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

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Abstract

Phenology is a key determinant of fitness, particularly in organisms with complex life cycles with dramatic transitions from an aquatic to a terrestrial life stage. Because optimum phenology is influenced by local environmental conditions, particularly temperature, phenotypic plasticity could play an important role in adaptation to seasonally variable environments. Here, we used a 18-generation longitudinal field dataset from a wild insect (the damselfly *Ischnura elegans*) and show that phenology has strongly advanced, coinciding with increasing temperatures in northern Europe. Using individual fitness data, we show this advancement is most likely an adaptive response towards a thermally-dependent moving fitness optimum. These field data were complemented with a laboratory experiment, revealing that developmental plasticity to temperature quantitatively matches the environmental dependence of selection and can explain the observed phenological advance. We expand the analysis to the macroevolutionary level, using a public database of over 1 million occurrence records on the phenology of Swedish damselfly and dragonfly species. Combining spatiotemporally matched temperature data and phylogenetic information, we estimated the phenological reaction norms towards temperature for 49 Swedish species. We show that thermal plasticity in phenology is more closely aligned with local adaptation for odonate species that have recently colonized northern latitudes, whereas there is more mismatch at lower latitudes. Our results show that phenological plasticity plays a key role in microevolutionary adaptation within in a single species, and also suggest that such plasticity may have facilitated post-Pleistocene range expansion at the macroevolutionary scale in this insect clade.

Impact Statement

Organisms with complex life cycles must time their life-history transitions to match environmental conditions favorable to survival and reproduction. The timing of these transitions – phenology – is therefore of critical importance, and phenology a key trait in adaptive responses to climate change. Here, we use field data from a single species and phylogenetic comparative from over 1 million individual damselfly and dragonfly records to show that plasticity in phenology underlies adaptation at both the microevolutionary scale (across generations in a single species) and the macroevolutionary scale (across deep time in a clade). Our results indicates that phenotypic plasticity has the potential to explain variation in phenology and adaptive response to climate change across disparate evolutionary time scales.

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 (1, 2). 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) (3-7). Thus, phenology is expected to be under net-stabilizing selection (8) with an optimum that is shaped by various

70 counteracting selection pressures that are in turn influenced by multiple biotic and abiotic
71 environmental variables, including temperature, precipitation, competition and resource
72 availability.

73 The location of phenological optima may fluctuate randomly in response to
74 environmental stochasticity across years in seasonally variable environments (9).
75 Concomitantly, such fitness optima may show directional change and track advancing
76 temperatures and resource abundances associated with anthropogenic climate change (10-12). In
77 both scenarios, phenological plasticity (phenotypic plasticity in the timing of life history
78 transitions) is expected to be a target of natural selection (13, 14). Individuals that adaptively
79 alter their developmental trajectory in response to available environmental cues regarding the
80 conditions occurring during or after the life history transition (15), will undergo metamorphosis
81 to closely match the phenological optimum and will therefore have a selective advantage (13).
82 Phenotypic plasticity in phenology is therefore an expected key evolutionary outcome of
83 adaptation to seasonally variable environments (16). This classical hypothesis has obtained some
84 qualitative support in a number of previous studies across a range of taxa that all suggest a key
85 role for plasticity in explaining between- and within population differences in phenological traits
86 (17-22), including studies of organisms with complex life cycles (23-25). Although there is
87 abundant evidence for the existence of phenological plasticity, whether such plasticity is
88 adaptive or not and the role of such plasticity in generating adaptation in natural populations is
89 largely unclear (26, 27).

90 Recent work has recast the concepts of plasticity, fitness, and environmental variation in
91 terms of estimable quantitative genetic parameters that together describe plasticity's potential
92 contribution to adaptive evolution. In this framework (Figure 1) plasticity, termed *b*, and the

93 environmental dependence of selection, termed *B*, jointly determine the extent that phenotypic
94 plasticity contributes to local adaptation (13). The strength of plasticity *b* describes the
95 relationship between expressed population mean phenology and the average environment
96 experienced by a population, and thus represents the population-mean reaction norm. The
97 environmental dependence of selection, *B,* describes the relationship between the optimum
98 phenology and the mean environment, and so represents the degree to which natural selection
99 changes as a function of environmental variation. Importantly, estimation and comparison of
100 these parameters can provide insight into plasticity's role in adaptation to spatio-temporal
101 environmental variation (9). New statistical and analytical approaches have allowed researchers
102 to leverage observational individual occurrence records to estimate these parameters indirectly,
103 via space-for-time substitution (23). A growing body of studies have employed these or similar
104 time-series based approaches (23, 28-30). This work (see also 8) indicates that plasticity can
105 indeed play a large role in explaining spatio-temporal variation in phenology across natural
106 populations in the wild.

107 It is, however, still unclear whether and to what extent plastic variation in phenology
108 matches our expectations for plasticity's role in evolution. In particular, two critical open
109 questions on phenological plasticity's role in evolution remain unanswered: 1) does phenotypic
110 plasticity correspond to environmental-dependence of phenological optima at the
111 microevolutionary scale, thus amplifying or accelerating adaptation? and 2) is the role of
112 plasticity in adaptation greater for lineages inhabiting variable and/or recently colonized
113 environments? Answering the first question requires long-term data on individual fitness in the
114 wild. Answering the second requires comparative analysis of multiple species or lineages that
115 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) to answer the above two related questions on phenological plasticity's role in adaptation. Odonates are semi-aquatic insects with a phylogenetically conserved complex life cycle characterized by metamorphosis from aquatic nymph (henceforth, 'larvae') to aerial reproductive adult (31). Together, our results suggest that phenological plasticity is indeed adaptive and may play a key role in local adaptation at the microevolutionary scale within a single species, and that the role of plasticity in evolution transcends the species level and can explain macroevolutionary divergence. We suggest that phenological plasticity may have played a key role in the colonization of northern areas and adaptation to the colder climates at high latitudes during the post-Pleistocene range expansion of this insect clade into Scandinavia.

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 during the reproductive season of *I. elegans*, beginning in late May when they start to emerge from the aquatic larval stage and continuing throughout the major part of the flying season until the end of July. Field work took place daily, except during days of low temperature (< 14 °C), heavy wind and rain, when adult damselflies are not active. Each population was visited at intervals between one and two weeks. See Willink and Svensson (32) for more details about general field and laboratory work procedures.

139 *I. elegans* adults were captured in the field. Copulating pairs were kept separate from
140 single-caught individuals in individual plastic containers. In the laboratory, all individuals were
141 sexed and their copulation status (mated or not) was recorded. In this study, we used data on one
142 major male fitness component: male mating success (a binomially distributed variable) to
143 estimate the strength of selection on phenology through mating success. Our previous work has
144 shown that this fitness measure is correlated with female fitness (fecundity) in these populations,
145 i. e. male and female phenotypic selection estimates are significantly and positively correlated
146 with each other across these populations (33). Hence, male mating success is a reasonable proxy
147 of mean population fitness as a whole. Our surveys yielded data on phenology (date of capture)
148 from 47,615 individuals (28,978 males, 18,637 females) during this 18 year period (2000-2017).
149 As the majority of individuals of *I. elegans* in these populations in southern Sweden are
150 univoltine and emerge as adults after one year in the larval stage, these 18 years correspond to
151 approximately the same number of generations.
152
153 *Laboratory experiment on thermal plasticity in development time*
154 We reared larvae of individual *I. elegans* from the egg stage, through hatching and
155 metamorphosis to adults, under two different larval temperature environments: 20 °C and 24 °C.
156 Maternal egg clutches (full sib clutches) were obtained from females caught *in copula* in the
157 field in the general population survey described above. Females caught *in copula* were set up for
158 oviposition in individual plastic cups, and provided with a wet filter paper for egg laying surface.
159 Two days later, the female was removed, the eggs were scanned and counted from the digital
160 images. See Svensson and Abbott (34) and Svensson et al. (35) for further methodological details
161 about laboratory procedures. Individual eggs and larvae from a total of 101 full-sib clutches were

