



Temperature fine-tunes Mediterranean *Arabidopsis thaliana* life-cycle phenology geographically

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1 RESEARCH PAPER

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3 **Temperature fine-tunes Mediterranean *Arabidopsis***
4 ***thaliana* life-cycle phenology geographically**

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22 *Running head:* life-cycle phenology of *Arabidopsis thaliana*

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29 *Keywords:* adaptive evolution, environmental gradients, flowering time, geographically
30 weighted regressions, seed dormancy, sliding window analysis

31

32 **ABSTRACT**

- 33 • To understand how adaptive evolution in life-cycle phenology operates in plants, we need
34 to unravel the effects of geographical variation in putative agents of natural selection on
35 life-cycle phenology by considering all key developmental transitions and their co-
36 variation patterns. We address this goal by quantifying the temperature-driven and
37 geographically varying relationship between seed dormancy and flowering time in the
38 annual *Arabidopsis thaliana* across the Iberian Peninsula.
- 39 • We used data on genetic variation in two major life-cycle traits, seed dormancy
40 (DSDS50) and flowering time (FT), in a collection of 300 *A. thaliana* accessions from the
41 Iberian Peninsula. The geographically varying relationship between life-cycle traits and
42 minimum temperature, a major driver of variation in DSDS50 and FT, was explored with
43 geographically weighted regressions. The environmentally varying correlation between
44 DSDS50 and FT was analysed by means of sliding window analysis across a minimum
45 temperature gradient.
- 46 • The maximum local adjustments between minimum temperature and life-cycle traits were
47 obtained in south-western Iberian Peninsula, an area with the highest minimum
48 temperatures. In contrast, in off-south-western locations, the effects of minimum
49 temperature on DSDS50 were rather constant across the region, whereas those of
50 minimum temperature on FT were more variable with peaks of strong local adjustments
51 of GWR models in central and north-western Spain. Sliding window analysis identified a
52 minimum temperature turning point in the relationship between DSDS50 and FT around
53 a minimum temperature of 7.2°C. Above this minimum temperature turning point, the
54 variation in the FT/DSDS50 ratio became rapidly constrained and the negative correlation
55 between FT and DSDS50 did not increase any further with increasing minimum
56 temperatures.

57 • South-western Iberian Peninsula emerges as an area where variation in life-cycle
58 phenology appears to be restricted by the duration and severity of the hot summer
59 drought. The temperature-driven varying relationship between DSDS50 and FT detected
60 the environmental boundaries for the co-evolution between FT and DSDS50 in *A.*
61 *thaliana*. In a context of global warming, we conclude that the *A. thaliana* phenology
62 from south-western Iberian Peninsula, determined by early flowering and deep seed
63 dormancy, might become the most common life-cycle phenotype for this annual plant in
64 the region.

For Peer Review

65 **INTRODUCTION**

66 Pronounced environmental heterogeneity in soil fertility, climate seasonality and fire
67 frequency typically characterises Mediterranean-climate regions. Broad agreement exists that
68 environmental heterogeneity has chiefly accounted for the high regional plant diversity
69 observed in these scarce climate regions, i.e. almost 20% of the known vascular plant species
70 occur in less than 5% of the Earth's surface (Cowling *et al.* 1996; Lobo *et al.* 2001). In
71 addition, the Quaternary glacial history of some Mediterranean-climate regions, e.g. the
72 Mediterranean Basin, has also played a very important role in structuring their current
73 patterns of plant biodiversity (Comes and Kadereit 1998; Hewitt 1999; Hughes *et al.* 2006;
74 Médail and Diadema 2009). As a result of such inherent environmental heterogeneity, the
75 agents of natural selection are assumed to have been acting on a fine scale, which is also
76 regarded as an additional explanation for the high plant diversity of these regions (Cowling *et*
77 *al.* 1996). Thus, Mediterranean-climate regions are appropriate natural scenarios to undertake
78 evolutionary studies on the processes underlying plant differentiation.

79 One of these evolutionary processes deals with the adaptive adjustment of life-cycle
80 phenology, i.e. the seasonal timing of developmental events spanning from seed to seed, to
81 heterogeneous environments (Rathcke and Lacey 1985; Donohue 2005; Wilczek *et al.* 2009;
82 Huang *et al.* 2010; Chiang *et al.* 2013; Burghardt *et al.* 2015). Life-cycle phenology must be
83 regarded as a composite trait influenced by the interaction between genetic and environmental
84 factors and whose adaptive variation enhances long-term population fitness. Variation in the
85 life-cycle phenology components with the highest impact on fitness, i.e. the timing of seed
86 germination and the timing of reproduction, are expected to be related to the variation
87 exhibited by their putative agents of natural selection (Caicedo *et al.* 2004; Stinchcombe *et al.*
88 2004; Lempe *et al.* 2005; Shindo *et al.* 2005; Méndez-Vigo *et al.* 2011; Kronholm *et al.* 2012;
89 Manzano-Piedras *et al.* 2014; Vidigal *et al.* 2016). As climate broadly acts as a major

90 selective pressure on life-cycle phenology, adaptive patterns of variation in continuous
91 populations are commonly captured by distinctive geographical clines, revealing the interplay
92 between life-cycle phenology, genetic loci that govern fitness-related life-cycle traits, and
93 climatic factors (Aranzana *et al.* 2005; Atwell *et al.* 2010; Fournier-Level *et al.* 2011, 2013;
94 Hancock *et al.* 2011; Méndez-Vigo *et al.* 2011; Savolainen *et al.* 2013; Vidigal *et al.* 2016).

95 It is becoming increasingly accepted that the evolutionary implications of variation in
96 life-cycle phenology cannot be fully understood if its components are considered in isolation
97 (Toorop *et al.* 2012; Debieu *et al.* 2013; Burghardt *et al.* 2016). As a matter of fact, the
98 success of an organism when facing heterogeneous or novel environments depends on its
99 ability to coordinate adaptive shifts in fitness-related traits that are closely correlated with
100 each other (Montesinos-Navarro *et al.* 2011; Fischer *et al.* 2016). Recent studies on the annual
101 plant *Arabidopsis thaliana* indeed showed the influence that flowering time, which strongly
102 determines seed maturation conditions and post-dispersal temperatures, has on seed dormancy
103 induction and germination behaviour (Donohue 2005, 2009, 2014; Finch-Savage and
104 Leubner-Metzger 2006; Chiang *et al.* 2011; Springthorpe and Penfield 2015; Burghardt *et al.*
105 2015, 2016). Hence, seed dormancy and flowering time, given their impact on fitness, are
106 expected to be tightly correlated, eventually determining adaptive variation in life-cycle
107 phenology.

108 Such a correlation has recently been found in *A. thaliana*, indicating that seed
109 dormancy and flowering time are negatively correlated at both regional (Vidigal *et al.* 2016)
110 and global (Debieu *et al.* 2013) scales. Interestingly, patterns of co-variation between seed
111 dormancy and flowering time appear to vary geographically and independently of major
112 genetic population structure. This supports the view that geographical patterns of variation in
113 life-cycle phenology are adaptive (Chaine 2010; Debieu *et al.* 2013) and that correlations
114 between fitness-related traits need not be constant over space and probably over time (Fischer

115 *et al.* 2016). Nevertheless, gaining and in-depth understanding of the evolutionary potential of
116 co-variation between seed dormancy and flowering time might be more complicated than
117 expected. For example, Debieu *et al.* (2013) used a set of 112 *A. thaliana* accessions to show
118 how vegetative growth, flowering time and seed dormancy followed a latitudinal cline across
119 Europe and how co-variation patterns between these traits differed between North and South
120 Europe. However, only latitude appeared to have some explanatory power in their study,
121 explaining only up to 5% and 11% of the observed variation in seed dormancy and flowering
122 time, respectively (Debieu *et al.* 2013). In contrast, Vidigal *et al.* (2016), using a collection of
123 300 *A. thaliana* accessions exclusively from the Iberian Peninsula, observed that early-
124 flowering accessions with very high seed dormancy mostly occurred in south-western Iberian
125 Peninsula. In this case, the high number of geo-referenced accessions allowed the
126 identification of minimum temperature as a major driver of variation in these traits. Average
127 annual minimum temperature alone explained up to 44.8% and 39.5% of the genetic variation
128 in seed dormancy and flowering time, respectively (Vidigal *et al.* 2016). Overall, these results
129 stress the value of high-density regional collections of natural accessions to study large-scale
130 variation in *A. thaliana*'s life-cycle phenology. However, regional patterns can vary
131 substantially across the species' distribution range, which eventually determine the density of
132 populations needed to detect such patterns.

