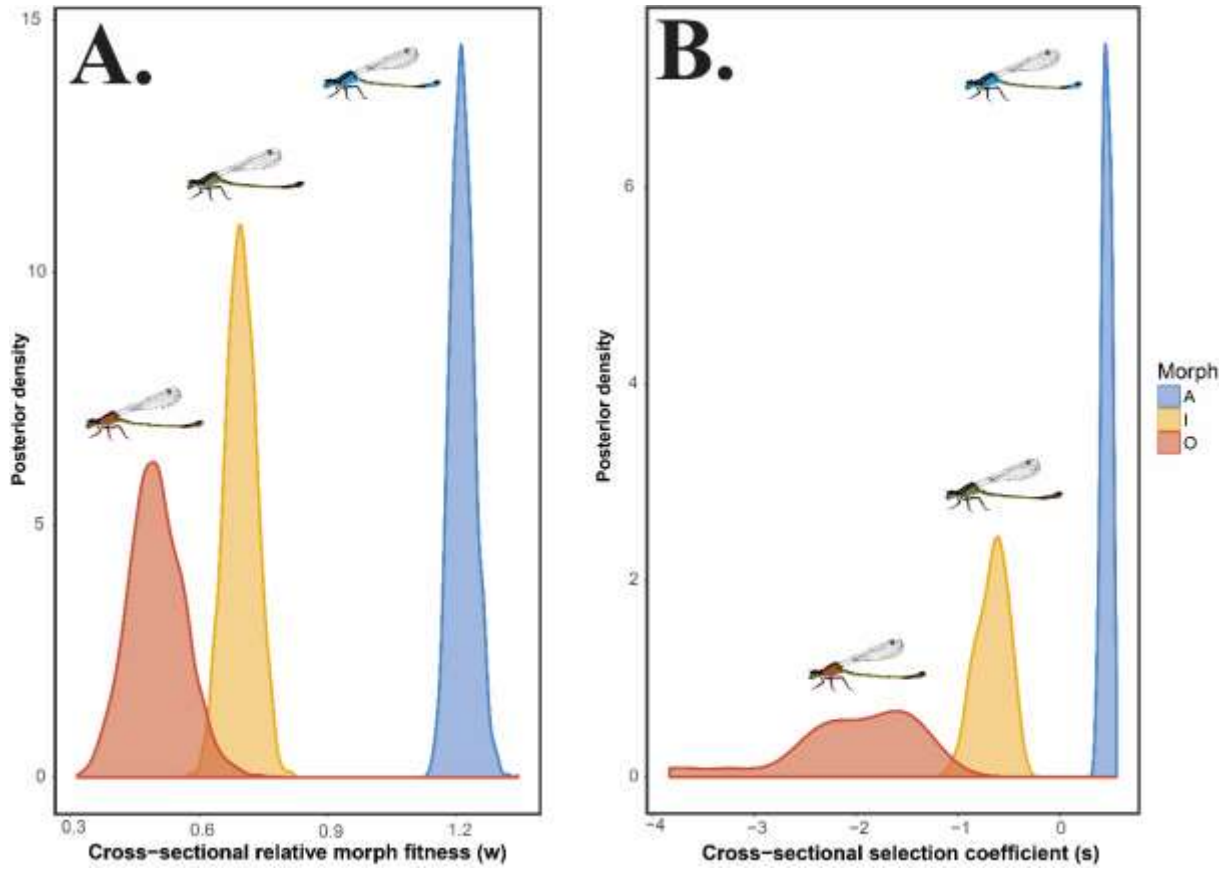
Fig. 3



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Fig. 4



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1098 **Supporting Information (SI) Appendix**

1099 **Table S1.** Relationship between latitude and frequency of the male mimic (androchrome
1100 female morph) in Europe.

1101	Fixed effect	D.f.	Estimate	SE	χ^2	<i>P</i>
1102	Latitude	1	0.137	0.015	81.358	< 0.001 ***
1103						
1104	Random effect	Levels		Variance		Standard Deviation
1105						
1106	Population	128		0.8018		0.8954
1107						

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1109 We used a generalized linear mixed model and the “glmer” function in the nlme/lme4 packages to analyze
1110 geographic variation in the frequency of the male mimicking female morph (A-females) in Europe (Fig. 2A,B). In
1111 this analysis, latitude was treated as a fixed factor, as here we were interested in explicitly estimating the
1112 relationship between latitude and androchrome frequency. In contrast, in a follow-up analysis, we were instead
1113 controlling for latitude by incorporating it as a random factor when we were investigating the role of local
1114 microclimatic variation and temperature on androchrome frequencies (Table S2). In the analysis presented above,
1115 we included population as a random factor to control for local and regional variation in morph frequencies within
1116 a given latitudinal band and also to control for possible effects of longitude.

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1124 **Table S2.** Effects of local microclimatic variation and temperature on biogeographic variation
 1125 in the proportion of the male-mimicking female colour morph.