162 reared across both environments. We started with 20 full siblings per environmental treatment
163 for a total of approximately 4000 larvae across this rearing experiment. Upon hatching,
164 individual larvae were transferred to plastic cups where they were housed individually in
165 temperature-controlled water baths. Larvae were fed *Artemia* daily and kept at a constant
166 midsummer light cycle (16:8). Towards the end of larval development and when the larvae
167 reached the last instar, each cup received a wooden stick as an emergence aid and cups were
168 covered with mesh to prevent escape of emerging adults. This experiment continued until all
169 individuals had either died as larvae or emerged as adults. Of the 4000 larvae, 523
170 (13%) successfully emerged as adults, with recorded emergence time for 521 individuals. Larval
171 development time was calculated as date of emergence as an adult minus the date of hatching,
172 consistent with our previous procedures (36).
173
174 *Occurrence records of Swedish Odonata*
175 We obtained public, spatiotemporally referenced observational records of adult Swedish
176 odonates identified to species level from the Swedish Species Observation System (Artportalen:
177 https://www.artportalen.se/). These data represent a combination of citizen science records, as
178 well as records reported by professional scientists and naturalists throughout Sweden, and these
179 data are also subsequently exported to the Global Biodiversity Information Facility (GBIF:
180 https://www.gbif.org/). We used data from a ten year period from 2006-2015, the range of years
181 for which we had accurate temperature data (see below) and for which substantial observational
182 records were available in this public database. We excluded rare taxa for which records were
183 unavailable in some years in this range, as well as taxa that were not included in our phylogeny
184 (see below). Our final dataset consists of 1,027,952 records from 49 species, which is the

185 majority of odonate species (75 %) that have so far been recorded in Sweden (currently a total of
186 65; 43 dragonflies (Anisoptera) and 22 damselflies (Zygoptera)).
187 We obtained spatiotemporally matched estimates of spring mean surface temperature
188 from the publicly accessible NOAA Global Forecast System Analysis (GFS ANL) database
189 (https://www.ncdc.noaa.gov/data-access/model-data/model-datasets/global-forcast-system-gfs).
190 The NOAA GFS ANL provides global weather data estimated four times per day gridded at .5°
191 resolution (corresponding to approximately 25km in Sweden), using a system of satellites and
192 globally-distributed weather stations. Comparison of these surface temperature estimates to
193 direct temperature records from a weather station in Malmö, Sweden, obtained from the Swedish
194 Meteorological and Hydrological Institute (https://www.smhi.se/en), indicates the GFS ANL
195 data are an accurate representation of surface temperature ($r > 0.99$ between temperature
196 records). For each record in our dataset of Swedish odonates, we obtained the mean surface
197 temperature (GFS ANL database variable: tmp2m) for the first 120 days of the year from
198 corresponding lat-long grid cell in the GFS ANL database. We focused on this period because it
199 corresponds to a substantial period of pre-metamorphosis larval development and covers the last
200 instar for all the species in our database. We also obtained mean temperature for the month of
201 April, although we here focus our analysis on the 120 day mean, as this turned out to be a better
202 predictor of phenology and provided better model fits (see Supplemental Material).
203 We obtained phylogenic information for all 49 species in our dataset, using a recently
204 published large molecular phylogeny of the odonates (37). We set branch lengths using Grafen's
205 (38) method to make our tree ultrametric and be able to use it as a covariance matrix in mixed
206 effects models.
207

208 **Methods – Statistical Analysis**

209 *Long term field study of Ischnura elegans*

210 We used a linear mixed effects model to assess phenological advance in the timing of the adult
211 aerial period in *I. elegans* during our 18 year study period. This model featured the date (in
212 Julian days; January 1 = day 1) of an observation of an individual as the response variable and
213 year as a fixed effect. Our model also included a random intercept for population to account for
214 spatial variation in phenology among the study populations.

215 To infer selection on phenology, we seek a statistical description of individual fitness as a
216 function of phenology, where the functional relationship between phenology and fitness varies
217 with spring temperature cues. We modify the Lande-Arnold (39) equation to include
218 temperature dependence in the form of directional selection on phenology:

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

219

220 where $\varphi = \frac{\phi}{\beta}$, ϕ is an interaction term as estimable in a regression model, w is individual
221 fitness, β is the linear selection gradient on phenology z, t is the temperature cue and γ is the
222 nonlinear selection gradient on phenology. Setting the first derivative of equation 1 with respect
223 to z to zero and solving yields the stationary point:

224

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

225

226 where θ_z is the optimum phenology (if selection is stabilizing, otherwise it is a fitness
227 minimum). We note that this quadratic description of environmental-dependence of selection

228 also approximates environmental dependence of a Gaussian fitness function (40). The first part
229 of the right hand side of equation 2 is an intercept equal to the fixed location of the optimum
230 obtained from the standard Lande-Arnold equation (41). The second term on the right hand side
231 describes a temperature-dependent slope. Thus, the simple modification of the Lande-Arnold
232 equation in 1 yields a statistical description of a linear relationship between temperature cue and
233 optimal phenology, where the slope of the relationship between temperature and θ_z is $\frac{\phi}{-\gamma}$, and
234 represents a direct estimate of the temperature dependence of selection (*B*).

235 We fit equation 1 to our *I. elegans* field dataset using a linear mixed effects model with
236 male copulation success as a response, i. e. a measure of sexual selection. For comparison, we
237 also fit a simplified model, with directional selection only (i.e., no temperature-dependence or
238 nonlinear terms). We focus on males only in this analysis, as copulation success is likely a more
239 critical determinant of male fitness than it is for females. We used absolute copulation success
240 as a response, as this resulted in more consistent model convergence and eases interpretation;
241 however, qualitatively equivalent conclusions were obtained using relativized (by subpopulation,
242 season or globally) copulation success. We estimated β, γ, and ϕ from the fixed effect
243 parameters (doubling the nonlinear regression coefficient; 42), and included random
244 (co)variation in β and γ among years to accommodate variation in selection independent of
245 temperature. Temperature was obtained as the mean of the first 120 days from the NOAA GFS
246 ANL database for the corresponding grid cell of our study populations, with the exception of
247 records from 2000-2005 for which we instead calculated this value from the Swedish
248 Meteorological and Hydrological Institute data since the GFS data was unavailable for this
249 period.
250

251 *Laboratory temperature experiment: raising damselfly families*

252 We used a mixed effects model to estimate the relationship between thermal developmental
253 environment of larvae (two temperature treatments) and emergence time from our laboratory
254 experiment. Our model featured emergence day as the response variable, temperature treatment
255 as a fixed effect, and random intercepts for tank and maternal full-sib family. We calculated
256 phenotypic variancies, additive genetic variances and heritability (h^2), the latter as twice the
257 family variance over the total variance of the random effects (the sum of tank, family, and
258 residual variance components). We assessed statistical significance of h^2 using a likelihood ratio
259 test of the family variance component. We also calculated standard error and 95% confidence
260 intervals by empirically constructing the sampling distribution of h^2 by taking 1 million draws
261 from a multivariate normal distribution centered on our original REML estimates of the variance
262 components with covariance equal to the Hessian matrix of the fitted model (43). We note that
263 our estimate of heritability is conditioned on the fixed effect of temperature treatment (44).

264

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

266 We used a bivariate mixed modelling approach (23, 29) to infer interspecific variation in thermal
267 reaction norms in phenology from our observational records of Swedish Odonata combined with
268 paired estimates of temperature in the first 120 days of the year. The premise behind this
269 approach is to use detrended covariation in temperature and phenology, within geographic grid
270 cells, as an estimate of population mean reaction norms. From the same model, covariation in
271 mean phenology and temperature across geographic grid cells provides an estimate of the
272 environmental dependence of selection (9). We thus fit the bivariate model

273

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

where ζ_i is the response vector of individual values of two variables: i = observed phenology or 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 we estimated the full 2x2 unstructured covariance matrix $\begin{bmatrix} \sigma^2_{phenology} & \sigma_{phenology,temp} \\ \sigma_{phenology,temp} & \sigma^2_{temperature} \end{bmatrix}$ 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. 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 13 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

295 (9). From the random effects, we can interpret $b \sim \sigma_{phenology,\ temperature}/\sigma^2_{temperature}$ where
296 σ and σ^2 are the elements of the random effect covariance matrix estimated across time points
297 within binned latitude ($a_{time(lat)}$). Thus, b is the de-trended regression of phenology across
298 temperature within latitudinal bins (23, 29). This bivariate model was fit separately for each
299 species in our dataset.

300 We then used the corresponding estimates of B and b in phylogenetic mixed models (45)
301 to explore the relationship between species mean latitude, species mean phenology, and the
302 strength and form of inferred phenological plasticity. We focus on species mean latitude because
303 we were interested in how plasticity has evolved with Northern range expansion. These models
304 included species as a random effect, with covariance among levels equal to the phylogenetic-
305 variance-covariance matrix from our species level phylogeny, standardized to a depth of 1 (i.e.,
306 using the correlation matrix). These models also included a residual term. We estimated
307 phylogenetic signal in the inferred reaction norms b using Pagel's lambda (46).