133 The main goal of this study is to reveal and quantify the temperature-driven and
134 geographically varying relationship between seed dormancy and flowering time in *A. thaliana*
135 across the Iberian Peninsula in West Mediterranean Basin, which is the *A. thaliana*'s
136 distribution area known to harbour the largest regional genomic diversity (Picó *et al.* 2008;
137 Cao *et al.* 2011; Weigel 2012; Brennan *et al.* 2014; The 1001 Genomes Consortium 2016).
138 We use recent data on genetic variation in seed dormancy and flowering time for the 300
139 Iberian *A. thaliana* accessions mentioned above (Vidigal *et al.* 2016). We hypothesise that the

140 negative correlation exhibited between seed dormancy and flowering time is spatially
141 heterogeneous across the Iberian Peninsula. It is already known that temperature strongly
142 shapes phenotypic variation in Iberian *A. thaliana* with warmer environments selecting for
143 early flowering (Méndez-Vigo *et al.* 2011; Manzano-Piedras *et al.* 2014) and high seed
144 dormancy (Vidigal *et al.* 2016). Beyond this general trend, however, we ignore how the
145 species fine-tunes life-cycle phenology across the region, since temperature sharply changes
146 along altitudinal and latitudinal clines. This requires a spatially-explicit approach to quantify
147 the effects of temperature variation, not only on seed dormancy and flowering time separately,
148 but on the relationship between these two major components. This knowledge will help better
149 understand how adaptive evolution in life-cycle phenology operates in plants (Debieu *et al.*
150 2013) as well as inferring how plants will manage to keep up with global climate change by
151 modifying their life-cycle phenology (Wilczek *et al.* 2014).

152

153 **MATERIAL AND METHODS**

154 **Source accessions**

155 The 300 Iberian *A. thaliana* accessions are part of a long-term project to generate a permanent
156 collection of natural populations from south-western Mediterranean Basin across Spain,
157 Portugal and Morocco for the integrative and comprehensive study of the species'
158 evolutionary ecology and functional genetics (Picó *et al.* 2008; Méndez-Vigo *et al.* 2011;
159 Brennan *et al.* 2014; Manzano-Piedras *et al.* 2014; Marcer *et al.* 2016; Vidigal *et al.* 2016).
160 For this study, we surveyed natural populations across the entire Iberian Peninsula (latitude
161 range = 36.52°N – 43.40°N, longitude range = 3.19°E – 8.54°W; altitude range = 1 – 2662
162 m.a.s.l.; between-population distance range = 1 – 1042 km) uninterruptedly between 2000 and
163 2010. We collected seed from several individuals per population depending on population
164 size. A few months after the field season of every sampling year, individuals were multiplied

165 by the single seed descent method in controlled conditions in the glasshouses at the Centro
166 Nacional de Biotecnología (Madrid, ES) and stored in dry conditions in cellophane bags at
167 room temperature in darkness. When possible, accessions were chosen based on their
168 flowering time and/or vernalization requirement during the multiplication experiments. This
169 is important for our study as we selected one individual with a common phenotype within its
170 population, increasing the odds of using those phenotypes best suited to their local
171 environments. By selecting a common phenotype per population, we are also buffering the
172 effects, if any, of changing environmental conditions during the sampling period (2000–2010)
173 on life-cycle traits. Here, we assume that temporal changes in environmental conditions
174 during this period of time have not substantially affected population mean values of major
175 life-cycle traits of interest, as suggested by preliminary data (F.X. Picó *et al.*, unpublished
176 material). Study accessions were genetically different from each other based on 250
177 polymorphic genome-wide neutral SNPs (Manzano-Piedras *et al.* 2014). In 2013, all
178 accessions were multiplied again in the greenhouses at Wageningen University (Wageningen,
179 NL) to synchronise their seed production, which is mandatory to obtain accurate seed
180 dormancy estimates (Vidigal *et al.* 2016).

181

182 **Life-cycle phenology**

183 *Arabidopsis thaliana*'s life-cycle phenology was characterised by variation in two major life-
184 cycle traits: seed dormancy and flowering time. For each accession, seed dormancy was
185 quantified as DSDS50, i.e. days of seed dry storage required to reach 50% of germination (see
186 He *et al.* 2014 for details on its calculation), whereas flowering time (FT hereafter) was
187 quantified as the number of days from the planting date until the anthesis of the first flower.
188 To estimate DSDS50, vernalized mother plants were first grown in standard conditions in a
189 glasshouse (20°C day/18°C night; 16 h light: 8 h darkness; 70% relative humidity) to

190 synchronise seed harvest among all accessions. Subsequently, seed was used to estimate
191 DSDS50 in an incubator at 22°C and constant light during 5 days (see Vidigal *et al.* 2016). FT
192 was estimated in a growth chamber at 21°C with a long-day photoperiod (16 h light: 8 h
193 darkness; see Méndez-Vigo *et al.* 2011). Experiments to estimate DSDS50 and FT lasted for a
194 maximum of 559 and 220 days, respectively (Méndez-Vigo *et al.* 2011; Vidigal *et al.* 2016).
195 Both seed dormancy and flowering time were estimated under controlled laboratory
196 conditions, providing the quantitative genetic component for both traits.

197

198 **Minimum temperature data**

199 Previous studies indicated that minimum temperature is the main contributing factor to the
200 observed patterns of variation in DSDS50 and FT in Iberian *A. thaliana* far above other
201 environmental factors (Méndez-Vigo *et al.* 2011; Manzano-Piedras *et al.* 2014; Vidigal *et al.*
202 2016). Given the predominant role of minimum temperature shaping patterns of phenotypic
203 variation in Iberian *A. thaliana*, for the sake of clarity and simplicity we used the average
204 annual minimum temperature (minimum temperature hereafter) as the explanatory variable in
205 all analyses. Additional analyses using variables related to precipitation, vegetation and soil,
206 alone or in combination with minimum temperature, neither improved the outcomes nor
207 added any value to the interpretation of the resulting patterns in a significant way (results not
208 shown). Minimum temperature from every population was obtained from the Digital Climatic
209 Atlas of the Iberian Peninsula (Ninyerola *et al.* 2000). Minimum temperature data came from
210 a spatial interpolation using temperature records from a total of 3608 meteorological stations
211 across Portugal and Spain during the period 1950–1999. Hence, although our *A. thaliana*
212 populations were sampled between 2000 and 2010, the minimum temperature data used in
213 this study reflect the environmental conditions where *A. thaliana* has probably been occurring
214 and adapting for long time in the Iberian Peninsula.