1126	Fixed effect	D.f.	Estimate	SE	χ^2	<i>P</i>
1127	Maximum temperature during	1	-0.203	0.045	20.593	<0.001***
1128	warmest month (Bio5)					
1129	Random effects	Levels		Variance		Standard Deviation
1130						
1131	Latitude block	7		0.2562		0.5062
1132	Population	128		0.7152		0.8457
1133						
1134						

1135 We used generalized linear mixed models and the “glmer” function in the nlme/lme4 packages to analyze
 1136 geographic variation in the frequency of the male mimicking female morph (A-females) in Europe (Fig. 2A,B)
 1137 and relate these local frequencies to Bioclim variables such as maximum temperature during the warmest month
 1138 (Fig. 2C). We included the population of origin and latitudinal position across Europe as random factors in this
 1139 mixed model to take into account the effect of spatial autocorrelation of local climates. Female morph was treated
 1140 as a binomial variable (probability of being an androchrome female = 1; not being an androchrome female = 0) in
 1141 this mixed model and temperature was a fixed effect. We related androchrome frequencies to the classical
 1142 bioclimatic variables (Bio1-Bio19) involving various measures of temperature and precipitation. The four best
 1143 models (judged by the lowest AIC scores) were all related to temperature: Bio1 (“Annual mean temperature”: AIC
 1144 = 931.6311), Bio2 (“Mean diurnal temperature”: AIC = 927.1970), Bio3 (“Isothermality”; AIC = 926.8641) and
 1145 Bio5 (“Maximum temperature during warmest month”; AIC = 923.0424). However, as all these temperature
 1146 variables are highly correlated with each other (*r* ranging from 0.67 to 0.86; all *P* <0.001), it is statistically difficult
 1147 to causally separate them, and we therefore only present the model containing the variable with the lowest AIC-
 1148 level (Bio5). Notably, Bio6 (“Mean temperature of the coldest month”; AIC = 938.0446) was not significantly
 1149 related to local androchrome frequencies (*P* = 0.72) suggesting that thermal conditions during winter (i. e. during
 1150 the larval stage) do not significantly explain biogeographic variation in adult androchrome frequencies. Instead,
 1151 morph frequencies are better explained by conditions during the warmest months, i. e. during the reproductive
 1152 season.

1153 **LEGENDS TO SUPPORTING FIGURES**

1154 **Figure S1.** Colour development and pigmentation during sexual development in three female
1155 colour morphs of the common bluetail damselfly (*Ischnura elegans*). All three female colour
1156 morphs have a similar blue abdomen patch as immatures that is shared with the males (left
1157 column). This blue abdomen patch is then gradually covered with dark pigment by the two
1158 sexually dimorphic gynochrome (female-like) morphs (I and O; middle and lower rows,
1159 respectively), whereas the androchrome (male-mimic) female morph (A) retains this blue
1160 abdomen patch during its entire life (upper row). At sexual maturity (right column) the I- and
1161 O-females have thus lost their blue abdomen patches which have been entirely covered with
1162 dark pigment (right column). The morphs also differ in the melanin patterning within the blue
1163 abdomen patch in their immature stage; note the arrow-shaped dark markings in the the I- and
1164 O-morph that is lacking in the A-morph (left column).

1165

1166 **Figure S2.** Relationship between local frequency of the male mimic (A-morph) and mean
1167 annual temperature (Bio1; cf. relationship between maximum temperature during warmest
1168 month (Bio5) and frequency of the male mimic in Fig. 2C; Table S2). The relationship between
1169 mean annual temperature and the local frequency of the male mimic is highly significant in a
1170 similar generalized mixed model as in Table S2 ($P = 0.004$), although not as strong as the
1171 relationship between maximum temperature of the warmest month and the frequency of the
1172 male mimic (AIC-values: 923.0424 for Bio5 and 931.6311 for Bio1, respectively).

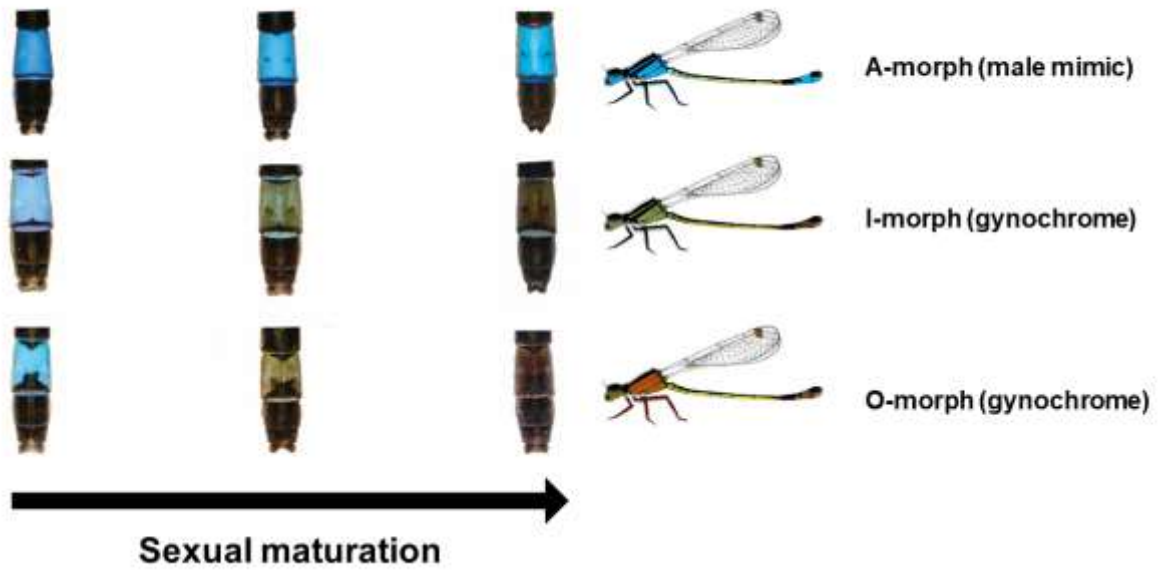
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Colour development and melanization in the three female morphs



