Phenotypic plasticity is aligned with phenological adaptation on both micro- and macroevolutionary timescales

Stephen P. De Lisle[1]*, Maarit I. Mäenpää[2], & Erik I. Svensson[1]

[1]Evolutionary Ecology Unit, Department of Biology
Lund University
Sölvegatan 37 223 62
Lund, Sweden
[2]Department of Zoology
Stockholm University
SE-106 91 Stockholm
*Email: stephen.de_lisle@biol.lu.se

Keywords: Phenology, phenotypic plasticity, microevolution, macroevolution, *Ischnura elegans*

Running title: Micro to macroevolution of phenological plasticity

Article Type: Letter

Figures/tables/boxes: 5/0/0

References: 83

Word count: 150 (abstract) 4999 (Main text)

Data: All data are provided on Dryad
https://datadryad.org/stash/share/FKHv4E_XmOX2f4yEi63pTibgzqmNFuXXhJLi_lnV3J0

Author Contributions: All authors contributed to study design and data collection. M.I.M and S.P.D. performed the laboratory experiment with input on the design from E.I.S., SPD compiled the comparative data, and E.I.S. provided the long term data from *I. elegans*. S.P.D. performed the statistical analyses and drafted the manuscript; all authors contributed to revising the manuscript.

Abstract

In seasonally-variable environments, phenotypic plasticity in phenology may be critical for adaptation to fluctuating environmental (temperature) conditions. Using an 18-generation longitudinal dataset from natural insect (damselfly) populations, we show that phenology has strongly advanced. Individual fitness data suggest this is likely an adaptive response towards a thermally-dependent fitness optimum. A laboratory experiment revealed that developmental plasticity qualitatively matches environmental-dependence of selection, partially explaining observed phenological advance. Expanding our analysis to the macroevolutionary level, we use a database of over 1-million occurrence records and spatiotemporally-matched temperature data from 49 Swedish Odonate species to infer macroevolutionary dynamics of phenology. Phenological plasticity was more closely aligned with adaptation for species that have recently colonized northern latitudes, with more phenological mismatch at lower latitudes. Our results show that phenological plasticity plays a key role in microevolutionary dynamics within a single species, and such plasticity may have facilitated post-Pleistocene range expansion in this insect clade.

Introduction

In many organism, life is characterized by dramatic life-history transitions between discrete stages that correspond to the unique demands of resource acquisition versus reproduction. For organisms with complex lifecycles, these life history transitions span disparate ecological niches, such as aquatic versus terrestrial environments, that demand irreversible metamorphosis to achieve such extreme ontogenetic niche shifts (Wilbur 1980; Werner & Gilliam 1984). The timing of such life history transitions, or phenology, is particularly crucial for organisms with complex life cycles that undergo their metamorphosis in seasonally variable environments. This is because success during a given life stage must depend not only on biotic factors such as predation, competition and seasonally-changing resources, but also on aspects of the abiotic environment, such as temperature, that vary across both time (generations) and space (between populations) (Hassal *et al.* 2007; Angilletta 2009; Plard *et al.* 2014; Visser *et al.* 2015; Bonamour *et al.* 2020). Thus, phenology is expected to be under net-stabilizing selection (Villemereuil *et al.* 2020) with an optimum that is shaped by various counteracting selection pressures that are in turn influenced by multiple biotic and abiotic environmental variables, including temperature, precipitation, competition and resource availability.

The location of phenological optima may fluctuate randomly in response to environmental stochasticity across years in seasonally variable environments (Hadfield 2016). Concomitantly, such fitness optima may show directional change and track advancing temperatures and resource abundances associated with anthropogenic climate change (Brashaw & Holzapfel 2001; Bradshaw & Holzapfel 2006; Parmesan 2006; Anderson *et al.* 2012; Thackeray & al. 2016). In both scenarios, phenological plasticity (phenotypic plasticity in the timing of life history transitions) is expected to be a target of natural selection (Chevin *et al.*

87 2010; Chevin & Lande 2015). Individuals that adaptively alter their developmental trajectory in
88 response to available environmental cues regarding the conditions occurring during or after the
89 life history transition (Whiteman 1994), will undergo metamorphosis to closely match the
90 phenological optimum and will therefore have a selective advantage (Chevin *et al.* 2010).
91 Phenotypic plasticity in phenology is therefore an expected key evolutionary adaptation to
92 seasonally variable environments (Levins 1968). This classical hypothesis has obtained
93 qualitative support in a number of studies across a range of taxa that all suggest a key role for
94 plasticity in explaining between- and within population differences in phenological traits
95 (Charmantier *et al.* 2008 ; Boutin & Lane 2014; Crozier & Hutchings 2014; Franks *et al.* 2014 ;
96 Nashoba & Kono 2020; Stamp & Hadfield 2020). Only a few of these studies includeorganisms
97 with complex life cycles (Phillimore *et al.* 2010; Stoks *et al.* 2014; Urban *et al.* 2014; Buckley &
98 Kingsolver 2019).

99 Recent work has recast the concepts of plasticity, fitness, and environmental variation in
100 terms of estimable quantitative genetic parameters that together describe plasticity's potential
101 contribution to adaptive evolution. In this framework (Figure 1) plasticity, termed *b*, and the
102 environmental dependence of selection, termed *B*, jointly determine the extent that phenotypic
103 plasticity contributes to local adaptation (Chevin *et al.* 2010). The strength of plasticity *b*
104 describes the relationship between expressed population mean phenology and the average
105 environment experienced by a population. This parameter (*b*) represents the population-mean
106 reaction norm. The environmental dependence of selection, *B,* describes the relationship between
107 the optimum phenology and the mean environment, and so represents the degree to which natural
108 selection changes as a function of environmental variation. Importantly, estimation and
109 comparison of these parameters can provide insight into plasticity's role in adaptation (ability to

track the optimum) to spatio-temporal environmental variation (Hadfield 2016). New statistical and analytical approaches have allowed researchers to leverage observational individual occurrence records to estimate these parameters indirectly, via space-for-time substitution (Phillimore *et al.* 2010). A growing body of studies have employed these or similar time-series based approaches (Phillimore *et al.* 2010; Crozier *et al.* 2011; Phillimore *et al.* 2012; Phillimore *et al.* 2016; del Mar Delgado *et al.* 2020; D. & Wilson 2021). This work (see also Villemereuil *et al.* 2020) indicates that plasticity can explain a substantial fraction of spatio-temporal variation in phenology across natural populations in the wild.

It is, however, still unclear whether and to what extent plastic variation in phenology matches our expectations for plasticity's role in evolution. In particular, two critical open questions on phenological plasticity's role in evolution remain unanswered: 1) does phenotypic plasticity correspond to environmental-dependence of phenological optima at the microevolutionary scale, thus amplifying or accelerating adaptation? and 2) is the role of plasticity in adaptation greater for lineages inhabiting variable and/or recently colonized environments? Answering the first question requires long-term data on individual fitness in the wild. Answering the second requires comparative analysis of multiple species or lineages that vary in ecology yet which share a similar and conserved complex life cycle.

Here we combine three independent, large-scale datasets from the insect order Odonata (damselflies and dragonflies). Odonates are semi-aquatic insects with a phylogenetically conserved complex life cycle characterized by metamorphosis from aquatic nymph (henceforth, 'larvae') to aerial reproductive adult (Stoks & Córdoba-Aguilar 2012). Our aim was to address the above two related questions on phenological plasticity's role in adaptation at short and deep timescales.

Methods – Data Collection

Long term study of Ischnura elegans

We analyzed data on individual phenology and fitness in a set of populations (approximately 16) of the damselfly *Ischnura elegans* in province of Skåne, southern Sweden, for 18 years (2000-2017). Each year, these populations were surveyed in a standardized approach during the reproductive season of *I. elegans*. All captured individuals were sexed and their copulation status (mated or not) was recorded. In this study, we used data on one major male fitness component: male mating success (a binomially distributed variable) to estimate the strength of selection on phenology through mating success.

Laboratory experiment on thermal plasticity in development time

We reared larvae of individual *I. elegans* from the egg stage, through hatching and metamorphosis to adults, under two different larval temperature environments: 20 °C and 24 °C. Maternal egg clutches (full sib clutches) were obtained from females caught *in copula* in the field in the general population survey described above. Individual eggs and larvae from a total of 101 full-sib clutches were reared in a split-clutch GxE design across both environments.

Occurrence records of Swedish Odonata

We obtained public, spatiotemporally referenced observational records of adult Swedish odonates identified to species level from the Swedish Species Observation System (Artportalen: https://www.artportalen.se/). We used data from a ten year period from 2006-2015, the range of years for which we had accurate temperature data and for which substantial observational records were available in this public database. We obtained spatiotemporally matched estimates

of spring mean surface temperature from the publicly accessible NOAA Global Forecast System Analysis (GFS ANL) database (https://www.ncdc.noaa.gov/data-access/model-data/model-datasets/global-forcast-system-gfs). We obtained the mean surface temperature from corresponding lat-long grid cell in the GFS ANL database for two time windows: the first 120 days of the year and the month of April. The first time window corresponds to a substantial period of pre-metamorphosis larval development and covers the last instar for all the species in our database, and so represents a long-term average temperature environment experienced during a key stage of development. The second time window represents a shorter time slice from late spring, just prior to the flight period of many early season species. Finally, we note that the scale and purpose of our analysis precludes use of a "sliding window" approach; Our interest is to compare developmental-temperature reaction norm variation across species (and so any such comparison requires use of similar or the same environmental cues), rather than to identify a specific time slice of spring temperature variation that is most important for a single species.

Methods – Statistical Analysis

Long term field study of Ischnura elegans

To infer selection on phenology, we modify the Lande-Arnold (Lande & Arnold 1983) equation to include temperature dependence in the form of directional selection on phenology:

$$w = \mathrm{a} + \beta \mathrm{z} * (1 + \varphi \mathrm{t}) + \frac{1}{2}\gamma \mathrm{z}^2 + \varepsilon \qquad 1$$

where $\varphi = \frac{\phi}{\beta}$, ϕ is an interaction term as estimable in a regression model. Setting the first derivative of equation 1 with respect to *z* to zero and solving yields the stationary point:

$$\theta_z = \frac{\beta}{-\gamma} + \frac{\phi}{-\gamma} t \qquad 2$$

178

179 where θ_z is the optimum phenology (if selection is stabilizing, otherwise it is a fitness

180 minimum). The slope of the relationship between temperature and θ_z is $\frac{\phi}{-\gamma}$, and represents a

181 direct estimate of the temperature dependence of selection (*B*).

182

183 *Laboratory temperature experiment: raising damselfly families*

184 We used a mixed effects model to estimate the relationship between thermal developmental

185 environment of larvae (two temperature treatments) and emergence time from our laboratory

186 experiment. Our model featured emergence day as the response variable, temperature treatment

187 as a fixed effect, and random intercepts for tank and maternal full-sib family

188

189 *Occurrence records of Swedish Odonata and phylogenetic comparative analyses*

190 We used a bivariate mixed modelling approach (Phillimore *et al.* 2010; Phillimore *et al.* 2012) to

191 infer interspecific variation in thermal reaction norms in phenology from our observational

192 records of Swedish Odonata combined with paired estimates of temperature in the first 120 days

193 of the year. We fit the bivariate model

194

$$\zeta_i = \mu_i + \beta_{1,i} * y + \beta_{2,i} * x + a_{lat} + a_{time(lat)} + \varepsilon \qquad 3$$

195

196 where ζ_i is the response vector of individual values of two variables: *i* = observed phenology or

197 spring temperature. β is a fixed effect regression coefficient between continuous latitude (*y*) or

year (x) and variable i. Two nested random effects are included in this model: a_{lat} is a random effect describing (co)variation across binned latitude, and $a_{time(lat)}$ is a random effect describing co(variation) across binned annual seasons within latitudinal grids. For each a and the residual ε we estimated the full 2x2 unstructured covariance matrix $\begin{bmatrix} \sigma^2_{phenology} & \sigma_{phenology,temp} \\ \sigma_{phenology,temp} & \sigma^2_{temperature} \end{bmatrix}$, for a total of three 2x2 random effect covariance matrices. We focus on analysis of latitudinal spatial variation because Sweden spans a far greater latitudinal than longitudinal range, and corresponding species distributions within Sweden reflect substantial latitudinal span but little longitudinal span.

From this model we can interpret the ratio of the fixed effect parameter estimates, particularly $\frac{\beta_{lat,\ \text{phenology}}}{\beta_{lat,\ \text{temperature}}}$ as approximating the environmental dependence of the optimum, B (Hadfield 2016). From the random effects, we can interpret $b \sim \sigma_{phenology,\ temperature} / \sigma^2_{temperature}$ where σ and σ^2 are estimated across time points within binned latitude ($a_{time(lat)}$). This bivariate model was fit separately for each species in our dataset. We then used the corresponding estimates of B and b in phylogenetic mixed models (Hadfield & Nakagawa 2010) to explore the relationship between species mean latitude, species mean phenology, and the strength and form of inferred phenological plasticity. Our models accommodated uncertainty in our estimates of B and b.