215

216 **Statistical analyses**

217 The hypothesised geographically varying relationship between life-cycle phenology traits and
218 minimum temperature was explored with geographically weighted regression (GWR) models
219 (Fotheringham *et al.* 2002) using SAM software (Rangel *et al.* 2010). First, we performed a
220 GWR to test the effect of minimum temperature on DSDS50 and another GWR to test the
221 effect of minimum temperature on FT. GWR is a spatially-explicit explanatory regression
222 where regression parameters are estimated at each sampled location i as follows:

223

$$224 \quad y_i = \beta(v_i, v_i)x_i + \varepsilon_i,$$

225

226 where y_i is the dependent variable, x_i is the independent variable, ε_i is the Gaussian error,
227 (v_i, v_i) are the geographical coordinates, and $\beta(v_i, v_i)$ is the GWR varying coefficient at each
228 location i . GWR performed a local regression at each of the 300 locations by taking a fraction
229 (10 – 15%) of the neighbouring locations into account using an optimal bandwidth size. For
230 each location, the selected bandwidth was optimised by using a Gaussian spatial weighting
231 function minimising the Akaike information criterion (AICc). This procedure generally
232 enhances the goodness-of-fit of GWR by setting the optimal number of neighbouring
233 locations to perform local regressions as well as by controlling for border and sampling
234 effects. GWR and OLS (ordinary least squares) results were also compared with an ANOVA
235 to check that the explanatory power of GWR was better than that of OLS. The lack of
236 autocorrelation of the residuals was checked with a spatial correlogram plotting Moran's I
237 values of GWR residuals. Thus, GWR provided local estimates of the adjustment of DSD50
238 and FT values to minimum temperatures in Iberian *A. thaliana* populations, i.e. local adjusted
239 pseudo- R^2 values.

240 After assessing the relationship between minimum temperature and DSDS50 and FT
241 in two separate GWR models, we uncovered the environmentally varying correlation between
242 DSDS50 and FT by means of sliding window analysis (as in Debieu *et al.* 2013). Sliding
243 window analyses are used to display the dynamic relationship between variables across a
244 continuous space. In this study, we used a temperature sliding window to account for the co-
245 variation between life-cycle traits across a minimum temperature gradient. We selected a
246 temperature sliding window instead of a geographical sliding window because minimum
247 temperature does a better job than geography in characterising the environment of Iberian *A.*
248 *thaliana* populations. For example, geographically close populations can exhibit pronounced
249 differences in minimum temperature if they are located along the same altitudinal gradient
250 (Montesinos *et al.* 2009; Méndez-Vigo *et al.* 2011). Hence, all 300 *A. thaliana* populations
251 were first ranked according to their minimum temperatures (range = $-0.7^{\circ} - 13.0^{\circ}\text{C}$). For
252 every population along the minimum temperature gradient and regardless of its geographical
253 location, we sub-sampled all populations whose minimum temperatures fell within a
254 temperature interval around it. We tested different temperature breadths and eventually
255 selected a temperature interval of 7°C centred on the minimum temperature characterising
256 each population. The temperature breadth determines the number of populations falling within
257 the temperature interval as well as the truncation of the sampling window for those
258 populations at the low and high ends of the temperature gradient. Consistent patterns were
259 detected with narrower temperature breadths (results not shown) but the chosen one optimised
260 the patterns observed.

261 For each of the 300 values of the minimum temperature gradient, we computed the
262 correlation between DSDS50 and FT as well as the mean and SD of the FT/DSDS50 ratio
263 using all the accessions falling within the temperature interval of 7°C (mean \pm SD number of
264 sampling accessions per minimum temperature = 194.6 ± 53.1). It must be noted that we used

265 more than 100 accessions in 93% of the analyses (279 of 300 analyses). The FT/DSDS50
266 ratio provided an integrated measure of variation between the two life-cycle traits because
267 accessions with early flowering times and high seed dormancy had low FT/DSDS50 ratios,
268 whereas late flowering accessions with low seed dormancy exhibited high FT/DSDS50 ratios.
269 The significance of the correlations between DSDS50 and FT for every minimum temperature
270 was tested with the Dutilleul's modified t test, using the R package SpatialPack (Osorio and
271 Vallejos 2014), to take any spatial autocorrelation in the data into account. The Dutilleul's
272 modified t test corrects the variance of the test statistic and the degrees of freedom according
273 to the extent of spatial autocorrelation of each variable of the correlation (Dutilleul *et al.*
274 1993). Our large sample size ($N = 300$) ensures a high accuracy of the results, as required in
275 spatially-explicit statistical tests (Legendre *et al.* 2002).

276

277 RESULTS

278 The geographically varying relationship between life-cycle phenology traits, i.e. DSDS50 and
279 FT, and minimum temperature was explored with two separate geographically weighted
280 regression (GWR) models. For DSDS50 and minimum temperature, the GWR results showed
281 that local models based on GWR performed better than the global model based on the
282 classical ordinary least squares (OLS) regression model ($F_{5,9, 292,1} = 6.68$, $P < 0.0001$;
283 ANOVA; adjusted $R^2 = 0.41$ and 0.34 for GWR and OLS regression, respectively). All local
284 models were significant ($P < 0.001$ in all cases) and all local standardised coefficients of
285 regression were positive (range of $r = 0.33 - 0.90$), indicating that minimum temperature and
286 DSDS50 were positively correlated in all cases, i.e. accessions from warmer locations
287 exhibited higher seed dormancy. The local adjusted pseudo- R^2 values (range of pseudo- $R^2 =$
288 $0.18 - 0.50$) clearly increased along a north-south latitudinal gradient reaching the highest
289 values in south-western Iberian Peninsula (Fig. 1A).

290 For FT and minimum temperature, the GWR results also showed that local models
291 performed better than the global OLS model ($F_{9,4, 288,6} = 8.90$, $P < 0.0001$; ANOVA; adjusted
292 $R^2 = 0.49$ and 0.36 for GWR and OLS regression, respectively). All local models were
293 significant ($P < 0.003$ in all cases) and all local standardised coefficients of regression were
294 negative (range of $r = -0.31 - -1.00$). Therefore, minimum temperature and FT were
295 negatively correlated in all cases, i.e. accessions from warmer locations flowered earlier. The
296 local adjusted pseudo- R^2 values for FT were more variable (range of pseudo- $R^2 = 0.13 - 0.65$)
297 and exhibited more geographical complexity than those estimated for DSDS50 (Fig. 1B). In
298 particular, south-western Iberian Peninsula was again an area where the adjustment between
299 minimum temperature and FT peaked, but strong adjustment peaks in other nuclei of
300 accessions in central and north-western Spain were also detected (Fig. 1B).

301 The environmentally varying relationship between DSDS50 and FT was analysed with
302 a temperature sliding window analysis. The results depicted the co-variation between DSD50
303 and FT along a minimum temperature gradient. The mean (\pm SD) of the FT/DSDS50 ratio
304 computed from all accessions falling within the temperature interval of 7°C along the
305 minimum temperature gradient varied from a low of 0.26 ± 0.21 , i.e. accessions with early
306 flowering time and high seed dormancy, to a high of 2.10 ± 0.79 , i.e. accessions with late
307 flowering time and low seed dormancy (Fig. 2A). As expected, the mean of the FT/DSDS50
308 ratio progressively decreased with increasing minimum temperatures (Fig. 2A). In contrast,
309 the slope of the relationship between minimum temperature and the SD of the FT/DSDS50
310 ratio exhibited a turning point at a minimum temperature around 7.2°C : the SD of the
311 FT/DSDS50 ratio decreased faster with minimum temperatures above this turning point (Fig.
312 2B).

313 The temperature sliding window analysis also indicated that the correlation between
314 FT and DSDS50 was not significant for populations with minimum temperatures below 3.6°C

315 and above 12.3°C ($N = 42$ out of 300; Fig. 2C). In between these two thresholds, the
316 correlation between FT and DSDS50 along the minimum temperature axis was significant and
317 negative, but progressively increased with increasing minimum temperatures around 7.3°C
318 (range of r values before the turning point = -0.18 – -0.39; Fig. 2C). From that minimum
319 temperature onwards, the significant correlation between FT and DSDS50 first had relatively
320 constant coefficients close to the maximum observed value to end with a more variable
321 behaviour with increasing minimum temperature (range of r values after the turning point = -
322 0.30 – -0.41; Fig. 2C). It is worth noting that the turning point in the relationship between FT
323 and DSDS50 along a minimum temperature gradient was also observed with additional
324 sliding window analyses depicting the correlation between each life-cycle trait and minimum
325 temperature along the minimum temperature gradient (Fig. S1). These results showed that the
326 correlation between DSDS50 and minimum temperature and between FT and minimum
327 temperature also reached a major turning point very close to those reported above.