308 All mixed models were fit in SAS using either the hpmixed (for bivariate models, due to
309 exceptional sample sizes) or glimmix (for all others) procedures. SAS and R scripts to reproduce
310 our analyses and figures, as well as all raw data, are provided at:
311 https://github.com/spdelisle/OdonatePhenology, with the exception of the dataset of matched
312 phenology and temperature records (which exceeds github's size limit), which can currently be
313 found as a .sas dataset at: https://www.dropbox.com/s/ah3w6geewv2p7k7/swed_big.sas?dl=0.
314 All datasets have also been deposited as .csv files on Dryad
315 https://datadryad.org/stash/share/FKHv4E_XmOX2f4yEi63pTibgzqmNFuXXhJLi_lnV3J0.
316

317 **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 approximately .58 days per year, corresponding to a predicted total advance of 10.44 days during the entire 18-year period.

Using data on male mating success (N = 21,384 individuals for which we also had annual temperature data, consisting of 5,487 mated males and 15,897 non-mated males), we find evidence of statistically significant but seasonally variable (Figure 3B) sexual selection on phenology. Overall, we found evidence of significant stabilizing selection on phenology (estimates of parameters in equation 1, in units of raw Julian days: γ = -0.00016, χ_1^2 = 4.51, P = 0.0336; β = 0.001308, χ_1^2 = 1.18, P = 0.2), along with significant temperature-dependent component to directional selection on phenology (ϕ = -0.00156, χ_1^2 = 6.90, P = 0.0086). Fitting a simplified model with directional selection only and random variation among years to variance-standardized data indicates standardized directional selection gradients varied substantially among seasons (overall estimate β = 0.027, SE = 0.021; see figure S1). The resulting estimate of the relationship between θ_z and spring mean temperature indicates a temporal advance in the optimum phenology with increasing temperature (Figure 3C). Our point estimate of *B*, the slope of the relationship between temperature and θ_z , given by the ratio of the parameter estimate $\frac{\phi}{-\gamma}$, indicates an advance of approximately 10 days in the phenological optimum for each degree centigrade of spring warming (Figure 3C). We reach qualitatively equivalent conclusions using an independent dataset of 1695 observations from the Artportalen

340 database when we inferred B indirectly from interspecific data on phenology (Supplemental
341 material, Figure S2).
342 We obtained individual and family emergence time records from 521 individuals from
343 101 full-sib genetic families across two temperature treatments in our laboratory rearing
344 experiment. We found significant acceleration of development in response to our experimental
345 manipulation of temperature ($F_{1, 367} = 65.61$, $P < 0.0001$, Figure 3A), with an estimated reaction
346 norm of approximately -17 days per 1 °C warming of the developmental environment. This
347 laboratory estimate therefore corresponds closely with our estimate of the environmental
348 dependence of θ_z estimated from the field data in the natural populations of *I. elegans* (Figure
349 3C). We found statistically significant broad-sense heritability in the timing emergence (H^2 =
350 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
351 = 326; $CV_A = 5.3\%$). Our finding of a strongly negative thermal reaction norm in larval
352 development time that aligns with the environmental dependence of selection on phenology was
353 supported by an independent, indirect inference of *b* using observational data (Figure S2).
354 We analyzed paired temperature and phenology data from 1,027,952 records from 49
355 species of Swedish Odonates (see Table S1). Reaction norm estimates varied across species,
356 although most estimates where within the range of five days per °C of spring temperature
357 variation (Figure 4, Table S1). The damselfly *Sympecma fusca* and the dragonfly *Aeshna serrata*
358 were the two species with the most negative reaction norms in phenology (Fig. 4), i. e. they
359 responded most strongly in terms of advancement. Notably, there were also several species that
360 showed positive reaction norm slopes (Fig. 4). These analyses show that temperature during the
361 last instar larval stage can either advance or delay emergence, depending on species, and some
362 species were relatively insensitive to spring temperature (Fig. 4). On average, the estimated

363 thermal reaction norms explained 9% of the de-trended within-latitude variation in phenology
364 (Table S1; full model fits provided in the supplemental material). We also found evidence of a
365 significant phylogenetic signal in the phenological reaction norm (Pagel's $\lambda = 1.002$ LR chi
366 square = 18.67, $P<0.001$), revealing that closely related species have similar magnitude of
367 thermal plasticity in phenology (Figure 4). Estimated reaction norms were correlated with mean
368 phenology across species, with early season species exhibiting more negative reaction norms and
369 late season species exhibiting positive reaction norms ($F_{1, 46} = 8.10$, $P = 0.0066$, Figure 5A). We
370 also found a trend of more negative reaction norm slopes for the southern species in a
371 phylogenetic mixed model with latitude as a predictor of estimated reaction norm ($F_{1, 45} = 3.49$, P
372 = 0.07, the two outlier species *Sympecma fusca* and *Aeshna serrata* excluded from this analysis,
373 Figure 5B).
374 The alignment between the estimated phenological reaction norms, b, and the estimated
375 environmental dependence of selection, B, described as |B-b|, varied significantly with species
376 mean latitude ($F_{1, 47} = 9.27$, $P = 0.0038$, Figure 5C). Species at northern latitudes had thermal
377 reaction norms that were more closely aligned with their estimated environmental dependence of
378 phenological optima. Thus, plasticity and selection were more closely aligned in the northern
379 species, suggesting that plasticity might be more adaptive in northern than in southern species.
380

381 **Discussion**

382 Here we combine three independent datasets, together representing well over 1 million
383 individual insect records, to test two related questions on the role of phenotypic plasticity in the
384 evolution of phenology. First, we demonstrate pronounced recent phenological advance in a
385 single damselfly species, *Ischnura elegans*, using a long term study of individuals in the wild.

386 We addressed the question of whether temperature-dependent phenological plasticity
387 corresponds to a temperature dependence in the optimum phenology. We find that thermal
388 plasticity in developmental time in the laboratory is substantial and matches the relationship
389 between temperature and the optimum phenology of the same species estimated in the field. This
390 suggests an important role for thermal plasticity in adaptation to seasonal environmental
391 variability, and also suggests plasticity could contribute to advancing phenology that corresponds
392 to recent anthropogenically-caused climate change in Sweden (47). At the macroevolutionary
393 scale, we used a large interspecific database of individual emergence records of 49 species of
394 odonates spanning the extensive latitudinal gradient of Sweden. We combined these occurrence
395 records with spatiotemporally matched estimates of spring mean temperature to examine the
396 possible role of phenotypic plasticity in species divergence in phenology. We found that these
397 species-specific mean phenological reaction norms are more closely aligned with indirect
398 inferences of the environmental dependence of the optimum for northern than for southern
399 species. Our combined intra- and interspecific results indicate that temperature-dependent
400 plasticity in phenology underlies both microevolutionary adaptation to contemporary climate
401 change as well as the macroevolutionary expansion into harsh climatic conditions at high
402 latitudes that took place when species colonized these areas after the last Ice Age in
403 Fennoscandia, about 10,000 years before present.
404 We found a phenological advance in a single species (*Ischnura elegans*) of 0.58
405 days/year, almost six years per decade or 10.44 days over the entire 18-year study period (Fig.
406 1). This is a dramatic phenological advance during the first two decades of the century in
407 Sweden. In fact, this is almost twice as large advancement of phenology as was documented
408 previously for *I. elegans* in a study of British Odonata for the period 1960-2004 (0.58 vs. 0.33;

409 48). Across all of these 25 British species of Odonata, the average phenological advancement per
410 decade was 1.51 days (48), again considerably lower than the estimated advancement of 5.8 days
411 per decade for *I. elegans* in Sweden. Moreover, the average advancement across these British
412 Odonata species was 3.08 days per °C (48), considerably less than our estimate of the advanced
413 phenology of *I. elegans* in Sweden. Thus, although our findings of phenological advance are
414 consistent with previous study in Britain, the new results presented here suggest temperature-
415 dependent phenological advance in odonates may be more extreme than previously appreciated.
416 We find close correspondence between point estimates of the environmental dependence
417 of selection in *Ischnura elegans* and the population mean reaction norm, using three independent
418 datasets and approaches. Our direct estimate of *B* using data on individual fitness from the long-
419 term study on *I. elegans* and our indirect estimates using a public database of individual
420 occurrence records for 49 different species both suggest that the optimum emergence time
421 advances substantially for every centigrade degree of spring warming. Our direct estimate of the
422 phenological reaction norm matches closely with our direct estimate of the environmental
423 dependence of selection, and the correspondence is also found using a bivariate mixed model
424 approach to indirectly estimate these parameters (see Supplemental Material). Although absolute
425 values of *B* and *b* differ substantially across these two approaches (direct vs indirect), the
426 qualitative conclusion – that phenotypic plasticity can explain the close match between local
427 optima set by environmental variation – is equivalent across these different approaches and
428 datasets. This correspondence is encouraging and suggests that our space-for-time substitution
429 approach to estimating environmental dependence of optimal phenologies may be robust to
430 inevitable violation of the assumptions (e.g., local adaptation and limited dispersal) that underlie
431 the theory behind the method (9). We are unaware of other comparisons between these