Results

We found evidence of significant phenological advance during the 18 year study period of *I. elegans* in the field ($F_{1, 47526} = 1231.38$, $P < 0.0001$, Figure 2B). Although there was significant among-year heterogeneity, on average the emergence date has advanced by .58 days (SE =

220 0.017) per year, corresponding to a predicted total advance of 10.44 days across the entire 18-
221 year period.

222 Using data on male mating success (N = 21,384 individuals for which we also had
223 annual temperature data, consisting of 5,487 mated males and 15,897 non-mated males), we find
224 evidence of statistically significant but annually variable (Figure 3B) sexual selection on
225 phenology. Overall, we found evidence of significant stabilizing selection on phenology
226 (estimates of parameters in equation 1, in units of raw days: γ = -0.00016, SE = 0.000072, χ_1^2 =
227 4.51, P = 0.0336; β = 0.001308, SE = 0.000972, χ_1^2 = 1.18, P = 0.2; binomial glmm: γ χ_1^2 =
228 6.99, P = 0.0177; β χ_1^2 = 2.16, P = 0.16), along with a significant temperature-dependence of
229 selection on phenology (ϕ = -0.00156, SE = 0.000593, χ_1^2 = 6.90, P = 0.0086; binomial glmm:
230 χ_1^2 = 5.02, P = 0.0251). Fitting a simplified model with directional selection only and random
231 variation among years to variance-standardized data indicates standardized directional selection
232 gradients varied substantially among seasons (ΔAIC = 265, overall estimate β = 0.027, SE =
233 0.021; see figure S1). The resulting estimate of the relationship between θ_z and spring mean
234 temperature indicates a temporal advance in the optimum phenology with increasing temperature
235 (Figure 3C). Our point estimate of *B*, the slope of the relationship between temperature and θ_z ,
236 given by the ratio of the parameter estimate $\frac{\phi}{-\gamma}$, indicates an advance of 10.24 days
237 (Bootstrapped 95% CI: -66.7995 – -0.59705) in the phenological optimum for each degree
238 Celsius of spring warming (Figure 3C). We also found evidence of negative *B* using an
239 independent dataset of 1695 observations from the Artportalen database when we inferred B
240 indirectly from interspecific data on phenology, although this indirect estimate was significantly
241 shallower than direct estimate we obtained from this long-term population study (Supplemental
242 material, Figure S2).

We obtained individual and family emergence time records from 521 individuals from 101 full-sib genetic families across two temperature treatments in our laboratory experiment. We found significant acceleration of development in response to our experimental manipulation of temperature ($F_{1, 367} = 65.61$, $P < 0.0001$, Figure 3A), with an estimated reaction norm of -17.38 (SE = 2.15) days per 1 °C warming of the developmental environment. This laboratory estimate therefore corresponds in sign, and to some extent in magnitude, with our estimate of the environmental dependence of θ_z estimated from the field data in the natural populations of *I. elegans* (Figure 3C). We found statistically significant broad-sense heritability in the timing emergence ($H^2 = 0.24$, $\chi_1^2 = 17.54$, $P < 0.0001$; SE: 0.078, 95% CI: 0.079-0.39; $V_a = 993$ SE: 245, $V_p = 4156$ SE = 326; $CV_A = 5.3\%$). Our finding of a negative thermal reaction norm in larval development time that aligns with the environmental dependence of selection on phenology was supported by an independent, indirect inference of *b* using observational data (although the magnitude of these estimates differs substantially; Figure S2).

We analyzed paired temperature and phenology data from 1,027,952 records from 49 species of Swedish Odonates (see Table S1). We explored two temperature windows as predictors of phenological variation: the mean temperature of the first 120 days of the year, and the mean April temperature. We found substantial support for the 120 day window, as these models consistently fit better than the April mean models for nearly all species (Figure S3). We then kept the 120 day average models for downstream analysis.

Reaction norm estimates varied across species, although most estimates where within the range of ± five days per °C of spring temperature variation (Figure 4A, Table S1). Notably, there were a mix of both positive and negative reaction norm estimates and many species that showed positive reaction norm slopes (Fig. 4). These analyses show that temperature during the last

instar larval stage can either advance or delay emergence, depending on species, and some species were relatively insensitive to spring temperature (Fig. 4A). On average, the estimated thermal reaction norms explained 1.4% of the all variation in phenology (Table S1). In comparison to our estimates of b, estimates of B exhibited substantial sampling variance (Table S1).

We found evidence of a significant phylogenetic signal in the phenological reaction norm (Phylogenetic h^2 = 0.96 ΔDIC = 239, posterior distribution in Figure 4B), revealing that closely related species have similar magnitude of thermal plasticity in phenology (Figure 4). Estimated reaction norms were correlated with mean phenology across species, with early season species exhibiting more negative reaction norms and late season species exhibiting positive reaction norms (posterior mean coefficient: 0.03192, 95% CI: 0.01915 – 0.04435, pMCMC < 0.001, Figure 5A). We also found a significant negative relationship between reaction norm slope and latitude in a phylogenetic mixed model with latitude as a predictor of estimated reaction norm (posterior mean coefficient: 0.40298, 95% CI: 0.03198 – 0.78868, pMCMC = 0.036, Figure 5B).

The alignment between the estimated phenological reaction norms, b, and the estimated environmental dependence of selection, B, scaled by the magnitude of B (i.e., |B-b|/|B|), varied significantly with species mean latitude (posterior mean coefficient: -0.054454, 95% CI: -0.115952 – -0.004226, pMCMC = 0.048; with outlier dropped, posterior mean coefficient: -0.055290, 95% CI: -0.105413 – 0.004944, pMCMC = 0.046; Figure 5C). When |B-b|/|B| is less than unity plasticity can be considered adaptive; when above unity plasticity can be described as maladaptive (Phillimore *et al.* 2016). Species at northern latitudes had thermal reaction norms that were more closely aligned with their estimated environmental dependence of phenological

optima, resulting in adaptive plasticity at northern latitudes (Figure 5C). Finally, plasticity *b* explained a greater amount of detrended variation in phenology (calculated as $p*([\sigma_{phenology,temp}/\sqrt{\sigma^2_{phenology}}\sqrt{\sigma^2_{temp}}]^2)$ were the term in parentheses is at the level of time(lat) and *p* is the proportion of the total variance in phenology that is explained by the time(lat) level) in Northern than in Southern species (posterior mean coefficient: -0.6489, 95% CI: - 1.1821 – - 0.1102, pMCMC = 0.018, Figure 5D).

Discussion

Here we combine three independent datasets, together representing well over 1 million individual insect records, to test two related questions on the role of phenotypic plasticity in the evolution of phenology. First, we demonstrate pronounced recent phenological advance in a single damselfly species, *Ischnura elegans*, using a long term study of individuals in the wild. We find that thermal plasticity in developmental time in the laboratory is substantial, corresponding in sign and to some degree in magnitude with the relationship between temperature and the optimum phenology of the same species estimated in the field. This suggests an important role for thermal plasticity in adaptation to seasonal environmental variability, and also suggests plasticity could contribute to advancing phenology that corresponds to recent anthropogenically-caused climate change in Sweden (SMHI 2015). At the macroevolutionary scale, we used a large interspecific database of individual emergence records of 49 species of odonates spanning the extensive latitudinal gradient of Sweden. Combining these occurrence records with spatiotemporally-matched estimates of spring mean temperature, we found that species-specific mean phenological reaction norms are more positive and more closely aligned with indirect inferences of the environmental dependence of the optimum for northern than for

312 southern species. We also find that plasticity contributes more to variation in phenology at
313 Northern latitudes. Our combined intra- and interspecific results indicate that temperature-
314 dependent plasticity in phenology underlies both microevolutionary adaptation to contemporary
315 climate change as well as the macroevolutionary expansion into harsh high-latitude climatic
316 conditions that took place when species colonized these areas after the last Ice Age in
317 Fennoscandia, about 10,000 years before present.

318 We found a phenological advance in a single species (*Ischnura elegans*) of 0.58
319 days/year, almost six days per decade or 10.44 days over the entire 18-year study period (Fig. 1).
320 This is a dramatic phenological advance during the first two decades of the century in Sweden.
321 In fact, this is almost twice as large advancement of phenology as was documented previously
322 for *I. elegans* in a study of British Odonata for the period 1960-2004 (0.58 vs. 0.33; Hassall *et al.*
323 2007). Thus, although our findings of phenological advance are consistent with previous study in
324 Britain, the new results presented here suggest temperature-dependent phenological advance in
325 odonates may be more extreme than previously appreciated.

326 We find some correspondence between point estimates of the environmental dependence
327 of selection in *Ischnura elegans* and the population mean reaction norm, using three independent
328 datasets and approaches. Our direct estimate of B using data on individual fitness from the long-
329 term study on *I. elegans* and our indirect estimates using a public database of individual
330 occurrence records both suggest that the optimum emergence time advances substantially for
331 every centigrade degree of spring warming in *I. elegans*. Our direct estimate of the phenological
332 reaction norm matches with our direct estimate of the environmental dependence of selection,
333 and the correspondence is also found using a bivariate mixed model approach to indirectly
334 estimate these parameters (see Supplemental Material). Although absolute values of B and b

differ substantially across these two approaches (direct vs indirect), the qualitative conclusion – that phenotypic plasticity can explain the close match between local optima set by environmental variation – is similar across these different approaches and datasets. One the one hand, this correspondence in qualitative interpretation (alignment of *B* and *b*) is encouraging and suggests that our space-for-time substitution approach to estimating environmental dependence of optimal phenologies may be robust to inevitable violation of the assumptions (e.g., local adaptation and limited dispersal) that underlie the theory behind the method (Hadfield 2016). On the other hand, we found a significant disparity in the magnitude of both *B* and *b* using these different approaches; our estimates of *B* and *b* were significantly steeper in our direct assessment (Figure S2). A likely explanation for this is that our direct estimate of these parameters was focused on a more limited section of the geographic range, compared to our indirect estimate using data from *I. elegans* across the latitudinal breadth of Sweden. In another comparison between these types of approaches, Phillimore et al. (Phillimore *et al.* 2016) found relatively close correspondence between different estimates of *B* in great tits (see also van Benthem *et al.* 2017 for a comparison of other related approaches). The results presented here echo past suggestions (Phillimore *et al.* 2016) that further validation of indirect estimates of *B* is warranted.

Our laboratory breeding experiment revealed a strong effect of temperature at larval stage on development and emergence time, with an advance of 17 days per degree Celsius. The fact that this estimate of the mean reaction norm was steeper than our estimate of the environmental-dependence of selection might suggest 'hyper plasticity' (Stamp & Hadfield 2020) wherein plastic responses have the potential to exceed fluctuations in the optimum (see Radersma et al. 2020). Nevertheless, our laboratory experiment indicates that the strength of temperature-dependent plasticity is qualitatively similar to our estimate of the environmental dependence of

358 selection and is broadly aligned with the direction of temporal phenological advance in response
359 to rising temperatures in natural populations (Fig. 3C). Indeed, the estimated population reaction
360 norm in *I. elegans* suggests that an adaptive fit between the thermal optimum and mean
361 phenology could be explained entirely by phenotypic plasticity. This indicates that *I. elegans*
362 may already be well-adapted to cope with changing climates and increasing temperature
363 compared to many other animal taxa, such as birds (Radchuk *et al.* 2019). Previous studies on
364 gene expression of heat- and cold shock proteins in *I. elegans* adults originating from different
365 parts of the geographic range indicate that adaptive thermal plasticity plays an important role in
366 the northward range expansion of this species (Lancaster *et al.* 2015; Lancaster *et al.* 2016), in
367 addition to genetic adaptation (Dudaniec *et al.* 2018).

368 Our laboratory experiment also revealed that development time was modestly heritable,
369 with a point estimate of $H^2 = 0.24$. This is considerably lower than a previous estimate of the
370 heritability of development time in this species (0.52), estimated in a single thermal environment
371 (Abbott & Svensson 2010). The modest level of heritability of development that we document
372 here would require a persistent variance-standardized directional selection gradient of
373 approximately $\beta = -.13$ to result in the observed annual phenological advance of 0.58 days/year
374 (approximately .03 within-year standard deviations). This would be relatively strong selection
375 compared to available evidence from field studies on phenotypic selection (Kingsolver *et al.*
376 2001). Although our results and analyses do indeed suggest that selection can be strong, we also
377 find little evidence of persistent directional selection of the magnitude required to produce such a
378 dramatic response. In fact, across all years, net selection on phenology is actually slightly
379 positive rather than negative as would be expected if earlier phenology was consistently favored
380 (Fig. S1). Therefore, it is unlikely that genetic evolution alone can entirely explain the strong

381 phenological advance of more than 10 days across the entire 18-year period, given the substantial
382 among-year variation (Figure 2) of *I. elegans* in southern Sweden. Instead, our analyses suggest
383 that phenotypic plasticity may have an important role in driving both inter-annual variation in
384 phenology and the progressively earlier flight seasons that we have documented here. These data
385 suggest that *I. elegans* can track changing phenological optima through plasticity, which will in
386 effect reduce or counteract directional selection for earlier phenology, a conclusion that was also
387 reached in a recent study of 21 bird and mammal species with long-term data (Villemereuil *et al.*
388 2020). More generally, various forms of phenotypic plasticity have been proposed to "buffer"
389 organisms against external stress from temperature extremes (Huey *et al.* 2003).