328 The representation of two groups of populations, whose minimum temperature is
329 above and below the turning point for the correlation between FT and DSDS50, onto a
330 minimum temperature map, provides hints on the geographical and ecological differentiation
331 of *A. thaliana* governed by minimum temperature (Fig. 3). In particular, *A. thaliana*
332 populations with minimum temperatures below the turning point are mostly located in the
333 northern half of the Iberian Peninsula, including the main mountain ranges (e.g. Pyrenees,
334 Cantabrian, Central and Iberian ranges), the northern plateau, and Sierra Nevada in South
335 Spain with the highest mountain of the Iberian Peninsula (Fig. 3). In contrast, *A. thaliana*
336 populations with minimum temperatures above the turning point occur in the southern half of
337 the Iberian Peninsula as well as in coastal locations around the region (Fig. 3). As a result, the
338 two groups of *A. thaliana* populations occurring below ($N = 191$) and above ($N = 109$) the
339 minimum temperature turning point for the correlation between FT and DSDS50 significantly

340 differed in altitude (mean \pm SE altitudes = 973.7 ± 24.0 and 459.4 ± 24.1 m.a.s.l. for the
341 former and the latter, respectively; $F_{1,298} = 198.61$, $P < 0.0001$; one-way ANOVA). In
342 addition, higher elevation populations with minimum temperatures below the turning point
343 also exhibited a higher percentage of wild habitat than lower elevation populations with
344 minimum temperatures above the turning point (mean \pm SE percentages of wild habitat = 64.4
345 ± 2.6 and $45.3 \pm 3.7\%$ for the former and the latter, respectively; $F_{1,298} = 18.95$, $P < 0.0001$;
346 one-way ANOVA). This makes sense as human activity with more dramatic impacts on
347 natural plant communities has traditionally been concentrated at lower elevations. In contrast,
348 the two groups of populations did not differ in total annual precipitation, mean annual solar
349 radiation, or soil pH ($P > 0.10$ in all cases; one-way ANOVA). Overall, these results stress the
350 explanatory power of minimum temperature for variation in life-cycle phenology in Iberian *A.*
351 *thaliana*.

352

353 **DISCUSSION**

354 Mediterranean-climate regions represent outstanding natural scenarios to address multitude of
355 evolutionary issues in plants. Adaptive variation in life-cycle phenology is particularly
356 appropriate because major events of life-cycle phenology are markedly influenced by climatic
357 factors, whose seasonality, in fact, defines the Mediterranean climate. It is clear that we need
358 to unravel the effects of climatic factors, not only on life-cycle traits separately, but on the
359 life-cycle phenology as a whole (Post *et al.* 2008; Debieu *et al.* 2013; Burghardt *et al.* 2016).
360 This is supported by increasingly abundant studies on *A. thaliana* as an evolutionary model
361 system, focused on the tight interplay between flowering time and seed germination
362 (Donohue 2005, 2009, 2014; Finch-Savage and Leubner-Metzger 2006; Chiang *et al.* 2011;
363 Toorop *et al.* 2012; Debieu *et al.* 2013; Wilczek *et al.* 2014; Springthorpe and Penfield 2015;
364 Burghardt *et al.* 2015, 2016) as well as on the effects of seasonal environmental variation on

365 key developmental-timing genes affecting multiple developmental transitions (Chiang *et al.*
366 2009; Atwell *et al.* 2010; Chen *et al.* 2014; Huo *et al.* 2016). Nevertheless, the separate
367 analyses of the geographically varying relationship between minimum temperature and *A.*
368 *thaliana*'s life-cycle traits still provide valuable elements that are worth considering. For
369 example, the GWR results indicated that south-western Iberian Peninsula represents an area
370 with strong local adjustments for both FT and DSDS50 (Fig. 1). This confirms the capital role
371 of minimum temperatures in shaping up life-cycle phenology towards early flowering time
372 and strong seed dormancy as the environment becomes warmer (Méndez-Vigo *et al.* 2011;
373 Manzano-Piedras *et al.* 2014; Vidigal *et al.* 2016).

374 Interestingly, our analyses indicated that for the rest of the Iberian Peninsula there
375 were notorious differences in the geographically varying effect of minimum temperature on
376 each of the study life-cycle traits. On the one hand, the positive relationship between
377 minimum temperature and DSDS50 remained rather moderate and homogeneous for
378 accessions from off-south-western locations (Fig. 1A). Overall, these results suggest that
379 minimum temperature increases its intensity as an agent of natural selection for DSDS50
380 towards south-western Iberian Peninsula, i.e. an area with the highest minimum temperatures.
381 Based on this, we hypothesise that the windows of opportunity for germination also follow a
382 north-south gradient, becoming narrower towards south-western Iberian Peninsula as a result
383 of the longer and more severe summer droughts in that area. Although field demographic
384 studies from northern *A. thaliana* populations indicated that germination peaks in early/mid
385 autumn, and to a lesser extent between late winter and early spring (Montesinos *et al.* 2009;
386 Picó 2012), we still lack germination data from southern populations. Therefore, further field
387 work is needed to quantify in detail the geographical patterns of variation in seed germination
388 behaviour in natural *A. thaliana* populations across the warmest area of the Iberian Peninsula
389 to test this hypothesis.

390 On the other hand, the negative relationship between minimum temperature and FT in
391 off-south-western locations was far more variable, with additional areas of pronounced local
392 adjustments of the GWR models, especially in central and north-western Spain (Fig. 1B). In
393 this case, orography might explain these results because central and north-western Spain are
394 mountainous areas with remarkable altitudinal gradients, which imply a rapid change in
395 ecological conditions over relatively short distances. As a matter of fact, altitudinal gradients
396 represent an important source of phenotypic and adaptive variation in *A. thaliana* in different
397 regions across its distribution range (Montesinos *et al.* 2009; Méndez-Vigo *et al.* 2011;
398 Montesinos-Navarro *et al.* 2011; Picó 2012; Suter *et al.* 2014; Luo *et al.* 2015a, 2015b; Tyagi
399 *et al.* 2016; but see Günther *et al.* 2016). We have previously shown that decreasing
400 temperatures with increasing altitude in Iberian mountains are related to late flowering in *A.*
401 *thaliana* through the obligate vernalization requirement (Méndez-Vigo *et al.* 2011). This is a
402 flowering-inductive effect of low temperature that appears to be restricted to altitudes above
403 800 m (Méndez-Vigo *et al.* 2011), locations with typically cooler climates that are practically
404 absent in south-western Iberian Peninsula (Marcer *et al.* 2016). Hence, the preponderance of a
405 vernalization requirement to trigger flowering at high altitude cool environments and the lack
406 thereof in generally warmer lowland environments illustrate the variety and complexity of the
407 genetic mechanisms underlying adaptive variation in FT in *A. thaliana* (Koornneef *et al.*
408 1998; Simpson and Dean 2002; Alonso-Blanco *et al.* 2009; Wilczek *et al.* 2009).