432 approaches (but see 49 for a comparison of other related approaches). The results presented here
433 echoes past suggestions (30) that further validation of indirect estimates of *B* is warranted.
434 Our laboratory breeding experiment revealed a strong effect of larval temperature on
435 development and emergence time, with an advance of 17 Julian days per degree Celsius.
436 Although this might suggest 'hyper plasticity' (17) wherein the population mean reaction norm
437 might exceed the environmental dependence of selection, we note that our laboratory experiment
438 consisted of constant thermal conditions within each treatment for the duration of development.
439 In natural settings, variation in temperature occurring on top of mean temperature differences
440 could reduce the expression of plasticity (and thus the mean reaction norm), or in other cases
441 inhance it. Moreover, our laboratory environment lacked other environmental cues, such as
442 photoperiod, that may be used in addition to or conjunction with temperature to determine
443 individual developmental rate, which may result in apparent hyper plasticity in analysis of a
444 single cue (14). Nevertheless, our laboratory experiment does indicate that the strength of
445 temperature-dependent plasticity qualitatively matches our estimate of the environmental
446 dependence of selection and is aligned with the direction of temporal phenological advance in
447 response to rising temperatures in natural populations. Indeed, the estimated population reaction
448 norm in *I. elegans* suggests that an adaptive fit between the thermal optimum and mean
449 phenology could be explained entirely by phenotypic plasticity. This indicates that *I. elegans*
450 may already be well-adapted to cope with changing climates and increasing temperature
451 compared to many other animal taxa, such as birds (50) and is able to closely track moving
452 phenological optima. Consistent with this finding, gene expression analyses and heat- and cold
453 shock experiments in *I. elegans* adults from different parts of the geographic range indicate that

454 adaptive thermal plasticity does play an important role in the northward range expansion of this
455 species (51, 52), in addition to genetic adaptation (53).
456 Our laboratory experiment also revealed that development time was modestly heritable,
457 with a point estimate of $H^2 = 0.24$. This is considerably lower than a previous estimate of the
458 heritability of development time in this species (0.51), estimated in a single thermal environment
459 (54). The modest level of heritability of development that we document here would require a
460 persistent variance-standardized selection gradient of approximately $\beta = -.13$ to result in the
461 observed annual phenological advance of 0.58 days/year (approximately .03 within-year standard
462 deviations). This would be relatively strong selection compared to available evidence
463 documented in meta-analyses of field studies on phenotypic selection (55). Although our results
464 and analyses do indeed suggest that selection can be strong, we also find little evidence of
465 persistent directional selection of the magnitude required to produce such a dramatic response. In
466 fact, across all years, net selection on phenology is actually slightly positive rather than negative
467 as would be expected if earlier phenology was consistently favored (Fig. S1). Therefore, it is
468 unlikely that genetic evolution alone can entirely explain the strong phenological advance of
469 more than 10 days during the entire 18-year period, given the substantial among-year variation
470 (Figure 2) of *I. elegans* in southern Sweden. Instead, our analyses suggest that phenotypic
471 plasticity may have a key role in driving both inter-annual variation in phenology and the
472 progressively earlier flight seasons that we have documented here. These data suggest that *I.*
473 *elegans* can track the changing phenological optima through plasticity, which will in effect
474 reduce or counteract directional selection for earlier phenology, a conclusion that was also
475 reached in a recent study of 21 bird and mammal species with long-term data (8). More
476 generally, various forms of phenotypic plasticity have been proposed to "buffer" organisms

477 against external stress from temperature extremes (56), and recent studies have shown that
478 plasticity and selection might sometimes be closely aligned (57, 58).

479 Theory suggests that the fitness benefit of phenological plasticity depends upon the
480 existence of environmental cues that accurately predict the conditions that determine fitness in
481 the next life stage (14). Here we have focused on spring mean temperature as a potential
482 environmental cue (or correlate thereof). This is justified in substantial evidence linking
483 temperature and corresponding thermal tolerance to various aspects of adult fitness in insects in
484 general (59, 60), and our study species *I. elegans* in particular (51-53, 61). Moreover, our
485 laboratory experiment confirms that larval developmental temperature is a key predictor of
486 emergence time into the transition to the adult terrestrial life-stage. Across all our interspecific
487 dataset, all but one species in our comparative analysis over-winter as either aquatic larvae or
488 eggs, and so are exposed to spring temperatures at a similar developmental stage. The only
489 exception to this rule is the winter damselfly *Sympecma fusca*, which overwinters as an adult in
490 vegetation refugia. Consistent with this, *S. fusca* was a clear outlier in our estimates of
491 phenological reaction norms, exhibiting the most extreme negative estimate of *b* (Fig. 4A). This
492 suggests that this species might be quicker to respond to spring temperature cues in the transition
493 to the aerial life-stage, compared to species that overwinter in their aquatic larval stage or as
494 eggs. This fits with previous research showing that temperature-phenology reaction norms tend
495 to be strongest in insects that over winter as adults (62). Across all the species included in this
496 study, it is likely that other environmental cues are also important for determining developmental
497 rate; for instance, aspects of the biotic environment, such as density of con- or heterospecific
498 competitors and resource availability. These unknown additional cues could explain much of the
499 residual variance in phenology as well as the lack of alignment of inferred reaction norms and

500 inferred optima in some southerly taxa. As an example, plasticity in response to resource
501 availability, instead of temperature, is a main driver of advanced phenology red squirrels despite
502 the appearance of directional selection on heritable phenology (63). Nevertheless, our focus on
503 temperature is relevant in light of anthropogenic climate change (11) and the general consensus
504 that temperature is a stronger determinant of fitness in ectotherms than in endotherms (64).
505 The degree of alignment between the phenological reaction norms and environmental
506 dependence of selection changed across the latitudinal range of these 49 species in Sweden (Fig.
507 4C). This indicates that adaptive phenological plasticity has played an important role in the
508 northern range expansion of this clade. Insect colonization of the Scandinavian peninsula
509 occurred relatively recently following the retreat of the large Fenno-Scandian ice sheet, which
510 covered the region from the last glacial maximum until its recession at the end of the Pleistocene
511 approximately 10,000 years ago (65, 66). Thus, the odonate species that currently occur in
512 northern Sweden have established themselves in this region only recently. Our findings of
513 stronger alignment between the fitness optima and plasticity in northern species in our
514 phylogenetic comparative analysis (Fig. 5) is thus consistent with the theoretical prediction (67,
515 68) that plasticity is important for adaption to novel and variable environments, such as near
516 range limits (51, 52). Conversely, although such adaptive alignment between plasticity and
517 phenological optima were observed in northern species, the southern species might currently
518 show some degree of non-adaptive or even maladaptive plasticity in relation to temperature as
519 they show less alignment (Fig. 4C). Recent research indicate that phenotypic plasticity can be
520 adaptive, non-adaptive or even maladaptive, depending on traits and environmental context (17,
521 57, 58, 69).

522 By combining data from multiple and independent sources of data, including individual
523 variation in flight periods, fitness data, and data on thermal environmental variation, we have
524 revealed a key role for phenotypic plasticity in explaining intra- and interspecific variation in
525 phenology. Importantly, through our focus on a group of insects with a conserved and complex
526 life cycle, we were able to expand our intraspecific microevolutionary analyses of a single
527 species to the macroevolutionary level, to asses phenological plasticity's role in post-Pleistocene
528 macroevolution of insect phenology and its temperature-dependence. Our work indicates that
529 temperature-dependent phenological plasticity can at least partly track changing optima due to
530 recent climate change, and that such adaptive phenological plasticity may also have facilitated
531 historical range expansion into variable and extreme northern environments.
532
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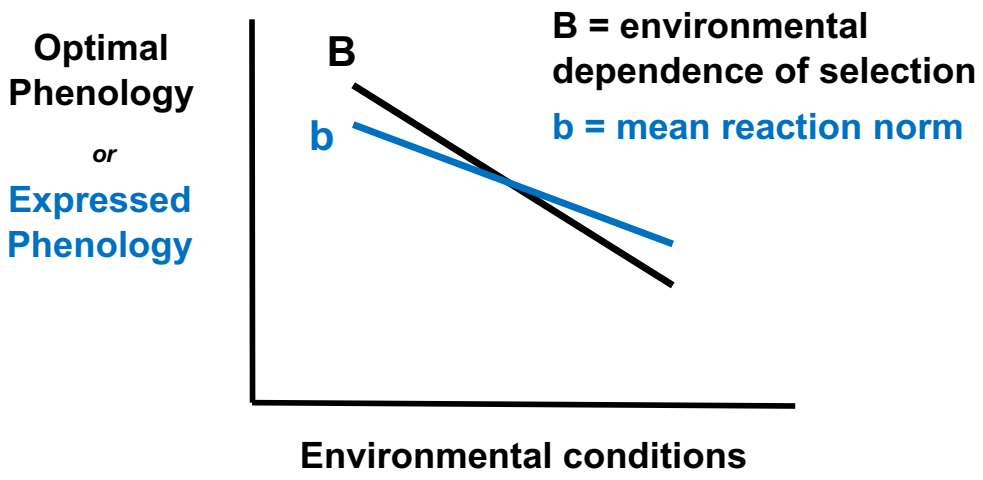


Figure 1. The environmental dependence of selection and the mean reaction norm jointly determine the role that phenotypic plasticity plays in adaptation. The x-axis represents an environmental variable important for individual fitness; the y-axis represents phenology, the timing of a key life history 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 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.