390 We found significant, phylogenetically-structured among-species variation in inferred
391 reaction norms in an analysis that explicitly accounted for uncertainty in these species-specific
392 estimates. Our point estimate indicates a phylogenetic heritability of near unity, indicating that
393 closely related species share similar phenological responses to temperature variation. The
394 significant phylogenetic signal in the reaction norm estimates is consistent with the idea that
395 species differences in ecology and physiology play important roles in generating reaction norm
396 variation across this clade, most likely because closely related species show a high degree of
397 niche conservatism (Wiens & Graham 2005; Svensson 2012; Wellenreuther *et al.* 2012). Our
398 findings add to a growing body of evidence from other taxa (Davis *et al.* 2010; Davies *et al.*
399 2013; Rafferty & Nabity 2017), indicating strong phylogenetic signal in phenological response to
400 climatic change.

401 Theory suggests that the fitness benefit of phenological plasticity depends upon the
402 existence of environmental cues that accurately predict the conditions that determine fitness in
403 the next life stage (Chevin & Lande 2015). Here we have focused on spring mean temperature

404 as a potential environmental cue (or correlate thereof). This is justified by substantial empirical
405 evidence linking temperature and corresponding thermal tolerance to various aspects of adult
406 fitness in insects in general (Punzalan *et al.* 2008; Garcia-Roa *et al.* 2020), and our study species
407 *I. elegans* in particular (Lancaster *et al.* 2015; Lancaster *et al.* 2016; Dudaniec *et al.* 2018;
408 Svensson *et al.* 2020b). Moreover, our laboratory experiment confirms that larval developmental
409 temperature is a predictor of emergence time into the transition to the adult aerial life-stage.
410 Across all our interspecific dataset, all but one species in our comparative analysis over-winter as
411 either aquatic larvae or eggs, and so are exposed to spring temperatures at a similar
412 developmental stage. The only exception to this rule is the winter damselfly *Sympecma fusca*,
413 which overwinters as an adult in vegetation refugia. Consistent with this, *S. fusca* exhibited one
414 of the most extreme negative estimate of *B* (Table S1), as well as a negative estimate of *b* (Fig.
415 4A). This fits with previous research showing that temperature-phenology reaction norms tend to
416 be strongest in insects that over winter as adults (Forrest 2016). Across all the species included
417 in this study, other environmental cues are also likely to be important for determining
418 developmental rate; including aspects of the biotic environment, such as density of con- or
419 heterospecific competitors, resource availability, and photoperiod. These unknown additional
420 cues could explain much of the residual variance in phenology. Nevertheless, our focus on
421 temperature is relevant in light of anthropogenic climate change (Parmesan 2006) and the
422 general consensus that temperature is a strong determinant of fitness in ectotherms (Angiletta
423 2009).

424 The degree of alignment between the phenological reaction norms and environmental
425 dependence of selection, and the contribution of plasticity to variation in phenology, changed
426 across the latitudinal range of these 49 species in Sweden (Fig. 5). This indicates that adaptive

427 phenological plasticity has played an important role in the northern range expansion of this
428 clade. Insect colonization of the Scandinavian peninsula occurred relatively recently following
429 retreat of the Fenno-Scandian ice sheet, which covered the region from the last glacial maximum
430 until its recession at the end of the Pleistocene approximately 10,000 years ago (Hewitt 1999,
431 2000). Thus, odonate species that currently occur in northern Sweden have established
432 themselves in this region more recently than the ones in southern Sweden. Our findings of
433 stronger evidence of adaptive plasticity in northern species in our phylogenetic comparative
434 analysis (Fig. 5) is consistent with theoretical predictions (Lande 2009; Chevin & Lande 2011)
435 that plasticity might be particularly important in adaption to novel and variable environments,
436 such as near range limits (Lancaster *et al.* 2015; Lancaster *et al.* 2016). Conversely, we found
437 that southern species currently show some degree of non-adaptive or even maladaptive plasticity
438 in relation to temperature as they show values of scaled |B-b| greater than unity (Fig. 5C). A
439 caveat here is that our estimates of *B* (both direct and indirect) exhibit substantial sampling
440 variance (Table S1; Results, Figure 3C), due in large part to ratio effects that amplify effects of
441 latitudinal regression coefficients / selection coefficients that compose the indirect/direct
442 estimate of *B*, respectively. Even our direct estimate of *B* from *I. elegans*, inferred using a large
443 dataset of individual component fitness measures, exhibited high sampling variance, which
444 highlights the challenges of estimating and interpreting these parameters even in the presence of
445 a large dataset.

446 Despite these challenges, our analyses are broadly consistent with recent research and
447 indicate that phenotypic plasticity can be adaptive (Noble *et al.* 2019; Stamp & Hadfield 2020;
448 Svensson *et al.* 2020a; Johansson *et al.* 2021), although we note that the much observed
449 plasticity in our study is not necessarily adaptive. Interestingly, there was some degree of mis-

alignment between *b* and *B* in the southern species *I. elegans* (Fig. 3C), which is broadly consistent with the greater mis-alignment in southern, compared to northern species at the macroevolutionary level (Fig. 5C). More generally, plasticity can be adaptive and selected against in unpredictable environments (Leung *et al.* 2020) and recent meta-analyses have documented that the degree of plasticity sometimes exceeds genetic differentiation, revealing non-adaptive or maladaptive plasticity (Radersma *et al.* 2020; Stamp & Hadfield 2020).

By combining multiple and independent datasets, we have revealed a potential role for phenotypic plasticity in explaining intra- and interspecific variation in phenology. Our combined intraspecific and phylogenetic-comparative analyses enabled us to evaluate the role of phenological plasticity in post-Pleistocene micro- and macroevolution of the temperature-dependence of insect phenology. Our work indicates that temperature-dependent phenological plasticity can at least partly track changing optima due to recent climate change, and that such adaptive phenological plasticity may also have facilitated historical range expansion into variable and extreme northern environments.

Acknowledgements We thank the many student interns that assisted with collection of the long-term *I. elegans* data, and especially Rachel Thomson and Tilly Pembury Smith who helped carry out the laboratory breeding experiment during 2017-2018. Deborah Goedert and Ally Phillimore provided critical feedback on the manuscript. Funding was provided by a postdoctoral scholarship grant to M.M. from The Emil Aaltonen Foundation, grants from the Royal Swedish Academy of Sciences and the Royal Physiographical Society of Lund to S.P.D., and research grants from The Swedish Research Council (VR; grant no. 2016-03356), Gyllenstiernska Krapperupstiftelsen (grant no. KR2018—0038), Lunds Djurskyddsfond, "Olle Engqvist Byggmästare Foundation", John Templeton Foundation (grant no. 60501) and Stina Werners Foundation to E.I.S.

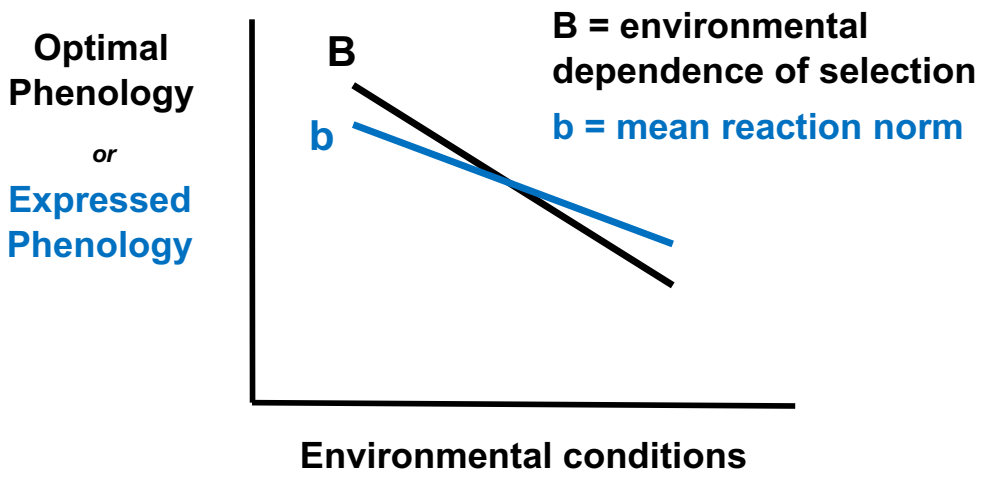


Figure 1. The environmental dependence of selection and the mean reaction norm jointly determine the role that phenotypic plasticity plays in adaptation. The x-axis represents an environmental variable important for individual fitness; the y-axis represents phenology, the timing of a key life history transition. The slope of the regression of the optimal phenology on environmental variation, B, represents the degree to which selection on phenology depends on the environment, shown in black. The degree to which expressed population mean phenology depends on environmental variation is represented by the population mean reaction norm, b, shown in blue. The degree to which B and b align determines the potential contribution of phenotypic plasticity to adaptation to environmental variation. Importantly, plasticity b is

adaptive when it moves a population closer to the optimum than it would otherwise be following an environmental shift.

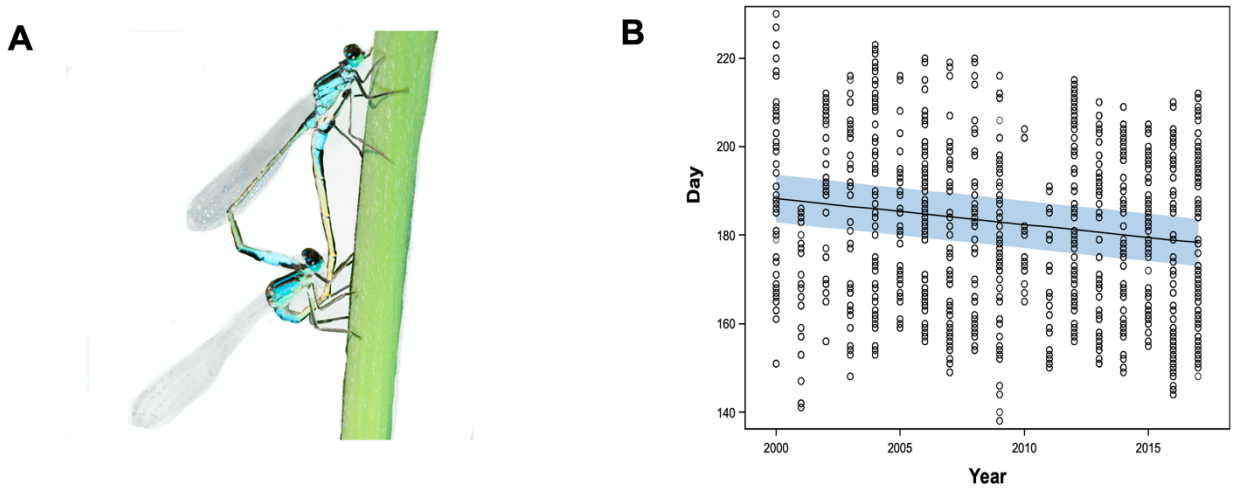
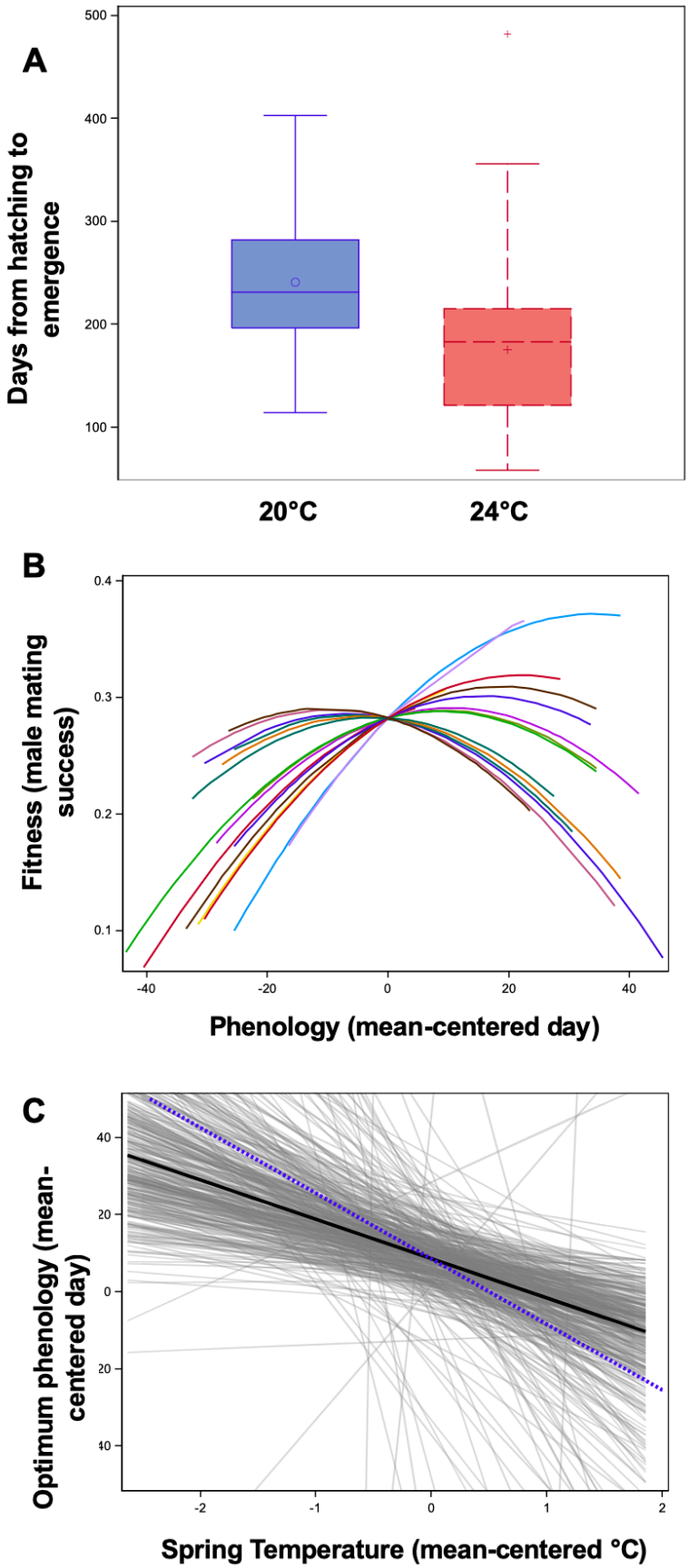


Figure 2. Reproductive advance in *I. elegans* in Southern Sweden. *Ischnura elegans*, a common European damselfly, exhibits an aerial reproductive adult stage (**A**) that follows a prolonged aquatic larval growth stage. Long term study of a set of populations in southern Sweden over 18 years indicates a significant advance in the timing of the aerial adult phase (**B**). Conclusions were unaffected by exclusion of data from the year 2000. Blue shading in B shows 95% confidence limit of the prediction.