409 On top of these geographically varying relationships between minimum temperature
410 and life-cycle traits, in this study we took a step further towards understanding how the
411 relationship between FT and DSDS50 varied across a minimum temperature gradient. The
412 results of the temperature sliding window analysis indicated that FT and DSDS50 co-varied
413 negatively with minimum temperature, in agreement with the behaviour of each life-cycle
414 trait with minimum temperature shown in this study, as well as with previous observations

415 (Debieu *et al.* 2013; Vidigal *et al.* 2016). The novel contribution of the sliding temperature
416 analysis is that it allowed the visualization and quantification of the heterogeneity of the
417 relationship between FT and DSDS50 along the minimum temperature gradient. Of particular
418 relevance is the existence of a minimum temperature turning point in the relationship between
419 FT and DSDS50, which has interesting ecological and evolutionary implications to better
420 understand life-cycle phenology, not only for *A. thaliana*, but for several other annuals and
421 short-lived perennials strongly relying on fast life cycles to succeed in their environments
422 (García *et al.* 2008).

423 For example, *A. thaliana* populations with minimum temperatures above the turning
424 point exhibited faster decreasing variability in the FT/DSDS50 ratio with respect to increasing
425 minimum temperature (Fig. 2B). This means that variation in *A. thaliana*'s life-cycle
426 phenology becomes progressively constrained as minimum temperatures become higher. In
427 Iberian warm environments, the duration and severity of the summer drought force *A.*
428 *thaliana* to complete its life cycle quickly, which can only be accomplished by becoming
429 dormant during the long hot summer together with early flowering before temperatures
430 become too high again. In fact, *A. thaliana* populations from south-western Iberian Peninsula
431 shed seed between mid April and early May and, in the warmest locations, between late
432 February and mid March (C. Alonso-Blanco and F.X. Picó, personal observation). Such early
433 flowering and fruiting imply that the maternal and post-dispersal environments encountered
434 by seeds might also promote deeper physiological seed dormancy, ensuring late germinations
435 when the summer drought is over with the first autumn rains.

436 Another implication of the existence of a minimum temperature turning point in the
437 relationship between FT and DSDS50 involves the strength of the correlation between the two
438 life-cycle traits. The turning point identified a minimum temperature threshold from which
439 the correlation coefficient between FT and DSDS50 cannot increase any further (Fig. 2C).

440 Hence, the temperature sliding window analysis provides a tool to detect the environmental
441 boundaries for the co-evolution between FT and DSDS50 for *A. thaliana* in Mediterranean
442 environments. In particular, below the minimum temperature threshold, *A. thaliana* shows
443 substantial phenotypic space to adjust its life-cycle phenology by progressively advancing
444 flowering time and increasing seed dormancy with increasing minimum temperature. Above
445 the minimum temperature threshold, *A. thaliana* might have reached the limits to adjust its
446 life-cycle phenology as described, which otherwise does not prevent the species to succeed in
447 warmer environments. Interestingly, populations characterised by minimum temperatures
448 above the turning point occur in southern Iberian Peninsula and in warmer coastal areas from
449 all over the Iberian Peninsula, whereas those below the turning point mostly occur in the
450 northern mountainous Iberian Peninsula (Fig. 3). Thus, unravelling the phenotypic limits for
451 the co-variation between key life-cycle traits and circumscribing such limits into a
452 geographical and ecological space, in our case strongly characterised by variation in
453 minimum temperature, might be more enlightening than depicting patterns of variation along
454 geographical or environmental gradients if we aim to extend our understanding of adaptive
455 evolution in plants.

456 In this study, we have shown that the genetic co-variation between flowering time and
457 seed dormancy is geographically fine-tuned by minimum temperature in Mediterranean *A.*
458 *thaliana*. However, it remains to be seen to what extent the timing of flowering initiation
459 affects the timing of seed germination, e.g. maternal environmental effects on seed dormancy
460 and germination patterns, or, conversely, whether the timing of seed germination affects the
461 timing of flowering initiation, e.g. environmental cues for flowering affecting rosettes from
462 seeds germinated at different times (see Donohue 2009). In any case, our results represent a
463 first step towards the development of spatially-explicit models to assess the evolutionary
464 dynamics of life-cycle phenology as well as the impact of climate change on adaptive

465 variation in *A. thaliana* (see non-spatially-explicit recent models for *A. thaliana* in Burghardt
466 *et al.* 2015; Fournier-Level *et al.* 2016). This is important because it has been predicted that
467 Mediterranean-climate regions, and in particular the Mediterranean Basin, will be
468 dramatically affected by global climate change before the end of the century, with a
469 generalised warming and an increase of the duration and severity of the summer drought
470 (Klausmeyer and Shaw 2009; Gómez-Navarro *et al.* 2010; Jacobeit *et al.* 2014). In this
471 context, our results suggest that the phenology exhibited by south-western Iberian *A. thaliana*
472 determined by early flowering and deep seed dormancy, might become the most common life
473 cycle of this annual plant in the climate scenarios forecasted for this region.

474

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487

488 **REFERENCES**

- 489 Alonso-Blanco C., Aarts M.G.M., Bentsink L., Keurentjes J.J.B., Reymond M., Vreugdenhil
490 D., Koornneef M. (2009) What has natural variation taught us about plant development,
491 physiology, and adaptation? *Plant Cell*, **21**, 1877–1896.
- 492 Aranzana M.J., Kim S., Zhao K., Bakker E., Horton M., Jakob K., *et al.* (2005) Genome-wide
493 association mapping in *Arabidopsis* identifies previously known flowering time and
494 pathogen resistance genes. *PLoS Genetics*, **1**, e60.
- 495 Atwell S., Huang Y.S., Vilhjalmsson B.J., Willems G., Horton M., Li Y., *et al.* (2010)
496 Genome-wide association study of 107 phenotypes in *Arabidopsis thaliana* inbred lines.
497 *Nature*, **465**, 627–631.
- 498 Brennan A.C., Méndez-Vigo B., Haddioui A., Martínez-Zapater J.M., Picó F.X., Alonso-
499 Blanco C. (2014) The genetic structure of *Arabidopsis thaliana* in the south-western
500 Mediterranean range reveals a shared history between North Africa and southern Europe.
501 *BMC Plant Biology*, **14**, 17.
- 502 Burghardt L.T., Metcalf C.J., Wilczek A.M., Schmitt J., Donohue K. (2015) Modeling the
503 influence of genetic and environmental variation on the expression of plant life cycles
504 across landscapes. *American Naturalist*, **185**, 212–227.
- 505 Burghardt L.T., Metcalf C.J., Donohue K. (2016) A cline in seed dormancy helps conserve
506 the environment experienced during reproduction across the range of *Arabidopsis thaliana*.
507 *American Journal of Botany*, **103**, 47–59.
- 508 Caicedo A.L., Stinchcombe J.R., Olsen K.M., Schmitt J., Purugganan M.D. (2004) Epistatic
509 interaction between *Arabidopsis FRI* and *FLC* flowering time genes generates a latitudinal
510 cline in a life history trait. *Proceedings of the National Academy of Sciences*, **101**, 15670–
511 15675.

- 512 Cao J., Schneeberger K., Ossowski S., Günther T., Bender S., Fitz J., *et al.* (2011) Whole-
513 genome sequencing of multiple *Arabidopsis thaliana* populations. *Nature Genetics*, **43**,
514 956–963.
- 515 Chen J., Tsuda Y., Stocks M., Källman T., Xu N., Kärkkäinen K., *et al.* (2014) Clinal
516 variation at phenology-related genes in spruce: parallel evolution in *FTL2* and *Gigantea*?
517 *Genetics*, **197**, 1025–1038.
- 518 Chiang G.C., Barua D., Kramer E.M., Amasino R.M., Donohue K. (2009) Major flowering
519 time gene, flowering locus C, regulates seed germination in *Arabidopsis thaliana*.
520 *Proceedings of the National Academy of Sciences*, **106**, 11661–11666.
- 521 Chiang G.C., Bartsch M., Barua D., Nakabayashi K., Debieu M., Kronholm I., *et al.* (2011)
522 *DOG1* expression is predicted by the seed-maturation environment and contributes to
523 geographical variation in germination in *Arabidopsis thaliana*. *Molecular Ecology*, **20**,
524 3336–3349.
- 525 Chiang G.C., Barua D., Dittmar E., Kramer E.M., de Casas R.R., Donohue K. (2013)
526 Pleiotropy in the wild: the dormancy gene *DOG1* exerts cascading control on life cycles.
527 *Evolution*, **67**, 883–893.
- 528 Chuine I. (2010) Why does phenology drive species distribution? *Philosophical Transactions*
529 *of the Royal Society B*, **365**, 3149–3160.
- 530 Comes H.P., Kadereit J.W. (1998) The effect of Quaternary climatic changes on plant
531 distribution and evolution. *Trends in Plant Science*, **3**, 432–438.
- 532 Cowling R.M., Rundel P.W., Lamont B.B., Arroyo M.K., Arianoutsou M. (1996) Plant
533 diversity in Mediterranean-climate regions. *Trends in Ecology and Evolution*, **11**, 362–366.
- 534 Debieu M., Tang C., Stich B., Sikosek T., Effgen S., Josephs E., *et al.* (2013) Co-variation
535 between seed dormancy, growth rate and flowering time changes with latitude in
536 *Arabidopsis thaliana*. *PLoS One*, **8**, e61075.