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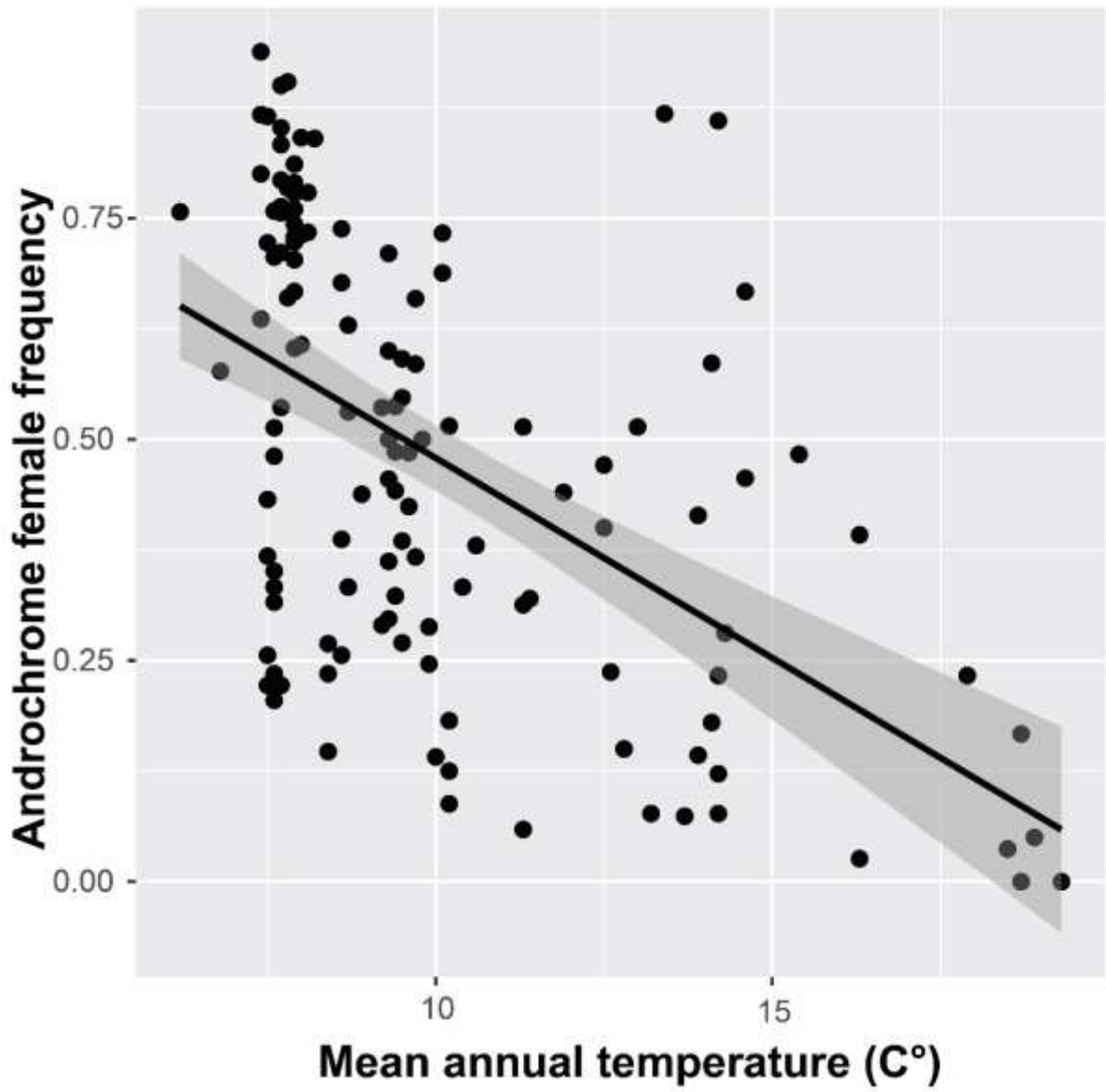
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