A
Days from hatching to emergence
500
400
300
200
100
20°C
24°C
B
Fitness (male mating success)
0.4
0.3
0.2
0.1
-40
-20
0
20
40
Phenology (mean-centered day)
C
Optimum phenology (mean-centered day)
40
20
0
20
40
-2
-1
0
1
2
Spring Temperature (mean-centered °C)

518

Figure 3. Temperature dependent selection and plasticity are aligned in *I. elegans*. In a laboratory experiment, increased larval developmental temperature was associated with accelerated time to metamorphosis as adults. **A**. Yaxis shows days from hatching to emergence. In a long term study of *I. elegans* in the wild, selection on phenology (measured as male mating success) varied across years but overall, selection was net-stabilizing, **B**, with optima that vary slightly across years (lines show model fits from the fixed effects across years; each color represents a unique year). The estimated optimum was negative temperature dependent, **C**. Panel C shows the linear relationship between θ_z and spring mean temperature (equation 2 in text); black line shows original ML estimates, grey lines show 400 samples from a multivariate normal distribution centered at the ML estimates with covariance equal to the covariance matrix of fixed effected from the fitted model (i.e. a parametric bootstrap). Dashed blue line shows the estimated reaction norm from the plasticity experiment (see panel A).

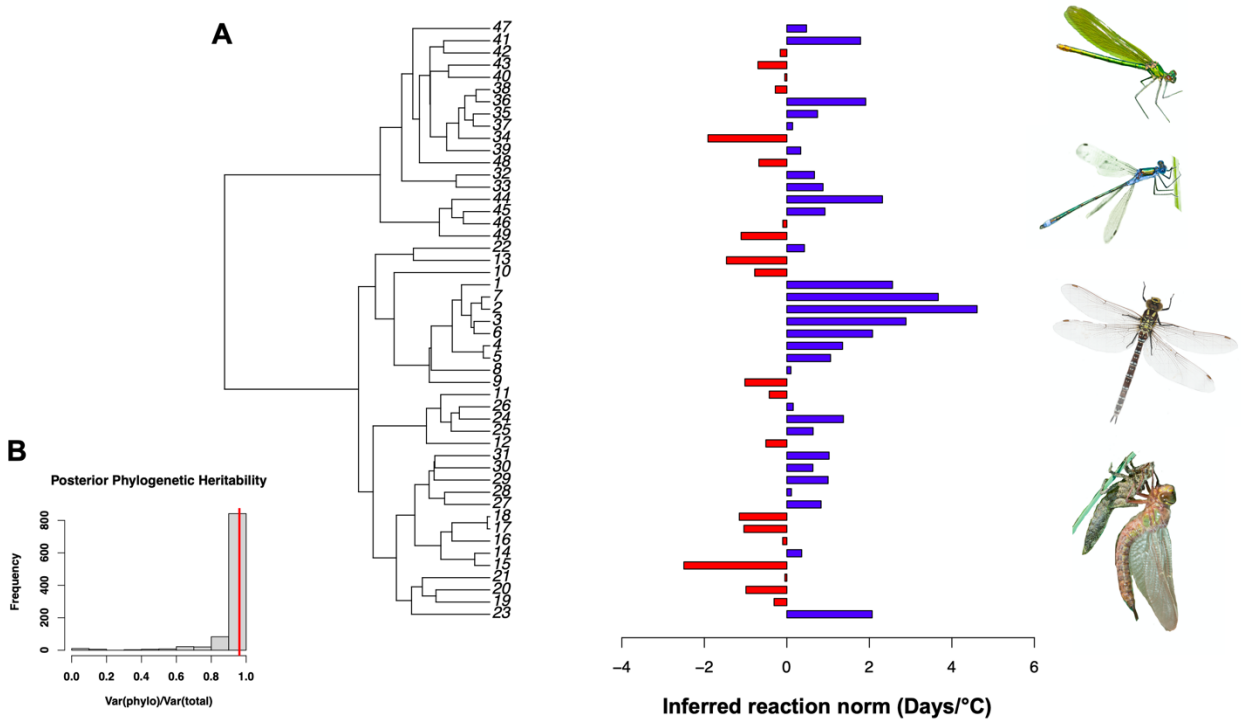


Figure 4. Inferred temperature reaction norms for phenology across Swedish Odonates. A, Estimated reaction norms for temperature dependent phenology show variation among 49 damselfly and dragonflyspecies, although most are relatively shallow and the phylogenetic signal is high and statistically significant. Species images (photos by S. De Lisle, top down): *Calopteryx splendens* (ID 32), *Lestes dryas* (ID 44), *Aeshna cyanea* (ID 1), *Brachytron praetense* (ID 9). Full species names for each ID number are provided in Table S1. Panel **B** shows the posterior distribution and point estimate (red line) for the phylogenetic heritability of the phenological reaction norm.

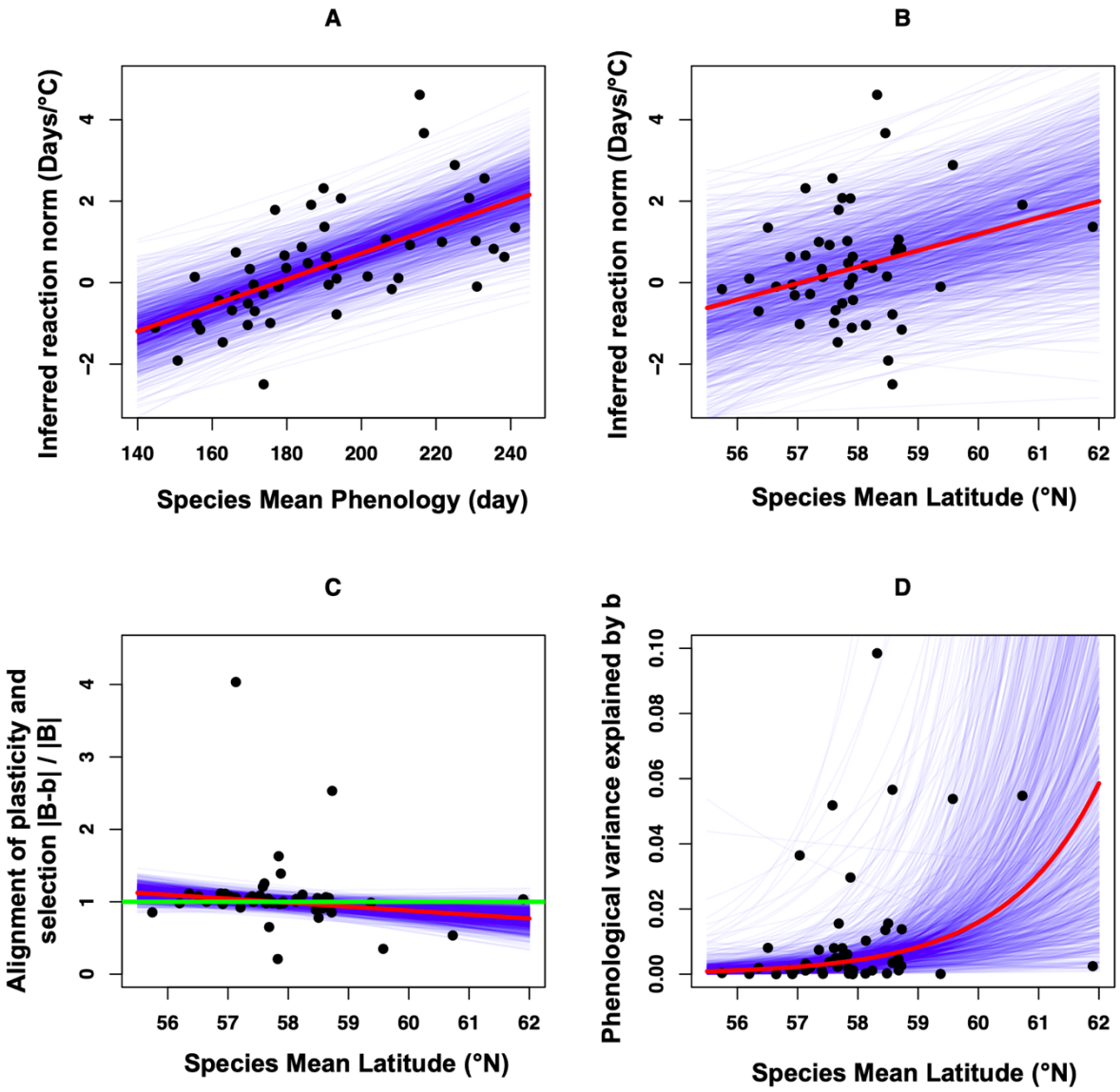


Figure 5. Macroevolutionary dynamics of phenological plasticity. Early-emerging odonate species have more negative thermal reaction norms than late-emerging species. Species that emerge late in the season instead tend to exhibit positive reaction norms to winter/spring temperature, **A**. There is evidence that negative reaction norms, reflecting accelerated development in response to warm temperatures, evolve in southern latitudes **B**. Across species,

the alignment between inferred reaction norm slope, b, and the inferred environmental dependence of the optimum phenology B, becomes greater (smaller absolute difference scaled by the magnitude of B) for species with Northern geographic ranges **C**. Green line in C equals unity; values of $|B-b|/|B| < 1$ indicate adaptive plasticity and values > 1 maladaptive plasticity. Inferred plasticity explains a greater proportion of the detrended variation in phenology in Northern species, Panel **D**. Together these data suggest that reaction norms have diversified at the macroevolutionary scale and that this diversity may have played a an important role during Odonate range expansion into northern latitudes. In all panels, red lines show posterior mean model fits, blue lines show the posterior distribution. All models accounted for phylogenetic relationships and uncertainty in the species-specific estimates of the *B* and *b*. In panel **C** an extreme single outlier (*Aeshna grandis*) is not shown in the figure, but inclusion/exclusion of this species had no effect on general conclusions (see text).

587

588 **Literature Cited**

589 1.

590 Abbott, J. & Svensson, E.I. (2005). Phenotypic and genetic variation in emergence and

591 development time of a trimorphic damselfly. *Journal of Evolutionary Biology*, 18, 1464-

592 1470.

593 2.

594 Abbott, J. & Svensson, E.I. (2010). Morph-specific variation in intersexual genetic correlations

595 in an intraspecific mimicry system. *Evolutionary Ecology Research*, 12, 105-118.

596 3.

597 Anderson, J.T., Inouye, D.W., McKinney, A.M., Colautti, R.I. & Mitchell-Olds, T. (2012).

598 Phenotypic plasticity and adaptive evolution contribute to advancing flowering

599 phenology in response to climate change *Proceedings of the Royal Society of London*

600 *Series B.*, 279, 3843-3852.

601 4.

602 Angiletta, M. (2009). *Thermal Adaptation*. Oxford University Press, Oxford.

603 5.

604 Angilletta, M.J. (2009). *Thermal Adaptation: A Theoretical and Empirical Synthesis*. Oxford

605 University Press, Oxford.

606 6.

607 Anholt, B.R. (1994). Cannibalism and early instart survival in a larval damselfly. *Oecologia*, 99,

608 60-65.

609 7.