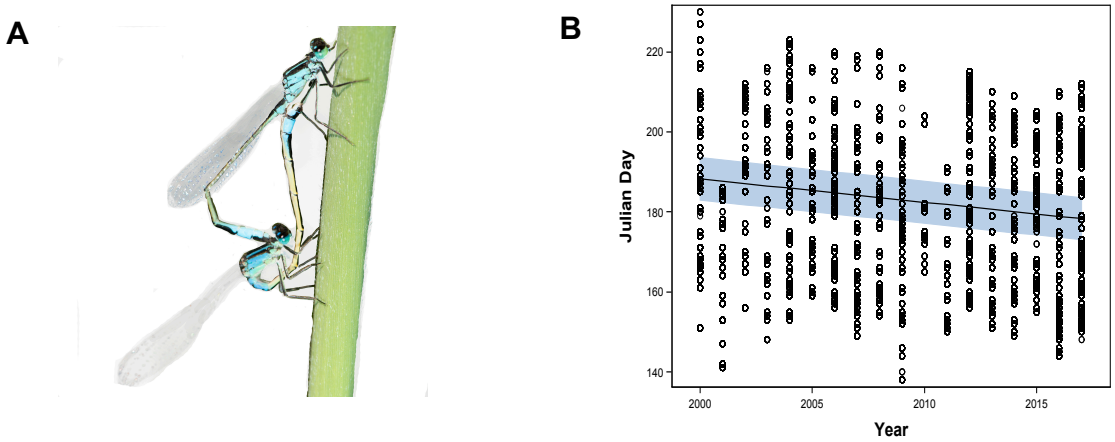
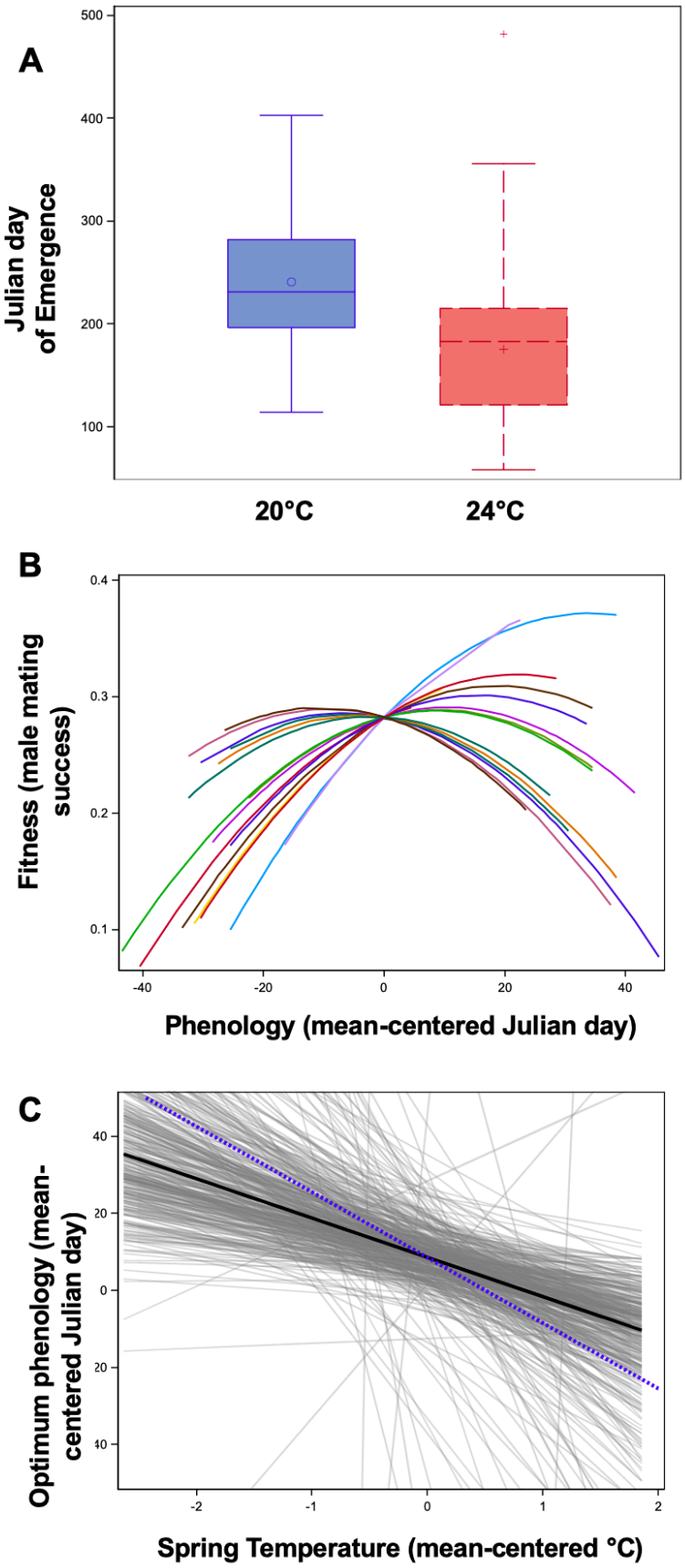


Figure 2. Reproductive advance in *I. elegans* in Southern Sweden. *Ischnura elegans*, a common European damselfly, exhibits an aerial reproductive adult stage (**A**) that follows a prolonged aquatic larval growth stage. Long term study of a set of populations in southern Sweden over 18 years indicates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


A
Julian day of 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 Julian day)
C
Optimum phenology (mean-centered Julian day)
40
20
0
20
40
-2
-1
0
1
2
Spring Temperature (mean-centered °C)