- 537 Donohue K. (2005) Niche construction through phenological plasticity: life history dynamics
538 and ecological consequences. *New Phytologist*, **166**, 83–92.
- 539 Donohue K. (2009) Completing the cycle: maternal effects as the missing link in plant life
540 histories. *Philosophical Transactions of the Royal Society B*, **364**, 1059–1074.
- 541 Donohue K. (2014) Why ontogeny matters during adaptation: developmental niche
542 construction and pleiotropy across the life cycle in *Arabidopsis thaliana*. *Evolution*, **68**,
543 32–47.
- 544 Dutilleul P. (1993) Modifying the *t* test for assessing the correlation between two spatial
545 processes. *Biometrics*, **49**, 305–314.
- 546 Finch-Savage W.E., Leubner-Metzger G. (2006) Seed dormancy and the control of
547 germination. *New Phytologist*, **171**, 501–523.
- 548 Fischer E.K., Ghalambor C.K., Hoke K.L. (2016) Plasticity and evolution in correlated suites
549 of traits. *Journal of Evolutionary Biology*, **29**, 991–1002.
- 550 Fotheringham A.S., Brunson C., Charlton M. (2002) *Geographically Weighted Regression:*
551 *The Analysis of Spatially Varying Relationships*. John Wiley & Sons, Inc., Hoboken, NJ,
552 USA: 284 pp.
- 553 Fournier-Level A., Korte A., Cooper M.D., Nordborg M., Schmitt J., Wilczek A.M. (2011) A
554 map of local adaptation in *Arabidopsis thaliana*. *Science*, **334**, 86–89.
- 555 Fournier-Level A., Wilczek A.M., Cooper M.D., Roe J. L., Anderson J., Eaton D., *et al.*
556 (2013) Paths to selection on life history loci in different natural environments across the
557 native range of *Arabidopsis thaliana*. *Molecular Ecology*, **22**, 3552–3566.
- 558 Fournier-Level A., Perry E.O., Wang J.A., Braun P.T., Migneault A., Cooper M.D., *et al.*
559 (2016) Predicting the evolutionary dynamics of seasonal adaptation to novel climates in
560 *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences*, **113**, E2812–
561 E2821.

- 562 García M.B., Picó F.X., Ehrlén J. (2008) Life span correlates with population dynamics in
563 perennial herbaceous plants. *American Journal of Botany*, **95**, 258–262.
- 564 Gómez-Navarro J.J., Montávez J.P., Jimenez-Guerrero P., Jerez S., García-Valero J.A.,
565 González-Rouco J.F. (2010) Warming patterns in regional climate change projections over
566 the Iberian Peninsula. *Meteorologische Zeitschrift*, **19**, 275–285.
- 567 Günther T., Lampei C., Barilar I., Schmid K.J. (2016) Genomic and phenotypic
568 differentiation of *Arabidopsis thaliana* along altitudinal gradients in the North Italian Alps.
569 *Molecular Ecology*, **25**, 3574–3592.
- 570 Hancock A.M., Brachi B., Faure N., Horton M.W., Jarymowycz L.B., Sperone F.G., *et al.*
571 (2011) Adaptation to climate across the *Arabidopsis thaliana* genome. *Science*, **334**, 83–
572 86.
- 573 He H., Vidigal D.S., Snoek L.B., Schnabel S., Nijveen H., Hilhorst H., *et al.* (2014)
574 Interaction between parental environment and genotype affects plant and seed performance
575 in *Arabidopsis*. *Journal of Experimental Botany*, **65**, 6603–6615.
- 576 Hewitt G.M. (1999) Post-glacial re-colonization of European biota. *Biological Journal of the*
577 *Linnean Society*, **68**, 87–112.
- 578 Huang X., Schmitt J., Dorn L., Griffith C., Effgen S., Takao S., *et al.* (2010) The earliest
579 stages of adaptation in an experimental plant population: strong selection on QTLs for
580 seed dormancy. *Molecular Ecology*, **19**, 1335–1351.
- 581 Hughes P.D., Woodward J.C., Gibbard P.L. (2006) Quaternary glacial history of the
582 Mediterranean mountains. *Progress in Physical Geography*, **30**, 334–364.
- 583 Huo H., Wei S, Bradford K.J. (2016) *DELAY OF GERMINATION1 (DOG1)* regulates both
584 seed dormancy and flowering time through microRNA pathways. *Proceedings of the*
585 *National Academy of Sciences*, 113, E2199–E2206.

- 586 Jacobeit J., Hertig E., Seubert, S., Lutz K. (2014) Statistical downscaling for climate change
587 projections in the Mediterranean region: methods and results. *Regional Environmental*
588 *Change*, **14**, 1891–1906.
- 589 Klausmeyer K.R., Shaw M.R. (2009) Climate change, habitat loss, protected areas and the
590 climate adaptation potential of species in Mediterranean ecosystems worldwide. *PLoS One*,
591 **4**, e6392.
- 592 Koornneef M., Alonso-Blanco C., Peeters A.J.M., Soppe W. (1998) Genetic control of
593 flowering time in *Arabidopsis*. *Annual Review of Plant Physiology and Plant Molecular*
594 *Biology*, **49**, 345–370.
- 595 Kronholm I., Picó F.X., Alonso-Blanco C., Goudet J., de Meaux J. (2012) Genetic basis of
596 adaptation in *Arabidopsis thaliana*: local adaptation at the seed dormancy QTL *DOG1*.
597 *Evolution*, **66**, 2287–2302.
- 598 Legendre P., Dale M.R.T., Fortin M.J., Gurevitch J., Hohn M., Myers D. (2002) The
599 consequences of spatial structure for the design and analysis of ecological field surveys.
600 *Ecography*, **25**, 601–615.
- 601 Lempe J., Balasubramanian S., Sureshkumar S., Singh A., Schmid M., Weigel D. (2005)
602 Diversity of flowering responses in wild *Arabidopsis thaliana* strains. *PLoS Genetics*, **1**,
603 0109–0118.
- 604 Lobo J.M., Castro I., Moreno J.C. (2001) Spatial and environmental determinants of vascular
605 plant species richness distribution in the Iberian Peninsula and Balearic Islands. *Biological*
606 *Journal of the Linnean Society*, **73**, 233–253.
- 607 Luo Y., Widmer A., Karrenberg S. (2015a) The roles of genetic drift and natural selection in
608 quantitative trait divergence along an altitudinal gradient in *Arabidopsis thaliana*.
609 *Heredity*, **114**, 220–228.