610 Bonamour, S., Chevin, L.M., Reale, D., Teplitsky, C. & Charmantier, A. (2020). Age-dependent
611 phenological plasticity in a wild bird. *Journal of Animal Ecology*, in press.
612 8.
613 Boutin, S. & Lane, J.E. (2014). Climate change and mammals: evolutionary versus plastic
614 responses *Evolutionary Applications*, 7, 29-41.
615 9.
616 Bradshaw, W.E. & Holzapfel, C.M. (2006). Evolutionary response to rapid climate change.
617 *Science*, 312, 1477-1478.
618 10.
619 Brashaw, W.E. & Holzapfel, C.M. (2001). Genetic shift in photoperiodic response correlated
620 with global warming. *Proceedings of the National Academy of Sciences*, 98, 14509-
621 14511.
622 11.
623 Buckley, L.B. & Kingsolver, J.G. (2019). Environmental variability shapes evolution, plasticity
624 and biogeographic responses to climate change. *Global Ecology and Biogeography*, 28,
625 1456-1468.
626 12.
627 Charmantier, A., McCeery, R.H., Cole, L.R., Perrins, C., Kruuk, L.E.B. & Sheldon, B.C. (2008).
628 Adaptive phenotypic plasticity in response to climate change in a wild bird population.
629 *Science*, 320, 800-803.
630 13.
631 Chevin, L.M. & Lande, R. (2011). Adaptation to marginal habitats by evolution of increased
632 phenotypic plasticity *Journal of Evolutionary Biology* 24, 1462-1476.

633 14.
634 Chevin, L.M. & Lande, R. (2015). Evolution of environmental cues for phenotypic plasticity
635 *Evolution*, 69, 2767-2775.
636 15.
637 Chevin, L.M., Lande, R. & Mace, G.M. (2010). Adaptation, plasticity, and extinction in a
638 changing environment: Towards a predictive theory. *PLoS Biology* 8 e1000357.
639 16.
640 Chevin, L.M., Visser, M.E. & Tufto, J. (2015). Estimating the variation, autocorrelation, and
641 environmental sensitivity of phenotypic selection. *Evolution*, 69, 2319-2332.
642 17.
643 Crozier, L.G. & Hutchings, J.A. (2014). Plastic versus evolutionary responses to climate change
644 in fish *Evolutionary Applications*, 7, 68-87.
645 18.
646 Crozier, L.G., Scheuerell, M.D. & Zabel, R.W. (2011). Using time series analysis to characterize
647 evolutionary and plastic responses to environmental change: A case study of shift
648 towards earlier migration date in sockeye salmon *The American Naturalist*, 178, 755-773.
649 19.
650 D., G. & Wilson, R.J. (2021). Intra- and interspeicifc variation in responses of insect phenology
651 to climate. *Journal of Animal Ecology*, 90, 248-259.
652 20.
653 Davies, T.J., Wolkovich, E.M., Kraft, N.J.B., Salamin, N., Allen, J.M., Ault, T.R. *et al.* (2013).
654 Phylogenetic conservatism in plant phenology. *Journal of Ecology* 101, 1520-1530.
655 21.

656 Davis, C.C., WIllis, C.G., Primack, R.B. & Miller-Rushing, A.J. (2010). The importance of
657 phylogeny to the study of phenological response to global climate change. *Philosophical*
658 *Transactions of The Royal Society B.*, 365, 3201-3213.
659 22.
660 De Block, M., Pauwels, K., Ven Den Broeck, M., De Meester, L. & Stoks, R. (2013). Local
661 genetic adaptation generates latitude-specific effects of warming on predator-prey
662 interactions. *Global Change Biology*, 19, 689-696.
663 23.
664 del Mar Delgado, M., Roslin, T., Tikhonov, G., Meyke, E., Lo, C., Gurarie, E. *et al.* (2020).
665 Differences in spatial versus temporal reaction norms for spring and autumn phenological
666 events. *Proceedings of the National Academy of Sciences*, 117, 31249-31258.
667 24.
668 Dinh, K.D., Janssens, L., Debecker, S., De Jonge, M., Lambret, P., Nilsson-Örtman, V. *et al.*
669 (2013). Susceptibility to a metal under global warming is shaped by thermal adaptation
670 along a latitudinal gradient. *Global Change Biology*, 19, 2625-2633.
671 25.
672 Dudaniec, R.Y., Yong, C.J., Lancaster, L.T., Svensson, E.I. & Hansson, B. (2018). Signatures of
673 local adaptation along environmental gradients in a range-expanding damselfly (Ischnura
674 elegans). *Molecular Ecology*, 27, 2576-2593.
675 26.
676 Forrest, J.R.K. (2016). Complex responses of insect phenology to climate change *Current*
677 *Opinion in Insect Science* 17 49-54.
678 27.

679 Franks, S.J., Weber, J.J. & Aitken, S. (2014). Evolutionary and plastic responses to climate
680 change in terrestrial plant populations *Evolutionary Applications*, 7, 123-139.
681 28.
682 Garcia-Roa, R., Garcia-Gonzalez, F., Noble, D.W.A. & Carazo, P. (2020). Temperature as a
683 modulator of sexual selection. *Biological Reviews*, in press
684 29.
685 Gosden, T.P. & Svensson, E.I. (2008). Spatial and temporal dynamics in a sexual selection
686 mosaic. *Evolution*, 62, 845-856.
687 30.
688 Hadfield, J.D. (2016). The spatial scale of local adaptation in a stochastic environment. *Ecology*
689 *Letters*, 19, 780-788.
690 31.
691 Hadfield, J.D. & Nakagawa, S. (2010). General quantitative genetic methods for comparative
692 biology: Phylogenies, taxonomies, meta-analysis and multi-trait models for continuous
693 and categorical characters. *Journal of Evolutionary Biology*, 23, 494-508.
694 32.
695 Hassal, C., Thompson, D.J., French, G.C. & Harvey, I.F. (2007). Historical changes in the
696 phenology of British Odonata are related to climate. *Global Change Biology*, 13, 933-
697 941.
698 33.
699 Hassall, C., Thompson, D.J., French, G.C. & Harvey, I.F. (2007). Historical changes in the
700 phenology of British Odonata are related to climate. *Global Change Biology*, 13, 933-
701 941.

702 34.

703 Hewitt, G. (1999). Postglacial re-colonization of European biota. *Biological Journal of the*
704 *Linnean Society*, 68, 87-112.

705 35.

706 Hewitt, G. (2000). The genetic legacy of the Quarternary ice ages. *Nature*, 405, 907-913.

707 36.

708 Houle, D. & Meyer, K. (2015). Estimating sampling error of evolutionary statistics based on
709 genetic covariance matrices using maximum likelihood. *Journal of Evolutionary Biology*,
710 28, 1542-1549.

711 37.

712 Huey, R.B., Hertz, P.E. & Sinervo, B. (2003). Behavioral drive versus behavioral inertia in
713 evolution: a null model approach. *The American Naturalist*, 161, 357-366.

714 38.

715 Johansson, F., Watts, P.C., Sniegula, S. & Berger, D. (2021). Natural selection mediated by
716 seasonal time constraints increases the alignment between evolvability and
717 developmental plasticity. *Evolution*, in press.

718 39.

719 Kingsolver, J.G., Hoekstra, H.E., Hoekstra, J.M., Berrigan, D., Vignieri, S.N., Hill, C.E. *et al.*
720 (2001). The strength of phenotypic selection in natural populations. *American Naturalist*,
721 157, 245-261.

722 40.

723 Lancaster, L.T., Dudaniec, R.Y., Chauhan, P., Wellenreuther, M. & Svensson, E.I. (2016). Gene
724 expression under thermal stress varies across a geographical range expansion front.
725 *Molecular Ecology*, 25, 1141-1156.
726 41.
727 Lancaster, L.T., Dudaniec, R.Y., Hansson, B. & Svensson, E.I. (2015). Latitudinal shift in
728 thermal niche breadth results from thermal release during a climate-mediated range
729 expansion. *Journal of Biogeography* 42, 1953-1963.
730 42.
731 Lande, R. (2009). Adaptation to an extraordinary environment by evolution of phenotypic
732 plasticity and genetic assimilation *Journal of Evolutionary Biology*, 22, 1435-1446.
733 43.
734 Lande, R. & Arnold, S.J. (1983). The measurement of selection on correlated characters.
735 *Evolution*, 37, 1210-1226.
736 44.
737 Lane, J.E., McAdam, A.G., McFarlane, S.E., Williams, C.T., Humphries, M.M., Coltman, D.W.
738 *et al.* (2018). Phenological shifts in North American red squirrels: diesntangling the roles
739 of phenotypic plasticity and microevolution. *Journal of Evolutionary Biology*, 31, 810-
740 821.
741 45.
742 Leung, C., Rescan, M., Grulois, D. & Chevin, L.M. (2020). Reduced phenotypic plasticity
743 evolves in less predictable environments. *Ecology Letters*, 23, 1664-1672.
744 46.
745 Levins (1968). *Evolution in changing environments*. Princeton University Press, Princeton, NJ.

47.

Livingstone, D.M. & Lotter, A.F. (1998). The relationship between air and water temperatures in lakes of the Swiss Plateau: a case study with paleolimnological implications. *Journal of Paleolimnologists*, 19, 181-198.

48.

Lynch, M. & Walsh, B. (1998). *Genetics and the analysis of quantitative traits*. Sinaur Assosciates Inc., Sunderland, MA.

49.

Nashoba, A.R. & Kono, T.J.Y. (2020). Selection and plasticity both account for interannual variation in life-history phenology in an annual praries legume. *Ecology and Evolution*, 10, 940-951.

50.

Noble, D.W.A., Radersma, R. & Uller, T. (2019). Plastic responses to novel environments are biased towards phenotype dimensions with high additive genetic variation. *Proceedings of the National Academy of Sciences*, 116, 13452-13461.

51.

Pagel, M. (1999). Inferring the historical patterns of biological evolution. *Nature*, 401, 877-884.

52.

Parmesan, C. (2006). Ecological and Evolutionary Responses to Recent Climate Change. *Annual Review of Ecology and Systematics*, 37, 637-669.

53.

767 Phillimore, A.B., Hadfield, J.D., Jones, O.R. & Smithers, R.J. (2010). Differences in spawning
768 date between populations of common frog reveal local adaptaion *Proceedings of the*
769 *National Academy of Sciences*, 107, 8292-8297.
770 54.
771 Phillimore, A.B., Leech, D.I., Pearce-Higgens, J.W. & Hadfield, J.D. (2016). Passerines may be
772 suffieciently plastic to track temperature-mediated shifts in optimum lay date. *Global*
773 *Change Biology* 22, 3258-3272.
774 55.
775 Phillimore, A.B., Stålhandske, S., Smithers, R.J. & Bernard, R. (2012). Dissecting the
776 contributions of plasticity and local adaptation to the phenology of a butterfly and its host
777 plants. *The American Naturalist*, 180, 655-670.
778 56.
779 Phillips, P.C. & Arnold, S.J. (1989). Visualizing multivariate selection. *Evolution*, 43, 1209-
780 1222.
781 57.
782 Plard, F., Gaillard, J.-M., Coulson, T., Hewison, A.J.M., Delorme, D. & Warnant, C. (2014).
783 Mismatch Between Birth Date and Vegetation Phenology Slows the Demography of Roe
784 Deer. *PLoS Biology*, 12, e1001828.
785 58.
786 Punzalan, D., Rodd, F.H. & Rowe, L. (2008). Sexual selection mediated by the thermoregulatory
787 effects of male colour pattern in the ambush bug Phymata americana. *Proceedings B*,
788 275, 483-492.
789 59.

790 Radchuk, V., Reed, T., Teplitsky, C., Mvd, P., Charmantier, A. & al., e. (2019). Adaptive
791 responses of animals to climate change are most likely insufficient. *Nature*
792 *Communications*, 10, 3109.
793 60.
794 Radersma, R., Noble, D.W.A. & Uller, T. (2020). Plasticity leaves a phenotypic signautre during
795 local adaptation. *Evolution Letters*, 4, 360-370.
796 61.
797 Rafferty, N.E. & Nabity, P.D. (2017). A global test for phylogenetic signal in shifts in flowering
798 time under climate change *Journal of Ecology* 105, 627-633.
799 62.
800 SMHI (2015). Climate Indicators - Temperature Available at:
801 https://www.smhi.se/en/climate/climate-indicators/climate-indicators-temperature-
802 1.91472.
803 63.
804 Stamp, M.A. & Hadfield, J.D. (2020). The relative importance of plasticitiy versus genetic
805 differntiation in explaining between population differences; a meta analysis *Ecology*
806 *Letters* doi: 10.1111/ele.13565.
807 64.
808 Stinchcombe, J.R., Agrawal, A.F., Hohenlohe, P.A., Arnold, S.J. & Blows, M.W. (2008).
809 Estimating nonlinear selection gradients using quadratic regression coefficients: double
810 or nothing? *Evolution*, 62, 2435-2440.
811 65.

Stoks, R. & Córdoba-Aguilar, A. (2012). Evolutionary ecology of odonata: A complex life cycle perspective *Annual Review of Entomolgy*, 57, 249-265.

66.

Stoks, R., Geerts, A.N. & De Meester, L. (2014). Evolutionary and plastic responses of freshwater invertebrates to climate change: realized patterns and future potential *Evolutionary Applications*, 7, 42-55.

67.

Svensson, E.I. (2012). Non-ecological speciation, niche conservatism and thermal adaptation: how are they connected? . *Organisms, Diversity, and Evolution* 12, 229-240.

68.

Svensson, E.I. & Abbott, J. (2005). Evolutionary dynamics and population biology of a polymorphic insect. *Journal of Evolutionary Biology*, 18, 1503-1514.

69.

Svensson, E.I., Abbott, J. & Hardling, R. (2005). Female polymorphism, frequency dependence, and rapid evolutionary dynamics in natural populations. *The American Naturalist*, 165, 567-576.