564

565 **Figure 3. Temperature dependent selection and plasticity are aligned in *I. elegans*.** In a
566 laboratory experiment, increased larval developmental temperature was associated with
567 accelerated time to metamorphosis into an aerial adult, **A**. Y axis days from hatching to
568 emergence. In a long term study of *I. elegans* in the wild, selection varied across years but was
569 net-stabilizing, **B**, with optima that vary across years (lines show model fits from the fixed
570 effects across years; each color represents a unique year). The estimated optimum was negative
571 temperature dependent, **C**. Panel C shows the linear relationship between θ_z and spring mean
572 temperature (equation 2 in text); black line shows original ML estimates, grey lines show 100
573 samples from a multivariate normal distribution centered at the ML estimates with covariance
574 equal to the covariance matrix of fixed effected from the fitted model. Dashed blue line shows
575 the estimated reaction norm from the plasticity experiment (see panel A).
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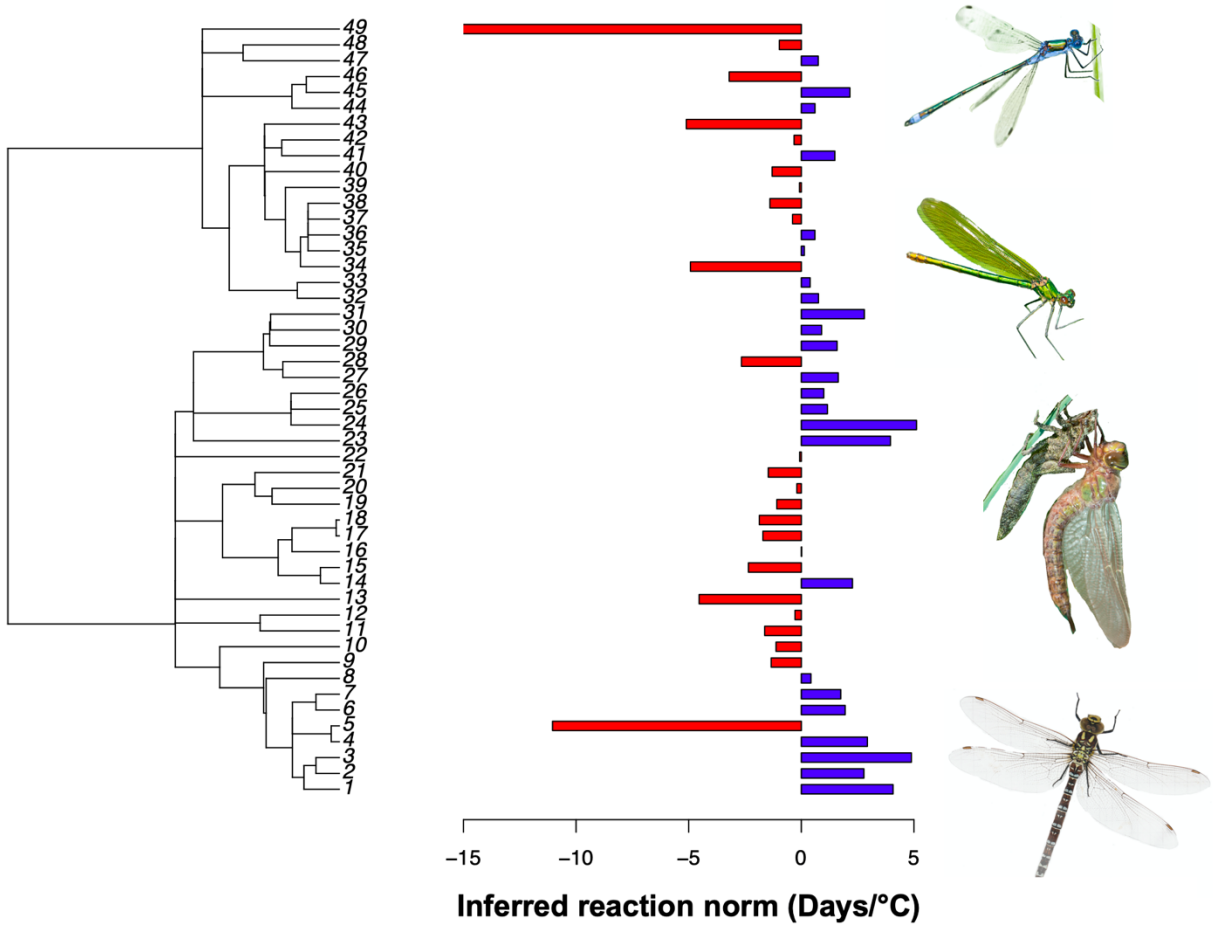
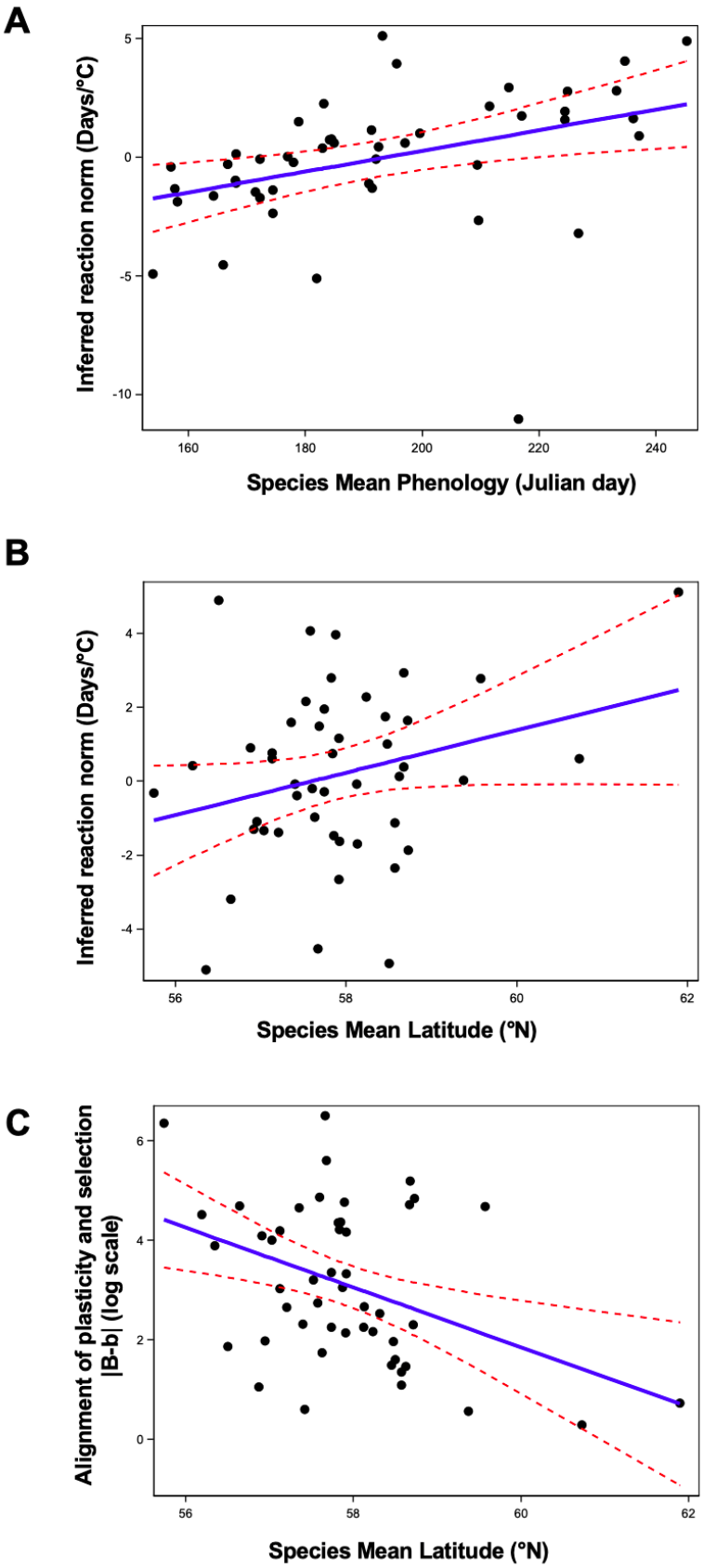


Figure 4. Inferred temperature reaction norms for phenology across Swedish Odonates. Estimated reaction norms for temperature dependent phenology show variation among species, although most are relatively shallow and phylogenetic signal is high and statistically significant. *Sympecma fusca*, 49, was truncated for clarity (b = -110.69). Species images (photos by S. De Lisle, top down): *Lestes dryas* (ID 44), *Calopteryx splendens* (ID 32), *Brachytron praetense* (ID 9), *Aeshna cyanea* (ID 1). Full species names for each ID number are provided in Table S1.


A
Inferred reaction norm (Days/°C)
5
0
-5
-10
160
180
200
220
240
Species Mean Phenology (Julian day)
B
Inferred reaction norm (Days/°C)
4
2
0
-2
-4
56
58
60
62
Species Mean Latitude (°N)
C
Alignment of plasticity and selection
|B-b| (log scale)
6
4
2
0
56
58
60
62
Species Mean Latitude (°N)

600

Figure 5. Macroevolutionary dynamics of phenological plasticity. Species with flight periods early in the year exhibit more negative thermal reaction norms, while those that emerge late in the season 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) for species with Northern geographic ranges **C**. Together these data suggest that reaction norms have diversified at the macroevolutionary scale and that this diversity may have played a particularly important role during expansion into northern latitudes.

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807 **Supplemental Material**

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. R^2_b is the variance in phenology explained by plasticity within grid cells. See text for further details.

Taxon	species ID #	Mean temp	Mean phenology	Mean Latitude	B	b	R^2_b
Aeshna cyanea	1	0.90	234.72	57.58	-11.38	4.07	0.25
Aeshna grandis	2	0.34	224.90	59.58	109.70	2.78	0.21
Aeshna juncea	3	1.43	245.27	56.51	-1.59	4.89	0.25
Aeshna mixta	4	-0.13	214.83	58.68	181.84	2.93	0.13
Aeshna serrata	5	0.41	216.44	58.32	1.43	-11.04	0.44
Aeshna subarctica	6	0.63	224.42	57.74	-7.57	1.95	0.02
Aeshna viridis	7	0.45	217.06	58.46	-2.66	1.75	0.04
Anax imperator	8	2.58	192.54	56.20	91.59	0.43	0.00
Brachytron pratense	9	1.20	157.68	57.03	-55.71	-1.34	0.09
Cordulegaster boltonii	10	-0.11	190.87	58.57	2.75	-1.12	0.02
Cordulia aenea	11	0.41	164.30	57.92	-66.13	-1.63	0.07
Epitheca bimaculata	12	0.95	166.71	57.74	28.19	-0.28	0.00
Gomphus vulgatissimus	13	0.81	165.96	57.67	-670.66	-4.54	0.20
Leucorrhinia albifrons	14	0.98	183.21	58.24	-6.42	2.27	0.07
Leucorrhinia caudalis	15	0.25	174.51	58.57	-5.34	-2.35	0.18
Leucorrhinia dubia	16	0.03	177.04	59.37	-1.75	0.02	0.00
Leucorrhinia pectoralis	17	0.40	172.30	58.13	12.64	-1.70	0.07
Leucorrhinia rubicunda	18	0.16	158.16	58.73	125.02	-1.86	0.06
Libellula depressa	19	1.17	168.22	56.95	6.12	-1.09	0.03
Libellula fulva	20	1.31	178.01	57.60	-129.90	-0.20	0.00
Libellula quadrimaculata	21	0.52	171.52	57.85	76.68	-1.47	0.06
Onychogomphus forcipatus	22	0.46	192.14	58.13	-9.53	-0.07	0.00
Orthetrum coerulescens	23	0.58	195.60	57.88	-17.14	3.96	0.22
Somatochlora arctica	24	-0.12	193.21	61.90	7.19	5.12	0.08
Somatochlora flavomaculata	25	0.72	191.30	57.92	28.90	1.16	0.02
Somatochlora metallica	26	0.10	199.56	58.48	-6.12	1.00	0.01
Sympetrum danae	27	0.60	236.08	58.72	-8.32	1.64	0.03
Sympetrum flaveolum	28	0.47	209.63	57.91	5.81	-2.66	0.04
Sympetrum sanguineum	29	1.05	224.38	57.35	106.20	1.59	0.06
Sympetrum striolatum	30	1.60	237.06	56.88	3.78	0.90	0.00
Sympetrum vulgatum	31	0.81	233.27	57.83	-75.24	2.80	0.09