- 610 Luo Y., Dong X., Yu T., Shi X., Li Z., Yang W., *et al.* (2015b) A single nucleotide deletion in
611 *Gibberellin20-oxidase1* causes alpine dwarfism in *Arabidopsis*. *Plant Physiology*, **168**,
612 930–937.
- 613 Manzano-Piedras E., Marcer A., Alonso-Blanco C., Picó F.X. (2014) Deciphering the
614 adjustment between environment and life history in annuals: lessons from a
615 geographically-explicit approach in *Arabidopsis thaliana*. *PLoS One*, **9**, e87836.
- 616 Médail F., Diadema K. (2009) Glacial refugia influence plant diversity patterns in the
617 Mediterranean Basin. *Journal of Biogeography*, **36**, 1333–1345.
- 618 Méndez-Vigo B., Picó F.X., Ramiro M., Martínez-Zapater J.M., Alonso-Blanco C. (2011)
619 Altitudinal and climatic adaptation is mediated by flowering traits and *FRI*, *FLC* and
620 *PHYC* genes in *Arabidopsis*. *Plant Physiology*, **157**, 1942–1955.
- 621 Montesinos A., Tonsor S.J., Alonso-Blanco C., Picó F.X. (2009) Demographic and genetic
622 patterns of variation among populations of *Arabidopsis thaliana* from contrasting native
623 environments. *PLoS One*, **4**, e7213.
- 624 Montesinos-Navarro A., Wig J., Picó F.X., Tonsor S.J. (2011) *Arabidopsis thaliana*
625 populations show clinal variation in a climatic gradient associated with altitude. *New*
626 *Phytologist*, **189**, 282–294.
- 627 Ninyerola M., Pons X., Roure J.M. (2000) A methodological approach of climatological
628 modelling of air temperature and precipitation through GIS techniques. *International*
629 *Journal of Climatology*, **20**, 1823–1841.
- 630 Osorio F., Vallejos R. (2014) SpatialPack: Package for analysis of spatial data. R package
631 version 0.2-3. URL: CRAN.R-project.org/package=SpatialPack.
- 632 Picó F.X., Méndez-Vigo B., Martínez-Zapater J.M., Alonso-Blanco C. (2008) Natural genetic
633 variation of *Arabidopsis thaliana* is geographically structured in the Iberian Peninsula.
634 *Genetics*, **180**, 1009–1021.

- 635 Picó F.X. (2012) Demographic fate of *Arabidopsis thaliana* cohorts of autumn- and spring-
636 germinated plants along an altitudinal gradient. *Journal of Ecology*, **100**, 1009–1018.
- 637 Post E.S., Pedersen C., Wilmer C.C., Forchhammer M.C. (2008) Phenological sequences
638 reveal aggregate life history response to climatic warming. *Ecology*, **89**, 363–370.
- 639 Rangel T.F., Diniz-Filho J.A.F., Bini L.M. (2010) SAM: A comprehensive application for
640 spatial analysis in macroecology. *Ecography*, **33**, 46–50.
- 641 Rathcke B., Lacey E.P (1985) Phenological patterns of terrestrial plants. *Annual Review of*
642 *Ecology, Evolution and Systematics*, **16**, 179–214.
- 643 Savolainen O., Lascoux M., Merilä J. (2013) Ecological genomics of local adaptation. *Nature*
644 *Genetics Reviews*, **14**, 807–820.
- 645 Shindo C., Aranzana M.J., Lister C., Baxter C., Nicholls C., Nordborg M., *et al.* (2005) Role
646 of *FRIGIDA* and *FLOWERING LOCUS C* in determining variation in flowering time of
647 *Arabidopsis*. *Plant Physiology*, **138**, 1163–1173.
- 648 Simpson G.G., Dean C. (2002) *Arabidopsis*, the rosetta stone of flowering time? *Science*, **296**,
649 285–289.
- 650 Springthorpe V., Penfield S. (2015) Flowering time and seed dormancy control use external
651 coincidence to generate life history strategy. *eLife*, **4**, e05557.
- 652 Stinchcombe J.R., Weinig C., Ungerer M., Olsen K.M., Mays C., Halldorsdottir S.S., *et al.*
653 (2004) A latitudinal cline in flowering time in *Arabidopsis thaliana* modulated by the
654 flowering time gene *FRIGIDA*. *Proceedings of the National Academy of Sciences*, **101**,
655 4712–4717.
- 656 Suter L., Rüegg M., Zemp N., Hennig L., Widmer A. (2014) Gene regulatory variation
657 mediates flowering responses to vernalization along an altitudinal gradient in *Arabidopsis*.
658 *Plant Physiology*, **166**, 1928–1942.

- 659 The 1001 Genomes Consortium (2016) 1,135 Genomes reveal the global pattern of
660 polymorphism in *Arabidopsis thaliana*. *Cell*, **166**, 481–491.
- 661 Toorop P.E., Cuerva R.C., Begg G.S., Locardi B., Squire G.R., Iannetta P.P. (2012) Co-
662 adaptation of seed dormancy and flowering time in the arable weed *Capsella bursa-*
663 *pastoris* (shepherd's purse). *Annals of Botany*, **109**, 481–489.
- 664 Tyagi A., Singh S., Mishra P., Singh A., Tripathi A.M., Jena S.N., *et al.* (2016) Genetic
665 diversity and population structure of *Arabidopsis thaliana* along an altitudinal gradient.
666 *AoB PLANTS*, **8**, plv145.
- 667 Vidigal D.S., Marques A.C.S.S., Willems L.A.J., Buijs G., Méndez-Vigo B., Hilhorst
668 H.W.M., *et al.* (2016) Altitudinal and climatic associations of seed dormancy and
669 flowering traits evidence adaptation of annual life cycle timing in *Arabidopsis thaliana*.
670 *Plant, Cell and Environment*, **39**, 1737–1748.
- 671 Weigel D. (2012) Natural variation in *Arabidopsis*: from molecular genetics to ecological
672 genomics. *Plant Physiology*, **158**, 2–22.
- 673 Wilczek A.M., Roe J.L., Knapp M.C., Cooper M.D., Lopez-Gallego C., Martin L.J., *et al.*
674 (2009) Effects of genetic perturbation on seasonal life history plasticity. *Science*, **323**, 930–
675 934.
- 676 Wilczek A.M., Cooper M.D., Korves T.M. Schmitt J. (2014). Lagging adaptation to warming
677 climate in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences*, **111**,
678 7906–7913.

679 **FIGURE LEGENDS**

680

681 **Fig. 1.** (A) Map showing the geographical position of accessions indicating their GWR local
682 adjusted pseudo- R^2 values for DSDS50 and minimum temperature. (B) Map showing the
683 geographical position of accessions indicating their GWR local adjusted pseudo- R^2 values for
684 FT and minimum temperature.

685

686 **Fig. 2.** (A) Relationship between minimum temperature and the mean of the FT/DSDS50
687 ratio. (B) Relationship between minimum temperature and the standard deviation of the
688 FT/DSDS50 ratio. Regression lines are given for accessions below and above the turning
689 point at 7.2°C, which maximises the R^2 values of the two groups of accessions. (C)
690 Relationship between minimum temperature and the correlation coefficients between FT and
691 DSDS50. Filled dots indicated significant correlation coefficients based on Dutilleul's
692 modified t test. Regression lines are given for accessions with significant correlation
693 coefficients below and above the turning point at 7.3°C, which maximises the R^2 values of the
694 two groups of accessions. In all three cases, values were obtained from temperature sliding
695 window analyses and ranked along the minimum temperature gradient.

696

697 **Fig. 3.** Minimum temperature map for the Iberian Peninsula indicating the geographical
698 position of *A. thaliana* populations with minimum temperatures below (hollow dots) and
699 above (filled dots) the turning point of 7.3°C for the correlation between FT and DSDS50
700 along the minimum temperature gradient (see Fig. 2C).