70.

Svensson, E.I., Gomez-Llano, M.A. & Waller, J.T. (2020a). Selection on phenotypic plasticity favors thermal canalization. *Proceedings of the National Academy of Sciences*, 117, 29767-29774.

71.

Svensson, E.I., Willink, B., Duryea, M.C. & Lancaster, L.T. (2020b). Temperature drives pre-reproductive selection and shapes the biogeography of a female polymorphism. *Ecology Letters* 23, 149-159.

835 72.
836 Thackeray, S.J. & al., e. (2016). Phenological sensitivity to climate across taxa and trophic
837 levels. *Nature* 535, 241-245.
838 73.
839 Urban, M.C., Richardson, J.L. & Freidenfelds, N.A. (2014). Plasticity and genetic adaptation
840 mediate amphibian and reptile responses to climate change *Evolutionary Applications*, 7,
841 88-103.
842 74.
843 van Benthem, K.J., Bruijning, M., Bonnet, T., Jongejans, E., Postma, E. & Ozgul, A. (2017).
844 Disentangling evolutionary, plastic and demographic processes underlying trait
845 dynamics: a review of four frameworks. *Methods in Ecology and Evolution* 8, 75-85.
846 75.
847 Villemereuil, P., Charmantier, A., Arlt, D., Bize, P., Brekke, P., Brouwer, L. *et al.* (2020).
848 Fluctuating optimum and temporally variable selection on breeding date in brids and
849 mammals. *Proceedings of the National Academy of Sciences*, 117, 31969-31978.
850 76.
851 Visser, M.E., Gienapp, P., Husby, A., Morrisey, M., de la Hera, I. & al., e. (2015). Effects of
852 Spring Temperatures on the Strength of Selection on Timing of Reproduction in a Long-
853 Distance Migratory Bird *PLoS Biology*, e1002120.
854 77.
855 Waller, J.T. & Svensson, E.I. (2017). Body size evolution in an old insect order: no evidence for
856 Cope's rule in spite of fitness benefits of large size. *Evolution* 71, 2178-2193.
857 78.

Wellenreuther, M., Larson, K.W. & Svensson, E.I. (2012). Climatic niche divergence or conservatism? Environmental niches and range limits in ecologically similar damselflies. *Ecology* 93, 1353-1366.

79.

Werner, E.E. & Gilliam, J.F. (1984). The ontogenetic niche and species interactions in size-structured populations. *Annual Review of Ecology and Systematics*, 15, 393-425.

80.

Whiteman, H.H. (1994). Evolution of facultative paedomorphosis in salamanders. *The Quarterly Review of Biology*, 69, 205-221.

81.

Wiens, J.J. & Graham, C.H. (2005). Niche conservatism: integrating ecolution, ecology, and conservation biology *Annual Review of Ecology, Evolution, and Systematics*, 26, 519-539.

82.

Wilbur, H.M. (1980). Complex Life Cycles. *Annual Review of Ecology and Systematics*, 11, 67-93.

83.

Willink, B. & Svensson, E.I. (2017). Intra- and intersexual differences in parasite resistance and female fitness tolerance in a polymorphic insect. *Proceedings of the Royal Society of London Series B.*, 28420162407.

881

882 **Supplemental Material**

883 **Detailed Methods- Data Collection**

884 *Long term study of Ischnura elegans*

885 We analyzed data on individual phenology and fitness in a set of populations (approximately 16)
886 of the damselfly *Ischnura elegans* in province of Skåne, southern Sweden, for 18 years (2000-
887 2017). This set of populations spans an area of approximately 40x40 km. Each year, these
888 populations were surveyed in a standardized approach during the reproductive season of *I.*
889 *elegans*, beginning in late May when they start to emerge from the aquatic larval stage and
890 continuing throughout the major part of the flying season until the end of July. Field work took
891 place daily, except during days of low temperature (< 14 °C), heavy wind and rain, when adult
892 damselflies are not active. Each population was visited at intervals between one and two weeks,
893 within the lifespan limits for adult *I. elegans* and corresponding to approximately 5 sampling
894 visits per site per year (Willink & Svensson 2017). Sampling was conducted for approximately
895 60 minutes at each site during a sampling session. See Willink and Svensson (2017) for more
896 details about general field and laboratory work procedures.

897 *I. elegans* adults were captured in the field. Copulating pairs were kept separate from
898 single-caught individuals in individual plastic containers. In the laboratory, all individuals were
899 sexed and their copulation status (mated or not) was recorded. In this study, we used data on one
900 major male fitness component: male mating success (a binomially distributed variable) to
901 estimate the strength of selection on phenology through mating success. Our focus on male
902 component fitness was due to two reasons: first, statistical power, in that we obtained far more
903 estimates of male mating success across seasons, and the nonlinear, random-regression

904 approaches (see below) to estimating variation in selection are data hungry. Second, our previous
905 work has shown that this fitness measure is correlated with female fitness (fecundity) in these
906 populations, corresponding to male and female phenotypic selection estimates that are
907 significantly and positively correlated with each other across these populations (Gosden &
908 Svensson 2008). This finding indicates that a broad patterns of selection acting through male
909 component fitness are indeed reflective of patterns of selection acting through female fecundity
910 in this system. We also note that female multiple mating is rare due to mate guarding in copula.
911 Hence, male mating success is a reasonable proxy of mean population fitness as a whole. Our
912 surveys yielded data on phenology (date of capture) from 47,615 individuals (28,978 males,
913 18,637 females) during this 18 year period (2000-2017). As the majority of individuals of *I.*
914 *elegans* in these populations in southern Sweden are univoltine and emerge as adults after one
915 year in the larval stage, these 18 years correspond to approximately the same number of
916 generations.
917
918 *Laboratory experiment on thermal plasticity in development time*
919 We reared larvae of individual *I. elegans* from the egg stage, through hatching and
920 metamorphosis to adults, under two different larval temperature environments: 20 °C and 24 °C.
921 These temperatures were chosen for two reasons. First, they mimic typical and elevated summer
922 conditions in Sweden, respectively, under a projected climatic shift of 4°C (De Block *et al.* 2013;
923 Dinh *et al.* 2013). Second, past work in odonates has indicated that temperature differences on
924 this scale can manifest significant affects on larval development (Dinh *et al.* 2013). Our choice
925 of only two temperature treatments also precludes estimation of more complex, nonlinear
926 reaction norms. Although this is a limitation of our design, we note that because statistical

927 theory (Phillimore *et al.* 2010; Chevin *et al.* 2015) behind reaction norm inference at other levels
928 of analysis in our study is limited to the case of linearity, we are most interested in the linear
929 approximation of the reaction norm. Maternal egg clutches (full sib clutches) were obtained in
930 2017 from females caught *in copula* in the field in the general population survey described
931 above. Females caught *in copula* were set up for oviposition in individual plastic cups at room
932 temperature, and provided with a wet filter paper for egg laying surface. Two days later, the
933 female was removed, the eggs were scanned and counted from the digital images. See Svensson
934 and Abbott (2005) and Svensson et al. (2005) for further methodological details about laboratory
935 procedures. Individual eggs and larvae from a total of 101 full-sib clutches were reared in a split-
936 clutch GxE design across both environments. We started with 20 full siblings per environmental
937 treatment for a total of approximately 4000 larvae across this rearing experiment. Upon hatching,
938 individual larvae were transferred to plastic cups where they were housed individually in
939 temperature-controlled water baths. Larvae were fed *Artemia* daily and kept at a constant
940 midsummer light cycle (16:8). Towards the end of larval development and when the larvae
941 reached the last instar, each cup received a wooden stick as an emergence aid and cups were
942 covered with mesh to prevent escape of emerging adults. This experiment continued until all
943 individuals had either died as larvae or emerged as adults. Of the 4000 larvae, 523 (13%)
944 successfully emerged as adults, with recorded emergence time for 521 individuals. Although
945 mortality in this experiment may appear nominally high, it is consistent with the high larval
946 mortality observed in other odonates (Anholt 1994; Stoks & Córdoba-Aguilar 2012), and we
947 note that over 500 adults recruited from 101 females corresponds to an absolute fitness (discrete
948 time per capita growth rate) ≈ 5, an exceptionally high number. Larval development time was

949 calculated as date of emergence as an adult minus the date of hatching, consistent with our
950 previous procedures (Abbott & Svensson 2005).
951
952 *Occurrence records of Swedish Odonata*
953 We obtained public, spatiotemporally referenced observational records of adult Swedish
954 odonates identified to species level from the Swedish Species Observation System (Artportalen:
955 https://www.artportalen.se/). These data represent a combination of validated citizen science
956 records, as well as records reported by professional scientists and naturalists throughout Sweden,
957 and these data are also subsequently exported to the Global Biodiversity Information Facility
958 (GBIF: https://www.gbif.org/). We used data from a ten year period from 2006-2015, the range
959 of years for which we had accurate temperature data (see below) and for which substantial
960 observational records were available in this public database. We excluded observations of
961 larvae, as well as for rare taxa for which records were unavailable in some years in this range, as
962 well as taxa that were not included in our phylogeny (see below). Our final dataset consists of
963 1,027,952 records from 49 species, which is the majority of odonate species (75 %) that have so
964 far been recorded in Sweden (currently a total of 65; 43 dragonflies (Anisoptera) and 22
965 damselflies (Zygoptera)).
966 We obtained spatiotemporally matched estimates of spring mean surface temperature
967 from the publicly accessible NOAA Global Forecast System Analysis (GFS ANL) database
968 (https://www.ncdc.noaa.gov/data-access/model-data/model-datasets/global-forcast-system-gfs).
969 The NOAA GFS ANL provides global weather data estimated four times per day gridded at .5°
970 resolution (corresponding to approximately 25km in Sweden), using a system of satellites and
971 globally-distributed weather stations. Comparison of these surface temperature estimates to

direct temperature records from a weather station in Malmö, Sweden, obtained from the Swedish Meteorological and Hydrological Institute (https://www.smhi.se/en), indicates the GFS ANL data are an accurate representation of surface temperature ($r > 0.99$ between temperature records). For each record in our dataset of Swedish odonates, we obtained the mean surface temperature (GFS ANL database variable: tmp2m) from corresponding lat-long grid cell in the GFS ANL database for two time windows: the first 120 days of the year and the month of April. The first time window corresponds to a substantial period of pre-metamorphosis larval development and covers the last instar for all the species in our database, and so represents a long-term average temperature environment experienced during a key stage of development. The second time window represents a shorter time slice from late spring, just prior to the flight period of many early season species. We focused on mean temperature, because daily fluctuations in air temperature are unlikely to correlate strongly with water temperature, although average temperatures are a strong predictor of average water temperatures (Livingstone & Lotter 1998). We note that surface air temperatures are strong a strong predictor of water temperatures in freshwater bodies, particularly in the upper 1m of the water column (Livingstone & Lotter 1998) where odonate larvae are most abundant. Finally, we note that the scale and purpose of our analysis precludes use of a "sliding window" approach; Our interest is to compare developmental-temperature reaction norm variation across species (and so any such comparison requires use of similar or the same environmental cues), rather than to identify a specific time slice of spring temperature variation that is most important for a single species.

We obtained phylogenic information for all 49 species in our dataset, using a recently published large dated molecular phylogeny of the odonates (Waller & Svensson 2017). This

994 tree is available publicly from the Odonate Phenotypic Database

995 (http://www.odonatephenotypicdatabase.org/shiny/odonates/).

996

997 **Detailed Methods- Data Analysis**

998 *Long term field study of Ischnura elegans*

999 We used a linear mixed effects model to assess phenological advance in the timing of the adult

1000 aerial period in *I. elegans* during our 18 year study period. This model featured the date (in

1001 ordinal day of the year; January 1 = day 1) of an observation of an individual as the response

1002 variable and year as a fixed effect. Our model also included a random intercept for population to

1003 account for spatial variation in phenology among the study populations; our model did not

1004 explore spatial autocorrelation, which we expect to be low as populations differ primarily in

1005 microenvironment that is uncorrelated with geography.

1006 To infer selection on phenology, we seek a statistical description of individual fitness as a

1007 function of phenology, where the functional relationship between phenology and fitness varies

1008 with spring temperature cues. We modify the Lande-Arnold (Lande & Arnold 1983) equation to

1009 include temperature dependence in the form of directional selection on phenology:

$$w = \mathrm{a} + \beta \mathrm{z} * (1 + \varphi \mathrm{t}) + \frac{1}{2}\gamma \mathrm{z}^2 + \varepsilon \qquad 1$$

1010

1011 where $\varphi = \frac{\phi}{\beta}$, ϕ is an interaction term as estimable in a regression model, *w* is individual

1012 fitness, β is the linear selection gradient on phenology *z*, *t* is the temperature cue and γ is the

1013 nonlinear selection gradient on phenology. Setting the first derivative of equation 1 with respect

1014 to *z* to zero and solving yields the stationary point:

1015

$$\theta_z = \frac{\beta}{-\gamma} + \frac{\phi}{-\gamma} t \qquad 2$$

1016

1017 where θ_z is the optimum phenology (if selection is stabilizing, otherwise it is a fitness
1018 minimum). We note that this quadratic description of environmental-dependence of selection
1019 also approximates environmental dependence of a Gaussian fitness function (Chevin *et al.*
1020 2015). The first part of the right hand side of equation 2 is an intercept equal to the fixed location
1021 of the optimum obtained from the standard Lande-Arnold equation (Phillips & Arnold 1989).
1022 The second term on the right hand side describes a temperature-dependent slope. Thus, the
1023 simple modification of the Lande-Arnold equation in 1 yields a statistical description of a linear
1024 relationship between temperature cue and optimal phenology, where the slope of the relationship
1025 between temperature and θ_z is $\frac{\phi}{-\gamma}$, and represents a direct estimate of the temperature dependence
1026 of selection (*B*).