Calopteryx splendens	32	1.05	184.48	57.13	-65.05	0.76	0.01
Calopteryx virgo	33	0.34	182.96	58.67	111.47	0.39	0.00
Coenagrion armatum	34	0.36	153.94	58.50	0.02	-4.92	0.08
Coenagrion hastulatum	35	0.06	168.22	58.63	-4.18	0.13	0.00
Coenagrion johanssoni	36	-1.11	184.94	60.73	1.93	0.61	0.01
Coenagrion lunulatum	37	1.15	157.04	57.42	-2.23	-0.39	0.00
Coenagrion puella	38	1.09	174.47	57.21	-15.63	-1.39	0.04
Coenagrion pulchellum	39	0.76	172.28	57.40	-10.17	-0.08	0.00
Enallagma cyathigerum	40	0.87	191.40	56.91	58.65	-1.29	0.03
Erythromma najas	41	0.43	178.93	57.68	272.77	1.49	0.02
Erythromma viridulum	42	2.69	209.41	55.74	572.93	-0.32	0.00
Ischnura pumilio	43	2.83	182.00	56.35	-53.77	-5.11	0.06
Lestes dryas	44	0.95	197.02	57.13	21.27	0.61	0.00
Lestes sponsa	45	0.61	211.48	57.53	26.84	2.16	0.12
Lestes virens	46	2.15	226.74	56.64	-112.34	-3.20	0.05
Platycnemis pennipes	47	0.79	184.15	57.84	68.47	0.75	0.01
Pyrrhosoma nymphula	48	0.42	168.05	57.63	4.68	-0.98	0.02
Sympecma fusca	49	1.18	155.54	57.90	6.57	-110.69	1.00

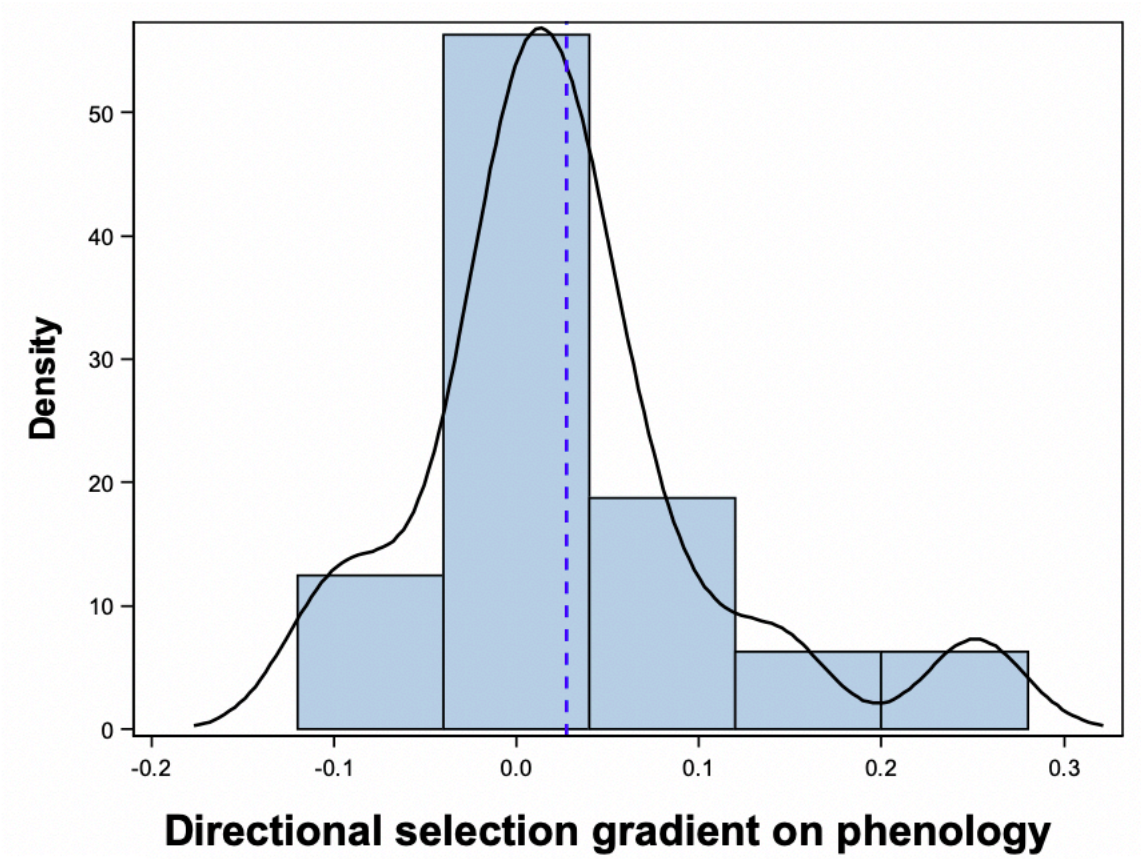

Figure S1. Variation in standardized directional selection gradients. Histogram shows BLUP estimates of annual directional selection on phenology, from a model with copulation success as response and globally-standardized (sd = 1) Julian day as a fixed effect. Blue dashed line indicates the overall (main 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.

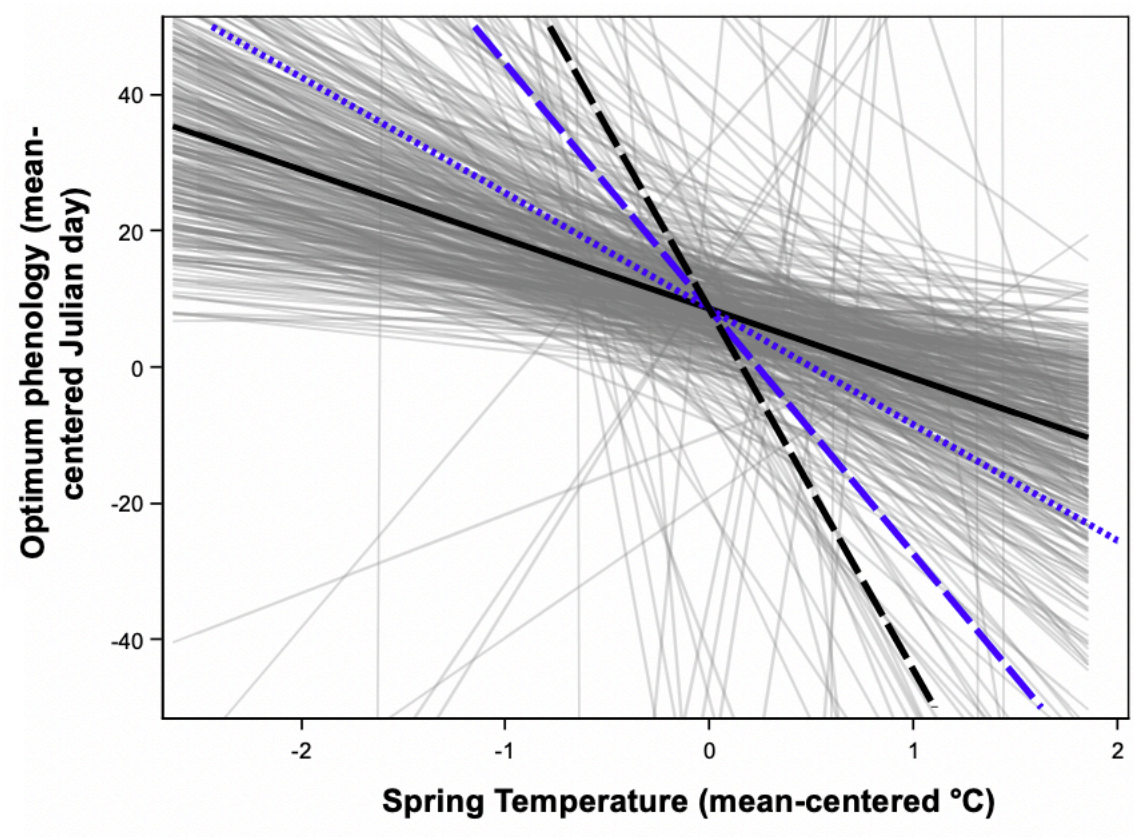

Figure S2. Comparison of parameter estimates from bivariate mixed effects reaction norm model and direct estimates of B and b. Long-dashed blue and black lines are (respectively) the estimates of the population mean reaction norm, b, and the temperature-dependence of the optimum phenology, B, estimated 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

837 estimated in a laboratory experiment, solid black line is the estimate of B from individual fitness

838 data from wild-caught damselflies.