701

702

703 **SUPPORTING INFORMATION**

704

705 **Fig. S1.** (A) Relationship between minimum temperature and the correlation coefficients
706 between DSDS50 and minimum temperature. (B) Relationship between minimum
707 temperature and the correlation coefficients between FT and minimum temperature. Filled
708 dots indicated significant correlation coefficients based on Dutilleul's modified t test. The
709 major turning point of each panel was detected at 7.4 and 6.9°C for DSDS50 and FT,
710 respectively. In all three cases, values were obtained from temperature sliding window
711 analyses and ranked along the minimum temperature gradient.

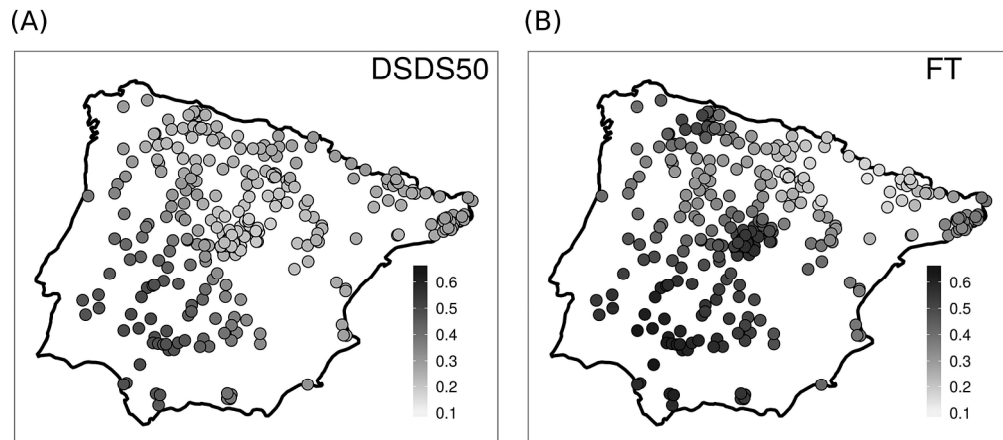


Fig. 1. (A) Map showing the geographical position of accessions indicating their GWR local adjusted pseudo-R2 values for DSDS50 and minimum temperature. (B) Map showing the geographical position of accessions indicating their GWR local adjusted pseudo-R2 values for FT and minimum temperature.

Fig. 1

297x209mm (300 x 300 DPI)

review

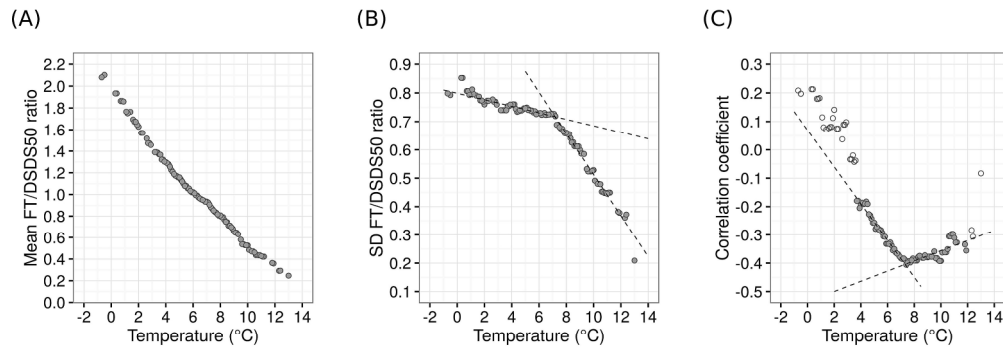


Fig. 2. (A) Relationship between minimum temperature and the mean of the FT/DSDS50 ratio. (B) Relationship between minimum temperature and the standard deviation of the FT/DSDS50 ratio. Regression lines are given for accessions below and above the turning point at 7.2°C, which maximises the R² values of the two groups of accessions. (C) Relationship between minimum temperature and the correlation coefficients between FT and DSDS50. Filled dots indicated significant correlation coefficients based on Dutilleul's modified t test. Regression lines are given for accessions with significant correlation coefficients below and above the turning point at 7.3°C, which maximises the R² values of the two groups of accessions. In all three cases, values were obtained from temperature sliding window analyses and ranked along the minimum temperature gradient.

Fig. 2

297x209mm (300 x 300 DPI)

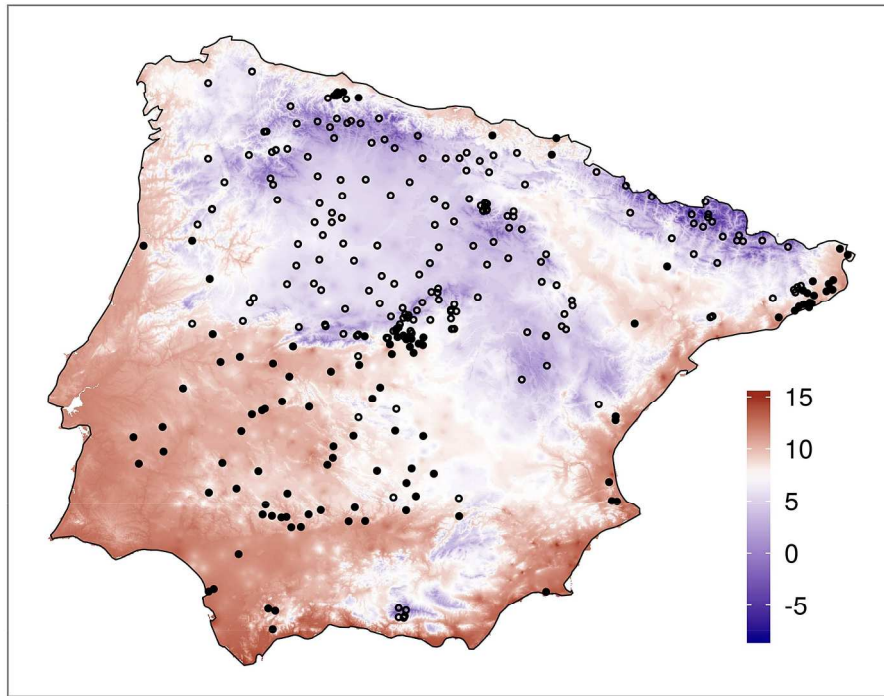
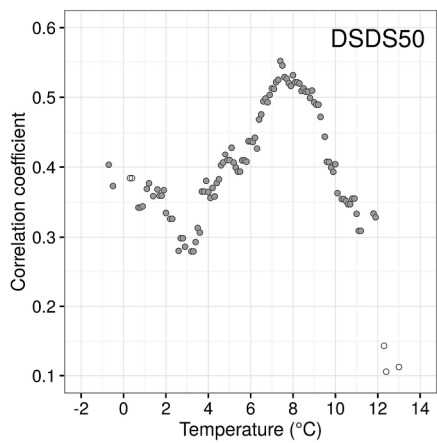


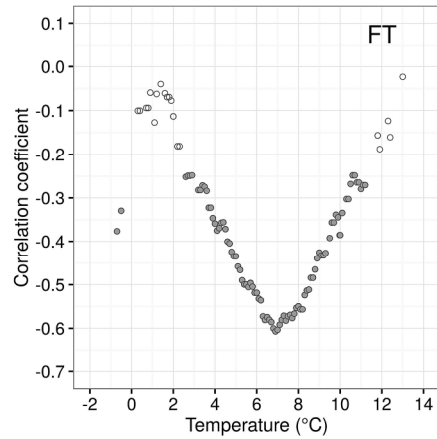
Fig. 3. Minimum temperature map for the Iberian Peninsula indicating the geographical position of *A. thaliana* populations with minimum temperatures below (hollow dots) and above (filled dots) the turning point of 7.3°C for the correlation between FT and DSDS50 along the minimum temperature gradient (see Fig. 2C).

Fig. 3
297x209mm (300 x 300 DPI)

(A)



(B)



297x209mm (300 x 300 DPI)

Review