1027 We fit equation 1 to our *I. elegans* field dataset using a linear mixed effects model with
1028 male copulation success as a response, i. e. a measure of sexual selection. For comparison, we
1029 also fit a simplified model, with directional selection only (i.e., no temperature-dependence or
1030 nonlinear terms). Although environmental effects on non-linear terms have not received
1031 theoretical or empirical attention (Chevin *et al.* 2015; Villemereuil *et al.* 2020), we nonetheless
1032 found no evidence for such effects and so focus on temperature effects on linear gradients. We
1033 focus on males only in this analysis, as copulation success is likely a more critical determinant of
1034 male fitness than it is for females; and also note that selection via male and female fitness
1035 components are strongly correlated across space and time in the wild (Gosden & Svensson
1036 2008). We used absolute copulation success as a response, as this resulted in more consistent
1037 model convergence and eases interpretation; however, qualitatively equivalent conclusions were

1038 obtained using relativized (by subpopulation, season or globally) copulation success. We
1039 estimated β, γ, and ϕ from the fixed effect parameters (doubling the nonlinear regression
1040 coefficient; Stinchcombe *et al.* 2008), and included random (co)variation in β and γ among years
1041 to accommodate variation in selection independent of temperature and possible relationships
1042 between temperature and height of the fitness function emerging from equation 1. We fit an
1043 unstructured covariance matrix as we found no evidence for temporal autocorrelation in selection
1044 (ΔAIC = 135 for first order autoregressive covariance structure). We fit a gaussian-distributed
1045 model for consistency with theory and to provide estimates of the parameters in equation 1
1046 (Chevin *et al.* 2015), although we also provide p-values from an equivalent mixed model
1047 assuming binomial error. Temperature was obtained as the mean of the first 120 days from the
1048 NOAA GFS ANL database for the corresponding grid cell of our study populations, with the
1049 exception of records from 2000-2005 for which we instead calculated this value from the
1050 Swedish Meteorological and Hydrological Institute data since the GFS data was unavailable for
1051 this period. We found close correspondence for the two years in which we had data from both of
1052 these sources (120 day mean, 2006: 1.061°C SMHI, 0.75°C NOAA; 2007: 5.89°C SMHI,
1053 5.02°C NOAA).
1054
1055 *Laboratory temperature experiment: raising damselfly families*
1056 We used a mixed effects model to estimate the relationship between thermal developmental
1057 environment of larvae (two temperature treatments) and emergence time from our laboratory
1058 experiment. Our model featured emergence day as the response variable, temperature treatment
1059 as a fixed effect, and random intercepts for tank and maternal full-sib family. We calculated
1060 phenotypic variances, additive genetic variances and heritability (h^2), the latter as twice the

1061 family variance over the total variance of the random effects (the sum of tank, family, and
1062 residual variance components). We assessed statistical significance of h^2 using a likelihood ratio
1063 test of the family variance component. We also calculated standard error and 95% confidence
1064 intervals by empirically constructing the sampling distribution of h^2 by taking 1 million draws
1065 from a multivariate normal distribution centered on our original REML estimates of the variance
1066 components with covariance equal to the Hessian matrix of the fitted model (Houle & Meyer
1067 2015). We note that our estimate of heritability is conditioned on the fixed effect of temperature
1068 treatment (Lynch & Walsh 1998).
1069
1070 *Occurrence records of Swedish Odonata and phylogenetic comparative analyses*
1071 We used a bivariate mixed modelling approach (Phillimore *et al.* 2010; Phillimore *et al.* 2012) to
1072 infer interspecific variation in thermal reaction norms in phenology from our observational
1073 records of Swedish Odonata combined with paired estimates of temperature in the first 120 days
1074 of the year. The premise behind this approach is to use detrended covariation in temperature and
1075 phenology, within geographic grid cells, as an estimate of population mean reaction norms.
1076 From the same model, covariation in mean phenology and temperature across latitudinal
1077 geographic grid cells provides an estimate of the environmental dependence of selection
1078 (Hadfield 2016). We thus fit the bivariate model
1079

$$\zeta_i = \mu_i + \beta_{1,i} * y + \beta_{2,i} * x + a_{lat} + a_{time(lat)} + \varepsilon \qquad 3$$

1080
1081 where ζ_i is the response vector of individual values of two variables: i = observed phenology or
1082 spring temperature. β is a fixed effect regression coefficient between continuous latitude (y) or

year (x) and variable i. Two nested random effects are included in this model: a_{lat} is a random effect describing (co)variation across binned latitude, and $a_{time(lat)}$ is a random effect describing co(variation) across binned annual seasons within latitudinal grids. For each a and the residual ε we estimated the full 2x2 unstructured covariance matrix $\begin{bmatrix} \sigma^2_{phenology} & \sigma_{phenology,temp} \\ \sigma_{phenology,temp} & \sigma^2_{temperature} \end{bmatrix}$, for a total of three 2x2 random effect covariance matrices. Thus, in this model space (latitude) and time (year) are included as both continuous fixed effects, as well as binned levels in nested random effects.

We focus on analysis of latitudinal spatial variation because Sweden spans a far greater latitudinal than longitudinal range, and corresponding species distributions within Sweden reflect substantial latitudinal span but little longitudinal span. Our dataset of odonate observations spanned latitudes from 55° – 69°, corresponding to the full latitudinal range of Sweden. We note, however, that longitudinal variation, when present, will be captured in variation in estimated spring mean temperatures for observations recorded from differing longitudes within the same latitudinal resolution, and thus is a source of residual within-bin variation accommodated in our model. For estimation of our mixed model random effects, we binned latitude by half degree increments, with the exception of extreme northern latitudes (over 62°) due to limited data from these sparsely populated Swedish regions. Our dataset contained 14 latitudinal bins with each species containing observations represented in at least 4 adjacent bins.

From this model we can interpret the ratio of the fixed effect parameter estimates, particularly $\frac{\beta_{lat,\ \text{phenology}}}{\beta_{lat,\ \text{temperature}}}$ as approximating the environmental dependence of the optimum, B (Hadfield 2016). From the random effects, we can interpret $b \sim \sigma_{phenology,\ temperature} / \sigma^2_{temperature}$ where σ and σ^2 are the elements of the random effect covariance matrix estimated

1105 across time points within binned latitude ($a_{time(lat)}$). Thus, b is the de-trended regression of
1106 phenology across temperature within latitudinal bins (Phillimore *et al.* 2010; Phillimore *et al.*
1107 2012). This bivariate model was fit separately for each species in our dataset.

1108 We then used the corresponding estimates of B and b in phylogenetic mixed models
1109 (Hadfield & Nakagawa 2010) to explore the relationship between species mean latitude, species
1110 mean phenology, and the strength and form of inferred phenological plasticity. We focus on
1111 species mean latitude because we were interested in how plasticity has evolved with Northern
1112 range expansion. These models included species as a random effect, with covariance among
1113 levels equal to the phylogenetic-variance-covariance matrix from our species level phylogeny,
1114 standardized to a depth of 1 (i.e., using the correlation matrix). These models also included a
1115 residual term. We accommodated uncertainty in our estimates of B and b in these phylogenetic
1116 mixed models by modelling the posterior variance of the species-specific estimates as a non-
1117 fitted random effect. We also estimated phylogenetic signal ("phylogenetic heritability") in the
1118 inferred reaction norms b as the phylogenetic variance over the sum of the phylogenetic and
1119 residual variance (Pagel 1999; Hadfield & Nakagawa 2010) from a mixed model with b as the
1120 response, an intercept, and species as a random effect (in addition to the posterior variance in b,
1121 as above).

1122 All bivariate and phylogenetic mixed models were fit by Bayesian MCMC using
1123 MCMCglmm in R and assuming weak priors. All other models were fit in SAS using the
1124 glimmix procedure. SAS and R scripts to reproduce our analyses and figures, as well as all raw
1125 data, are provided at: https://github.com/spdelisle/OdonatePhenology. All datasets have also
1126 been deposited as .csv files on Dryad
1127 https://datadryad.org/stash/share/FKHv4E_XmOX2f4yEi63pTibgzqmNFuXXhJLi_lnV3J0.

Table S1. Summary statistics for 49 species of Swedish Odonata. B is the indirect estimate of the environmental dependence of the optimum, b is the indirect estimate of the temperature-phenology reaction norm. R2 is the variance in phenology explained by plasticity. Parameter values are posterior modes; standard errors are the square root of the posterior variance See text for further details.

	Species ID #	Mean Phenology	Mean Latitude	Mean Temperature	B	SE_B	b	SE_b	R2	SE_R2	|B-b|/|B|	SE_|B-b|/|B|
Aeshna cyanea	1	233.0	57.6	1.442	-12.406	4.095	2.5608	0.6320	0.0518	0.026012	1.206422	0.0844
Aeshna grandis	2	215.6	58.3	0.646	-0.003	0.345	4.6119	0.8505	0.0985	0.025757	1615.617342	412.9258
Aeshna juncea	3	225.0	59.6	-0.289	4.442	0.574	2.8877	0.7127	0.0538	0.027019	0.349861	0.1680
Aeshna mixta	4	241.2	56.5	1.975	-16.852	1.153	1.3509	0.7323	0.0081	0.009180	1.080163	0.0441
Aeshna serrata	5	206.5	58.7	0.171	48.812	8.887	1.0600	1.7186	0.0012	0.006108	0.978285	0.0362
Aeshna subarctica	6	228.9	57.7	1.192	290.459	7623.444	2.0766	1.3736	0.0053	0.008642	0.992851	0.0076
Aeshna viridis	7	216.7	58.5	0.263	32.910	2.436	3.6705	1.1661	0.0135	0.011257	0.888469	0.0351
Anax imperator	8	193.3	56.2	2.529	4.809	1.908	0.0994	0.9901	0.0001	0.012450	0.979327	11.3041
Brachytron pratense	9	155.9	57.0	1.523	13.764	6.111	-1.0166	0.4538	0.0364	0.028240	1.073857	0.0723
Cordulegaster boltonii	10	193.3	58.6	-0.027	-9.118	0.897	-0.7787	0.7096	0.0035	0.008274	0.914595	0.0767
Cordulia aenea	11	161.8	57.9	0.783	-19.209	1.434	-0.4265	0.6294	0.0012	0.005941	0.977796	0.0331
Epitheca bimaculata	12	169.6	57.7	0.411	-28.628	1055.377	-0.5089	1.0138	0.0080	0.028895	0.982223	0.0676
Gomphus vulgatissimus	13	162.8	57.7	0.973	35.321	2.908	-1.4637	0.9210	0.0022	0.003554	1.041438	0.0268
Leucorrhinia albifrons	14	179.8	58.2	0.870	-3.666	0.534	0.3605	0.9089	0.0011	0.013577	1.098343	0.2528
Leucorrhinia caudalis	15	173.8	58.6	0.247	-27.909	7.367	-2.4957	0.7459	0.0566	0.039911	0.910575	0.0328
Leucorrhinia dubia	16	177.8	59.4	-0.260	-8.546	1.022	-0.1009	0.7446	0.0001	0.005767	0.988188	0.0888
Leucorrhinia pectoralis	17	169.5	58.1	0.830	26.900	3.789	-1.0400	0.6179	0.0103	0.011496	1.038663	0.0244
Leucorrhinia rubicunda	18	156.8	58.7	0.177	0.753	0.490	-1.1531	0.6833	0.0138	0.019647	2.530831	184.0942
Libellula depressa	19	166.2	57.0	1.854	2.794	1.590	-0.3093	0.6055	0.0013	0.010124	1.110712	22.2029
Libellula fulva	20	175.6	57.6	1.097	3.919	1.652	-0.9911	1.0366	0.0080	0.015304	1.252859	0.9418
Libellula quadrimaculata	21	171.1	57.9	1.005	-1.558	0.431	-0.0468	0.4086	0.0002	0.008075	0.969989	0.6462
Onychogomphus forcipatus	22	192.1	58.1	0.496	-236.086	64.794	0.4264	1.1429	0.0002	0.001650	1.001806	0.0051
Orthetrum coerulescens	23	194.5	57.9	0.925	-5.314	1.082	2.0682	0.7418	0.0297	0.020072	1.389179	0.1711
Somatochlora arctica	24	190.1	61.9	-2.928	-41.123	3.064	1.3711	1.8187	0.0025	0.009623	1.033340	0.0437