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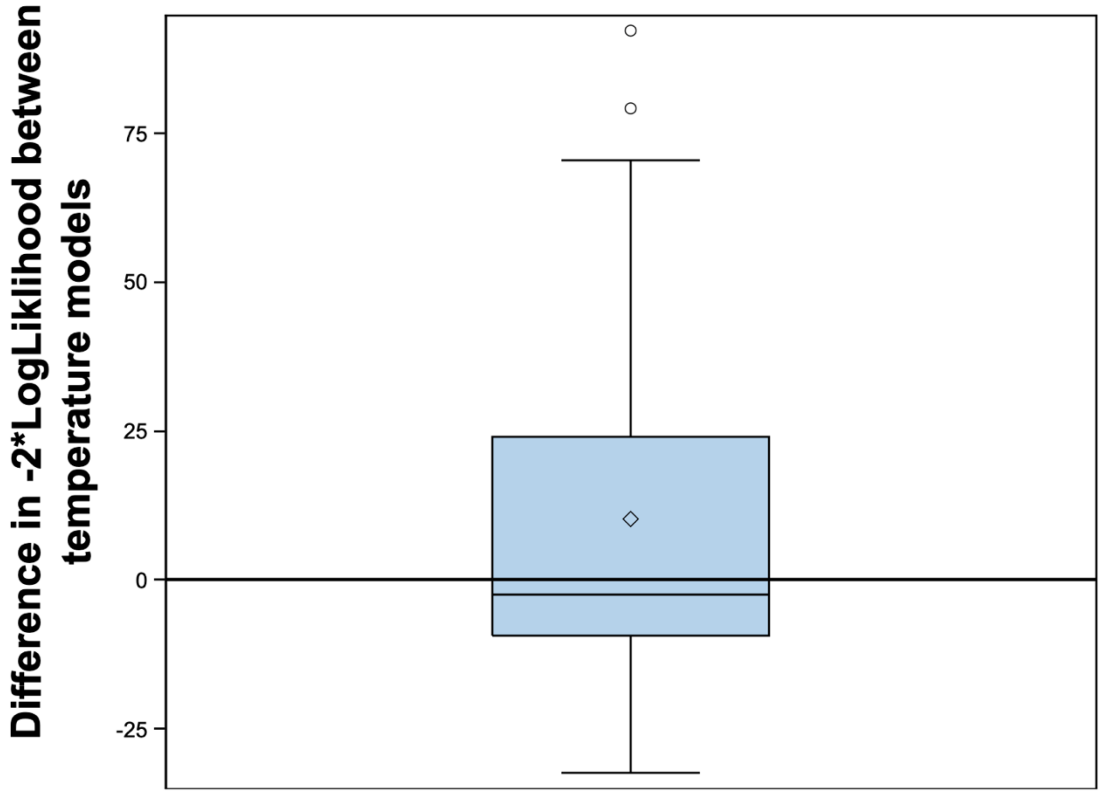

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843 **Figure S3 Comparison of model fits between identical bivariate mixed models** fit to

844 temperature and phenology data using either the mean of the first 120 Julian days of the year or

845 the mean April temperature. The difference in -2 log likelihood shows that on average across the

846 49 species studied that the mean 120 days fit on average 10.17 (SE = 4.38, DF = 48 P = 0.0245)

847 log likelihood units better than mean April temperature. Note that these are identical datasets

848 (equal N) and identical models, differing only in the values of mean temperature and so

849 comparing the likelihoods in this way provides one approach to assessing fit. We found no

850 evidence of any phylogetic signal in which data set fit better (Pagel's λ = 6.67295e-05, P ≈ 1).

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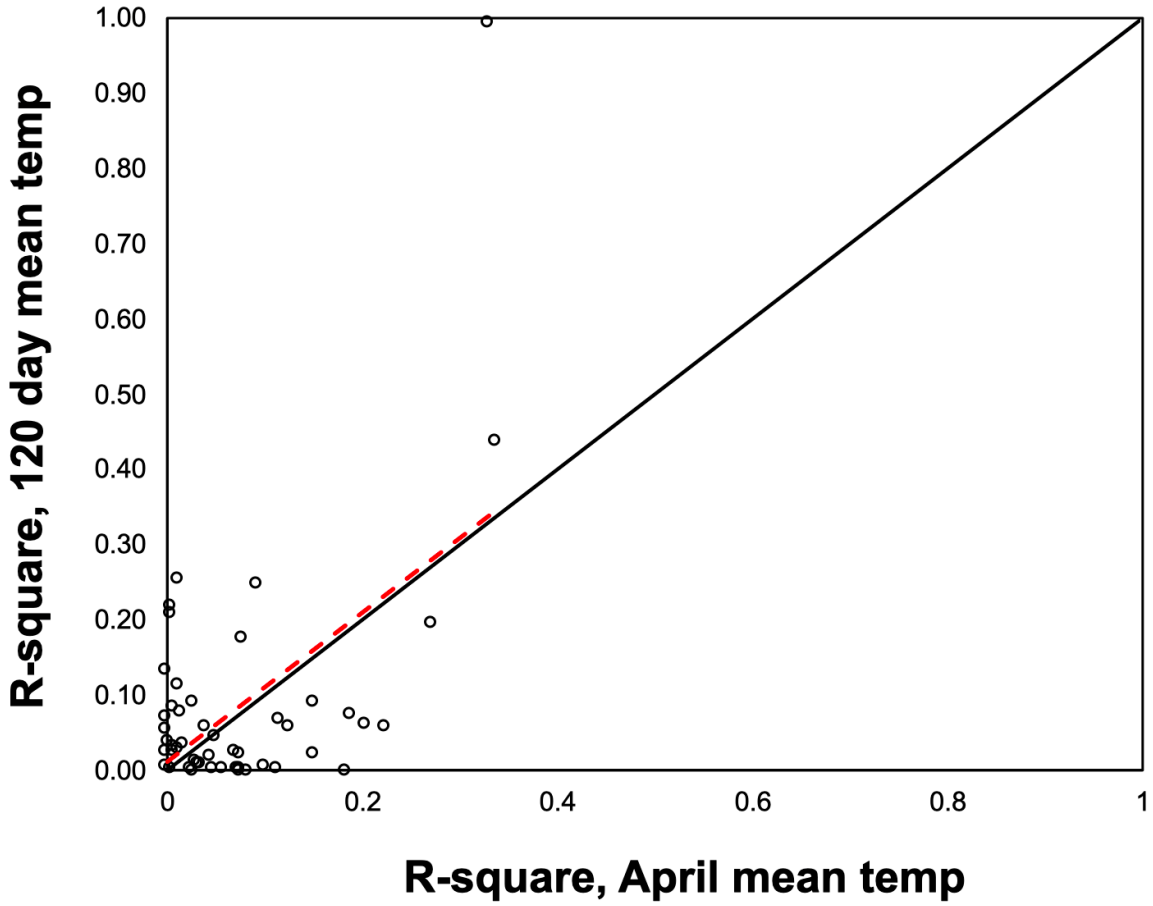

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855 **Figure S4. Comparison of R^2 of b estimated using two values for spring mean temperature**.

856 Values are the squares of the correlation coefficients estimated as the covariance between

857 temperature and phenology across time within binned latitudinal grid cells divided by the

858 product of the square roots of the variance in temperature and phenology. On average, reaction

859 norm from the mean 120 days explained slightly more variation (9%) than did that estimated

using April temperature only (7.5%). Red dashed line shows the regression slope between the two estimates.