Somatochlora flavomaculata	25	190.5	57.9	0.810	184.057	5219.785	0.6361	0.7264	0.0012	0.005339	0.996544	0.0064
Somatochlora metallica	26	201.7	58.5	0.325	-3.016	1.194	0.1539	0.6943	0.0002	0.008832	1.051026	0.4046
Sympetrum danae	27	235.4	58.7	0.577	5.620	0.368	0.8295	0.6413	0.0025	0.003751	0.852406	0.1093
Sympetrum flaveolum	28	209.9	57.9	1.027	-58.311	5.308	0.1120	1.1227	0.0000	0.000851	1.001921	0.0198
Sympetrum sanguineum	29	221.6	57.4	1.452	-21.563	1.009	1.0001	0.7844	0.0075	0.009955	1.046382	0.0365
Sympetrum striolatum	30	238.3	56.9	1.856	-5.498	15.219	0.6316	1.2205	0.0013	0.010595	1.114881	1.0232
Sympetrum vulgatum	31	230.6	57.8	1.142	1.295	1.082	1.0242	0.7465	0.0061	0.008825	0.209107	29.8587
Calopteryx splendens	32	179.4	57.1	1.653	-0.220	1.310	0.6685	0.8075	0.0033	0.009491	4.034132	9.7274
Calopteryx virgo	33	184.0	58.7	0.208	-16.654	1.325	0.8775	0.5640	0.0046	0.005008	1.052692	0.0346
Coenagrion armatum	34	150.7	58.5	0.187	-8.651	3.439	-1.9120	2.3426	0.0156	0.059467	0.778982	0.2984
Coenagrion hastulatum	35	166.3	58.6	0.311	-11.758	0.463	0.7438	0.6654	0.0036	0.008095	1.063258	0.0566
Coenagrion johanssoni	36	186.5	60.7	-1.658	1.245	0.377	1.9119	1.0268	0.0547	0.051148	0.535081	0.5853
Coenagrion lunulatum	37	155.3	57.4	1.281	100.991	3427.786	0.1397	0.7116	0.0001	0.004499	0.998617	0.0086
Coenagrion puella	38	173.8	57.2	1.514	-3.466	1.194	-0.2790	0.5345	0.0021	0.012848	0.919508	0.2366
Coenagrion pulchellum	39	170.1	57.4	1.390	-4.426	0.650	0.3369	0.4939	0.0011	0.006491	1.076123	0.1158
Enallagma cyathigerum	40	191.2	56.9	1.677	-1.596	0.502	-0.0496	0.4573	0.0000	0.004072	0.968954	0.2893
Erythromma najas	41	176.8	57.7	1.216	5.112	0.595	1.7870	0.7510	0.0155	0.010195	0.650404	0.1576
Erythromma viridulum	42	208.1	55.7	2.723	-1.092	0.531	-0.1602	1.5354	0.0003	0.041118	0.853396	35.0739
Ischnura pumilio	43	171.4	56.4	2.697	6.200	2.925	-0.6999	2.6840	0.0020	0.033518	1.112892	2.2247
Lestes dryas	44	189.8	57.1	1.833	-40.366	2.767	2.3172	1.9056	0.0011	0.002662	1.057406	0.0462
Lestes sponsa	45	213.0	57.5	1.223	-10.889	0.572	0.9240	0.6146	0.0038	0.006110	1.084856	0.0554
Lestes virens	46	231.0	56.6	2.259	184.289	25.457	-0.0969	1.6426	0.0000	0.003050	1.000526	0.0093
Platycnemis pennipes	47	185.6	57.8	1.058	-0.759	7.638	0.4768	0.7596	0.0019	0.010137	1.628500	1.5016
Pyrrhosoma nymphula	48	165.3	57.6	1.342	-31.494	5.328	-0.6777	0.5652	0.0049	0.006708	0.978481	0.0195
Sympecma fusca	49	144.7	57.9	1.232	-172.664	96.182	-1.1082	2.7190	0.0007	0.005742	0.993582	0.0150

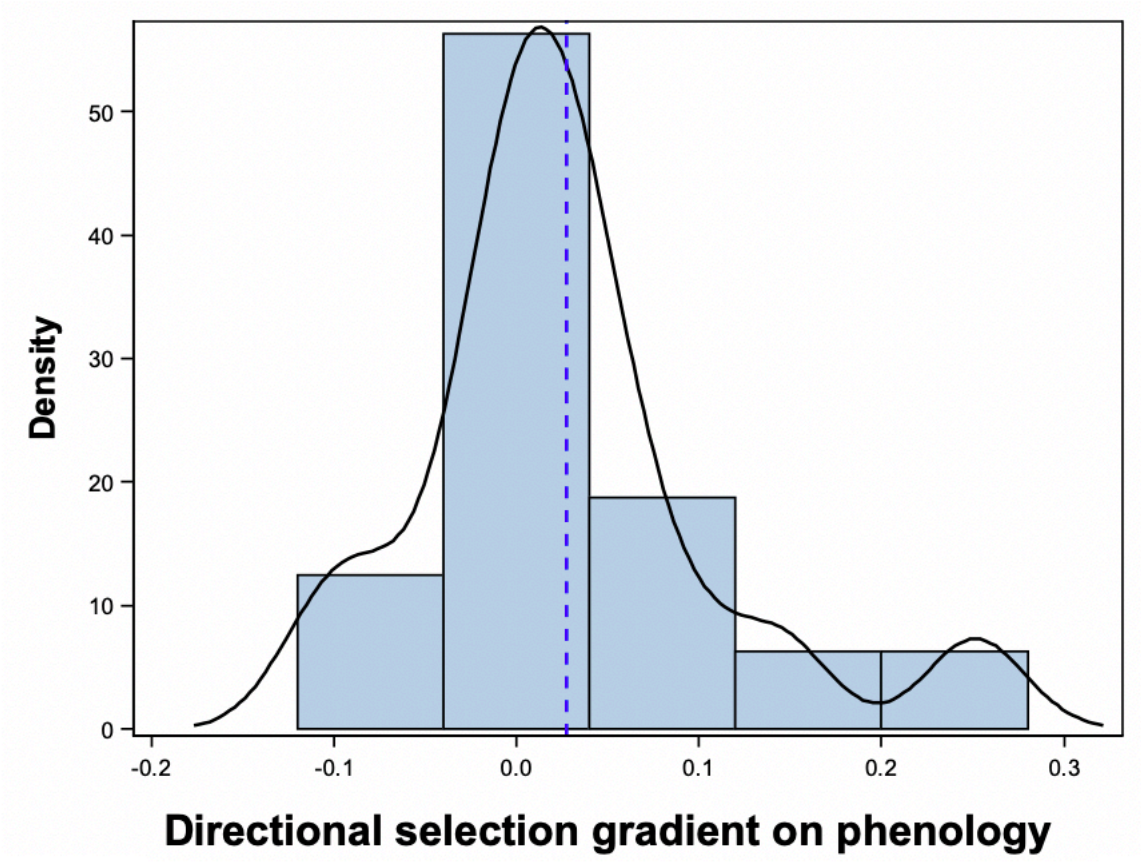
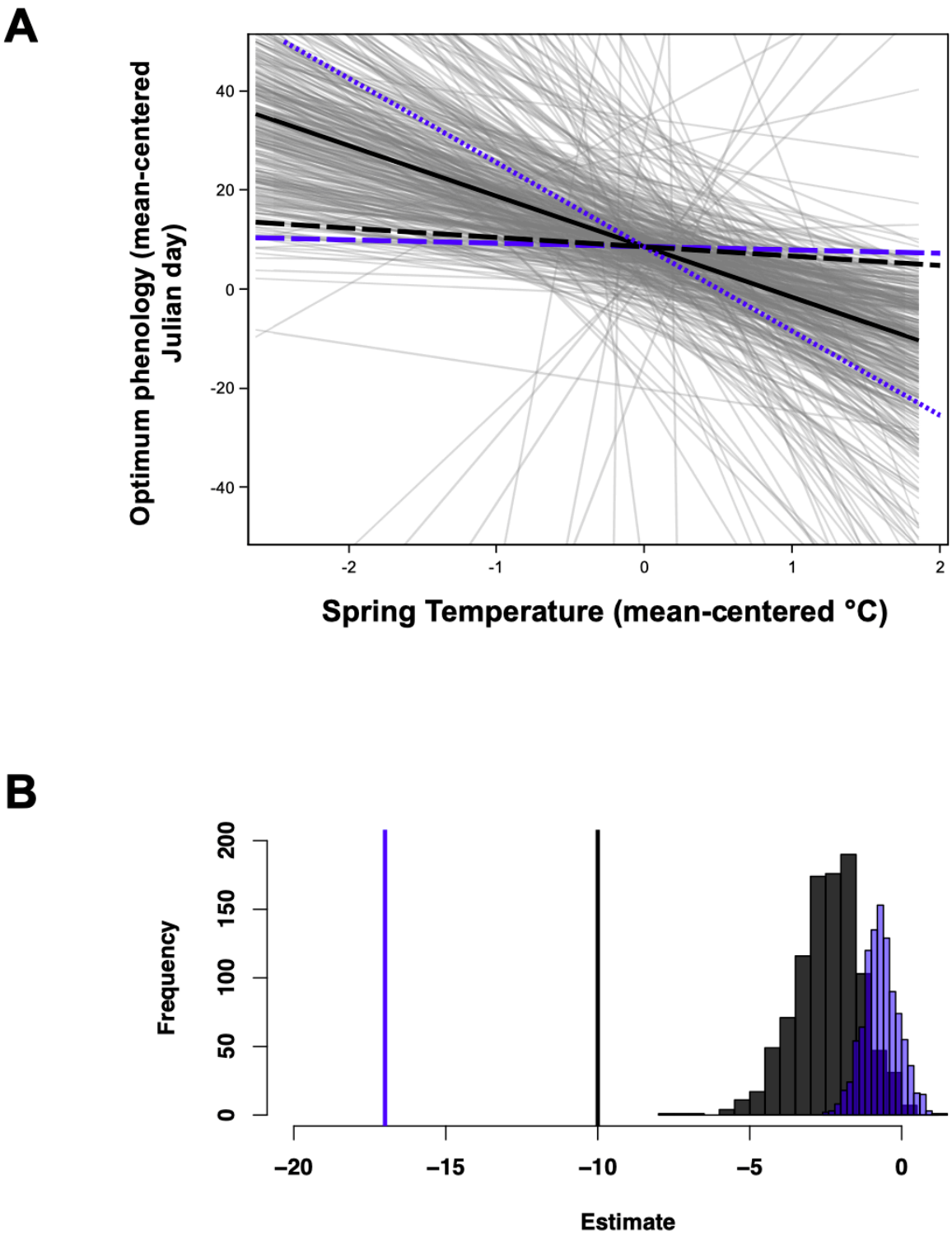


Figure S1. Variation in standardized directional selection gradients. Histogram shows BLUP estimates of annual directional selection on phenology, from a model with copulation success as response and globally-standardized (sd = 1) day as a fixed effect. Blue dashed line indicates the overall (main effect) estimate of directional selection across all 18 study years. Data come from a mixed effects model with random variation in slope and intercept among years.

1140



1141

1142

Figure S2. Comparison of parameter estimates from bivariate mixed effects reaction norm model and direct estimates of B and b. In panel A, Long-dashed blue and black lines are (respectively) the estimates of the population mean reaction norm, b, and the temperature-dependence of the optimum phenology, *B*, estimated from a bivariate mixed model with fixed and random effects of space and time (see text) using observational data from the Swedish Artportalen and NOAA GFS ANL. Other graph features are as in Figure 3C; although sampling effects result in slight differences in the grey lines showing error in B. Short-dashed blue line is the reaction norm b estimated in a laboratory experiment, solid black line is the estimate of B from individual fitness data from wild-caught damselflies. Panel B shows the posterior distributions of the observational estimates of *B* (black) and *b* (blue), along with the direct point estimates of these parameters. The direct estimate of *B* (*b*) differs significantly from the indirect measure of *B* (*b*).

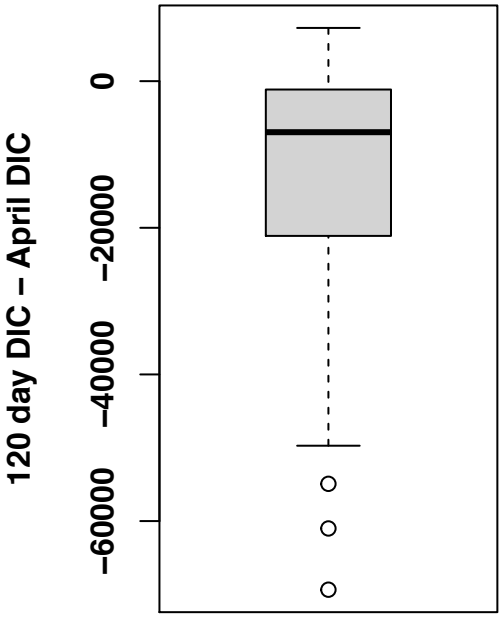


Figure S3 Comparison of model fits between identical bivariate mixed models fit to temperature and phenology data using either the mean of the first 120 days of the year or the mean April temperature. The difference in Deviance shows that on average across the 49 species studied that the mean 120 days fit on average 14322 (95% CI: -19713.136 – -8932.425, P < 0.0001) DIC units better than mean April temperature. Note that these are identical datasets (equal N) and identical models, differing only in the values of mean temperature and so comparing the likelihoods in this way provides one approach to assessing fit.